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STATE OF THE ART REVIEW

Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications

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ABSTRACT

Resistance of the Enterobacteriaceae to antibiotics, especially of the β lactam type, is increasingly dominated by the mobilization of continuously expressed single genes that encode efficient drug modifying enzymes. Strong and ubiquitous selection pressure has seemingly been accompanied by a shift from "natural" resistance, such as inducible chromosomal enzymes, membrane impermeability, and drug efflux, to the modern paradigm of mobile gene pools that largely determine the epidemiology of modern antibiotic resistance. In this way, antibiotic resistance is more available than ever before to organisms such as *Escherichia coli* and *Klebsiella pneumoniae* that are important causes of major sepsis. Modulation of the phenotype by host bacteria makes gene transmission less obvious and may in part explain why tracking and control of carbapenem resistance has been particularly problematic in the Enterobacteriaceae. This review discusses the underlying principles and clinical implications of the mobility and fixation of resistance genes and the exploitable opportunities and potential threats arising from apparent limitations on diversity in these mobile gene pools. It also provides some illustrative paradoxes and clinical corollaries, as well as a summary of future options.

Introduction

Antibiotic resistance typically occurs within a few years of the introduction of a new antibiotic. Such resistance is not surprising because most modern antibiotics are derived directly or indirectly from microbial products. To mitigate this problem pre-existing resistance mechanisms may be identified in target pathogens even before the introduction of a new antibiotic.[1] Bacterial DNA sequences in the human gut indicate the presence of proteins that are similar to important antibiotic resistance enzymes,[2] and genes that are similar to "modern" antibiotic resistance genes are found in the environment and in samples dating back millions of years.[3 4] This suggests an almost unlimited capacity within the global microbiome to resist any new antibiotic, which existed long before the evolution of modern humans (fig 1).

This review will focus on the management and control of antibiotic resistance in medically important Gram negative bacteria, particularly *Escherichia coli* and *Klebsiella pneumoniae*, which are the main agents implicated in severe sepsis and septic shock. It will examine the mechanisms by which resistance becomes established in bacterial populations and the basic principles underlying rational management, including antimicrobial stewardship and infection control surveillance. The review deals mainly with resistance to β lactam antibiotics because of the continuing clinical importance of these agents, particularly for the management of severe infection, and the emerging threat of carbapenem resistance in the Enterobacteriaceae (genera such as *Escherichia*, *Klebsiella*, *Citrobacter*, *Serratia*, *Salmonella*, *Enterobacter*, and *Proteus*). References to comparator bacteria illustrate important differences; table 1 provides a classification of common pathogenic Gram negative bacteria and the agents used to treat associated infections.

Sources and selection criteria

The references used in this review were identified through PubMed and Medline searches of articles published between 1980 and 2015 and through our personal libraries. Search terms included "mechanisms" and "resistance", "ecology" and "resistance", "horizontal gene transfer", and "treatment" and "carbapenem resistance". We prioritized early and definitive references, but cited recent high quality reviews when multiple references were relevant. We prioritized references relating to those antibiotic resistance mechanisms associated with *E coli* and *K pneumoniae* and those that are known to be transmissible and most pertinent to the widely used aminoglycoside and β lactam antibiotics, particularly third generation cephalosporin and carbapenem antibiotics.

Incidence and prevalence

Antibiotic resistance in human isolates

The 2014 World Health Organization report on global resistance provided a snapshot of Africa, the Americas, the eastern Mediterranean, Europe, South East Asia, and the western Pacific.[5] The carriage of extended spectrum β lactamases (ESBLs; see Glossary) confers resistance to

GLOSSARY AND ACRONYMS

Antibiotic selection pressure: The pressure to adapt that is exerted on bacterial populations by antibiotics that threaten their survival

β lactams and β lactamases: β lactams are the most widely used type of antibiotic (penicillins, cephalosporins, and related compounds such as carbapenems), especially in hospitals. Recognition of increasingly widespread resistance to the early drugs was followed by chemical modification of the penicillins and the introduction of new classes such as cephalosporins (first, second, third, and later "generations"), cephamycins (often grouped with second generation cephalosporins), monobactams (such as aztreonam), and carbapenems (such as meropenem). The third generation cephalosporins (such as cefotaxime, ceftriaxone, ceftazidime) are the archetypal "extended spectrum β lactam" antibiotics. The functional Ambler classification divides β lactam hydrolyzing enzymes into class A (such as extended spectrum β lactamases), class C (AmpC enzymes), and class D (OXA) serine proteases and the Ambler class B metalloenzymes (such as NDM). By convention, β lactamase enzymes are described in upper case (for example, CTX-M-15) and their genes in appended subscript to an italicized lower case *bla* prefix (for example, $bla_{CTX\text{-}M\text{-}15}$)

Biofilms: Bacterial growth style of an extracellular matrix plus organisms (often multiple species) that display multiple phenotypes and growth characteristics

Breakpoint/MIC/MBC: Breakpoints are used to define antibiotic susceptibility and resistance in clinical microbiology. The lowest antibiotic concentration at which bacterial growth is inhibited in optimal conditions in vitro is the minimal inhibitory concentration (MIC) and that at which no viable cells can be recovered is the minimal bactericidal concentration (MBC). If the MIC for a given drug-bacterial combination does not exceed the predefined standardized susceptibility breakpoint, the bacteria are deemed to be susceptible.

Conjugative plasmid: Large (>60 kbp) replicative extrachromosomal element (usually circular) that encodes the capacity to be transmitted to receptive bacterial cells

Constitutive promoter: A region of DNA upstream of a gene that induces expression of that gene at a constant level; this is a common feature of mobile genetic elements that have "captured" a resistance gene (see Gene capture)

CRE and CPE: The terms CRE (carbapenem resistant Enterobacteriaceae) and CPE (carbapenemase producing Enterobacteriaceae) are not synonymous. Production of carbapenemases may not result in clinically relevant carbapenem resistance. Similarly, β lactamases not regarded as typical carbapenemases may produce carbapenem resistant phenotypes in certain settings

CTX-M: Most common class of ESBL enzymes, recognized for cefotaxime (CTX) hydrolysis and first isolated in Munich

Derepressed mutant: A cell containing a mutation that decreases the repression of gene expression; this is important in certain Enterobacteriaceae that produce AmpC enzymes

ESBL (extended spectrum β lactamases): The classic ESBLs are Ambler class A enzymes capable of hydrolyzing third generation cephalosporin antibiotics. The term is sometimes more loosely applied to any enzyme that hydrolyzes third generation cephalosporins including members of all other classes of β lactamases even, arguably, those known for hydrolyzing carbapenems, the carbapenemases

Epidemiological cut-off (ECOFF): The upper limit of the normal distribution of MICs in a given bacterial population (see breakpoint/MIC/MBC)

Fitness costs: The general adverse consequences for growth and survival that may result from a mutation or adaptation to a specific circumstance (such as the acquisition, loss, or alteration of an important gene or genes)

Gene capture: Certain genes seem to have been excised from their original genetic locus by genetic elements that promote excision from the chromosome or by packaging in a way that results in the mobilization of that gene into a transmissible gene pool and, usually, efficient expression of the protein encoded by that gene. See also "Mobile genetic elements"

Homologous recombination: Nucleotide sequences are exchanged between highly similar or identical DNA molecules, sometimes introducing new unrelated DNA sequences that are flanked by these regions of highly similar DNA

Inocula and the inoculum effect: The inoculum is the starting amount of viable bacteria in any growth environment; the effectiveness of many antibiotics varies with the inoculum, with larger inocula having seemingly higher MICs than smaller inocula. This "inoculum effect" is the result of a high density of bacteria producing extracellular antibiotic resistance proteins (such as hydrolyzing enzymes) that concentrate locally to destroy more antibiotic than individual cells or smaller inocula could. In the laboratory testing (unlike in vivo), the inoculum for susceptibility testing is standardized, partly for this reason

KPC: the *Klebsiella pneumoniae* carbapenemase is found in particular *K pneumoniae* strains and is generally associated with high levels of antibiotic resistance in those strains

MBL/metallo β lactamases: The Ambler class B family of cation (usually zinc) dependent enzymes (such as IMP, imipenemase; NDM, New Delhi metallo β lactamase) hydrolyze most β lactam-type molecules, including cephamycins (second generation cephalosporins) and carbapenems (such as meropenem) but usually not monobactams (such as aztreonam)

Microbiota: The entire population of microorganisms within a given ecological system, such as the gut or the ocean

Microbiome: The entire microbe associated genetic content within a given ecological system, including genes and non-gene elements

Mobile genetic elements: Transposons and insertion sequences and similar small genetic units capable of transferring DNA such as antibiotic resistance genes

Plasmid host range: The diversity of bacterial types that a given plasmid can enter and stably reproduce within; this is therefore a key determinant of plasmid epidemiology

Plasmid replicon: The reproductive unit, typically referring to those genes specifically dedicated to plasmid replication; specific differences are used to define plasmid (replicon) types and incompatibility groups

Plasmid incompatibility: The mutual intolerance of similar plasmids in a given cell, predictable largely on the basis of replicon type, and commonly used to group plasmids (for example, IncF, IncI)

Plasmid addiction systems: Bacterial toxicity upon plasmid loss after cell division (sometimes called post-segregational killing, PSK) is commonly a result of long acting residual toxins that are more stable than plasmid provided antitoxins (sometimes called toxin-antitoxin, TA, systems). Plasmid addiction systems are widespread in conjugative plasmids and are relatively predictable for a given replication (incompatibility) type; they are important in stabilizing antibiotic resistance traits in the accessory genome of bacteria

Porins: Protein channels in the outer membrane that permit transport of solutes, often by passive diffusion, and constitute an important gateway to the cell from the outside

many penicillins and cephalosporins (table 2) and often co-occurs with mechanisms that confer resistance to other types of antibiotics. The prevalence of ESBL-type resistance in both *E coli* and *K pneumoniae* varies widely across countries (fig 2) and is presumably related to factors such as antibiotic availability and restriction, waste and water management, and the general standard of living and healthcare.[5-8]

Some parts of the world report that 60% or more of *E coli* and *K pneumoniae* are resistant to important β lactam hospital antibiotics, such as third generation cephalosporins (for example, cefotaxime), and travelers regularly import resistance into countries with lower prevalence.[28-31] The use of β lactam and aminoglycoside antibiotics (such as gentamicin) has long been widespread and resistance has been managed with

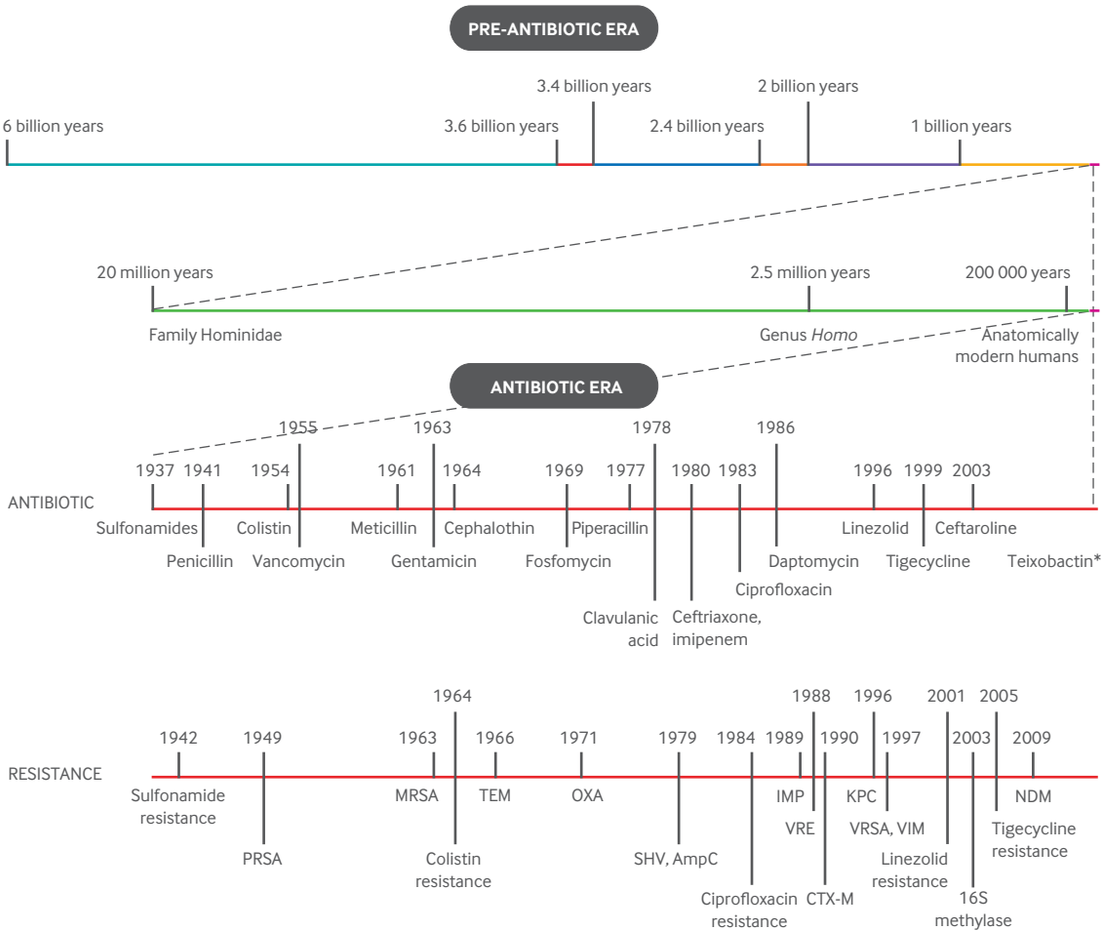

Fig 1 | Timeline of bacterial evolution. The introduction of each antibiotic has been quickly followed by recognition of an adaptation (including a phenotype or mechanism, as marked on resistance timeline) to resist it. *Not in clinical use. AmpC=ampicillin hydrolyzing; CTX-M=cefotaximase, first identified in Munich; IMP=imipenemase; KPC=*Klebsiella pneumoniae* carbapenemase; MRSA=meticillin resistant *Staphylococcus aureus*; NDM=New Delhi metallo β lactamase; PRSA=penicillin resistant *S aureus*; TEM, SHV, and OXA=common and diverse groups of β lactam hydrolyzing enzymes; VRE=vancomycin resistant enterococci; VRSA=vancomycin resistant *S aureus*; VIM=Verona integron encoded metallo β lactamase

carbapenems (such as meropenem and doripenem) and fluoroquinolones (such as ciprofloxacin and levofloxacin).

Fluoroquinolone resistance is now also present in at least half of reported clinical isolates of *E coli* from many locations around the world however,[32] and some European, South East Asian, and eastern Mediterranean regions report similar prevalence rates for carbapenem resistance.[5] The co-occurrence of resistance to extended spectrum β lactams, carbapenems, aminoglycosides, and fluoroquinolones is well described.

Even in countries with strict controls on antibiotic use and where antibiotic resistance is uncommon, resistance seems to be rising. In Canada and Australia, the prevalence of ESBL phenotypes in *E coli* has roughly doubled from around only 3% five years ago.[33-35] Ciprofloxacin resistance in clinical isolates of *E coli* increased from 5.4% in 2010 to 6.9% in 2012 in Australia, and from 21% to 27% between 2007 and 2011 in Canada, where fluoroquinolone usage has been somewhat higher than in Australia.[33-35]

Subgroups with high prevalence of resistance

"New" antibiotic resistance threats are often first recognized in travelers returning to countries with well developed health systems and laboratory diagnostics,[36] and screening of those returning after healthcare exposure is widely recommended.[29 37] Returning Dutch and Swedish international travelers were reported to have ESBL colonisation rates of 24%, from a very low baseline in those countries, and 10-fold higher colonisation rates of *E coli* that contained ESBL were reported in asymptomatic military personnel stationed in Afghanistan compared with those stationed in the United States.[38-40] Long term residents of aged care facilities, who have frequent exposure to healthcare and antibiotics and experience common cross-transmission of bacteria, also tend to have a high prevalence of antibiotic resistance.[41-45]

Much of the available antibiotic resistance data are from clinical (typically, hospital) contexts, in which antibiotic exposure and cross-transmission are expected to be increased, especially in resource limited settings. However, prevalence varies between populations in a

Table 1 | Taxonomy and treatment of infection with common pathogenic Gram negative bacteria

Phylum	Family	Example genus	Antibiotic classes active*	Commonly used antibiotics*
Proteobacteria	Enterobacteriales	*Escherichia*, *Klebsiella*	Penicillins, cephalosporins	Ampicillin, piperacillin, cephazolin, ceftriaxone
		Enterobacter, *Citrobacter*, *Serratia*	Carbapenems, aminoglycosides, fluoroquinolones	Meropenem, gentamicin, ciprofloxacin
	Pseudomonales	Pseudomonas	Penicillins, carbapenems, aminoglycosides, fluoroquinolones	Piperacillin, meropenem, gentamicin, ciprofloxacin
	Moraxellaceae	Acinetobacter	Penicillins, carbapenems	Piperacillin, meropenem
Bacteroidetes	Bacteroidaceae	Bacteroides	Nitroimidazoles, penicillins	Metronidazole, piperacillin

*Examples of most commonly used agents, in the absence of specific resistance. A β lactam inhibitor (such as tazobactam) is typically combined with piperacillin to overcome common β lactamases.

region. For example, recent studies of urine samples from asymptomatic patients report no carbapenem resistance and that only 20% of *E coli* and less than 10% of *K pneumoniae* had ESBL-type resistance in rural settings near Delhi,[46] where high levels of antibiotic resistance are commonly reported in clinical isolates. This has implications for the design of surveillance and it implies that antimicrobial stewardship and infection control initiatives still have much to offer.

Antibiotic resistance in isolates from environmental and agricultural sources

Antimicrobials in animal husbandry and the effluent and waste from hospitals and factories are probably important drivers of resistance.[47] The feces of humans and animals contaminate the environment, and drinking and environmental water supplies may harbor highly resistant *E coli* in both resource poor and rich countries.[48-54] Antibiotic resistant human pathogens are also common in food and in food chain animals,[55-61] and household pets may carry similar multi-resistant isolates to humans.[62 63] Wild animals are often affected,[64] particularly scavengers such as seagulls, which are important vectors of antibiotic resistance including in known human pathogens.[65-67]

Defining antibiotic resistance

The designation of a pathogen as antibiotic susceptible or resistant is a key role of the diagnostic microbiology laboratory. This is done primarily by defining the minimum inhibitory concentration (MIC; see Glossary) at which bacterial growth is inhibited under standardized conditions in vitro.[68] Consensus "breakpoints" define susceptible, resistant, and (sometimes) intermediate MIC ranges for specific bacteria. They are based on whether the MIC can be reliably achieved in a patient with regard to drug dosing (pharmacokinetics) and mode of action (pharmacodynamics) and could therefore be reasonably expected to result in therapeutic success.[69] These decisions may be further informed by the determination of an "epidemiological cut-off" (see Glossary), which describes the normal distribution of the MIC within a population.[70 71]

A resistant MIC predicts antibiotic failure but a susceptible MIC is no guarantee of success. Therapeutic failure despite in vitro susceptibility can be caused by reduced antibiotic penetration or activity (in sites such as abscesses or beyond the blood-brain barrier) and sometimes by high local concentrations of a hydrolyzing enzyme produced by dense populations of bacteria (the "inoculum effect"; see Glossary).[71] In addition, bacteria are generally most vulnerable to antibiotics when they are rapidly growing and metabolizing (for example, in the diagnostic laboratory) because most antibiotics target these processes. Unfortunately, however, the growth stage of an organism in a clinically important infection may differ greatly from that in which antibiotic susceptibility is determined in vitro.

Bacterial biofilms (see Glossary) are common in the clinical environment on abiotic surfaces including catheters and implanted prostheses, as well as infected natural surfaces such as bone, cartilage, and heart valves. Biofilms often contain populations in growth phases

Table 2 | Selected important β lactamases in the Enterobacteriaceae

Commonly affected β lactams	Common terminology	Examples	Clinical inhibitors	Laboratory inhibitors	Effective β lactams	Commonly implicated species
PEN, 1GC	Penicillinases (Ambler class A)	TEM, SHV	Clavulanate, tazobactam, sulbactam, avibactam	Clavulanate, tazobactam, sulbactam	TZP, 3GC, 4GC, MEM	*E coli* (P), *Klebsiella spp* (C) (P)
PEN, 1GC 3GC, ATZ	ESBLs (Ambler class A)	CTX-M			MEM, (TZP, 4GC)*	ESBL: most medically important Enterobacteriaceae (P)
PEN, 1GC, 2GC† 3GC, ATZ	AmpC β lactamases (Ambler class C)	CMY-2† (P); inducible AmpC (C)	Avibactam	Boronic acids, cloxacillin	MEM, (TZP, 4GC)*	AmpC: *Enterobacter*, *Serratia*, *Citrobacter*, *Providencia*, *Morganella*, *Pseudomonas*, *Acinetobacter* (C), most medically important Enterobacteriaceae (P)
PEN, 1GC, 2GC† 3GC	OXA-48-type‡ β lactamases (Ambler class D)	OXA-48/181‡	Avibactam	None	(MEM, ATZ)*	OXA, MBL, KPC: most medically important Enterobacteriaceae (P)
PEN, 1GC, 2GC† (MBL)* 3GC, 4GC MEM	*K pneumoniae* carbapenemases (Ambler class A)Metallo β lactamases (Ambler class B)	KPC	Avibactam	Boronic acids	(MEM)*	OXA‡, MBL: *Pseudomonas*, *Acinetobacter* (C)
		IMP, VIM, NDM	ATZ	EDTA, mercaptopurine, dipicolonic acid	ATZ, (MEM)*	

Abbreviations: 1/2/3/4GC=1st/2nd/3rd/4th generation cephalosporins; AmpC=ampicillin hydrolyzing enzymes; ATZ=monobactam antibiotics (such as aztreonam); CMY-2=cephamycinase; CTX-M=cefotaximase, first identified in Munich; ESBLs=extended spectrum β lactamases; IMP=imipenemase; KPC=*Klebsiella pneumoniae* carbapenemase; MBLs=metallo β lactamases; MEM=carbapenems (such as meropenem); NDM=New Delhi metallo β lactamase; PEN=penicillins; TEM, SHV, and OXA=common and diverse groups of β lactam hydrolyzing enzymes; TZP=penicillin β lactamase inhibitor combinations (such as piperacillin-tazobactam); VIM=Verona integron encoded metallo β lactamase; (P)/(C), commonly plasmid/chromosomally encoded.
*May vary with enzyme or drug combination or coexisting mechanisms that contribute to the phenotype.
†Cephamycins, often grouped with 2GC, are hydrolyzed by MBLs and AmpC β lactamases (with the exception of ACC). Hydrolysis of cephamycins is used to help differentiate AmpC enzymes from ESBLs in the diagnostic laboratory.
‡Most OXAs do not hydrolyze MEM, but *Acinetobacter* has class D (OXA) mediated carbapenem resistance and OXA-48 is an emerging problem in the Enterobacteriaceae.

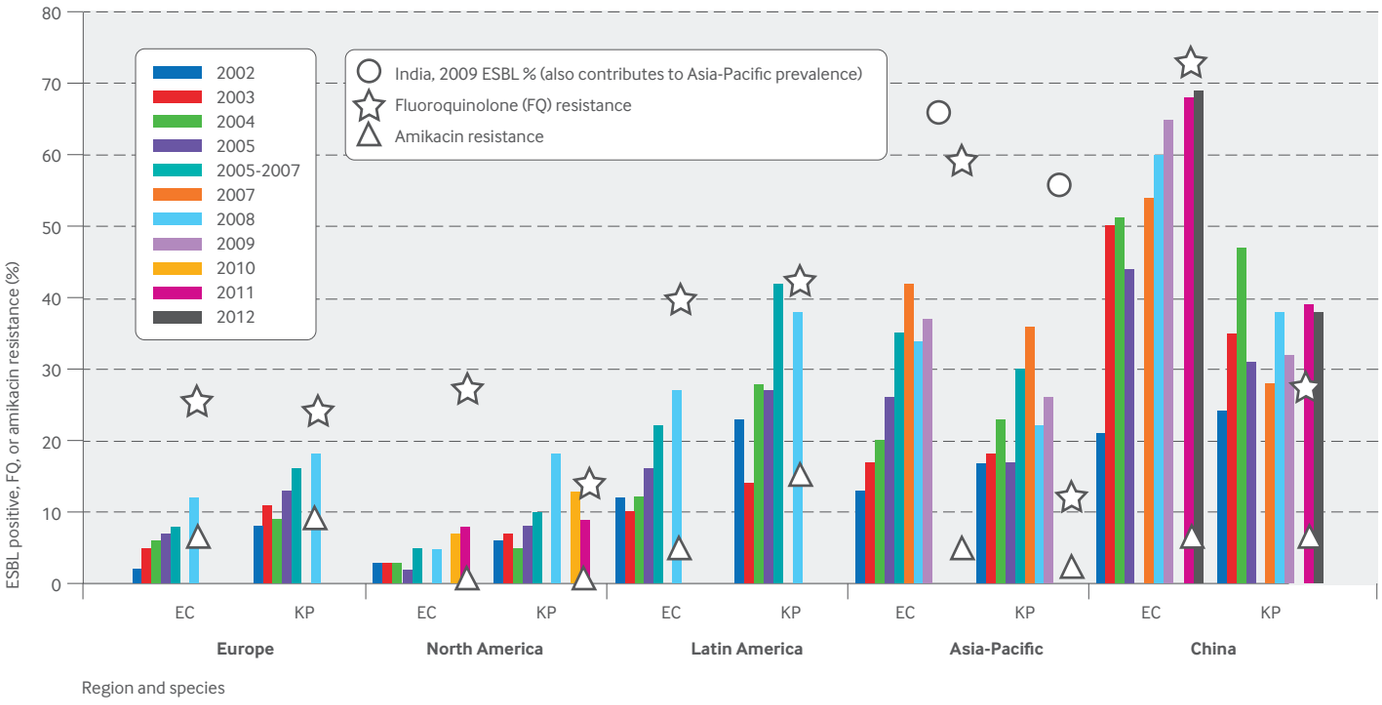

Fig 2 | Antibiotic resistance by region. Increasing rates of extended spectrum β lactamase (ESBL) carriage in *Klebsiella pneumoniae* (KP) and *Escherichia coli* (EC) and high rates of fluoroquinolone resistance are seen in all regions, whereas amikacin resistance remains relatively rare. Data were collated from the SMART studies of intra-abdominal and urinary tract infection.[9-26] Fluoroquinolone and aminoglycoside resistance data for the Asia-Pacific region come from Mendes and colleagues[27]

that make them less vulnerable to antibiotics that target growth and remodeling processes. This disparity between the susceptibility of vegetative organisms and those in biofilms often results in relapse of infection a few days after antibiotics are stopped, typically after a good initial response (fig 3).[72 73]

Bacterial adaption in Gram positive and Gram negative bacteria

Clinical outcomes have been most closely related to the MIC, rather than the minimum bactericidal concentration (MBC) beyond which viable cells cannot be recovered in standardized protocols with moderate inocula (see Glossary), but adaptive change occurs at all levels of exposure that do not result in bacterial death. There may be great differences between MIC and MBC, which vary with organism, drug, and growth conditions as different populations arise among the progeny of a single organism to suit changed conditions.[74 75] Acquired antibiotic resistance thus results from normal adaptive capacities that are a balance of compromises or evolutionary trade-offs,[76-78] and that do not necessarily require acquisition of new genetic material.

Bacterial strategies for coping with antibiotic selection pressure (see Glossary) include target modification, drug exclusion or expulsion, and drug modification. Table 3 and fig 4 compare these strategies for some of the important and common antibiotic classes used for medically important Gram negative and positive bacteria. The Gram stain highlights important biological differences that make adaptive strategies more predictable for given organism-drug combinations. Gram positive organisms have a simpler peptidoglycan-rich cell wall than Gram negative bacteria, and their lifestyles are often more externalised (for example, *Staphylococcus aureus* persists on dry surfaces). By contrast, Gram negative bacteria typically have a lifestyle in which liquid phase motility, the management of permeable channels through their hydrophobic outer envelope, and the exchange of information (including genetic information) with near neighbors may be much more important.

Role of the outer envelope in Gram negative bacteria

Gram negative bacteria that tolerate an environment in which external toxins are abundant and osmotic pressures variable (such as *Acinetobacter baumannii, Pseudomonas aeruginosa*) are usually much less permeable than members of the Enterobacteriaceae that operate competitively in dense communities in the relatively protected environment of the mammalian gut. Transmembrane pumps are important for coping with drugs that act intracellularly (such as aminoglycosides, quinolones, tetracyclines, and macrolides) but provide little protection against drugs such as β lactam antibiotics, which act in the periplasmic space between the inner cytoplasmic (plasma) membrane and the hydrophobic outer membrane.[79]

A range of relatively non-specific pores (porins) in the hydrophobic outer membrane are the main barrier to β lactam antibiotics in Gram negative bacteria (fig 4). The outer membrane of environmentally hardy *A baumannii* is around seven times less permeable to carbapenem and cephalosporin antibiotics than that of *P aeruginosa* and up to 100 times less permeable than *E coli*, largely due to

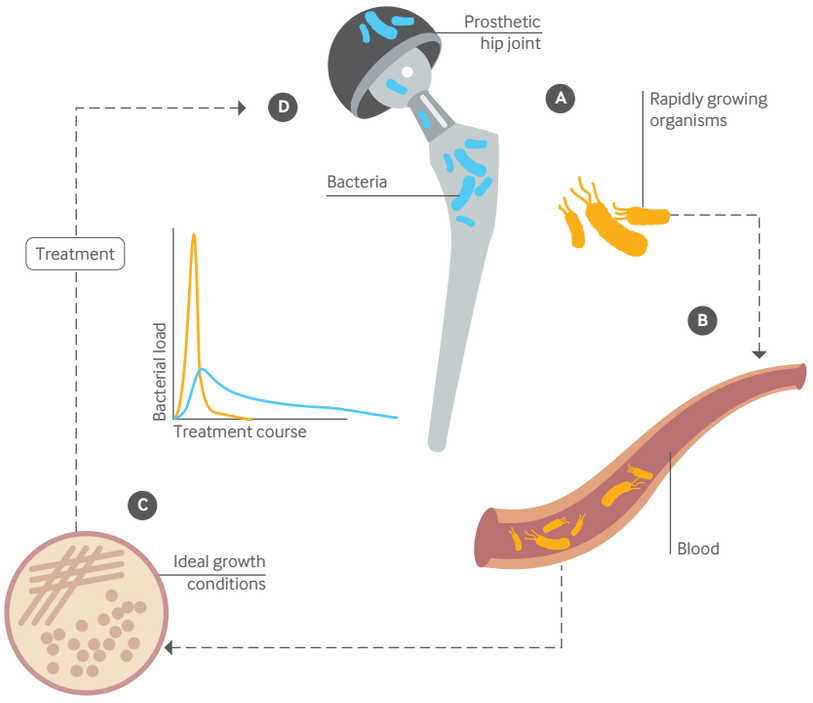

Fig 3 | Differences in growth states and antibiotic susceptibility. Bacteria in a biofilm state (blue bacteria) growing on a prosthetic hip joint (A) release occasional rapidly growing organisms (yellow) into the blood (B). Antimicrobial susceptibility testing of these isolates in ideal growth conditions (C) may overestimate the susceptibility of organisms in the biofilm. This is because rapidly growing bacteria are easily killed by standard therapy (yellow curve, centre) whereas bacteria in less susceptible growth states may survive initial therapy (blue curve, centre). The shape of these curves and the associations between them vary with bacterial and drug types

differences in the type and number of outer membrane porins.[80] Such differences have a predictable effect on antibiotic resistance strategies (fig 5).[80 81] In general, Gram negative organisms best adapted to survive in hospital drains or on equipment (*Acinetobacter, Pseudomonas*, and even *Enterobacter* spp) are most likely to combine reduced outer membrane permeability and periplasmic hydrolysis with efflux systems[82] to produce strong resistance phenotypes (fig 5).

Intrinsic mechanisms of drug modification

Drug modification is a major mechanism of resistance. Chromosomally encoded penicillin hydrolyzing (ampicillin hydrolyzing, AmpC) enzymes have general structural similarities to the ubiquitous penicillin binding proteins that have an important role in cell wall remodeling. These enzymes are commonly found in medically important Gram negative bacteria in the orders Enterobacteriales and Pseudomonadales. Chromosomal AmpC enzymes are variably induced on exposure to β lactam antibiotics, such as cephalosporins, and other agents such as aztreonam. AmpC enzymes are less active against some modified penicillins such as oxacillin, but are also less effectively inhibited by the classic β lactamase inhibitors (such as clavulanate).

Strong AmpC inducing antibiotics that are also good AmpC substrates (such as ampicillin, first generation cephalosporins) are clearly ineffective in vitro and therefore create no confusion when tested in the diagnostic laboratory. By contrast, weak inducers that are relatively poor substrates (such as third generation cephalosporins) can appear effective in vitro but occasionally fail in vivo due to selection of derepressed mutants (see Glossary) that significantly overproduce the enzyme compared with wild-type organisms (see ECOFF in Glossary).

Chromosomal AmpC induction is complex and variable,[83] but the important clinical point is that derepression-type mutations that lead to stable high level expression of AmpC-type enzymes are not unusual and can lead to clinical failure.[84] Consequently, relevant authorities urge vigilance for the development of resistance to cephalosporins while treating infections caused by *Enterobacter*, *Serratia*, and *Citrobacter* spp in particular. These bacteria are often reported by microbiology laboratories as resistant despite in vitro susceptibility, on the presumption that this mechanism is present.[85]

Acquired mechanisms for drug modification

A range of genes encoding drug modifying traits have emerged into the mobile gene pool, expressed from constitutive promoters (see Glossary) at similar and apparently optimized levels, and many move easily between bacteria like *E coli* and *K pneumoniae*.[86] Despite the broad protection that accrues from other mechanisms such as drug exclusion or specific removal from the cell, even environmentally adapted bacteria like *Acinetobacter* and *Pseudomonas* spp commonly acquire additional drug modifying traits in this way, implying that this is an efficient adaptive strategy.

The means of horizontal transfer of genetic traits varies with bacterial lifestyle. For example, marine organisms such as *Vibrio cholerae* are likely to receive advantageous traits from formally packaged bacterial viruses (transduction by bacteriophages).[87-89] However, organisms that live in the human gastrointestinal tract may be more likely to directly take up free DNA (transformation) or plasmids, including self transmitting (conjugative) plasmids (see Glossary) (fig 4).[90]

Resistance genes are made available in this gene pool after being mobilized from the chromosome of a range of organisms through the "copy and paste" replication mechanisms of small genetic elements such as transposons, integrons, and insertion sequences (see Glossary: "mobile genetic elements").[91-93] The subsequent transfer of these gene packages into efficient vehicles such as conjugative plasmids allows their dissemination into pathogenic strains, provided the donor and recipient are "ecologically linked" through a shared habitat (such as the gastrointestinal tract) or a chain of organisms that link the donor to the final organism (fig 6).[94 95]

The "capture" of the original gene in this way is rare and its subsequent mobilization on to a suitable vehicle such as a plasmid equally so. The ultimate success of a gene in the mobile gene pool relates to its capacity for transfer to other successful mobile vehicles and the access of these vehicles into successful host bacteria.[71 97 98] This enhanced mobility (for example, within a conjugative plasmid) is crucial to the success of a resistance gene.[99] Mutations that subsequently occur within the resistance gene may not be competitive enough to

Table 3 | Different resistance mechanisms for three important antibiotic classes

Important mechanisms	Typical examples	Comment
β lactam-type antibiotics		
Drug modification	β lactamases	Often transmissible within a mobile gene pool (P); phenotype may be augmented by reduced permeability or efflux
Membrane permeability (C)	OprD (for IPM in *Pseudomonas aeruginosa*); OmpK36 (*Klebsiella pneumoniae*)	Important for β lactam resistance in less permeable organisms and for carbapenem resistance in Enterobacteriaceae
Efflux (P)	MexAB-OprM (for MPM in *P aeruginosa*)	Efflux pumps are generally more important in environmentally adapted organisms than in typical Enterobacteriaceae; substrate specificity may also be important
Aminoglycosides		
Drug modification	Specific acetylases, aminoacyl transferases, and phosphotransferases	Enterobacteriaceae such as *Escherichia coli* (P); others such as *P aeruginosa,Acinetobacter* (C)
Target modification	16S rRNA methylases (such as ArmA, Rmt enzymes)	Enterobacteriaceae and environmental organisms both significantly affected; very high-level resistance (P)
Efflux	AcrD (*E coli*); MexXY (*P aeruginosa*)	Environmental organisms affected to a greater extent than Enterobacteriaceae (C)
Fluoroquinolones		
Target modification	DNA gyrase (*gyrA*) mutation; topoisomerase IV (*parC*) mutation	Enterobacteriaceae and environmental organisms both significantly affected (C)
Target mimicry	Pentapeptide repeat proteins (Qnr proteins)	Enterobacteriaceae and environmental organisms (transmissible *qnr* genes) (P)
Efflux	AcrAB-TolC (C), QepA (P)	Enterobacteriaceae and environmental organisms (non-transmissible, except QepA)

MPM=meropenem and IPM=imipenem (carbapenem antibiotics); (P)/(C)=usually plasmid/chromosomally encoded.

persist.[100] Alternatively, they may give rise to more successful variants—for example, the widespread bla_{TEM} and $bla_{CTX\text{-}M}$ gene families seem to have evolved in situ after their initial mobilization to confer a broad and diverse range of advantageous phenotypes.[101 102]

The rarity of successful gene capture and mobilization events constitutes an ecological bottleneck (fig 6) that results in a relatively small pool of genes that can be acquired and transmitted to and between human pathogens.[94 103] As a result, some resistance genes are globally disseminated whereas others are never seen more than once in a clinical setting. For example, genes encoding resistance to third generation cephalosporins (www.lahey.org/Studies) or aminoglycosides are diverse and numerous,[104] but few are commonplace,[105] and this enables genotypic tests with high predictive values.[106 107]

The development of multi-resistance regions

Resistance to several antibiotics can be carried on individual resistance plasmids or on the chromosome. The accumulation of resistance genes around an initial insertion event in a region of DNA that acts as a "founder element" creates dynamic and diverse multi-resistance regions in chromosomes and plasmids.[91] There are two important corollaries of the accretion of resistance genes into multi-resistance regions, both of which promote their ecological success. The first is that the increasing prevalence of common genetic sequences may increase the ease with which new resistance genes are incorporated—for example, by homologous recombination (see Glossary) or as a result of their enrichment with gene capture systems (see Glossary). The second is that each multi-resistance region has pluripotent resistance potential, so that exposure to one drug selects for resistance to many.[98]

For example, gentamicin resistance genes are commonly found with the ESBL gene $bla_{CTX\text{-}M\text{-}15}$ on plasmids in *E coli*,[108] and treatment with gentamicin will therefore often (co-)select organisms that are resistant to both gentamicin and extended spectrum β lactams. This makes the choice of an alternative antibiotic to minimize selection for a particular gene or phenotype increasingly difficult, but it is essential to understand these associations to aid effective antimicrobial stewardship.

Implications for infection management and control

Variable expression of resistance traits

Porins, efflux pumps, and inducible AmpC enzymes, are actively regulated for optimal efficiency, but horizontally acquired genes are generally constitutively expressed from promoters within the insertion sequence or gene capture system itself.[86] Acquired aminoglycoside modifying enzymes produce a clinically important aminoglycoside resistant phenotype as reliably in *E coli* as in *P aeruginosa*, but this is not so for other resistance traits. For example, variants of the quinolone resistance trait QnrB are common on antibiotic resistance plasmids in *E coli* but do not produce a clinically significant quinolone resistant phenotype alone.[109] This "conditional" phenotype is commonly associated with enzymes that hydrolyze β lactams, particularly, carbapenem antibiotics, and is an important consideration for most contemporary antibiotic prescribers.

Most major β lactamases such as the CTX-M-type ESBL enzymes hydrolyze their primary targets (for example, cefotaxime) so well as to result in clinically important antibiotic resistance without the need for additional mechanisms that remove the antibiotic from the bacterial cell or restrict antibiotic entry to the cell. Consequently, they are predictably associated with a clinically significant level of antibiotic resistance even in highly antibiotic permeable species such as *E coli*. MIC variations all exceed the susceptibility breakpoint but the variations in MIC normally go unreported by diagnostic laboratories, the organisms being described simply as either "susceptible" or "resistant."

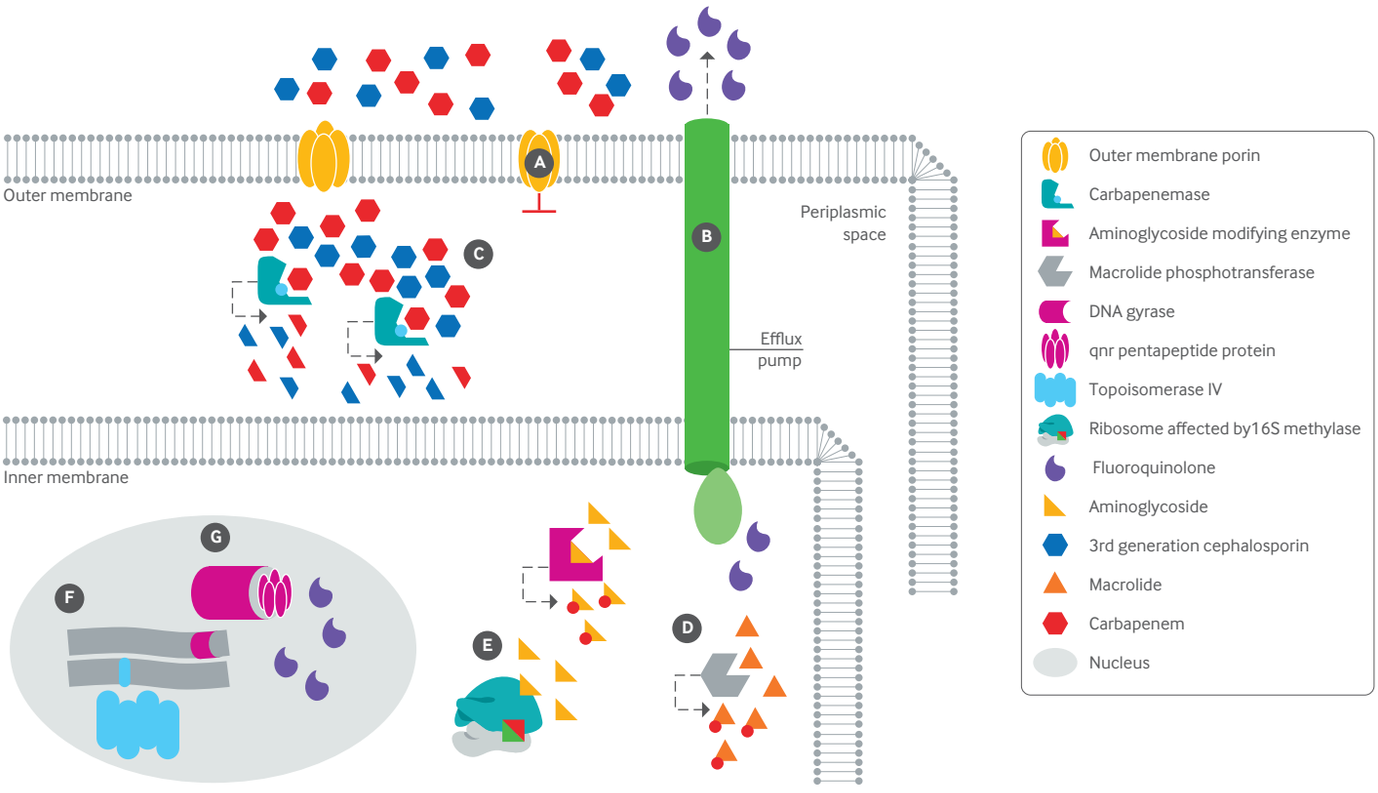

Fig 4| Important mechanisms of antibiotic resistance in the Enterobacteriaceae. Porin deficiencies or alterations (A) reduce antibiotic access and efflux pumps (B) may actively transport antibiotics out of the cell. β lactamases (C) acting in the periplasmic space hydrolyse β lactam antibiotics and thereby prevent disruption of the cell wall. Intracellular (for example, aminoglycoside modifying) enzymes (D) alter antibiotics. 16S rRNA methylases (E) prevent aminoglycoside binding. Mutations in targeted DNA gyrase and topoisomerase IV genes (F) render fluoroquinolones ineffective. Pentapeptide Qnr proteins (G) prevent fluoroquinolones from effectively binding to DNA gyrase through target mimicry

This dichotomous reporting creates confusion in the case of carbapenemase producing Enterobacteriaceae. For example, in *E coli* and *K pneumoniae* that produce the metallo β lactamase (MBL) enzyme IMP-4, the MIC for carbapenem antibiotics is commonly below the breakpoint value, except in the presence of an augmenting factor (such as a porin defect).[110 111] Similarly, the OXA-24 enzyme is associated with marked carbapenem resistance in low permeability *A baumannii* but not with levels of antibiotic resistance in *E coli* that would suggest a risk of treatment failure.[112] By contrast, the "*K pneumoniae* carbapenemase" (KPC) enzyme is almost always linked to marked carbapenem resistance, and this is possibly because it is most commonly found in *K pneumoniae* strains in which this phenotype is augmented (see below).

In addition, structural similarities between carbapenems and other β lactam antibiotics mean that many carbapenems are susceptible to attack by ESBL and AmpC enzymes, although not very efficiently and usually not resulting in clinically relevant increases in carbapenem MICs that would be expected to be associated with treatment failure. This means that although carbapenemases generally result in much higher carbapenem MICs in the same host strain, ESBL and AmpC enzymes expressed in a porin deficient host may be more common causes of clinically significant carbapenem resistance where carbapenemases are rare.

The plasticity of the "accessory genome"

Bacteria that frequently share genes often do so through the exchange of plasmids, which provide access to the enormous genetic potential within the microbiome in places such as the human gastrointestinal tract.[2 113-116] In addition to common small non-mobilizable plasmids, this "accessory genome" often includes several different large, transmissible, low copy number plasmids of 60-200 kb in each bacterial cell, even in wild animals without particular antibiotic resistance.[117] This accessory genome thus comprises 10% or more of the total genome of species such as *E coli* and *K pneumoniae*. Many of these plasmids are conjugative (self transmissible) or mobilizable (with the help of a conjugative plasmid). Conjugative plasmids can quickly convert life threatening bacteremic sepsis from being antibiotic susceptible to resistant after only one or two antibiotic doses by direct acquisition of the plasmid by the pathogen or by expansion of subpopulation(s) in which that plasmid resides.[118]

Discussion of the relatedness of plasmids and of more specific approaches to define relationships between plasmids[119] is beyond the scope of this review but the plasmid replicon type (see Glossary) can be regarded as a key determinant of both (in)compatibility and of host range (that is, the capacity for a plasmid to become stably established in a given bacterial population). Mutual incompatibility of plasmids that share the same replication system has been used for decades as a convenient typing

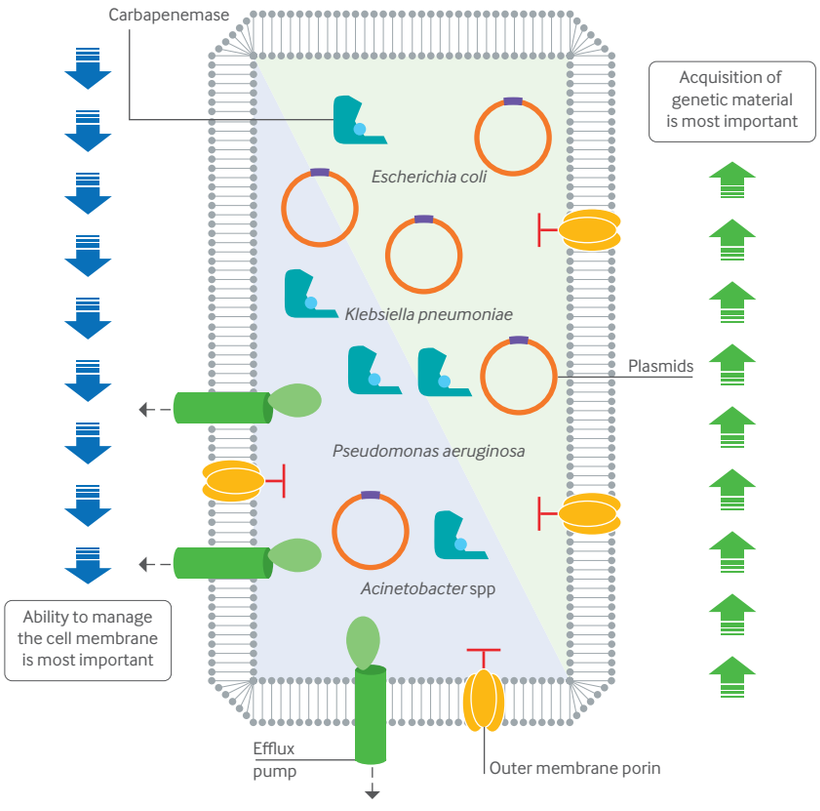

Fig 5| Variable resistance strategies in Gram negative bacteria. Organisms living in toxic or occasionally hyperosmolar environments often have mechanisms for restricting antibiotic influx and exporting antibiotics. By contrast, those that live in a relatively less noxious and more stable environment in the gut may be more adapted to compete by acquiring genetic material from neighbors. The antibiotic resistance mechanisms in a given bacterial type can be predicted to some extent by its lifestyle

scheme, and polymerase chain reaction (PCR) based replicon typing is a popular surrogate.[120] Plasmid stability and "addiction" systems (see Glossary) that ensure persistence of the plasmid in a bacterial population are linked to the replicon itself, and the presence of specific plasmid addiction systems can generally be predicted by replicon type (see "Ecological fixation of antibiotic resistance" section).

The natural plasmid complement of a given bacterial cell does not necessarily contain antibiotic resistance genes, but these may be acquired from any DNA locus (including another plasmid) as part of a mobile genetic element that has originally "captured" the gene from elsewhere. The original source of a gene in the mobile pool is not always known, however, and may be distant in time and environmental context from that of the bacterial population in which it is first recognized.

Successful genes, plasmids and clones

bla$_{CTX\text{-}M\text{-}15}$

The most successful of the ESBL genes, *bla*$_{CTX\text{-}M\text{-}15}$, seems to have been captured in its original form from *Kluyvera ascorbata*, a member of the Enterobacteriaceae that is rarely pathogenic in humans, by a mobile genetic element,[121 122] along with a small amount of other genetic material. It is efficiently expressed by a promoter that resides within the element that initially captured it, in this case the insertion sequence IS*Ecp1*. There are probably many reasons for its global spread,[101] because it is found on different plasmid types and on the chromosome in pathogenic *E coli* strains.[123-125] However, its success is due at least in part to an association with highly successful uropathogenic *E coli* (such as sequence type (ST)131) subclones that also harbor chromosomal fluoroquinolone resistance,[124 126-128] as well as its presence on IncF-type plasmids that are particularly common in *E coli*.[129 130] The development of a PCR based assay for rapid detection of *E coli* subtype ST131, for example, has added value because of the strong associations between known pathogenic strains and subtypes and resistance determinants that are tracked for the purposes of treatment and infection control.[131]

K pneumoniae ST258 and the KPC carbapenemase

Another example is that of *K pneumoniae* ST258 and the KPC carbapenemase.[132] The strong epidemiological association between gene and bacterium may relate to plasmids that are relatively *Klebsiella* specific (for example, InFII$_K$-type) on which the KPC resistance gene is commonly found. It is important to note that pathogenic *K pneumoniae* subtypes that carry the KPC gene[133-136] have often not only lost a functional OmpK35 "matrix" porin but also have a potentially important variation in the OmpK36 outer membrane "osmo" porin that is expected to augment the resistance phenotype.[134 137] *E coli* carrying the KPC gene are much less resistant than *K pneumoniae* ST258 with the same gene when both are present together.[134] KPC gene transmission between strains, species, and patients may therefore go undetected by phenotypic screening methods, as discussed later, although the spread of KPC probably relates primarily to its successful association with the *Klebsiella* strains in which the carbapenem resistance phenotype is marked.

Mobility within and between common pathogenic species such as *K pneumoniae* and *E coli* is also seen for transmissible carbapenem resistance traits linked to outbreaks in many countries.[138-140] However, the carbapenem resistant phenotype is disproportionately over-represented in *K pneumoniae* compared with *E coli*,[141] perhaps by up to 20-fold.[142] Relevant factors may include the relative ease with which reduced permeability is tolerated in the Klebsiellae and the ecological connectivity to soil and other environments from which "new" resistance genes may emerge.

Ecological fixation of antibiotic resistance

An important question is whether the persistence of resistant bacteria in the environment is due to low level antibiotic contamination, non-antibiotic selection, the stability of the resistance genes and transfer elements, or a combination of these factors.[143] Antibiotics in environments that are associated with humans (rivers, wastewater treatment plants, hospitals, aquaculture, farms), even at low concentrations, select for resistant organisms (and associated elements: plasmids, mobile genetic elements, genes),[144 145] and a range of determinants of success are to some extent common to all such adaptations in prokaryotic and eukaryotic systems.[146]

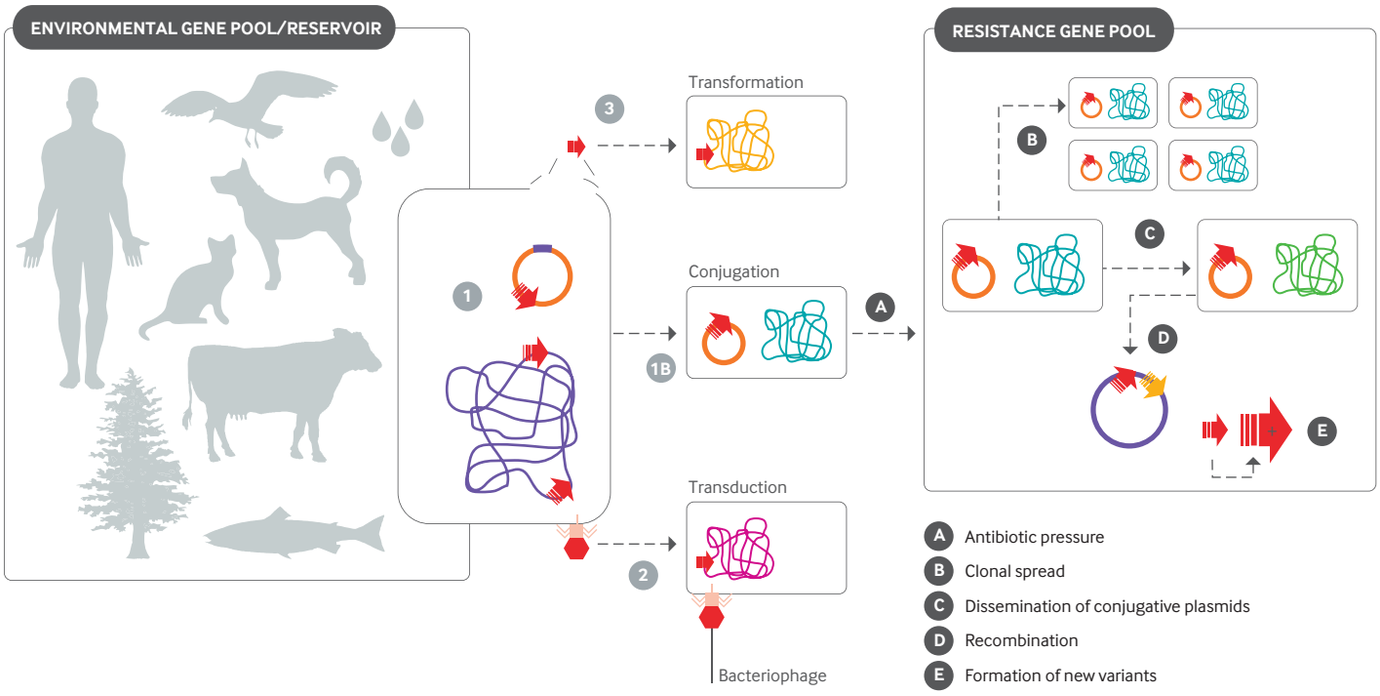

Fig 6| Ecology of antibiotic resistance. The processes of horizontal gene transfer—transduction, transformation, and conjugation—mobilize genes between different environments. Rare gene capture events mediated by small mobile genetic elements mobilize the gene (for example, from chromosome to plasmid or conjugative transposon; 1), which may in turn transfer to a new bacterial cell (1B). Packaging and transfer of a resistance gene by bacteriophages (transduction; 2) is probably very rare. Uptake of free DNA by competent bacteria (transformation; 3) may also occur. Under antibiotic pressure, resistant organisms have a selective advantage and become part of the resistance gene pool (A). They may then spread as a successful clone (B) from which conjugative plasmids may be disseminated widely (C). Small mobile elements may move by formal mechanisms or by simple recombination between multi-resistance regions (D) in plasmids or chromosomes. Rare mutations that arise in existing resistance genes can generate new variants with activity against many different antibiotics (E)[96]

Resistant organisms clearly persist asymptomatically in the microbiome without antibiotic selection, but travelers have been shown to clear these organisms over a few months after returning to a country with low resistance rates.[147 148] In settings where the prevalence of resistance is generally high, continued fecal carriage is more likely,[149] and some strains seem to be more likely to persist than others.[150] Thus, the likelihood of resistant organisms (or plasmids) being simply replaced by antibiotic susceptible organisms (or plasmids) of the same type and thereby diluted into the local microbiota is low when the background prevalence of antibiotic resistance or of organisms adapted to human colonization is high.

Important unknowns include the extent to which bacteria and plasmids lose redundant genes or promote delivery of strongly selected genes to more secure genomic locations—for example, well adapted plasmids or the chromosome. There may be critical thresholds relating to fitness costs (see Glossary), the background reservoir of diverse non-resistant plasmids, and the content of the resistance gene pool, beyond which a return to antibiotic susceptibility is unlikely even if antibiotic usage stopped completely.[151]

Reduced bacterial "fitness" is often described as a disadvantage of resistance plasmid carriage, but measures of "fitness" are highly context specific and are often defined in terms of growth rates in optimal conditions in vitro. Under these conditions the carriage of resistance plasmids may impose little fitness cost on a bacterial host or a cost that is quickly ameliorated and fitness even enhanced.[152-154] As indicated above, large, low copy, conjugative resistance plasmids stabilize themselves in bacterial populations by ensuring effective partitioning at cell division and by directly poisoning populations from which the plasmid is lost (plasmid "addiction").[155] These addiction systems are common in acquired genetic elements in bacteria and are widely recognized in conjugative plasmids.

An example of the type of addiction systems found in these large plasmids is the combination of a stable toxin and a more labile or shorter acting antitoxin, so that cell death occurs if the plasmid is lost and the antitoxin can no longer be produced.[156] Even small resistance plasmids without systems to ensure partitioning of plasmids into each new bacterial cell after division (or to kill off any new bacterial cells in which this fails) seem to co-evolve with bacterial populations to ensure persistence in the absence of antibiotic selection.[157 158]

It may therefore be helpful to consider the entire population of a single bacterial strain type as a genetic ecosystem, within which certain genetic niches are occupied by plasmids representing mutually exclusive examples of each various type. The main ecological parameters to consider in this paradigm are:

- Host range (the capacity to become established in different bacterial populations) of conjugative elements such as plasmids
- The mutual associations and incompatibilities of these conjugative elements
- The advantages for host bacteria that are associated with the acquisition and loss of these conjugative elements

Table 4 | Guidelines for surveillance and testing: patient screening and identification

Guideline	All admissions	High risk patients	Outbreak response	Surveillance sites	Laboratory method
CDC	Not specified	Not specified	Contacts	Stool, perirectal, perineal or inguinal, urinary catheters, wounds	Phenotypic
Australian	Not specified	Yes, including recent antibiotic therapy	Contacts	Multiple sites, including rectal and perianal	Not specified
ESCMID	During outbreaks	Consider weekly	Admission, discharge, and weekly	Stool, perirectal, perineal or inguinal, urinary catheters	Phenotypic

CDC=Centers for Disease Control and Prevention; ESCMID=European Society of Clinical Microbiology and Infectious Diseases.

Table 5 | Guidelines for carbapenemase surveillance and testing: isolate screening and identification

Guideline	MIC susceptibility breakpoints	Isolates for referral	Laboratory method
CLSI	MPM: ≤1 µg/mL, EPM: ≤0.5 µg/mL, IPM: ≤1 µg/mL	Intermediate or resistant to at least one carbapenem	Modified Hodge test for epidemiological and infection control purposes; susceptibility for clinical isolates according to clinical breakpoint MIC
EUCAST	MPM*: ≤2 µg/mL, EPM: ≤0.5 µg/mL, IPM ≤2 µg/mL (I)	Any isolate with MPM or EPM MIC >0.12 µg/mL or IPM MIC >1 µg/mL is recommended for further testing	Multiple combined disk tests to determine specific enzymatic basis or carbapenem hydrolysis (for example, CarbaNP), or both

Abbreviations: CLSI= Clinical and Laboratory Standards Institute; EPM=ertapenem; EUCAST=European Committee on Antimicrobial Susceptibility Testing; IPM=imipenem; MPM=meropenem; MIC=minimum inhibitory concentration.
*MPM advised for optimal balance of sensitivity and specificity.

- The capacity of host bacteria to ameliorate any associated fitness costs
- The capacity of host bacteria to exchange and recombine genetic material

In the case of the typical large conjugative and addictive antibiotic resistance plasmids, they are relatively fixed in bacterial accessory genomes. The dynamics of this system are key determinants of the epidemiology of modern transmissible antibiotic resistance in Gram negative bacteria, particularly the Enterobacteriaceae. The general concept of genetic ecology could be applied equally to other mobile genetic elements and the ecological constraints on their dissemination.

Surveillance guidelines

Despite the variation in antibiotic resistance that may be associated with host strain context, phenotypic screening for acquired resistance is often highly effective, especially in conjunction with genetic methods, when the transmissibility of the resistance trait is limited. When the transmissible resistance trait is widely disseminated among different species and the association between its presence and the particular phenotype breaks down, direct detection of the transmissible trait is needed.

Detection and sampling of transmissible carbapenem resistance

Currently the most problematic area seems to be that of detection of transmissible carbapenem resistance traits in the Enterobacteriaceae. It is important to distinguish between the detection of carbapenemase producing Enterobacteriaceae (CPE) that contain a carbapenem hydrolyzing enzyme and the carbapenem resistant Enterobacteriaceae (CRE) that have a non-susceptible MIC when considering this.

Existing guidelines for clinicians and laboratories on how best to efficiently detect and track transmissible carbapenemases (tables 4 and 5) are based on limited data and vary significantly.[85 159-162] Screening of a large patient group is time consuming and costly. Targeted screening of at risk people is a compromise that detects those most likely to carry resistant organisms and who are most at risk of adverse consequences. Available guidelines emphasize the need for screening contacts in the outbreak setting but vary with regard to screening of all high risk patients (Australian) or even all admissions (European Society of Clinical Microbiology and Infectious Diseases; ESCMID).

One pragmatic approach is simply to screen only for organisms that are evidently antibiotic resistant in vitro (such as KPC producing *K pneumoniae*, OXA-23 producing *A baumannii*) as would normally be done for non-transmissible resistance in other bacteria. This means that a transmissible trait will go undetected in organisms in which it produces only a low level of antibiotic resistance (such as IMP -producing *K pneumoniae* and KPC producing *E coli*), allowing a reservoir of transmissible resistance to go undetected. Such traits may then emerge unpredictably in different strains that have high MICs and may not respond to antibiotic treatment. Some authorities therefore contend that an acquired carbapenemase should always be reported, even in the context of a susceptible MIC.[163] Clearly, we need more detailed genetic epidemiological data for risk-benefit calculations that best inform cost effective screening policies.

The apparently limited diversity of the shared gene pool results in a high negative predictive value for targeting genetic traits that are known to be successful, but this approach will obviously not detect novel mechanisms.[106 107] Currently, major laboratory guidelines advise phenotypic investigation and confirmation of isolates that reach MIC thresholds associated with therapeutic failure.[85 159]

Detection of carbapenemase genes in the Enterobacteriaceae may be improved by lowering the MIC threshold of concern (for example, using a lower carbapenem concentration in growth media), by using surrogate phenotypes (for example, using cefotaxime resistance to detect MBL-type carbapenemases), or by using associated traits (such as co-transmitted aminoglycoside resistance). The ideal associated traits for screening purposes are closely genetically linked and consistently expressed. For example, high level amikacin resistance is unusual in most countries but is commonly associated with plasmid borne bla_{NDM}.[164] Such resistance should be regarded as a reason to test for bla_{NDM} in an organism such as *E coli* in which the carbapenem resistant phenotype may not be very striking.

Unfortunately, associated traits are too often absent from strains of interest or too often present in other strains to be reliable. Traditional susceptibility testing to screen for organisms that are resistant or nearly resistant to a particular antibiotic may help reduce the number of candidate organisms that need to be subjected to further testing by more direct (such as genetic) methods and a combined approach is probably optimal. Testing for specific hydrolytic capacity is also a useful direct method and has the obvious advantage of detecting the phenotype caused by a novel gene, but it may be less sensitive than specific nucleic acid detection for known genes.[165 166]

Monitoring the mobile resistance gene pool

When considering an outbreak of antibiotic resistant bacteria it is crucial to determine the mechanism(s) of dissemination. At one extreme is a resistance mutation in *Mycobacterium tuberculosis* that is attributable to a single nucleotide change, which creates a new clonal variant that subsequently spreads. The transmission chain is effectively tracked by comparative genomic analyses (the highest resolution of which is to determine the entire DNA sequence of each genome) because exchange of resistance genes is relatively unimportant.[167] A phenotype from a gene that tends to be linked to a specific bacterial type (such as KPC in ST258 *K pneumoniae*) can also be informatively studied by whole genome sequencing to obtain a transmission chain and to detect potential emerging bacterial types.[168 169]

At the other extreme is highly mobile plasmid borne resistance with a broad host range among the Enterobacteriaceae, in which the pattern of mobility determines the epidemiology of transmission. The adaptive capacity of bacteria is greatly enhanced by ready access to the large gene pool. For example, CMY-2 AmpC-like β lactamases constitutively (permanently) expressed from plasmids have become the dominant local source of an AmpC phenotype in *E coli*.[106 170 171] Once accessible to human pathogens through the shared mobile gene pool, dissemination of resistance trait(s) can proceed rapidly. Sharing of genetic traits is expected to be less common between distantly related organisms (such as *Acinetobacter* and *Escherichia* spp) than between members of the Enterobacteriaceae, which share many plasmid types.[172] Nevertheless, the plasmid host range (the range of bacterial types capable of receiving and supporting a given plasmid; see above) may include completely different classes of bacteria,[173] and plasmids play a vital role in connecting diverse species and ecosystems and in disseminating antibiotic resistance traits widely.[174]

The role of the bacterial host in increasing the prevalence of antibiotic resistance in a population, especially if that host is already successful in a particular ecological space for reasons other than antibiotic resistance, was discussed above for *E coli* ST131 with $bla_{\text{CTX-M-15}}$ and *K pneumoniae* ST258 with bla_{KPC}.[136] Horizontal plasmid transfer may also be enhanced when specific bacterial populations expand in the context of gut inflammation or antibiotic treatment.[175 176] A complete epidemiological picture of a mobile resistance trait would ideally contain detailed information about:

- The genetic context of the resistance gene(s) of interest (associated genes and mobile genetic elements, such as insertion sequences and transposons; whether the trait is likely to be present as a single gene or within a complex genetic unit)
- The vehicle(s) in which the resistance trait is present (for example, plasmid type and host range or whether it is only present on the chromosome)
- The prevalence of permissive strains (bacteria that could receive this gene or its vehicle and augment the phenotype through features such as porin variation(s)).

Microbiota and gene pools should be monitored within hospital contexts and also within the community. It is important to understand the interactions between these groups, especially dilution of resistance in the gut microbiota by incoming "healthy" microbes, and the policy implications for antimicrobial stewardship and infection control (see "Detection and sampling of transmissible carbapenem resistance" section).

The relations between specific vehicles of the resistance gene pool (especially conjugative plasmids) and the bacterial subtypes in which they are detected in antimicrobial resistance surveys (see "Antibiotic resistance in human isolates" section above) may be an important epidemiological determinant that is as yet poorly understood.

The role of antibiotic stewardship

Drug companies have turned away from unprofitable antibiotic development, even as international calls for action highlight the need for new antibiotics and for antimicrobial stewardship strategies "to preserve the integrity and effectiveness of the existing antimicrobial armamentarium."[5 177] Unfortunately, less than 25% of blood cultures are positive even in severe sepsis,[178] and timely information is often unavailable. Reliable and robust point of care diagnostics for sepsis and antibiotic resistance are essential tools to develop.

Antibiotic resistance rates may fall with reduced prescribing,[179] and the potential for appropriate antimicrobial stewardship to reduce antimicrobial resistance and improve individual patient outcomes is obvious.[180-183] However, assumptions that "narrow spectrum" antibiotics (defined in terms of medically important bacteria) have less impact on the microbiota have not been well tested and some of these assumptions may be incorrect.

It is commonly thought that "narrower spectrum" third generation cephalosporins are preferable to "broader spectrum" carbapenems or even piperacillin-tazobactam in clinical situations in which either would be equally effective. However, data relating to the ecological impact on the microbiota suggest that third generation cephalosporins and cefepime are more likely to result in subsequent infection with *P aeruginosa*, ESBL producing Enterobacteriaceae, and multiply resistant *S aureus* and *Clostridium difficile*.[184-186] It is currently unclear exactly what "appropriate" antibiotic prescribing is, and translational research is urgently needed in this area.[187] Indeed, it has been argued that an aggressive antibiotic based curative policy promotes the resistance it aims to avoid.[188] Our approach to antibiotic stewardship probably needs to be much more sophisticated.

The paradox of selective gut decontamination

Selective decontamination of the digestive tract uses non-absorbable antibiotics in the oropharynx and gut (selective oral decontamination) plus four days of intravenous antibiotics (usually the third generation cephalosporin, cefotaxime). This approach results in overall reduced rates of antibiotic usage, and the reported absolute overall mortality benefit of 3-6% exceeds that of other widely accepted medical interventions (such as urgent

angioplasty for myocardial infarction).[189 190] However, it is not widely practised outside the areas in which it is championed because of concerns about the development of antibiotic resistance in countries where the background prevalence rate of antibiotic resistance is high.[191] It may be that the additive effect of the intravenous antibiotic is the least important component,[192] and the use of a drug such as piperacillin-tazobactam might not have the adverse ecological effect that is expected from cefotaxime.

Emerging treatments

Management of ESBL infection has been well reviewed including the contentious issue of extended spectrum penicillin-β lactamase inhibitors (such as piperacillin-tazobactam) instead of carbapenems.[193] Consequently, we will focus again on CRE as the most challenging clinical problem.

Non-carbapenem based regimens are traditionally preferred for treating infections with CRE.[194] However, many carbapenemase producing isolates without an adjunctive resistance mechanism have carbapenem MICs that are only one or two dilutions above the clinical breakpoint, and are achievable in vivo. Observational studies of treatment of infections with CRE have looked at a heterogeneous mix of organisms and patients, and carbapenem containing regimens have been shown to confer a mortality benefit in the context of a MIC that is not conspicuously resistant.[195 196] Extended infusion regimens may restore efficacy even when the in vitro MIC is slightly above the resistance breakpoint,[197] and dual carbapenem therapy may be considered for KPC producing Enterobacteriaceae using ertapenem as an additional (sacrificial) substrate to saturate or overwhelm the capacity of the KPC enzyme to hydrolyze the principal therapeutic carbapenem.[198-200] The benefits of dual β lactam therapy warrant review,[201] whether they accrue from synergistic action at a single target site or from targeting different penicillin binding proteins (as for enterococcal therapy with ampicillin and cefotaxime or ceftriaxone).[202]

Newer antibiotics

New antibiotic classes for which resistance is expected to be slow or difficult to develop are welcome arrivals.[203] Agents that suppress virulence characteristics,[204-206] rather than kill the microbe, should also select less strongly for resistance and are attractive candidates for co-administration with current antimicrobials,[207-211] although none is immediately available.

Tigecycline

Tigecycline is a glycylcycline antibiotic in the tetracycline class that was approved in 2005 for intra-abdominal infections as well as complicated skin and skin structure infections. However, pharmacodynamic and pharmacokinetic properties make it less suitable as monotherapy for serious intra-abdominal infection and hospital acquired pneumonia.[212-214] Resistance is not uncommon among the Enterobacteriaceae, especially in species such as *Proteus* spp, which may exhibit carbapenem resistance on acquisition of a carbapenemase gene: a UK study of CRE showed less than half to be susceptible to tigecycline.[214]

Avibactam

Avibactam is a novel non-β lactam β lactamase inhibitor with activity against common KPC and "classic" ESBL enzymes (Ambler class A), AmpC enzymes (Ambler class C), and OXA-48 (Ambler class D), but not MBLs (Ambler class B). Ceftazidime-avibactam has performed well in phase II trials of intra-abdominal infection (with metronidazole) and urinary tract infection,[215] and the addition of avibactam to aztreonam has promise against MBLs such as NDM-1 (New Delhi metallo β lactamase 1), in which ESBL enzymes that would otherwise simply hydrolyze the aztreonam are common.[216]

Eravacycline

Eravacycline, a novel fluorocycline antibiotic, withstands usual tetracycline resistance mechanisms, has broad activity against enteric Gram negative pathogens including multi-resistant isolates and anaerobes, and may be as safe and effective as ertapenem in complicated intra-abdominal infection.[217 218]

Plazomicin

Plazomicin is a novel aminoglycoside that is resistant to modification by most currently described transferases and acetylases but is ineffective in the presence of the 16S rRNA methylases that are commonly acquired with NDM.[219 220] Other potential novel agents and inhibitors are discussed elsewhere.[221 222]

Antimicrobial peptides

Antimicrobial peptides are generally short amino acids that kill bacteria by multiple mechanisms, including membrane pore formation,[223 224] and they are synergistic with traditional antibiotics.[225] Although the fact that these peptides have multiple targets theoretically protects against the development of resistance, they are part of the innate immunity of plants and animals and multiple resistance mechanisms have been recognized.[226] Improvements in bioavailability and stability, and reduced toxicity, are needed to make them part of the deliverable antimicrobial armamentarium of the future.[223]

Older antibiotics

Colistimethate sodium

Colistimethate sodium (colistin), first described in 1954, interacts with lipopolysaccharide in the bacterial outer membrane. It is bactericidal in a concentration dependent manner and early establishment of adequate tissue levels by giving "loading doses" may increase efficacy and reduce nephrotoxicity (which affects 10-30% of recipients and may be related to total cumulative dose).[227 228] This drug may be comparable to tigecycline and carbapenems,[195] with which it is used in combination (or with aminoglycosides) as well as in monotherapy for infections caused by CRE.[228] However, dosing is difficult and clinically apparent resistance increases with drug exposure.[228 229] New compounds with similar bactericidal activity may be less toxic but show cross resistance with colistimethate sodium,[230 231] and a plasmid borne resistance trait has recently been recognized.[232]

Fosfomycin

Fosfomycin acts by inhibiting peptidoglycan synthesis in the cytosol.[233] The antibiotic is well tolerated and bactericidal levels are maintained in the urine for 72 hours after a single dose.[234] Intravenous fosfomycin has also been used successfully in critically ill patients, although almost always in combination with another active agent.[235 236] Resistance due to decreased uptake, target site, or drug modification may emerge during treatment.[233]

Non-antibiotic treatments

Lytic bacteriophages (phages)

Lytic bacteriophages (phages) are bacterial viruses that were discovered before penicillin [237-239] and developed in Soviet Russia after the second world war while antibiotics and antibiotic resistance developed in the West.[240] They are often highly target specific and synergistic with antibiotics,[241] and they may be valuable in antibiotic resistant and biofilm-type infections, infecting target bacteria and disappearing as they are consumed.[242] Empiric treatment usually requires a phage "cocktail" to overcome resistance, but problems associated with resistance, targeting, immunogenicity, and diffusion must be resolved before they can be used routinely.[241 243 244] Intrinsic bacterial defense systems such as the CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 attack complex, which ordinarily functions as an adaptive immune mechanism to combat bacteriophages and foreign DNA, can be re-engineered to specifically target resistance, virulence, or other autologous DNA sequences, including with phage delivery.[245-247]

Bacteriotherapy

Bacteriotherapy (fecal microbiota transplantation) to restore normal gut microflora is experiencing a resurgence of interest.[248-252] This interest has been driven largely by the increased incidence of severe *C difficile* associated diarrhea and colitis,[253 254] and by the recognition of opportunities for intervention in other diseases.[255] Certain antibiotics such as third generation cephalosporins have long been known to be associated with colonization and infection by opportunistic and antibiotic resistant pathogens including *C difficile*.[186] Differential colonization effects are evident within 48 hours of admission to intensive care,[185] and these antibiotics may be associated with less effective spontaneous recovery of phylum level balance within gut microflora compared with those without such associations.[184] A better understanding of the effect of probiotics and antibiotics on the gut microflora is therefore essential.[256] Increased understanding of the role of microbial production of, and competition for, key substrates and apparent successes in manipulating these dynamics with limited bacterial combinations to ameliorate dysbiosis hint at more elegant solutions than whole fecal microbiota transplantation in the future.[257-259]

Conclusions

Antibiotic resistance is a natural adaptive process in bacteria that pre-dates the evolution of modern humans but that may have been accelerated by the omnipresence of antibacterial compounds in medicine, agriculture, and the environment.[260] Public and clinical policy paradoxes in our approach to antibiotic stewardship and the prophylaxis of infection require a more sophisticated understanding of the effects of antibiotics on microbial populations and interactive microbial systems.

Current knowledge makes it easier to understand why carbapenem resistance in enteric bacteria still occasionally surprises us, given the complexities of co-selection, the phenotypically silent spread of mobile traits that encode it, and the variability of the bacterial factors that augment it. For now, our knowledge of the apparently restricted diversity in transmissible antibiotic resistance gene pools can be exploited for diagnostic and screening purposes. However, it highlights the possibility that mobile gene pools in major pathogens such as *E coli* and *K pneumoniae* may be being driven consistently toward loss of more diverse antibiotic susceptible elements, while the capacity to shed antibiotic resistance genes from the gene pool remains undefined. This in turn suggests the potential for an "ecological tipping point" in the mobile gene pool,[151] beyond which point the usual plasmids available to be acquired by our gut bacteria, even in the absence of any specific selection, may only be antibiotic resistance plasmids of limited diversity. It emphasizes the urgent need to better understand the pools of mobile resistance genes and their inter-relationships in host bacterial populations.

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1 Iredell J, Thomas L, Power D, et al. Tigecycline resistance in Australian antibiotic-resistant Gram-negative bacteria. *J Antimicrob Chemother* 2007;59:816-18.
2 Sommer MO, Dantas G, Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science* 2009;325:1128-31.
3 Forsberg KJ, Reyes A, Wang B, et al. The shared antibiotic resistome of soil bacteria and human pathogens. *Science* 2012;337:1107-11
4 Bhullar K, Waglechner N, Pawlowski A, et al. Antibiotic resistance is prevalent in an isolated cave microbiome. *PLoS One* 2012;7:e34953.
5 WHO. Antimicrobial resistance: global report on surveillance, 2014. http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf.
6 Geser N, Stephan R, Korczak BM, et al. Molecular identification of extended-spectrum-β-lactamase genes from Enterobacteriaceaeisolated from healthy human carriers in Switzerland. *Antimicrob Agents Chemother* 2012;56:1609-12.
7 Lonchel CM, Meex C, Gangoué-Piéboji J, et al. Proportion of extended-spectrum ß-lactamase-producing Enterobacteriaceae in community setting in Ngaoundere, Cameroon. *BMC Infect Dis* 2012;12:53.
8 Castanheira M, Farrell SE, Deshpande LM, et al. Prevalence of β-lactamase-encoding genes among Enterobacteriaceaebacteremia isolates collected in 26 US hospitals: report from the SENTRY antimicrobial surveillance program (2010). *Antimicrob Agents Chemother* 2013;57:3012-20.
9 Rossi F, Baquero F, Hsueh PR, et al. In vitro susceptibilities of aerobic and facultatively anaerobic Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: 2004 results from SMART (Study for Monitoring Antimicrobial Resistance Trends). *J Antimicrob Chemother* 2006;58:205-10.
10 Paterson DL, Rossi F, Baquero F, et al. In vitro susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART). *J Antimicrob Chemother* 2005;55:965-73.
11 Hawser SP, Bouchillon SK, Hoban DJ, et al. Epidemiologic trends, occurrence of extended-spectrum β-lactamase production, and performance of ertapenem and comparators in patients with intra-abdominal infections: analysis of global trend data from 2002-2007 from the SMART study. *Surg Infect* 2010;11:371-8.

12 Brink AJ, Botha RF, Poswa X, et al. Antimicrobial susceptibility of gram-negative pathogens isolated from patients with complicated intra-abdominal infections in South African hospitals (SMART Study 2004-2009): impact of the new carbapenem breakpoints. *Surg Infect* 2012;13:43-9.
13 Yang Q, Zhang H, Wang Y, et al. A 10 year surveillance for antimicrobial susceptibility of Escherichia coli and Klebsiella pneumoniaein community- and hospital-associated intra-abdominal infections in China. *J Med Microbiol* 2013;62(pt 9):1343-9.
14 Zhang H, Yang Q, Xiao M, et al. Antimicrobial susceptibility of Gram-negative bacteria causing intra-abdominal infections in China: SMART China 2011. *Chin Med J (Engl)* 2014;127:2429-33.
15 Yang Q, Wang H, Chen M, et al. Surveillance of antimicrobial susceptibility of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in China: the 2002-2009 Study for Monitoring Antimicrobial Resistance Trends (SMART). *Int J Antimicrob Agents* 2010;36:507-12.
16 Hoban DJ, Bouchillon SK, Hawser SP, et al. Susceptibility of gram-negative pathogens isolated from patients with complicated intra-abdominal infections in the United States, 2007-2008: results of the Study for Monitoring Antimicrobial Resistance Trends (SMART). *Antimicrob Agents Chemother* 2010;54:3031-4.
17 Hoban DJ, Lascols C, Nicolle LE, et al. Antimicrobial susceptibility of Enterobacteriaceae, including molecular characterization of extended-spectrum β-lactamase-producing species, in urinary tract isolates from hospitalized patients in North America and Europe: results from the SMART study 2009-2010. *Diagn Microbiol Infect Dis* 2012;74:62-7.
18 Ko WC, Hsueh PR. Increasing extended-spectrum β-lactamase production and quinolone resistance among Gram-negative bacilli causing intra-abdominal infections in the Asia/Pacific region: data from the Smart Study 2002-2006. *J Infect* 2009;59:95-103.
19 Hsueh PR, Snyder TA, Dinubile MJ, et al. In vitro susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region: 2004 results from SMART (Study for Monitoring Antimicrobial Resistance Trends). *Int J Antimicrob Agents* 2006;28:238-43.
20 Hawser S, Hoban D, Bouchillon S, et al. Antimicrobial susceptibility of intra-abdominal gram-negative bacilli from Europe: SMART Europe 2008. *Eur J Clin Microbiol Infect Dis* 2011;30:173-9.
21 Villegas MV, Blanco MG, Sifuentes-Osornio J, et al. Increasing prevalence of extended-spectrum-β-lactamase among Gram-negative bacilli in Latin America—2008 update from the Study for Monitoring Antimicrobial Resistance Trends (SMART). *Braz J Infect Dis* 2011;15:34-9.
22 Baquero F, Hsueh PR, Paterson DL, et al. In vitro susceptibilities of aerobic and facultatively anaerobic gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: 2005 results from Study for Monitoring Antimicrobial Resistance Trends (SMART). *Surg Infect* 2009;10:99-104.
23 Lu PL, Liu YC, Toh HS, et al. Epidemiology and antimicrobial susceptibility profiles of Gram-negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009-2010 results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). *Int J Antimicrob Agents* 2012;40(suppl):S37-43.
24 Hawser SP, Bouchillon SK, Hoban DJ, et al. In vitro susceptibilities of aerobic and facultative anaerobic Gram-negative bacilli from patients with intra-abdominal infections worldwide from 2005-2007: results from the SMART study. *Int J Antimicrob Agents* 2009;34:585-8.
25 Hawser SP, Bouchillon SK, Lascols C, et al. Susceptibility of European Escherichia coli clinical isolates from intra-abdominal infections, extended-spectrum β-lactamase occurrence, resistance distribution, and molecular characterization of ertapenem-resistant isolates (SMART 2008-2009). *Clin Microbiol Infect* 2012;18:253-9.
26 Chow JW, Satishchandran V, Snyder TA, et al. In vitro susceptibilities of aerobic and facultative gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2002 Study for Monitoring Antimicrobial Resistance Trends (SMART). *Surg Infect* 2005;6:439-48.
27 Mendes RE, Mendoza M, Banga Singh KK, et al. Regional resistance surveillance program results for 12 Asia-Pacific nations (2011). *Antimicrob Agents Chemother* 2013;57:5721-6.
28 Hilty M, Betsch BY, Bögli-Stuber K, et al. Transmission dynamics of extended-spectrum β-lactamase-producing Enterobacteriaceae in the tertiary care hospital and the household setting. *Clin Infect Dis* 2012;55:967-75.
29 Adler A, Shklyar M, Schwaber MJ, et al. Introduction of OXA-48-producing *Enterobacteriaceae* to Israeli hospitals by medical tourism. *J Antimicrob Chemother* 2011; published online 19 Sep; doi:10.1093/jac/dkr382.
30 Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β-lactamases. *Antimicrob Agents Chemother* 2010;54:24-38.
31 Wendt C, Schütt S, Dalpke AH, et al. First outbreak of Klebsiella pneumoniae carbapenemase (KPC)-producing K pneumoniae in Germany. *Eur J Clin Microbiol Infect Dis* 2010;29:563-70.
32 Doi Y, Park YS, Rivera JI, et al. Community-associated extended-spectrum β-lactamase-producing Escherichia coli infection in the United States. *Clin Infect Dis* 2013;56:641-8.
33 Lagacé-Wiens PR, Adam HJ, Low DE, et al. Trends in antibiotic resistance over time among pathogens from Canadian hospitals: results of the CANWARD study 2007-11. *J Antimicrob Chemother* 2013;68(suppl 1):i23-9.
34 Turnidge JD, Gottlieb T, Mitchell DH, et al. Community-onset Gram-negative Surveillance Program annual report, 2012. *Commun Dis Intell Q Rep* 2014;38:E54-8.
35 Turnidge JD, Gottlieb T, Mitchell DH, et al. Australian Group on Antimicrobial Resistance Community-onset Gram-negative Surveillance Program annual report, 2010. *Commun Dis Intell Q Rep* 2013;37:E219-23.
36 Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob Agents Chemother* 2009;53:5046-54.
37 Rogers BA, Aminzadeh Z, Hayashi Y, et al. Country-to-country transfer of patients and the risk of multi-resistant bacterial infection. *Clin Infect Dis* 2011;53:49-56.
38 Tängdén T, Cars O, Melhus Å, et al. Foreign travel is a major risk factor for colonization with Escherichia coli producing CTX-M-type extended-spectrum β-lactamases: a prospective study with Swedish volunteers. *Antimicrob Agents Chemother* 2010;54:3564-8.
39 Von Wintersdorff CJH, Penders J, Stobberingh EE, et al. High rates of antimicrobial drug resistance gene acquisition after international travel, the Netherlands. *Emerg Infect Dis* 2014;20:649-57.
40 Vento TJ, Cole DW, Mende K, et al. Multidrug-resistant Gram-negative bacteria colonization of healthy US military personnel in the US and Afghanistan. *BMC Infect Dis* 2013;13:1-11.
41 Shitrit P, Reisfeld S, Paitan Y, et al. Extended-spectrum β-lactamase-producing Enterobacteriaceae carriage upon hospital admission: prevalence and risk factors. *J Hosp Infect* 2013;85:230-32.
42 Rooney PJ, O'Leary MC, Loughrey AC, et al. Nursing homes as a reservoir of extended-spectrum β-lactamase (ESBL)-producing ciprofloxacin-resistant Escherichia coli. *J Antimicrob Chemother* 2009;64:635-41.
43 Luvsansharav UO, Hirai I, Niki M, et al. Fecal carriage of CTX-M β-lactamase-producing Enterobacteriaceae in nursing homes in the Kinki region of Japan. *Infect Drug Resist* 2013;6:67-70.
44 Oteo J, Navarro C, Cercenado E, et al. Spread of Escherichia coli strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. *J Clin Microbiol* 2006;44:2359-66.
45 O'Fallon E, Kandel R, Schreiber R, et al. Acquisition of multidrug-resistant Gram-negative bacteria: incidence and risk factors within a long-term care population. *Infect Control Hosp Epidemiol* 2010;31:1148-53.
46 Lohiya A, Kant S, Kapil A, et al. Pattern of antibiotic resistance among community derived isolates of enterobacteriaceae using urine sample: a study from northern India. *J Clin Diagnost Res* 2015;9:LC15-9.
47 Diwan V, Tamhankar AJ, Khandal RK, et al. Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. *BMC Public Health* 2010;10:414.
48 Amos GCA, Hawkey PM, Gaze WH, et al. Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. *J Antimicrob Chemother* 2014;69:1785-91.
49 Chen B, Zheng W, Yu Y, et al. Class 1 integrons, selected virulence genes, and antibiotic resistance in Escherichia coli isolates from the Minjiang River, Fujian Province, China. *Appl Environ Microbiol* 2011;77:148-55.
50 De Boeck H, Miwanda B, Lunguya-Metila O, et al. ESBL-positive Enterobacteriaisolates in drinking water [letter]. *Emerg Infect Dis* 2012;18:1019-20.
51 Coleman BL, Louie M, Salvadori MI, et al. Contamination of Canadian private drinking water sources with antimicrobial resistant Escherichia coli. *Water Res* 2013;47:3026-36.
52 Kamruzzaman M, Shoma S, Bari SMN, et al. Genetic diversity and antibiotic resistance in Escherichia coli from environmental surface water in Dhaka city, Bangladesh. *Diagn Microbiol Infect Dis* 2013;76:222-6.
53 Talukdar PK, Rahman M, Rahman M, et al. Antimicrobial resistance, virulence factors and genetic diversity of Escherichia coli isolates from household water supply in Dhaka, Bangladesh. *PLoS One* 2013;8:e61090.
54 Dhanji H, Murphy NM, Akhigbe C, et al. Isolation of fluoroquinolone-resistant O25b:H4-ST131 Escherichia coli with CTX-M-14 extended-spectrum β-lactamase from UK river water. *J Antimicrob Chemother* 2011;66:512-6.
55 Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, et al. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin Microbiol Infect* 2011;17:873-80.
56 Schmid A, Hörmansdorfer S, Messelhäusser U, et al. Prevalence of extended-spectrum β-lactamase-producing Escherichia coli on Bavarian dairy and beef cattle farms. *Appl Environ Microbiol* 2013;79:3027-32.
57 Sahoo KC, Tamhankar AJ, Sahoo S, et al. Geographical variation in antibiotic-resistant Escherichia coli isolates from stool, cow-dung and drinking water. *Int J Environ Res Public Health* 2012;9:746-59.
58 Frye JG, Jackson CR. Genetic mechanisms of antimicrobial resistance identified in Salmonella enterica, Escherichia coli, and Enteroccocus spp isolated from US food animals. *Front Microbiol* 2013;4:135.
59 Geser N, Stephan R, Hächler H. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Vet Res* 2012;8:21.
60 Horton RA, Randall LP, Snary EL, et al. Fecal carriage and shedding density of CTX-M extended-spectrum β-lactamase-producing Escherichia coli in cattle, chickens, and pigs: implications for environmental contamination and food production. *Appl Environ Microbiol* 2011;77:3715-19.

61 Rodriguez-Lazaro D, Ariza-Miguel J, Diez-Valcarce M, et al. Identification and molecular characterization of pathogenic bacteria in foods confiscated from non-EU flights passengers at one Spanish airport. *Int J Food Microbiol* 2015;209:20-5.
62 Schmiedel J, Falgenhauer L, Domann E, et al. Multiresistant extended-spectrum β-lactamase-producing Enterobacteriaceaefrom humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol* 2014;14:1471-2180.
63 Timofte D, Dandrieux J, Wattret A, et al. Detection of extended-spectrum-β-lactamase-positive Escherichia coli in bile isolates from two dogs with bacterial cholangiohepatitis. *J Clin Microbiol* 2011;49:3411-4.
64 Guenther S, Ewers C, Wieler LH. Extended-spectrum β-lactamases producingE coli in wildlife, yet another form of environmental pollution? *Front Microbiol* 2011;2:246.
65 Hasan B, Sandegren L, Melhus A, et al. Antimicrobial drug-resistant *Escherichia coli* in wild birds and free-range poultry, Bangladesh. *Emerg Infect Dis* 2012;18:2055-8.
66 Hernandez J, Johansson A, Stedt J, et al. Characterization and comparison of extended-spectrum β-lactamase (ESBL) resistance genotypes and population structure of Escherichia coli isolated from Franklin's Gulls (Leucophaeus pipixcan) and humans in Chile. *PLoS One* 2013;8:e76150.
67 Hasan B, Melhus A, Sandegren L, et al. The gull (Chroicocephalus brunnicephalus) as an environmental bioindicator and reservoir for antibiotic resistance on the coastlines of the Bay of Bengal. *Microb Drug Resist* 2014;30:30.
68 Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. *Clin Microbiol Rev* 2007;20:391-408.
69 Mouton JW, Brown DF, Apfalter P, et al. The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. *Clin Microbiol Infect* 2012;18:E37-45.
70 Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin Microbiol Infect* 2006;12:418-25.
71 Martinez JL, Coque TM, Baquero F. What is a resistance gene? Ranking risk in resistomes. *Nat Rev Microbiol* 2015;13:116-23.
72 Kester JC, Fortune SM. Persisters and beyond: mechanisms of phenotypic drug resistance and drug tolerance in bacteria. *Crit Rev Biochem Mol Biol* 2014;49:91-101.
73 Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet* 2001;358:135-8.
74 Kotte O, Volkmer B, Radzikowski JL, et al. Phenotypic bistability in Escherichia coli's central carbon metabolism. *Mol Syst Biol* 2014;10:736.
75 Arnoldini M, Vizcarra IA, Peña-Miller R, et al. Bistable expression of virulence genes in *Salmonella* leads to the formation of an antibiotic-tolerant subpopulation. *PLoS Biol* 2014;12:e1001928.
76 Ferenci T, Maharjan R. Mutational heterogeneity: a key ingredient of bet-hedging and evolutionary divergence? *Bioessays* 2015;37:123-30.
77 Maharjan R, Seeto S, Notley-McRobb L, et al. Clonal adaptive radiation in a constant environment. *Science* 2006;313:514-7.
78 Beardmore RE, Gudelj I, Lipson DA, et al. Metabolic trade-offs and the maintenance of the fittest and the flattest. *Nature* 2011;472:342-6.
79 Li XZ, Plesiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin Microbiol Rev* 2015;28:337-418.
80 Sato K, Nakae T. Outer membrane permeability of Acinetobacter calcoaceticus and its implication in antibiotic resistance. *J Antimicrob Chemother* 1991;28:35-45.
81 Fernández L, Hancock RE. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clin Microbiol Rev* 2012;25:661-81.
82 Nikaido H, Pagès JM. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. *FEMS Microbiol Rev* 2012;36:340-63.
83 Jacoby GA. AmpC β-lactamases. *Clin Microbiol Rev* 2009;22:161-82.
84 Choi SH, Lee JE, Park SJ, et al. Emergence of antibiotic resistance during therapy for infections caused by Enterobacteriaceae producing AmpC β-lactamase: implications for antibiotic use. *Antimicrob Agents Chemother* 2008;52:995-1000.
85 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 2014. http://ncipd.org/control/images/NCIPD_docs/CLSI_M100-S24.pdf.
86 Kamruzzaman M, Patterson JD, Shoma S, et al. Relative strengths of promoters provided by common mobile genetic elements associated with resistance gene expression in Gram-negative bacteria. *Antimicrob Agents Chemother* 2015;59:5088-91.
87 Muniesa M, Colomer-Lluch M, Jofre J. Potential impact of environmental bacteriophages in spreading antibiotic resistance genes. *Future Microbiol* 2013;8:739-51.
88 Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* 1996;272:1910-4.
89 Karaolis DK, Somara S, Maneval DR Jr, et al. A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature* 1999;399:375-9.
90 Johnston C, Martin B, Fichant G, et al. Bacterial transformation: distribution, shared mechanisms and divergent control. *Nat Rev Microbiol* 2014;12:181-96.
91 Partridge SR. Analysis of antibiotic resistance regions in Gram-negative bacteria. *FEMS Microbiol Rev* 2011;35:820-55.
92 Frost LS, Leplae R, Summers AO, et al. Mobile genetic elements: the agents of open source evolution. *Nat Rev Microbiol* 2005;3:722-32.
93 Liebert CA, Hall RM, Summers AO. Transposon Tn21, flagship of the floating genome. *Microbiol Mol Biol Rev* 1999;63:507-22.
94 Martínez JL. Bottlenecks in the transferability of antibiotic resistance from natural ecosystems to human bacterial pathogens. *Front Microbiol* 2011;2:265.
95 Wellington EMH, Boxall ABA, Cross P, et al. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect Dis* 2013;13:155-65.
96 Karim A, Poirel L, Nagarajan S, et al. Plasmid-mediated extended-spectrum β-lactamase (CTX-M-3 like) from India and gene association with insertion sequence ISEcp1. *FEMS Microbiol Lett* 2001;201:237-41.
97 Baquero F. From pieces to patterns: evolutionary engineering in bacterial pathogens. *Nat Rev Microbiol* 2004;2:510-8.
98 Walsh TR. Combinatorial genetic evolution of multiresistance. *Curr Opin Microbiol* 2006;9:476-82.
99 Tagg KA, Iredell JR, Partridge SR. Complete sequencing of IncI1 sequence type 2 plasmid pJIE512b indicates mobilization of bla_{CMY-2} from an IncA/C plasmid. *Antimicrob Agents Chemother* 2014;58:4949-52.
100 Weinreich DM, Delaney NF, DePristo MA, et al. Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science* 2006;312:111-14.
101 Cantón R, Coque TM. The CTX-M β-lactamase pandemic. *Curr Opin Microbiol* 2006;9:466-75.
102 Caniça MM, Lu CY, Krishnamoorthy R, et al. Molecular diversity and evolution of bla_{TEM} genes encoding β-lactamases resistant to clavulanic acid in clinical E coli. *J Mol Evol* 1997;44:57-65.
103 Martínez JL, Baquero F. Emergence and spread of antibiotic resistance: setting a parameter space. *Ups J Med Sci* 2014;119:68-77.
104 Shaw KJ, Rather PN, Hare RS, et al. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev* 1993;57:138-63.
105 Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 2010;74:417-33.
106 Ginn AN, Zong Z, Wiklendt AM, et al. Limited diversity in the gene pool allows prediction of third-generation cephalosporin and aminoglycoside resistance in Escherichia coli and Klebsiella pneumoniae. *Int J Antimicrob Agents* 2013;42:19-26.
107 Ginn AN, Wiklendt AM, Zong Z, et al. Prediction of major antibiotic resistance in Escherichia coli and Klebsiella pneumoniae in Singapore, USA and China using a limited set of gene targets. *Int J Antimicrob Agents* 2014;43:563-5.
108 Partridge SR, Zong Z, Iredell JR. Recombination in IS26 and Tn2 in the evolution of multiresistance regions carrying $bla_{CTX-M-15}$ on conjugative IncF plasmids from Escherichia coli. *Antimicrob Agents Chemother* 2011;55:4971-8.
109 Hooper DC, Jacoby GA. Mechanisms of drug resistance: quinolone resistance. *Ann N Y Acad Sci* 2015;1354:12-31.
110 Espedido B, Iredell J, Thomas L, et al. Wide dissemination of a carbapenemase plasmid among gram-negative bacteria: implications of the variable phenotype. *J Clin Microbiol* 2005;43:4918-19.
111 Hawkey PM, Xiong J, Ye H, et al. Occurrence of a new metallo-β-lactamase IMP-4 carried on a conjugative plasmid in Citrobacter youngae from the People's Republic of China. *FEMS Microbiol Lett* 2001;194:53-7.
112 Bou G, Oliver A, Martínez-Beltrán J. OXA-24, a novel class D β-lactamase with carbapenemase activity in an Acinetobacter baumannii clinical strain. *Antimicrob Agents Chemother* 2000;44:1556-61.
113 Allen HK, Donato J, Wang HH, et al. Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Microbiol* 2010;8:251-9.
114 Forsberg KJ, Patel S, Gibson MK, et al. Bacterial phylogeny structures soil resistomes across habitats. *Nature* 2014;509:612-6.
115 Salyers AA, Gupta A, Wang Y. Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends Microbiol* 2004;12:412-6.
116 Norman A, Hansen LH, Sørensen SJ. Conjugative plasmids: vessels of the communal gene pool. *Phil Trans R Soc B* 2009;364:2275-89.
117 Sherley M, Gordon DM, Collignon PJ. Species differences in plasmid carriage in the Enterobacteriaceae. *Plasmid* 2003;49:79-85.
118 Van Hal SJ, Wiklendt A, Espedido B, et al. Immediate appearance of plasmid-mediated resistance to multiple antibiotics upon antibiotic selection: an argument for systematic resistance epidemiology. *J Clin Microbiol* 2009;47:2325-7.
119 Garcillan-Barcia MP, Alvarado A, de la Cruz F. Identification of bacterial plasmids based on mobility and plasmid population biology. *FEMS Microbiol Rev* 2011;35:936-56.
120 Carattoli A, Bertini A, Villa L, et al. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005;63:219-28.
121 Rodríguez MM, Power P, Radice M, et al. Chromosome-encoded CTX-M-3 from Kluyvera ascorbata: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. *Antimicrob Agents Chemother* 2004;48:4895-7.
122 Humeniuk C, Arlet G, Gautier V, et al. β-Lactamases of Kluyvera ascorbata, probable progenitors of some plasmid-encoded CTX-M types. *Antimicrob Agents Chemother* 2002;46:3045-9.
123 Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: origin and diffusion. *Front Microbiol* 2012;3:110.
124 Price LB, Johnson JR, Aziz M, et al. The epidemic of extended-spectrum-β-lactamase-producing Escherichia coli ST131 is driven by a single highly pathogenic subclone, H30-Rx. *mBio* 2013;4:e00377-13.

125 Hirai I, Fukui N, Taguchi M, et al. Detection of chromosomal bla$_{CTX-M-15}$ in Escherichia coli O25b-B2-ST131 isolates from the Kinki region of Japan. *Int J Antimicrob Agents* 2013;42:500-6.
126 Denisuik AJ, Lagacé-Wiens PR, Pitout JD, et al. Molecular epidemiology of extended-spectrum β-lactamase-, AmpC β-lactamase- and carbapenemase-producing Escherichia coli and Klebsiella pneumoniae isolated from Canadian hospitals over a 5 year period: CANWARD 2007-11. *J Antimicrob Chemother* 2013;68(suppl 1):i57-65.
127 Johnson JR, Johnston B, Clabots C, et al. Escherichia coli sequence type ST131 as the major cause of serious multidrug-resistant E. coli infections in the United States. *Clin Infect Dis* 2010;51:286-94.
128 Petty NK, Ben Zakour NL, Stanton-Cook M, et al. Global dissemination of a multidrug resistant Escherichia coli clone. *Proc Natl Acad Sci USA* 2014;111:5694-9.
129 Marcadé G, Deschamps C, Boyd A, et al. Replicon typing of plasmids in Escherichia coli producing extended-spectrum β-lactamases. *J Antimicrob Chemother* 2009;63:67-71.
130 Boyd EF, Hill CW, Rich SM, et al. Mosaic structure of plasmids from natural populations of Escherichia coli. *Genetics* 1996;143:1091-100.
131 Clermont O, Dhanji H, Upton M, et al. Rapid detection of the O25b-ST131 clone of Escherichia coli encompassing the CTX-M-15-producing strains. *J Antimicrob Chemother* 2009;64:274-7.
132 Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. *Antimicrob Agents Chemother* 2001;45:1151-61.
133 Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2011;17:1791-8.
134 Partridge SR, Ginn AN, Wiklendt AM, et al. Emergence of bla$_{KPC}$ carbapenemase genes in Australia. *Int J Antimicrob Agents* 2015;45:130-6.
135 Kitchel B, Rasheed JK, Endimiani A, et al. Genetic factors associated with elevated carbapenem resistance in KPC-producing Klebsiella pneumoniae. *Antimicrob Agents Chemother* 2010;54:4201-7.
136 Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev* 2011;35:736-55.
137 Martínez-Martínez L. Extended-spectrum β-lactamases and the permeability barrier. *Clin Microbiol Infect* 2008;14:82-9.
138 Peleg AY, Franklin C, Bell JM, et al. Dissemination of the metallo-β-lactamase gene bla$_{IMP-4}$ among Gram-negative pathogens in a clinical setting in Australia. *Clin Infect Dis* 2005;41:1549-56.
139 Pournaras S, Protonotariou E, Voulgari E, et al. Clonal spread of KPC-2 carbapenemase-producing Klebsiella pneumoniae strains in Greece. *J Antimicrob Chemother* 2009;64:348-52.
140 Navon-Venezia S, Leavitt A, Schwaber MJ, et al. First report on a hyperepidemic clone of KPC-3-producing Klebsiella pneumoniae in Israel genetically related to a strain causing outbreaks in the United States. *Antimicrob Agents Chemother* 2009;53:818-20.
141 Hawser SP, Bouchillon SK, Hackel M, et al. Trending 7 years of in vitro activity of tigecycline and comparators against Gram-positive and Gram-negative pathogens from the Asia-Pacific region: Tigecycline Evaluation Surveillance Trial (TEST) 2004-2010. *Int J Antimicrob Agents* 2012;39:490-5.
142 Kaiser RM, Castanheira M, Jones RN, et al. Trends in Klebsiella pneumoniae carbapenemase-positive K. pneumoniae in US hospitals: report from the 2007-2009 SENTRY antimicrobial surveillance program. *Diagn Microbiol Infect Dis* 2013;76:356-60.
143 Salyers AA, Amábile-Cuevas CF. Why are antibiotic resistance genes so resistant to elimination? *Antimicrob Agents Chemother* 1997;41:2321-5.
144 Baquero F, Martínez J-L, Cantón R. Antibiotics and antibiotic resistance in water environments. *Curr Opin Biotechnol* 2008;19:260-5.
145 Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. *Nat Rev Microbiol* 2014;12:465-78.
146 Hughes D, Andersson DI. Evolutionary consequences of drug resistance: shared principles across diverse targets and organisms. *Nat Rev Genet* 2015;16:459-71.
147 Rogers BA, Kennedy KJ, Sidjabat HE, et al. Prolonged carriage of resistant E coli by returned travellers: clonality, risk factors and bacterial characteristics. *Eur J Clin Microbiol Infect Dis* 2012;31:2413-20.
148 Kennedy K, Collignon P. Colonisation with Escherichia coli resistant to "critically important" antibiotics: a high risk for international travellers. *Eur J Clin Microbiol Infect Dis* 2010;29:1501-6.
149 Kuenzli E, Jaeger VK, Frei R, et al. High colonization rates of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli in Swiss travellers to South Asia—a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. *BMC Infect Dis* 2014;14:528.
150 Titelman E, Hasan CM, Iversen A, et al. Faecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae is common 12 months after infection and is related to strain factors. *Clin Microbiol Infect* 2014;20:O508-15.
151 Baquero F. Chaos, complexity, and evolution. *ASM News* 2003;69:543-52.
152 Enne VI, Delsol AA, Davis GR, et al. Assessment of the fitness impacts on Escherichia coli of acquisition of antibiotic resistance genes encoded by different types of genetic element. *J Antimicrob Chemother* 2005;56:544-51.
153 Dahlberg C, Chao L. Amelioration of the cost of conjugative plasmid carriage in Eschericha coli K12. *Genetics* 2003;165:1641-9.
154 Silva RF, Mendonça SCM, Carvalho LM, et al. Pervasive sign epistasis between conjugative plasmids and drug-resistance chromosomal mutations. *PLoS Genet* 2011;7:e1002181.
155 Sengupta M, Austin S. Prevalence and significance of plasmid maintenance functions in the virulence plasmids of pathogenic bacteria. *Infect Immun* 2011;79:2502-9.
156 Yamaguchi Y, Park JH, Inouye M. Toxin-antitoxin systems in bacteria and archaea. *Annu Rev Genet* 2011;45:61-79.
157 San Millan A, Heilbron K, MacLean RC. Positive epistasis between co-infecting plasmids promotes plasmid survival in bacterial populations. *ISME J* 2014;8:601-12.
158 San Millan A, Peña-Miller R, Toll-Riera M, et al. Positive selection and compensatory adaptation interact to stabilize non-transmissible plasmids. *Nat Commun* 2014;5; doi:10.1038/ncomms6208.
159 Giske CG, Martinez-Martinez L, Canton R, et al. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version1. 2013. www.eucast.org/resistance_mechanisms.
160 Tacconelli E, Cataldo MA, Dancer SJ, et al. ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. *Clin Microbiol Infect* 2014;20(suppl 1):1-55.
161 Centers for Disease Control and Prevention. Guidance for control of carbapenem resistant Enterobacteriaceae. *CRE Toolkit* 2012. www.cdc.gov/hai/organisms/cre/cre-toolkit.
162 Australian Government. National Health and Medical Research Council. Australian guidelines for the prevention and control of infection in healthcare. 2010. www.nhmrc.gov.au/guidelines-publications/cd33.
163 Livermore DM, Andrews JM, Hawkey PM, et al. Are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly? *J Antimicrob Chemother* 2012;67:1569-77.
164 Poirel L, Schrenzel J, Cherkaoui A, et al. Molecular analysis of NDM-1-producing enterobacterial isolates from Geneva, Switzerland. *J Antimicrob Chemother* 2011;66:1730-3.
165 Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing Enterobacteriaceae. *J Antimicrob Chemother* 2013;68:487-9.
166 Maurer FP, Castelberg C, Quiblier C, et al. Evaluation of carbapenemase screening and confirmation tests with Enterobacteriaceaeand development of a practical diagnostic algorithm. *J Clin Microbiol* 2015;53:95-104.
167 Takiff HE, Feo O. Clinical value of whole-genome sequencing of Mycobacterium tuberculosis. *Lancet Infect Dis* 2015;15:1077-90.
168 Pecora ND, Li N, Allard M, et al. Genomically informed surveillance for carbapenem-resistant Enterobacteriaceaein a health care system. *mBio* 2015;6:e01030-15.
169 Mathers AJ, Stoesser N, Sheppard AE, et al. Klebsiella pneumoniae carbapenemase (KPC) producing K. pneumoniae at a single institution: insights into endemicity from whole genome sequencing. *Antimicrob Agents Chemother* 2015;59:1656-63.
170 Martínez JL. Antibiotics and antibiotic resistance genes in natural environments. *Science* 2008;321:365-7.
171 Tagg KA, Ginn AN, Jiang X, et al. Distribution of acquired AmpC β-lactamase genes in Sydney, Australia. *Diagn Microbiol Infect Dis* 2015;83:56-8.
172 Garcillan-Barcia MP, Ruiz del Castillo B, Alvarado A, et al. Degenerate primer MOB typing of multiresistant clinical isolates of E coli uncovers new plasmid backbones. *Plasmid* 2015;77:17-27.
173 Jain A, Srivastava P. Broad host range plasmids. *FEMS Microbiol Lett* 2013;348:87-96.
174 Guiney DG. Broad host range conjugative and mobilizable plasmids in Gram-negative bacteria. In: Clewell DB, ed. Bacterial conjugation. Plenum Press, 1993:75-103.
175 Stecher B, Denzler R, Maier L, et al. Gut inflammation can boost horizontal gene transfer between pathogenic and commensal Enterobacteriaceae. *Proc Natl Acad Sci USA* 2012;109:1269-74.
176 Stecher B, Maier L, Hardt WD. "Blooming" in the gut: how dysbiosis might contribute to pathogen evolution. *Nat Rev Microbiol* 2013;11:277-84.
177 Bartlett JG. A call to arms: the imperative for antimicrobial stewardship. *Clin Infect Dis* 2011;53(suppl 1):S4-7.
178 ARISE Investigators and the ANZICS Clinical Trials Group. Goal-directed resuscitation for patients with early septic shock. *N Engl J Med* 2014;371:1496-506.
179 Davey P, Brown E, Charani E, et al. Interventions to improve antibiotic prescribing practices for hospital inpatients. *Cochrane Database Syst Rev* 2013;4:CD003543.
180 Filice G, Drekonja D, Greer N, et al. Antimicrobial stewardship programs in inpatient settings: a systematic review. Department of Veterans Affairs, 2013. www.ncbi.nlm.nih.gov/books/NBK253513.
181 Butler CC, Dunstan F, Heginbothom M, et al. Containing antibiotic resistance: decreased antibiotic-resistant coliform urinary tract infections with reduction in antibiotic prescribing by general practices. *Br J Gen Pract* 2007;57:785-92.
182 Hunter PA, Dawson S, French GL, et al. Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. *J Antimicrob Chemother* 2010;65(suppl 1):i3-17.
183 Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance-the need for global solutions. *Lancet Infect Dis* 2013;13:1057-98.

184 Antonopoulos DA, Huse SM, Morrison HG, et al. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. *Infect Immun* 2009;77:2367-75.
185 Ginn AN, Wiklendt AM, Gidding HF, et al. The ecology of antibiotic use in the ICU: homogeneous prescribing of cefepime but not tazocin selects for antibiotic resistant infection. *PLoS One* 2012;7:e38719.
186 Paterson DL. "Collateral damage" from cephalosporin or quinolone antibiotic therapy. *Clin Infect Dis* 2004;38(suppl 4):S341-5.
187 Spellberg B, Blaser M, Guidos RJ, et al. Combating antimicrobial resistance: policy recommendations to save lives. *Clin Infect Dis* 2011;52(suppl 5):S397-428.
188 Read AF, Day T, Huijben S. The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy. *Proc Natl Acad Sci USA* 2011;108(suppl 2):10871-7.
189 Daneman N, Sarwar S, Fowler RA, et al. Effect of selective decontamination on antimicrobial resistance in intensive care units: a systematic review and meta-analysis. *Lancet Infect Dis* 2013;13:328-41.
190 Brener SJ, Mehran R, Brodie BR, et al. Predictors and implications of coronary infarct artery patency at initial angiography in patients with acute myocardial infarction (from the CADILLAC and HORIZONS-AMI Trials). *Am J Cardiol* 2011;108:918-23.
191 Cuthbertson BH, Campbell MK, MacLennan G, et al. Clinical stakeholders' opinions on the use of selective decontamination of the digestive tract in critically ill patients in intensive care units: an international Delphi study. *Crit Care* 2013;17:R266-6.
192 De Smet AM, Kluytmans JA, Blok HE, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. *Lancet Infect Dis* 2011;11:372-80.
193 Vardakas KZ, Tansarli GS, Rafailidis PI, et al. Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to Enterobacteriaceae producing extended-spectrum β-lactamases: a systematic review and meta-analysis. *J Antimicrob Chemother* 2012;67:2793-803.
194 Sbrana F, Malacarne P, Viaggi B, et al. Carbapenem-sparing antibiotic regimens for infections caused by Klebsiella pneumoniae carbapenemase-producing K pneumoniae in intensive care unit. *Clin Infect Dis* 2013;56:697-700.
195 Falagas ME, Lourida P, Poulikakos P, et al. Antibiotic treatment of infections due to carbapenem-resistant Enterobacteriaceae: systematic evaluation of the available evidence. *Antimicrob Agents Chemother* 2014;58:654-63.
196 Tzouvelekis LS, Markogiannakis A, Piperaki E, et al. Treating infections caused by carbapenemase-producing Enterobacteriaceae. *Clin Microbiol Infect* 2014;20:862-72.
197 Ho VP, Barie PS, Stein SL, et al. Antibiotic regimen and the timing of prophylaxis are important for reducing surgical site infection after elective abdominal colorectal surgery. *Surg Infect* 2011;12:255-60.
198 Bulik CC, Nicolau DP. Double-carbapenem therapy for carbapenemase-producing Klebsiella pneumoniae. *Antimicrob Agents Chemother* 2011;55:3002-4.
199 Giamarellou H, Galani L, Baziaka F, et al. Double-carbapenem regimen for carbapenemase-producing pandrug-resistant Klebsiella pneumoniae infections: is it really effective in humans? *Antimicrob Agents Chemother* 2013;57:2388-90.
200 Oliva A, D'Abramo A, D'Agostino C, et al. Synergistic activity and effectiveness of a double-carbapenem regimen in pandrug-resistant Klebsiella pneumoniae bloodstream infections. *J Antimicrob Chemother* 2014; published online 11 Feb; doi:10.1093/jac/dku027.
201 Rahme C, Butterfield JM, Nicasio AM, et al. Dual beta-lactam therapy for serious Gram-negative infections: is it time to revisit? *Diagn Microbiol Infect Dis* 2014;80:239-59.
202 Mainardi JL, Gutmann L, Acar JF, et al. Synergistic effect of amoxicillin and cefotaxime against Enterococcus faecalis. *Antimicrob Agents Chemother* 1995;39:1984-7.
203 Ling LL, Schneider T, Peoples AJ, et al. A new antibiotic kills pathogens without detectable resistance. *Nature* 2015;517:455-9.
204 Yang J, Hocking DM, Cheng C, et al. Disarming bacterial virulence through chemical inhibition of the DNA binding domain of an AraC-like transcriptional activator protein. *J Biol Chem* 2013;288:31115-26.
205 Koppolu V, Osaka I, Skredenske JM, et al. Small-molecule inhibitor of the Shigella flexneri master virulence regulator VirF. *Infect Immun* 2013;81:4220-31.
206 LaSarre B, Federle MJ. Exploiting quorum sensing to confuse bacterial pathogens. *Microbiol Mol Biol Rev* 2013;77:73-111.
207 Desroy N, Denis A, Oliveira C, et al. Novel HldE-K inhibitors leading to attenuated Gram negative bacterial virulence. *J Med Chem* 2013;56:1418-30.
208 Kvist M, Hancock V, Klemm P. Inactivation of efflux pumps abolishes bacterial biofilm formation. *Appl Environ Microbiol* 2008;74:7376-82.
209 Kern WV, Steinke P, Schumacher A, et al. Effect of 1-(1-naphthylmethyl)-piperazine, a novel putative efflux pump inhibitor, on antimicrobial drug susceptibility in clinical isolates of Escherichia coli. *J Antimicrob Chemother* 2006;57:339-43.
210 Schumacher A, Steinke P, Bohnert JA, et al. Effect of 1-(1-naphthylmethyl)-piperazine, a novel putative efflux pump inhibitor, on antimicrobial drug susceptibility in clinical isolates of Enterobacteriaceae other than Escherichia coli. *J Antimicrob Chemother* 2006;57:344-8.
211 Leitner I, Nemeth J, Feurstein T, et al. The third-generation P-glycoprotein inhibitor tariquidar may overcome bacterial multidrug resistance by increasing intracellular drug concentration. *J Antimicrob Chemother* 2011;66:834-9.
212 Prasad P, Sun J, Danner RL, et al. Excess deaths associated with tigecycline after approval based on noninferiority trials. *Clin Infect Dis* 2012;54:1699-709.
213 Devasahayam G, Scheld WM, Hoffman PS. Newer antibacterial drugs for a new century. *Expert Opin Investig Drugs* 2010;19:215-34.
214 Livermore DM, Warner M, Mushtaq S, et al. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *Int J Antimicrob Agents* 2011;37:415-9.
215 Lagacé-Wiens P, Walkty A, Karlowsky JA. Ceftazidime-avibactam: an evidence-based review of its pharmacology and potential use in the treatment of Gram-negative bacterial infections. *Core Evid* 2014;9:13-25.
216 Wang X, Zhang F, Zhao C, et al. In vitro activities of ceftazidime-avibactam and aztreonam-avibactam against 372 Gram-negative bacilli collected in 2011 and 2012 from 11 teaching hospitals in China. *Antimicrob Agents Chemother* 2014;58:1774-8.
217 Solomkin JS, Ramesh MK, Cesnauskas G, et al. Phase 2, randomized, double-blind study of the efficacy and safety of two dose regimens of eravacycline versus ertapenem for adult community-acquired complicated intra-abdominal infections. *Antimicrob Agents Chemother* 2014;58:1847-54.
218 Sutcliffe JA, O'Brien W, Fyfe C, et al. Antibacterial activity of eravacycline (TP-434), a novel fluorocycline, against hospital and community pathogens. *Antimicrob Agents Chemother* 2013;57:5548-58.
219 Galani I, Souli M, Daikos GL, et al. Activity of plazomicin (ACHN-490) against MDR clinical isolates of Klebsiella pneumoniae, Escherichia coli, and Enterobacter spp from Athens, Greece. *J Chemother* 2012;24:191-4.
220 Livermore DM, Mushtaq S, Warner M, et al. Activity of aminoglycosides, including ACHN-490, against carbapenem-resistant Enterobacteriaceaeisolates. *J Antimicrob Chemother* 2011;66:48-53.
221 Drawz S, Papp-Wallace K, Bonomo R. New β-lactamase inhibitors: a therapeutic renaissance in an MDR world. *Antimicrob Agents Chemother* 2014;58:1835-46.
222 Pucci MJ, Bush K. Investigational antimicrobial agents of 2013. *Clin Microbiol Rev* 2013;26:792-821.
223 Mandal SM, Roy A, Ghosh AK, et al. Challenges and future prospects of antibiotic therapy: from peptides to phages utilization. *Front Pharmacol* 2014;5:105.
224 Li W, Tailhades J, O'Brien-Simpson N, et al. Proline-rich antimicrobial peptides: potential therapeutics against antibiotic-resistant bacteria. *Amino Acids* 2014;46:2287-94.
225 Feng Q, Huang Y, Chen M, et al. Functional synergy of α-helical antimicrobial peptides and traditional antibiotics against Gram-negative and Gram-positive bacteria in vitro and in vivo. *Eur J Clin Microbiol Infect Dis* 2015;34:197-204.
226 Gruenheid S, Le Moual H. Resistance to antimicrobial peptides in Gram-negative bacteria. *FEMS Microbiol Lett* 2012;330:81-9.
227 Rao GG, Ly NS, Haas CE, et al. New dosing strategies for an "old" antibiotic: pharmacodynamics of front-loaded regimens of colistin at simulated pharmacokinetics of patients with kidney or liver disease. *Antimicrob Agents Chemother* 2014;58:1381-8.
228 Nation RL, Li J. Colistin in the 21st century. *Curr Opin Infect Dis* 2009;22:535.
229 Neonakis IK, Samonis G, Messaritakis H, et al. Resistance status and evolution trends of Klebsiella pneumoniae isolates in a university hospital in Greece: ineffectiveness of carbapenems and increasing resistance to colistin. *Chemotherapy* 2010;56:448-52.
230 Vaara M. Novel derivatives of polymyxins. *J Antimicrob Chemother* 2013;68:1213-9.
231 Quale J, Shah N, Kelly P, et al. Activity of polymyxin B and the novel polymyxin analogue CB-182,804 against contemporary Gram-negative pathogens in New York City. *Microb Drug Resist* 2012;18:132-6.
232 Liu YY, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2015; published online 18 Nov; doi:10.1016/S1473-3099(15)00424-7.
233 Karageorgopoulos DE, Miriagou V, Tzouvelekis LS, et al. Emergence of resistance to fosfomycin used as adjunct therapy in KPC Klebsiella pneumoniae bacteraemia: report of three cases. *J Antimicrob Chemother* 2012;67:2777-9.
234 Falagas ME, Kastoris AC, Kapaskelis AM, et al. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum β-lactamase producing, Enterobacteriaceae infections: a systematic review. *Lancet Infect Dis* 2010;10:43-50.
235 Michalopoulos AS, Livaditis IG, Gougoutas V. The revival of fosfomycin. *Int J Infect Dis* 2011;15:e732-9.
236 Pontikis K, Karaiskos I, Bastani S, et al. Outcomes of critically ill intensive care unit patients treated with fosfomycin for infections due to pandrug-resistant and extensively drug-resistant carbapenemase-producing Gram-negative bacteria. *Int J Antimicrob Agents* 2014;43:52-9.
237 Twort FW. The ultra-microscopic viruses. *Lancet* 1921;198:204.
238 Twort FW. An investigation on the nature of ultra-microscopic viruses. *Lancet* 1915;186:1241-3.

239 Hérelle F. Sur un microbe invisible antagoniste des bacilles dysentériques. *C R Acad Sci Ser D Paris* 1917:373-5.
240 Stone R. Bacteriophage therapy. Stalin's forgotten cure. *Science* 2002;298:728-31.
241 Ly-Chatain MH. The factors affecting effectiveness of treatment in phages therapy. *Front Microbiol* 2014;5:51.
242 Khawaldeh A, Morales S, Dillon B, et al. Bacteriophage therapy for refractory Pseudomonas aeruginosa urinary tract infection. *J Med Microbiol* 2011;60(pt 11):1697-700.
243 Burrowes B, Harper DR, Anderson J, et al. Bacteriophage therapy: potential uses in the control of antibiotic-resistant pathogens. *Expert Rev Anti Infect Ther* 2011;9:775-85.
244 Bragg R, van der Westhuizen W, Lee JY, et al. Bacteriophages as potential treatment option for antibiotic resistant bacteria. *Adv Exp Med Biol* 2014;807:97-110.
245 Barrangou R, Marraffini Luciano A. CRISPR-Cas systems: prokaryotes upgrade to adaptive immunity. *Mol Cell* 2014;54:234-44.
246 Gomaa AA, Klumpe HE, Luo ML, et al. Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. *mBio* 2014;5:e00928-13.
247 Citorik RJ, Mimee M, Lu TK. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. *Nat Biotech* 2014;32:1141-5.
248 Van der Waaij D, Berghuis JM. Determination of the colonization resistance of the digestive tract of individual mice. *J Hyg* 1974;72:379-87.
249 Wilson KH, Silva J, Fekety FR. Suppression of Clostridium difficile by normal hamster cecal flora and prevention of antibiotic-associated cecitis. *Infect Immun* 1981;34:626-8.
250 Heidt PJ, van der Waaij D, Vossen JM, et al. The use of a human donor flora for recontamination following antibiotic decontamination. *Prog Food Nutr Sci* 1983;7:53-63.
251 Tvede M, Rask-Madsen J. Bacteriotherapy for chronic relapsing Clostridium difficile diarrhoea in six patients. *Lancet* 1989;1:1156-60.
252 Youngster I, Sauk J, Pindar C, et al. Fecal microbiota transplant for relapsing Clostridium difficile infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. *Clin Infect Dis* 2014;58:1515-22.
253 Ananthakrishnan AN. Clostridium difficile infection: epidemiology, risk factors and management. *Nat Rev Gastroenterol Hepatol* 2011;8:17-26.
254 Kachrimanidou M, Malisiovas N. Clostridium difficile infection: a comprehensive review. *Crit Rev Microbiol* 2011;37:178-87.
255 Yurist-Doutsch S, Arrieta MC, Vogt SL, et al. Gastrointestinal microbiota-mediated control of enteric pathogens. *Annu Rev Genet* 2014;10:10.
256 Petschow B, Dore J, Hibberd P, et al. Probiotics, prebiotics, and the host microbiome: the science of translation. *Ann N Y Acad Sci* 2013:1306:1-17.
257 Ley RE. Harnessing microbiota to kill a pathogen: the sweet tooth of Clostridium difficile. *Nat Med* 2014;20:248-9.
258 Ng KM, Ferreyra JA, Higginbottom SK, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature* 2013;502:96-9.
259 Lawley TD, Clare S, Walker AW, et al. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. *PLoS Pathog* 2012;8:25.
260 Gillings MR, Paulsen IT, Tetu SG. Ecology and evolution of the human microbiota: fire, farming and antibiotics. *Genes (Basel)* 2015;6:841-57.