Antimicrobials: access and sustainable effectiveness 2

Understanding the mechanisms and drivers of antimicrobial resistance

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To combat the threat to human health and biosecurity from antimicrobial resistance, an understanding of its mechanisms and drivers is needed. Emergence of antimicrobial resistance in microorganisms is a natural phenomenon, yet antimicrobial resistance selection has been driven by antimicrobial exposure in health care, agriculture, and the environment. Onward transmission is affected by standards of infection control, sanitation, access to clean water, access to assured quality antimicrobials and diagnostics, travel, and migration. Strategies to reduce antimicrobial resistance by removing antimicrobial selective pressure alone rely upon resistance imparting a fitness cost, an effect not always apparent. Minimising resistance should therefore be considered comprehensively, by resistance mechanism, microorganism, antimicrobial drug, host, and context; parallel to new drug discovery, broad ranging, multidisciplinary research is needed across these five levels, interlinked across the health-care, agriculture, and environment sectors. Intelligent, integrated approaches, mindful of potential unintended results, are needed to ensure sustained, worldwide access to effective antimicrobials.

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This is the second in a **Series** of five papers about access to and sustainable effectiveness of antimicrobials

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Introduction

The increasing challenge to health care attributable to antimicrobial resistance, and the subsequent absence of access to effective antimicrobials, is of worldwide concern. There is a real threat that the public health gains from improved access to antimicrobials, including the improvements in childhood survival, could be undermined.[1] Understanding the scientific basis of antimicrobial resistance is essential to combating this public health threat. This understanding should cover the resistance mechanisms, enabling novel approaches to diagnostics and therapeutics, through to the drivers of antimicrobial resistance in society and the environment, essential for the development of appropriate interventional policies.[2–4] The many factors contributing to the present worldwide status of antimicrobial resistance are reviewed in this Series paper, with a particular focus on emergence of resistance, transmission, bacterial fitness, and potential for reversibility. The evidence for, and the role of, important drivers of antimicrobial resistance are considered and assessed in the context of the community (including the environment and agriculture) and in health-care systems. Please see appendix for a list of supplementary references. From this evidence, stakeholders can engage with issues specific to their area of practice, yet also be mindful of cross-sectoral interconnectivity and the need for a One Health approach to antimicrobial resistance.

Emergence of resistance

1) Why does resistance emerge within a micro-organism?

Through a darwinian selection process microorganisms have developed robust mechanisms to evade destruction from many toxic substances. Most antimicrobial drugs are naturally produced by microorganisms, including environmental fungi and saprophytic bacteria, or are synthetic modifications of them, with only a few drugs (eg, sulphonamides and fluoroquinolones) being wholly synthetic. The protective mechanisms that have evolved include preventing entry of or exporting the drug, producing enzymes that destroy or modify the antimicrobial, or making changes to the antimicrobial target. Therefore, antimicrobial resistance could be considered to simply represent the darwinian competition from natural microorganism-derived antimicrobial molecules. Functional meta-genomic studies of soil microorganisms have shown a widespread

Key messages

- The emergence of antimicrobial resistance is a natural evolutionary response to antimicrobial exposure. At a societal level, complex and interlinking drivers are increasing the prevalence of antimicrobial-resistant microorganisms, predominantly arising from use in human beings and agriculture and the pollution of the environment.
- Acquisition of antimicrobial resistance mechanisms does not necessarily compromise microbial fitness. Worldwide clonal spread and long-term persistence of resistant bacteria are also seen in the absence of direct antibiotic selection pressure.
- Reversibility of antimicrobial resistance after withdrawal of antimicrobial selective pressure is consequently not clear cut; minimisation of emergence of resistance to new and future agents is therefore essential.
- Gaining insight into the mechanisms of antimicrobial resistance, long-term persistence, and successful clonal spread, is fundamental to the development of novel targets for both diagnostic tests and therapeutic agents with integration of these into sustainable antimicrobial resistance strategies.
- Gaps in understanding and areas for innovation are clear, yet progress towards these goals is still urgently needed, with a careful awareness of any potential effect on access to effective antimicrobial treatment.
- There is no single solution and several, synergistic, overlapping, and complementing approaches will be needed, with a strong overarching shared goal to ensure and sustain access to effective antimicrobial therapies.

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See Online for appendix

diversity of genetic determinants conferring antibiotic resistance, of which only a fraction have been described in human pathogens.[5] One example where a naturally occurring resistance mechanism has had an effect on human health is the resistance developed against β-lactam antimicrobial drugs, in which the enzymes (β-lactamases) that inactivate these antimicrobial molecules have existed for millions of years.[6]

The production of antimicrobial molecules by saprophytic organisms were originally thought to inhibit the growth of neighbouring organisms, providing a competitive advantage in the local environment; however, some studies suggest a more complex interaction. First, the concentration of antimicrobial molecules in the soil seems to be too low to inhibit growth of other bacteria.[7] Second, evidence suggests that even sublethal concentrations of antimicrobials have substantial effects on bacterial physiology, increasing the rate of microbial adaptive evolution and possibly acting as signalling molecules influencing microbial and host gene expression.[8] Of particular note are some saprophytic bacteria that produce carbapenems (an important class of broad-spectrum antimicrobials in clinical use) in which the genes implicated in the synthesis of carbapenems might also have a role in the quorum-sensing apparatus (the mechanism through which a colony of microorganisms coordinates growth and gene expression) or formation of biofilms.[6] This process leads to further questions about the unintended effects of antimicrobial drugs, with our understanding of their potential effect on microorganisms, beyond their inhibitory action, remaining incomplete.[9]

Emergence of resistance to synthetic antimicrobials also occurs. This resistance has unfortunately been widely exemplified in the case of fluoroquinolones, for which in *Escherichia coli* isolated from patients in Europe, fluoroquinolone resistance is now at 10–40%.[10] Many resistance mechanisms have emerged including alteration of target (a DNA-gyrase), increased efflux (export of a drug out of the microorganism), fluoroquinolone inactivation (by an aminoglycoside N-acetyltransferase), and protection of the target by DNA-binding proteins (known as Qnr).[11]

Even though many microorganisms in the environment, and organisms including plants and animals, naturally produce antimicrobial substances, little evidence exists to suggest that this contributes significantly to the selection of antimicrobial-resistant microorganisms in their native environment.[7] Therefore, the many and varied human, animal, and agricultural uses of antimicrobials should be considered key worldwide drivers of antimicrobial resistance.[2,12,13]

2) Why does antimicrobial resistance emerge at the individual human level?

Neonates are rapidly colonised by Enterobacteriaceae after birth, irrespective of whether they are breast fed or not. A study in India of a cohort of breast fed babies noted that at 1-day old, 14·3% harboured Enterobacteriaceae that contained an enzyme that inactivates β-lactam drugs, an extended-spectrum β-lactamase (ESβL), yet this increased to 41·5% of babies by day 60.[14] The environment, drinking water, and food are probably the most important means for establishing the normal (healthy) gut microflora. Antimicrobial-resistant bacteria have been found in every environment examined so far including Antarctica, the sea, soil, drinking water,[15] and various food products.[16] This polymicrobial, variably antimicrobial-resistant, commensal microbiome (microorganisms that are not causing infection at that body site eg, the gastrointestinal tract or skin) is established at an early age.

In a pristine (ie, free from external antimicrobial selection pressure) ecosystem, antimicrobial-resistant and non-resistant species coexist in a stable balance.[17] The human microbiota is no exception, and commensal microorganism populations in human beings include species that are naturally resistant to some antimicrobials. Selective pressure is exerted by any condition (eg, antimicrobial exposure) that allows microorganisms with inherent resistance or newly acquired mutations or resistance genes to survive and proliferate.[6] Antimicrobial use exerts such selective pressure on commensal human microflora, and pathogens, increasing the risk of recovery of resistant organisms from patients.[12]

Use of antimicrobials in clinical medicine has exposed the human microbiota to unprecedented high concentrations of these drugs. In-vivo development of de-novo resistance within a human individual has been recorded during treatment courses with a range of antimicrobials including, worryingly, carbapenems.[18] In some patients, such as those with cystic fibrosis, there are increased rates of mutation in the infecting bacteria (ie, they are hypermutable). Some antimicrobials exacerbate this hypermutability, promoting selection of resistance.[19]

3) Why does resistance emerge at the population level in humans and animals?

Antimicrobials are among the most commonly prescribed drugs used in human medicine, yet up to 50% of all antimicrobials prescribed to people are considered unnecessary.[20] This use, misuse, or overuse of antimicrobial drugs is considered to be a major driving force towards antimicrobial resistance.[21,22]

In human beings, the concentration of antibiotic prescribing might be highest in inpatient settings, with 30–40% of patients on antibiotics in European hospitals.[23] However, the overall quantity of antimicrobial prescribing is highest in the community.[24] Even though guidelines recommend prudent use, needless prescriptions are seen even in countries with low rates of prescription.[25] However an overall reduction in prescriptions for antimicrobials has been reported in some settings over the past decade,

with a modest reduction in antimicrobial resistance recorded.[26] The role of educating prescribers is crucial in overcoming antimicrobial misuse or overuse and is seen to be effective in primary care[27] and secondary care.[28] Additionally, raising awareness of the fundamentals of antimicrobial use in the general public is equally essential.[29]

However, although the link between human antimicrobial use and resistance seems clear cut, this association is complex.[30] Confounding factors mean a uniform approach to understanding resistance cannot be taken. These factors include pathogen–drug interactions, pathogen-host interactions, mutation rates of the pathogen, emergence of successful antimicrobial-resistant clones, the transmission rates of pathogens between human beings, animals, and the environment, cross-resistance, and selection of co-resistance to unrelated drugs. Importantly, at the human population level, public health factors such as rates of vaccine uptake,[31] different systems of health care, the role of migration and tourism, sanitation, and population densities, also influence the prevalence of resistance.[30]

More antimicrobials are used in food production than in human beings,[22] with marked national differences in the number of antimicrobial drugs used in food-producing animals, varying a 100-fold from 4 mg to 400 mg of antimicrobial per kg of meat produced in European countries.[32] Various studies have shown that antimicrobial resistance has, at least in part, emerged as a result of the selective pressure exerted by antimicrobial use outside of human medicine, namely in veterinary medicine,[33] food-animal and fish production,[34,35] and agriculture.[22]

In summary, the role of antimicrobial use in driving the emergence of resistance is likely to be specific to each drug and to each microorganism, as is the effect of changes in this use. This means that policies need to be mindful of this complexity in addressing selection pressure and that an integrated approach is adopted across both the community (including agriculture and the environment) and health-care structures.

Transmission of resistance

1) How does transmission of resistance occur between micro-organisms?

In addition to selection of antimicrobial resistance through mutations in genes encoded on a microbe's chromosome, new genetic material can also be exchanged between organisms. This process can provide the host cell and its progeny with new genetic material encoding antimicrobial resistance and can occur through several mechanisms, of which perhaps the most important is plasmid transmission (figure 1).[15,36–40] Antimicrobials influence this, not only by exerting a selective pressure towards emergence of antimicrobial resistance, but also by inducing transfer of resistance determinants between microorganisms.[41]

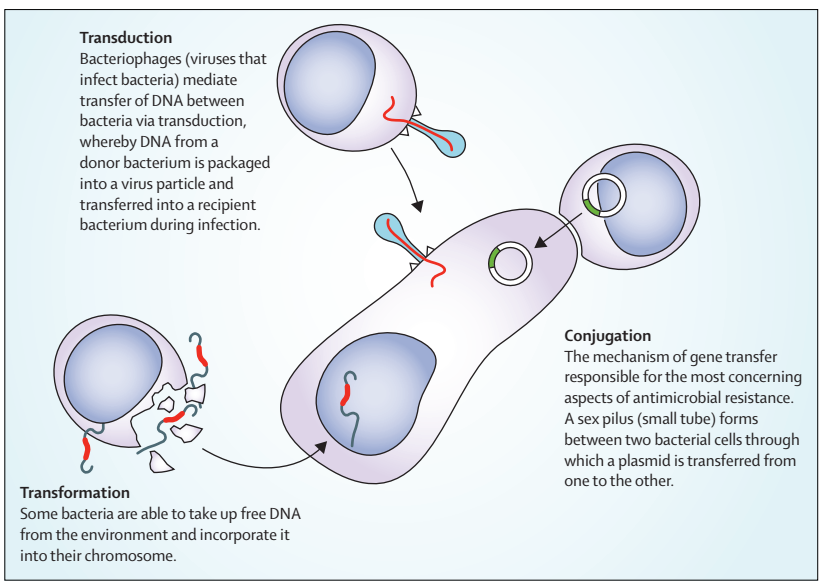

Figure 1: **Transmission of genetic material between microorganisms**
Genetic material is transferred between microorganisms through three main routes: (i) transformation—examples include recombination of foreign DNA from *Streptococcus mitis* to *Streptococcus pneumoniae,* conferring penicillin resistance via the formation of mosaic genes, and *Neisseria gonorrhoeae* where a mosaic *penA* gene is associated with ceftriaxone resistance;[38] (ii) transduction—where antimicrobial resistance genetic material has been identified in phage DNA isolated from waste water treatment,[26] and both extended-spectrum β-lactamase genes and *mecA* genes (the latter responsible for meticillin resistance in *Staphylococcus aureus*) have been identified in bacteriophage extracted from faecal samples at farms and abattoirs;[37] and (iii) conjugation—with plasmids being responsible for example for the global dissemination of genes encoding carbapenemases such as New Delhi metallo-β-lactamase,[15] as well as ESβLs.[39] Furthermore integrative chromosomal elements (ICEs) can transfer these resistance genes between plasmids and the bacterial host chromosome in a range of Gram-negative species and streptococci.[40]

2) How does human-human transmission drive resistance?

Modelling of transmission dynamics has improved understanding of how human–human transmission contributes to the spread of pathogens and antimicrobial resistance.[21,22] In the community, faecal–oral transmission, often through failures in sanitation, plays an important part, particularly for resistant Enterobacteriaceae.[42] Transmission can also occur through sexual encounters; for *Neisseria gonorrhoeae*, core groups have contributed to widespread dissemination of resistant clones.[43] Perhaps where the dynamics of transmission are best understood is in the context of health-care-associated infections. Using meticillin-resistant *Staphylococcus aureus* (MRSA) as an example, modelling suggests duration of patient stay and contamination of health-care workers' hands both contribute to continuing transmission.[44] Use of whole-genome sequencing has enabled more detailed epidemiological analysis, providing rapid, fine resolution sequencing to help with mapping outbreaks and delineating transmission points.[45] What is missing, perhaps, is a better understanding of how movement and flow of patients, and the built environment, affect resistance transmission within,[46] and between,[47] health-care institutions. Moreover, the inability to rapidly

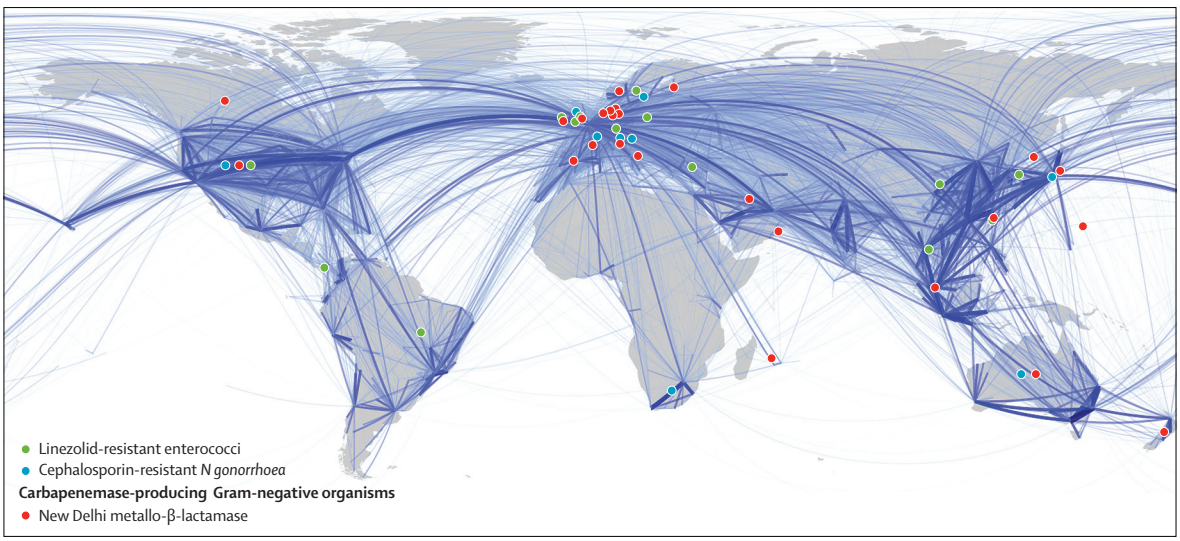

Figure 2: **Worldwide travel routes and emergence of antimicrobial resistance**

Although extended-spectrum β-lactamase-producing *Enterobacteriaceae* and MRSA are now nearly ubiquitous, certain novel types of resistance, among both Gram-negative and Gram-positive organisms, are of particular concern. The mechanisms of human-to-human transmission for these organisms are likely to be complex, but include association with travel. Data shown includes NDM-positive bacteria from patients with an epidemiological link to the Indian subcontinent,[52] linezolid-resistant enterococci,[53] and reported cefixime/ceftriaxone treatment failures for *Neisseria gonorrhoea*.[54] Flight path data developed by Dr Jonathan Read and Professor Tom Solomon, based on the number of commercial flight bookings made (number of travellers might be higher).

identify resistant microorganisms with appropriate and efficient diagnostic tests is likely to further contribute to continuing transmission in health-care settings.

During the last 10 years the human microbiota has acquired antimicrobial resistant Enterobacteriaceae on an unprecedented scale. In some parts of the world the carrier rate of ESβL-positive Enterobacteriaceae in the gut is more than 50%,[48] and travel has been clearly associated with increased risk of gut colonisation with these organisms. In a prospective study from the Netherlands, 8·6% of travellers were colonised with ESβL-producing Enterobacteriaceae before travel, but 30·5% acquired gut colonisation during travel, with independent risk factors being travel to south and east Asia.[49] More recently, and perhaps more worryingly, is the spread of carbapenem resistance mechanisms across the world, and between organisms, with New Delhi metallo-β-lactamase (NDM),[15] *Klebsiella pneumoniae* carbapenemase,[50] and OXA-48[51] enzymes being among those of greatest concern (figure 2).[52–54] Travel-related human–human spread has also been evident for Gram-positive organisms, notably in the spread of antimicrobial-resistant *Streptococcus pneumoniae* from Spain to Iceland.[55]

Population-based strategies to interrupt human–human transmission, including through interventions such as mass drug administration and vaccination, might alter the transmission dynamics of pathogens and resistance determinants. Evidence for this approach is strongest for vaccines, in which their use can result in reduction or elimination of the target organism, reducing the need for antimicrobial treatment and selection of antimicrobial resistance.[56] Targeting vaccines against strains with drug resistance has been suggested, and provision of even slightly higher rates of protection for drug-resistant over drug-sensitive strains might be an effective method in controlling antimicrobial resistance.[57] Finally, controlling antimicrobial resistance through use of targeted, vaccine-induced replacement strains has been proposed.[58]

3) What is the role of animals and the environment in driving transmission of resistance?

The potential for transmission of antimicrobial-resistant microbes from animals to human beings, and the association between use of antimicrobial growth promoters in farm animals and transmission of resistant bacteria, were recognised in the 1960s.[59] Antimicrobial resistance that arises in animal husbandry is now well established and affects zoonotic pathogens such as *Salmonella serovars*[60] and *Campylobacter* spp;[61] the mechanisms of resistance are indistinguishable in bacteria isolated from animals or human beings. Bacteria, and mobile genetic elements conferring resistance, linger on animal skin and in faeces and by various means can be transferred between bacteria, and bacteria can make their way to human beings.[62] Despite the subsequent accumulation of this and other evidence, and the publication of the seminal report by Swann on this issue more than 40 years ago,[30,63] a European ban on the use of antimicrobials for growth promotion in livestock did not occur until 2006, and outside the European Union such use still occurs widely, including in the USA. The effect on patterns of resistance from changing use of antimicrobials in animal health care continues to be well described.[21,22]

This interweaving of animal and human microbial ecosystems extends to both commensals and opportunistic

pathogens, including species such as *E coli*, enterococci, and *Staphylococcus aureus*. Evidence supporting transmission from livestock to human beings of ESβL and AmpC-β-lactamase genes on plasmids and of *E coli* clones, most likely through the food chain, have been reported.[64] Phylogenetic evidence from whole-genome analyses of 51 *Enterococcus faecium* strains goes further and supports the hypothesis that the epidemic hospital-adapted lineage emerged from a population that included mostly animal strains.[65] Human infections with MRSA have been classified by their putative sources, such as health-care associated or community associated. However, human MRSA cases associated with exposure to pigs (livestock-associated MRSA [LA-MRSA]) have been described,[66] as have MRSA skin and soft-tissue infections associated with proximity to crop field pig manure and livestock operations.[67]

The contribution of the environment to antimicrobial resistance is also concerning. Use of metals in agriculture (for example when copper is applied directly as a bactericide or fungicide),[68] and even natural occurrence of metals in certain geographical areas,[69] can select for resistance; of more concern is that many metals co-select for antimicrobial resistance.[7,70] Even the commonly used nitrogen fertilisers could affect the soil content of antibiotic resistance genes, causing shifts in the relative abundance of microorganisms.[5] The importance of sewage and waste processing in environment–human transmission is also clear. This stems from antimicrobials and antimicrobial metabolites entering not only from human waste processing, but also from pharmaceutical industry pollution. As a result, many potentially pathogenic antimicrobial resistance microbes have been isolated from pre-treatment and (importantly) post-treatment sewage systems.[42,71] The subsequent detection of antimicrobials and antimicrobial-resistant microbes in surface water and ground water[35,42] reinforces the environmental need for preventive action to be taken.

In summary, the worldwide acquisition, persistence, and transmission of antimicrobial-resistant microbes by people, animals, and the environment is hugely affected by no access to clean water, open rather than closed sewage systems, variation in health-care infection-control practices, inadequate provision of antimicrobials and diagnostics, farming systems with suboptimum regulation of antimicrobials, and high population densities (figure 3, appendix). Although some of these issues exist in high resource settings, they are likely to represent particularly important drivers of antimicrobial resistance in low-income and middle-income countries.

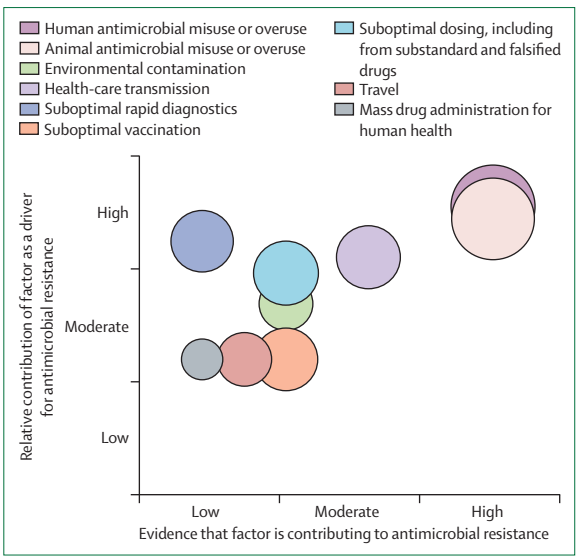

Figure 3: **Role of modifiable drivers for antimicrobial resistance: a conceptual framework**

An infographic to show the considered potential contribution of each factor as a driver for antimicrobial resistance. Associated relative contribution, supporting evidence, and potential population affected (diameter of bubble) was created from a two round Delphi method of contributing authors. Factors were identified from review of the national and international antimicrobial resistance literature. The Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) approach was used to identify the quality of the evidence (the study with the highest GRADE estimate was cited) supporting each driver as being contributory to the rise in antimicrobial resistance (appendix).

The complex issues of fitness and reversibility of antimicrobial resistance

1) Does antimicrobial resistance affect the fitness of micro-organisms?

There is a perception that antimicrobial-resistant microbes might be less fit (ie, less able to grow or cause an infection) than their antimicrobial susceptible counterparts. This situation would mean that reducing the burden of resistance might simply be achieved through removing the selective pressure of antimicrobials, leading to antimicrobial-resistant microbes losing out in darwinian competition with the susceptible strains. Unfortunately, this is frequently not the case and can be seen in two important classes of antimicrobials, the fluoroquinolones and the β-lactams.[72]

In the case of fluoroquinolones (man-made antimicrobials), clonal expansion has given rise to a worldwide high prevalence of resistance in several bacteria. This increase is exemplified by whole-genome sequencing of an epidemic MRSA strain (EMRSA-15; ST22)[73] and *Clostridium difficile* O27.[74] Furthermore, widespread dissemination of fluoroquinolone-resistant clones of *E coli* ST131 exists,[75] whereas fluoroquinolone-resistant *Salmonella enterica* serovar Typhi[72] and *N gonorrhoeae*[76] are not uncommon.

This development and worldwide spread of fluoroquinolone-resistant bacteria suggests resistance to this class of drugs is unlikely to be a burden to the bacterium. Mutations that alter the target of antimicrobials (such as *gyrA*, in the case of fluoroquinolones) can change bacterial physiology, potentially making them less fit. Yet compensatory mutations that restore fitness to wild-type levels might explain, in part,

***Panel 1*: Reversibility of antimicrobial resistance after withdrawal of antimicrobial selective pressure in human and animal populations: a complex picture**

Human health

Evidence of correlation between reduction of antimicrobial use and decrease in antimicrobial resistance is complex, yet it has been seen in some Gram-positive organisms. In a French prospective trial,[82] a reduction in antimicrobial use corresponded to significantly decreased pharyngeal colonisation of penicillin-non-susceptible *S pneumoniae*. In Finland, reductions in macrolide use led to a decrease in erythromycin resistance in *Streptococcus pyogenes*,[83] although this was possibly due to clonal replacement rather than reduced selection pressure. Correlation has also been seen in Gram-negative organisms. In bloodstream infections in the UK, non-susceptibility to cephalosporins and quinolones in *Enterobacteriaceae* has shown a modest decrease over the past decade, probably reflecting prescribing shifts.[26] However, the relative contribution of reductions in antimicrobial use and concomitant infection control interventions is difficult to quantify.

By contrast, evidence also exists where reduced antimicrobial use has not equated to a decrease in antimicrobial resistance. In a prospective trial in Sweden, an 85% reduction in trimethoprim consumption resulted in only a marginal slowing of the rise in trimethoprim resistance in uropathogenic *Escherichia coli*.[84] In the UK, a 98% decrease in cotrimoxazole consumption did not result in reduction of antimicrobial resistance, instead it was inexplicably followed by a 6% increase in sulphonamide resistance in clinical isolates of *E coli*.[85]

This absence of clear correlation between reduced use of antimicrobials and decreased antimicrobial resistance urgently needs a greater understanding to enable the design of effective interventions.

Animal health

Complexity is also evident in animal health when correlating reduced antimicrobial use to a decrease in antimicrobial resistance. In Denmark and Norway, after an avoparcin (a glycopeptide antimicrobial) agricultural ban, a marked reduction in the proportion of glycopeptide-resistant enterococci (GRE) was seen in broilers from poultry farms previously exposed to avoparcin.[86] However, although post-ban studies suggested that the amount of faecal GRE in broilers might have decreased significantly, the prevalence of animals colonised with GRE was still high several years after the ban.[11587] A similar situation was seen in pigs. In the UK, after the 1971 ban of tetracycline as an animal growth promoter, the proportion of tetracycline-resistant *E coli* isolated from the exposed pig population decreased, but the prevalence of colonised pigs remained at 100% several years later.[88]

Yet, importantly, reduction of animal antimicrobial use might not alter production volumes. The avoparcin ban for antimicrobial growth promotion noted above had no deleterious effect on production volume.[89] Similarly in Danish pigs, a 60% reduction in pig antimicrobial consumption had no negative effect on productivity.[90] However the European ban on antimicrobials as growth promoters has seen a modest increase in infections and an attributable increase in therapeutic use of antimicrobials in classes of direct importance to human health.[91]

Although reduction in animal antimicrobial use might not clearly decrease antimicrobial resistance, it will delay further development and spread, without adversely affecting production volumes. Policies to restrict novel classes to either animal or human health might further help prevent the crossover of resistance.

why clinical isolates resistant to this class of drugs have proliferated and spread. As a specific example, mutations detected in a fluoroquinolone-resistant strain of *E coli* were experimentally reconstructed. Strains with single mutations were less fit than the parental strain; however, two or more mutations in combination increased the fitness of the bacterium to similar or greater levels than that of the antimicrobial-susceptible strain.[77] Therefore, once selected, fluoroquinolone-resistant mutants are able to persist and thrive even in the absence of fluoroquinolone antimicrobials.[77]

The carriage of mobile genetic elements such as plasmids, which often contain several antimicrobial resistance genes, has also been proposed to reduce bacterial fitness. However, carriage of natural plasmids with no antimicrobial resistance genes is common, and might confer a benefit to hosts, thereby promoting expansion of plasmid-carrying strains.[78] For example, an IncK plasmid (named pCT), which carries one antimicrobial resistance gene (encoding the ESβL CTX-M-14) has no detectable fitness effect when introduced into new host strains and has spread worldwide in diverse *E coli* strains from animals, human beings, and the environment.[79] As such, absence of antimicrobials might not lead to a reduction in the prevalence of this type of third generation cephalosporin-resistance.[80]

2) Is emergence of resistance reversible?

One strategy to reduce the development and spread of antimicrobial resistance is to lower the selective pressure by limiting or suspending the use of antimicrobials. This strategy is based on the assumption that resistant microorganisms will be outnumbered by susceptible strains if the selective advantage of possessing the resistance determinant is diminished,[81] but as noted, this is not always true (panel 1). Mathematical models have increasingly been used to identify, predict, and help design intervention programmes for antimicrobial resistance.[92] These models support antimicrobial exposure as central to resistance emergence and spread, but importantly do

Panel 2: **Potential approaches to prolong the useful therapeutic life of antimicrobials available at present**

Maintain heterogeneity of antimicrobial agents

Excessively homogeneous antimicrobial use might contribute to selective pressure. Maintaining prescribing diversity can be achieved through several methods. One such method is drug cycling (replacing an antimicrobial belonging to one class with one or more belonging to different classes, sequentially, at the level of the unit or hospital). However, cycling might only be useful if implemented before resistance to the replacement drug has emerged or if resistance to the first drug imposes a fitness cost.[77] Another approach is drug mixing (diversification of antimicrobial prescription at the individual level allowing for patient variation), which maintains personalisation of infection treatment. However, implementing personalised medicine effectively would require accurate and rapid diagnosis of pathogens, antimicrobial resistance, and host factors.

Assure and ensure adequate serum drug concentrations

Subtherapeutic concentrations contribute to poor treatment responses and exert non-lethal selective pressures. Unfortunately, suboptimal drug exposures have many causes: use of poor quality drug (falsified, substandard, or degraded), systematic under-dosing (small infants, overweight adults, infrequent dosing), inadequate drug absorption (malnutrition and drug interactions), unusual large apparent volume of distribution (pregnancy), or particularly rapid clearance.[2] Taken individually, populations exposed to sub-therapeutic concentrations might seem small, but they represent a high proportion of the patients receiving antimicrobials in low-income and middle-income countries. Optimisation of dosage and guarantee of drug quality could reduce sub-therapeutic drug exposure and reduce this modifiable driver of resistance.

Repurposing of withdrawn and underused antimicrobial drugs

Repurposing previously discovered (often FDA-approved) pharmacotherapies might provide a potentially less economically risky pursuit than de-novo drug discovery. This approach has already been evident with the return of colistin and fosfomycin use for multidrug-resistant Gram-negative infections, repurposing of older drugs for bacteria such as *Acinetobacter baumannii*, and more widespread consideration of fusidic acid in clinical practice in some countries since the 1960s. Incentives advocating new drug discovery, including mechanisms to accelerate clinical trials, and making these drugs attractive to industry for production might also need to be adapted to such repurposed drugs.

Combination therapy

Combination therapy is use of several antimicrobials to which the targeted organisms do not show cross-resistance. This relies on microbial populations containing singly resistant mutants, but none that are resistant simultaneously to several drugs. However, the increasing prevalence of multidrug-resistant strains needs careful assessment to ensure efficacy of drug combinations. This strategy has been successful in preventing or delaying resistance in tuberculosis, HIV, and malaria. However, combination therapy successes for the organisms causing these diseases are not directly translatable to bacterial infections and have not been widely recommended so far, often because of the increased cost, but also from fear of incremental, unwanted disturbance of the microbiome. Furthermore, the differentials in half-life of drugs used in combination should be carefully considered, or unintentional monotherapy might ensue. In conclusion, the risk–benefit of combination therapy is unclear and further work is urgently needed to clarify these issues.

find correlations between reduction in antimicrobial use and reduction in antimicrobial resistance, at both the individual patient[93] and human population levels.[94]

Complete eradication of antimicrobial resistance in populations of microbes after reduced selective pressure from antimicrobials is not straightforward. Resistance determinants are easy for microbes to acquire and might persist at low, but detectable, levels for many years in the absence of particular antimicrobials,[95] and in turn, antimicrobial resistant microbes can persist for many years on human and animal skin and as faecal flora without any further exposure or selection pressure.[96] The absence of a clear correlation between reduced use of antimicrobials and decreased antimicrobial resistance can be explained by the interplay of several factors. One factor is context, such as whether the system is open (ie, continuous inflow and outflow, in which the incoming population has a differing frequency of antimicrobial resistance) or closed (ie, a community in which migration is restricted). At the level of the microbe, other factors include the nature of the resistance mechanisms (and level of fitness cost), the propensity for horizontal gene transfer and transmissible elements, and cross-selection and co-selection mechanisms.[77] Cross-selection and co-selection are highly pertinent because many bacteria are multidrug resistant owing to the presence of several antimicrobial resistance genes; therefore, only by reducing use of all drugs to which resistance is encoded will the prevalence of a multidrug-resistant microbe decrease, and only then if this is beneficial to the bacterium.

Approaches to optimising antimicrobial use

1) How should antimicrobials be used to preserve effectiveness and delay resistance in humans?

The published work on antimicrobial resistance across many different diseases converges on a remarkably consistent set of recommendations for prevention and containment.[10,20–22,97,98] These principles focus on improvement of diagnosis and prescription practices, reduction of antimicrobial use in animal husbandry, fish farming,

agriculture, and environmental exposure in general, development of new antimicrobials, guarantee of access to essential medicines of assured quality, and improvement of surveillance. Despite these generic principles being acknowledged, implementation has been slow.[99] However, some disease-specific approaches have been tried, and the evidence suggests that several approaches could be beneficial if tested and validated on a wide range of infectious diseases (panel 2).

In a world where patients often need urgent effective treatment, some antimicrobial optimisation strategies present practical challenges. Analysis of antimicrobial stewardship programmes, and their implementation in varied health-care settings, is an area of vigorous academic pursuit, and although many lessons have clearly been learnt,[28] gaps in our knowledge still exist.[100] The human and economic costs of overcoming these challenges, and filling these gaps, are likely to be small compared with unchecked resistance, which might mean drugs have to be withdrawn and replaced with newly developed alternatives, or worse, the inability to treat at all.[101] Furthermore, beyond optimisation of antimicrobial use, development and implementation of robust infection prevention and control initiatives at national and local levels should be established to curtail onwards transmission of antimicrobial-resistant microbes.[22]

2) How should antimicrobials be used to preserve effectiveness and delay resistance in animals?

The evidence for reversibility of antimicrobial resistance in the context of animal health has been noted as complex (panel 1); however, three principles are clear. First, antimicrobials used as animal growth promoters and for inappropriate routine infection prevention in herds should be banned. Second, access to non-medicated animal feed for farmers should be improved. Third, use of specific classes of antimicrobials should be restricted to either human beings or animals.[4]

Approaches to optimisation of antimicrobial use in both human and animal health should be integrated and coordinated, with shared learning, understanding, and participation in environmental interventions. Such a One Health approach, integrating human medicine, veterinary medicine, public health, and environmental science in specialties including surveillance, development of new diagnostics and therapeutics, and interlinking research and education should enable creation and implementation of more comprehensive and effective policies. Coordinated action and application of these might prolong the therapeutic life of present antimicrobials, and should be a high priority for all.

What gaps in our knowledge need addressing?

The need to address the research gaps in antimicrobial resistance has never been more keenly felt. However, identification of priorities, increase of research funding, and targeting research activity should be coordinated and cohesively addressed. The construction of a worldwide database of previous and present antimicrobial resistance projects has been advocated,[102] but irrespective of this, several areas have particular priority.

First, understanding of how to minimise the selection of antimicrobial resistance is fundamental, including understanding of how to optimise antimicrobial use. This approach requires detailed analysis to define optimum durations and dosage of therapy in specific patient groups (including infants, pregnant women, undernourished, obese, and co-infected patients).[103,104] The absence of basic knowledge about ideal prescribing regimens represents a significant gap. Furthermore, as noted (panel 2), some previously advocated strategies to combat antimicrobial resistance need further assessment, including investigation of antimicrobial prescribing combinations. Many of these matters can only be investigated through translational research, including novel educational methods,[105] and through implementation of international networking and collaborations. A reorganisation of antimicrobial resistance funding to support such translational work is needed.[102]

Second, in support of optimisation of antimicrobial use, improved targeting through rapid infection diagnostics should be enabled. Although this method has been widely advocated,[20,21,97] it has been slow to be implemented, due partly to technical and financial barriers,[22] but also issues around innovation adoption.[106] An example is next generation sequencing, which is likely to radically change microbiological diagnostics, but costs, data pipelines, and clinical confidence in interpreting results have yet to be resolved.[107] An alternative technological development already widely in use, namely matrix-assisted laser desorption/ionisation time-of-flight, allows rapid microorganism identification, and potentially also antimicrobial susceptibility testing.[108] These avenues are suitable for secondary care, but for primary care optimising antimicrobial use through near-patient inflammatory marker assays has growing evidence.[109] For low-income and middle-income countries, diagnostic options several-fold cheaper, with less need of logistic infrastructure, are needed and a focus on chromogenic tests might provide avenues for exploration.[110]

Third, although new drug discovery is essential (and through use of novel laboratory methods, drug discovery has taken a leap forward)[111] research on the quality of currently used antimicrobials is also urgently needed. The issue of substandard antimicrobials is a potentially significant driver of resistance and little is known on the international extent of the problem.[2] Engaging policy makers, prescribers, antimicrobial providers, and the public in ensuring access and assuring quality of antimicrobials should be an essential component of addressing antimicrobial resistance.

Fourth, understanding of how to effectively reduce the prevalence of resistant organisms and their transmission

underpins much of the research needed. In the context of human–human transmission, delineating not just effective, but cost-effective interventions to optimise antimicrobial use, reduce transmission, and prevent environmental contamination with antimicrobial resistance and antimicrobials is essential. To address this balance through minimisation of antimicrobial use in agriculture and aquaculture while meeting the ever-increasing worldwide food demands, is also a fundamental challenge. Adopting a One Health lens to identify gaps in understanding at these interfaces, and then construct integrated research strategies to bridge them, is likely to be a productive way forward. Inherent in this area of research is improvement towards a greater level of detail from surveillance (ie, fine resolution geographical and temporal information) to identify associations between antimicrobial use and misuse, and to clearly identify successful interventions. However, present surveillance data are rarely standardised or reported in a timely way and are often aggregated, making interpretation problematic; without access to individual patient outcomes data are of little use. Making primary data available internationally, and standardising which markers of resistance and species are tracked across international boundaries, might be one avenue to harmonise these efforts[22,97] and is potentially possible to a fine resolution[112] with automated methods.[113] Such information can provide early warning of emerging resistance and allow prompt implementation of efforts to preserve and maximise the useful therapeutic life of present antimicrobials. This coordinated approach has been successfully implemented for malaria and could be productively applied to other pathogens.

Finally, at the level of the microorganism, continued investigation is needed to generate new insights into the basic mechanisms of resistance, gene transfer, and adaptive bacterial evolution. This includes investigating the role of persistence and host–pathogen interactions and their contribution to antimicrobial resistance[114] and antimicrobial resistance reversal. Pursuing areas such as these might uncover new targets for improved therapeutics and diagnostics. Areas of particular therapeutic interest[97,115] include small molecules to attenuate bacterial virulence and disrupt biofilm formation,[116] bacteriophage therapy,[117] the potential for eco-biological approaches,[118] identification of drug targets that select for reduced bacterial fitness during development of antimicrobial resistance,[8] and enhancement of host immune responses[56–58] including host-directed therapy.[119]

Conclusion

Many of the drivers of antimicrobial resistance have a common origin in inappropriate use of antimicrobials in human and animal health care or in agriculture, or from environmental contamination. Although our understanding of antimicrobial resistance is far from complete, the existing evidence base is sufficient to allow targeted policies to be developed in several areas.[2–4] Such strategies to reduce antimicrobial resistance should consider the role and effect of many factors, including the resistance mechanisms, species of microorganism, the particular antimicrobial, as well as the setting and context. So far, all evidence suggests that no single solution exists and several overlapping and synergistic approaches will be needed. Furthermore these approaches should be coordinated at national and international levels, while engaging local stakeholders to ensure widespread implementation. The approaches should be mindful of any potential unintended consequences (including possible impact on access to necessary antimicrobials[2] and patient outcomes) and should share a strong overarching goal to ensure access to effective antimicrobial therapies for this generation and for the future.

Contributors

All authors contributed to the literature search, interpretation of published information, writing, and contribution to figure content for their respective areas of expertise. AHH and LSPM edited, structured, and coordinated the complete manuscript and the figures and panels. All authors reviewed and approved the final version of the manuscript.

Declaration of interests

AHH and LSPM have previously consulted for bioMérieux. All other authors declare no competing interest.

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For more on **malaria surveillance** see www.wwarn.org

References

1 Laxminarayan R, Matsoso P, Klugman KP, et al. Access to effective antimicrobials: a worldwide challenge. *Lancet* 2015; published online Nov 18. http://dx.doi.org/10.1016/S0140-6736(15)00474-2.

2 Mendelson M, Røttingen J-A, Gopinathan U, et al. Maximising access access to achieve appropriate human antimicrobial use in low-income and middle-income countries. *Lancet* 2015; published online Nov 18. http://dx.doi.org/10.1016/S0140-6736(15)00547-4.

3 Dar OA, Hasan R, Schlndt Jo, et al. Exploring the evidence base for national and regional policy interventions to combat resistance. *Lancet* 2015; published online Nov 18. http://dx.doi.org/10.1016/S0140-6736(15)00520-6.

4 Ardal C, Outterson K, Hoffman SJ, et al. International cooperation to improve access to and sustain effectiveness of antimicrobials. *Lancet* 2015; published online Nov 18. http://dx.doi.org/10.1016/S0140-6736(15)00470-5.

5 Forsberg KJ, Patel S, Gibson MK, et al. Bacterial phylogeny structures soil resistomes across habitats. *Nature* 2014; **509:** 612–16.

6 Aminov RI. The role of antibiotics and antibiotic resistance in nature. *Environ Microbiol* 2009; **11:** 2970–88.

7 Martinez JL. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proc R Soc Biol Sci* 2009; **276:** 2521–30.

8 Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. *Nat Rev Microbiol* 2014; **12:** 465–78.

9 Morita Y, Tomida J, Kawamura Y. Responses of *Pseudomonas aeruginosa* to antimicrobials. *Front Microbiol* 2014; **4:** 422.

10 European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2012. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm, 2013.

11 Redgrave LS, Sutton SB, Webber MA, Piddock LJV. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol* 2014; **22:** 438–45.

12 Bell BG, Schellevis F, Stobberingh E, Goossens H, Pringle M. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. *BMC Infect Dis* 2014; **14:** 13.

13 Landers TF, Cohen B, Wittum TE, Larson EL. A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Rep* 2012; **127:** 4–22.

14 Kothari C, Gaind R, Singh LC, et al. Community acquisition of β-lactamase producing Enterobacteriaceae in neonatal gut. *BMC Microbiol* 2013; **13:** 136.

15 Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* 2011; **11:** 355–62.

16 Rubin JE, Ekanayake S, Fernando C. Carbapenemase-producing organism in food, 2014. *Emerg Infect Dis* 2014; **20:** 1264–65.

17 Martinez JL. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ Pollut* 2009; **157:** 2893–902.

18 Elliott E, Brink AJ, van Greune J, et al. In vivo development of ertapenem resistance in a patient with pneumonia caused by *Klebsiella pneumoniae* with an extended-spectrum beta-lactamase. *Clin Infect Dis* 2006; **42:** e95–98.

19 Blázquez J, Oliver A, Gómez-Gómez J-M. Mutation and evolution of antibiotic resistance: antibiotics as promoters of antibiotic resistance? *Curr Drug Targets* 2002; **3:** 345–49.

20 Centres for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013. Atlanta, 2013. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf (accessed Jan 17, 2015).

21 Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance-the need for global solutions. *Lancet Infect Dis* 2013; **13:** 1057–98.

22 WHO. The evolving threat of antimicrobial resistance: options for action. Geneva, 2012. http://apps.who.int/iris/bitstream/10665/44812/1/9789241503181_eng.pdf (accessed Jan 17, 2015).

23 European Centre for Disease Prevention and Control. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals, 2011–2012. Stockholm, 2013. http://ecdc.europa.eu/en/healthtopics/Healthcare-associated_infections/point-prevalence-survey/Documents/healthcare-associated-infections-antimicrobial-use-PPS-summary.pdf (accessed Jan 17, 2015).

24 European Centre for Disease Prevention and Control. Surveillance of antimicrobial consumption in Europe 2012. Stockholm, 2014. http://ecdc.europa.eu/en/publications/Publications/antimicrobial-consumption-europe-esac-net-2012.pdf (accessed Jan 17, 2015).

25 Arnold S, Straus S. Interventions to improve antibiotic prescribing practices in ambulatory care. *Cochrane Database Syst Rev* 2009; **4:** CD003539.

26 Livermore DM, Hope R, Reynolds R, Blackburn R, Johnson AP, Woodford N. Declining cephalosporin and fluoroquinolone non-susceptibility among bloodstream *Enterobacteriaceae* from the UK: links to prescribing change? *J Antimicrob Chemother* 2013; **68:** 2667–74.

27 Little P, Stuart B, Francis N, et al, and the GRACE consortium. Effects of internet-based training on antibiotic prescribing rates for acute respiratory-tract infections: a multinational, cluster, randomised, factorial, controlled trial. *Lancet* 2013; **382:** 1175–82.

28 Davey P, Brown E, Charani E, et al. Interventions to improve antibiotic prescribing practices for hospital inpatients. *Cochrane Database Syst Rev* 2013; **4:** CD003543.

29 McNulty CAM, Cookson BD, Lewis MAO. Education of healthcare professionals and the public. *J Antimicrob Chemother* 2012; **67** (suppl 1)**:** i11–18.

30 Turnidge J, Christiansen K. Antibiotic use and resistance—proving the obvious. *Lancet* 2005; **365:** 548–49.

31 Grijalva CG. Decrease in antibiotic use, an added benefit of PCVs. *Lancet Infect Dis* 2014; **14:** 175–77.

32 European Medicines Agency: European Surveillance of Vetinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 25 EU / EEA countries. Third ESVAC report. London, 2013. doi:EMA/236501/2013. http://www.ema.europa.eu/docs/en_GB/document_library/Report/2014/10/WC500175671.pdf (accessed Jan 19, 2015).

33 Rantala M, Hölsö K, Lillas A, Huovinen P, Kaartinen L. Survey of condition-based prescribing of antimicrobial drugs for dogs at a veterinary teaching hospital. *Vet Rec* 2004; **155:** 259–62.

34 Schwarz S, Kehrenberg C, Walsh TR. Use of antimicrobial agents in veterinary medicine and food animal production. *Int J Antimicrob Agents* 2001; **17:** 431–37.

35 Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ Microbiol* 2006; **8:** 1137–44.

36 Parsley LC, Consuegra EJ, Kakirde KS, Land AM, Harper WF Jr, Liles MR. Identification of diverse antimicrobial resistance determinants carried on bacterial, plasmid, or viral metagenomes from an activated sludge microbial assemblage. *Appl Environ Microbiol* 2010; **76:** 3753–57.

37 Colomer-Lluch M, Imamovic L, Jofre J, Muniesa M. Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs, and poultry. *Antimicrob Agents Chemother* 2011; **55:** 4908–11.

38 Unemo M, Golparian D, Nicholas R, Ohnishi M, Gallay A, Sednaoui P. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother* 2012; **56:** 1273–80.

39 Dhanji H, Doumith M, Rooney PJ, et al. Molecular epidemiology of fluoroquinolone-resistant ST131 *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in nursing homes in Belfast, UK. *J Antimicrob Chemother* 2011; **66:** 297–303.

40 Wozniak RAF, Waldor MK. Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nat Rev Microbiol* 2010; **8:** 552–63.

41 Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* 2004; **427:** 72–74.

42 Wellington EMH, Boxall AB, 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.

43 Lewis DA. The role of core groups in the emergence and dissemination of antimicrobial-resistant *N Gonorrhoeae*. *Sex Transm Infect* 2013; **89** (suppl 4)**:** iv47–51.

44 Chamchod F, Ruan S. Modeling methicillin-resistant Staphylococcus aureus in hospitals: transmission dynamics, antibiotic usage and its history. *Theor Biol Med Model* 2012; **9:** 25.

45 Harris SR, Cartwright EJP, Török ME, et al. Whole-genome sequencing for analysis of an outbreak of meticillin-resistant Staphylococcus aureus: a descriptive study. *Lancet Infect Dis* 2013; **13:** 130–36.

46 Davis GS, Sevdalis N, Drumright LN. Spatial and temporal analyses to investigate infectious disease transmission within healthcare settings. *J Hosp Infect* 2014; **86:** 227–43.

47 Donker T, Wallinga J, Slack R, Grundmann H. Hospital networks and the dispersal of hospital-acquired pathogens by patient transfer. *PLoS One* 2012; **7:** e35002.

48 Woerther P-L, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev* 2013; **26:** 744–58.

49 Paltansing S, Vlot JA, Kraakman MEM, et al. Extended-spectrum Enterobacteriaceae among travelers from the Netherlands. *Emerg Infect Dis* 2014; **19:** 1206–13.

50 Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. *Lancet Infect Dis* 2013; **13:** 785–96.

51 Thomas CP, Moore LSP, Elamin N, et al. Early (2008-2010) hospital outbreak of Klebsiella pneumoniae producing OXA-48 carbapenemase in the UK. *Int J Antimicrob Agents* 2013; **42:** 531–36.

52 Johnson AP, Woodford N. Global spread of antibiotic resistance: the example of New Delhi metallo-b-lactamase (NDM)-mediated carbapenem resistance. *J Med Microbiol* 2013; **62:** 499–513.

53 Mendes RE, Deshpande LM, Jones RN. Linezolid update: stable in vitro activity following more than a decade of clinical use and summary of associated resistance mechanisms. *Drug Resist Updat* 2014; **17:** 1–12.

54 WHO. Report on global sexually transmitted infection surveillance 2013. Geneva, 2014. http://apps.who.int/iris/bitstream/10665/112922/1/9789241507400_eng.pdf?ua=1 (accessed Sept 30, 2015).

55 Kristinsson KG. Epidemiology of penicillin resistant pneumococci in Iceland. *Microb Drug Resist* 1995; **1:** 121–25.

56 Dagan R, Klugman KP. Impact of conjugate pneumococcal vaccines on antibiotic resistance. *Lancet Infect Dis* 2008; **8:** 785–95.

57 Joice R, Lipsitch M. Targeting imperfect vaccines against drug-resistance determinants: a strategy for countering the rise of drug resistance. *PLoS One* 2013; **8:** e68940.

58 Tekle YI, Nielsen KM, Liu J, et al. Controlling antimicrobial resistance through targeted, vaccine-induced replacement of strains. *PLoS One* 2012; **7:** e50688.

59 Anderson ES, Lewis MJ. Drug resistance and its transfer in *Salmonella typhimurium*. *Nature* 1965; **206:** 579–83.

60 Mølbak K. Spread of resistant bacteria and resistance genes from animals to humans--the public health consequences. *J Vet Med B Infect Dis Vet Public Health* 2004; **51:** 364–69.

61 Humphrey TJ, Jørgensen F, Frost JA, et al. Prevalence and subtypes of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. *Antimicrob Agents Chemother* 2005; **49:** 690–98.

62 Kruse H, Sørum H. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Appl Environ Microbiol* 1994; **60:** 4015–21.

63 Swann M. Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine. London, 1969.

64 Kluytmans JAJW, Overdevest ITMA, Willemsen I, et al. Extended-spectrum β-lactamase-producing *Escherichia coli* from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. *Clin Infect Dis* 2013; **56:** 478–87.

65 Lebreton F, Van Schaik W, Manson A. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *MBio* 2013; **4:** e00534–13.

66 Price LB, Stegger M, Hasman H, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio* 2012; **3:** e00305–11.

67 Casey JA, Curriero FC, Cosgrove SE, Nachman KE, Schwartz BS. High-density livestock operations, crop field application of manure, and risk of community-associated methicillin-resistant *Staphylococcus aureus* infection in Pennsylvania. *JAMA Intern Med* 2013; **173:** 1980–90.

68 Berg J, Tom-Petersen A, Nybroe O. Copper amendment of agricultural soil selects for bacterial antibiotic resistance in the field. *Lett Appl Microbiol* 2005; **40:** 146–51.

69 Knapp CW, McCluskey SM, Singh BK, Campbell CD, Hudson G, Graham DW. Antibiotic resistance gene abundances correlate with metal and geochemical conditions in archived Scottish soils. *PLoS One* 2011; **6:** e27300.

70 Seiler C, Berendonk TU. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Front Microbiol* 2012; **3:** 399.

71 Kristiansson E, Fick J, Janzon A, et al. Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. *PLoS One* 2011; **6:** e17038.

72 Baker S, Duy PT, Nga TVT, et al. Fitness benefits in fluoroquinolone-resistant *Salmonella typhi* in the absence of antimicrobial pressure. *eLife* 2013; **2:** e01229.

73 Holden MTG, Hsu L-Y, Kurt K, et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. *Genome Res* 2013; **23:** 653–64.

74 He M, Miyajima F, Roberts P, et al. Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*. *Nat Genet* 2013; **45:** 109–13.

75 Guo S, Brouwers HJM, Cobbold RN, et al. Fluoroquinolone-resistant extraintestinal pathogenic *Escherichia coli*, including O25b-ST131, isolated from faeces of hospitalized dogs in an Australian veterinary referral centre. *J Antimicrob Chemother* 2013; **68:** 1025–31.

76 Dillon J-AR, Parti RP. Fluoroquinolone resistance in *Neisseria gonorrhoeae*: fitness cost or benefit? *J Infect Dis* 2012; **205:** 1775–77.

77 Marcusson LL, Frimodt-Møller N, Hughes D. Interplay in the selection of fluoroquinolone resistance and bacterial fitness. *PLoS Pathog* 2009; **5:** e1000541.

78 Paytubi S, Aznar S, Madrid C, et al. A novel role for antibiotic resistance plasmids in facilitating Salmonella adaptation to non-host environments. *Environ Microbiol* 2013; **16:** 950–62.

79 Dhanji H, Khan P, Cottell JL, et al. Dissemination of pCT-like IncK plasmids harboring CTX-M-14 extended-spectrum β-lactamase among clinical *Escherichia coli* isolates in the United Kingdom. *Antimicrob Agents Chemother* 2012; **56:** 3376–77.

80 Cottell JL, Webber MA, Piddock LJV. Persistence of transferable extended-spectrum-β-lactamase resistance in the absence of antibiotic pressure. *Antimicrob Agents Chemother* 2012; **56:** 4703–06.

81 Andersson DI, Hughes D. Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiol Rev* 2011; **35:** 901–11.

82 Guillemot D, Varon E, Bernède C, et al. Reduction of antibiotic use in the community reduces the rate of colonization with penicillin G-nonsusceptible *Streptococcus pneumoniae*. *Clin Infect Dis* 2005; **41:** 930–38.

83 Seppälä H, Klaukka T, Vuopio-Varkila J, et al, and the Finnish Study Group for Antimicrobial Resistance. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. *N Engl J Med* 1997; **337:** 441–46.

84 Sundqvist M, Geli P, Andersson DI, et al. Little evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use. *J Antimicrob Chemother* 2010; **65:** 350–60.

85 Enne VI, Livermore DM, Stephens P, Hall LM. Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. *Lancet* 2001; **357:** 1325–28.

86 Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob Agents Chemother* 2001; **45:** 2054–59.

87 Borgen K, Simonsen GS, Sundsfjord A, Wasteson Y, Olsvik O, Kruse H. Continuing high prevalence of VanA-type vancomycin-resistant enterococci on Norwegian poultry farms three years after avoparcin was banned. *J Appl Microbiol* 2000; **89:** 478–85.

88 Smith HW. Persistence of tetracycline resistance in pig E. coli. *Nature* 1975; **258:** 628–30.

89 Emborg H, Ersbøll AK, Heuer OE, Wegener HC. The effect of discontinuing the use of antimicrobial growth promoters on the productivity in the Danish broiler production. *Prev Vet Med* 2001; **50:** 53–70.

90 Aarestrup FM, Jensen VF, Emborg H-D, Jacobsen E, Wegener HC. Changes in the use of antimicrobials and the effects on productivity of swine farms in Denmark. *Am J Vet Res* 2010; **71:** 726–33.

91 Vigre H, Larsen PB, Andreasen M, Christensen J, Jorsal SE. The effect of discontinued use of antimicrobial growth promoters on the risk of therapeutic antibiotic treatment in Danish farrow-to-finish pig farms. *Epidemiol Infect* 2008; **136:** 92–107.

92 Temime L, Hejblum G, Setbon M, Valleron AJ. The rising impact of mathematical modelling in epidemiology: antibiotic resistance research as a case study. *Epidemiol Infect* 2008; **136:** 289–98.

93 Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ* 2010; **340:** c2096.

94 Goossens H, Ferech M, Vander Stichele R, Elseviers M, and the ESAC Project Group. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* 2005; **365:** 579–87.

95 Johnsen PJ, Townsend JP, Bøhn T, Simonsen GS, Sundsfjord A, Nielsen KM. Factors affecting the reversal of antimicrobial-drug resistance. *Lancet Infect Dis* 2009; **9:** 357–64.

96 Sjölund M, Wreiber K, Andersson DI, Blaser MJ, Engstrand L. Long-term persistence of resistant Enterococcus species after antibiotics to eradicate *Helicobacter pylori*. *Ann Intern Med* 2003; **139:** 483–87.

97 Department of Health, Department for Environment Food and Rural Affairs. UK Five year Antimicrobial Resistance Strategy 2013 to 2018. London, 2013. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/244058/20130902_UK_5_year_AMR_strategy.pdf (accessed July 14, 2015).

98 WHO. Strategic and Technical Advisory Group on Antimicrobial Resistance. Report of the first meeting Geneva, Sept 19–20, 2013 http://www.who.int/drugresistance/stag/amr_stag_meetingreport0913.pdf (accessed July 14, 2015).

99 zur Wiesch PA, Kouyos R, Engelstädter J, Regoes RR, Bonhoeffer S. Population biological principles of drug-resistance evolution in infectious diseases. *Lancet Infect Dis* 2011; **11:** 236–47.

100 Davey P, Peden C, Charani E, Marwick C, Michie S. Time for action-Improving the design and reporting of behaviour change interventions for antimicrobial stewardship in hospitals: Early findings from a systematic review. *Int J Antimicrob Agents* 2015; **45:** 203–12.

101 Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. London, 2014. http://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf (accessed Dec 12, 2014).

102 Head MG, Fitchett JR, Cooke MK, et al. Systematic analysis of funding awarded for antimicrobial resistance research to institutions in the UK, 1997–2010. *J Antimicrob Chemother* 2014; **69:** 548–54.

103 Falagas ME, Athanasoulia AP, Peppas G, Karageorgopoulos DE. Effect of body mass index on the outcome of infections: a systematic review. *Obes Rev* 2009; **10:** 280–89.

104 Barker CIS, Standing JF, Turner MA, McElnay JC, Sharland M. Antibiotic dosing in children in Europe: can we grade the evidence from pharmacokinetic/pharmacodynamic studies—and when is enough data enough? *Curr Opin Infect Dis* 2012; **25:** 235–42.

105 Castro-Sánchez E, Charani E, Moore LSP, Gharbi M, Holmes AH. "On call: antibiotics"—development and evaluation of a serious antimicrobial prescribing game for hospital care. In: Schouten B, Fedtke S, Schijven M, Vosmeer M, Gekker A, eds. Games for Health 2014: Proceedings of the 4th conference on gaming and playful interaction in healthcare. Wiesbaden, Springer Vieweg: 2014.

106 Kyratsis Y, Ahmad R, Holmes A. Technology adoption and implementation in organisations: comparative case studies of 12 English NHS Trusts. *BMJ Open* 2012; **2:** e000872.

107 Köser CU, Ellington MJ, Cartwright EJP, et al. Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. *PLoS Pathog* 2012; **8:** e1002824.

108 Hrabák J, Chudácková E, Walková R. Matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. *Clin Microbiol Rev* 2013; **26:** 103–14.

109 Schuetz P, Müller B, Christ-Crain M, et al. Procalcitonin to initiate or discontinue antibiotics in acute respiratory tract infections. *Cochrane Database Syst Rev* 2012; **9:** CD007498.

110 Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 2012; **18:** 1503–07.

111 Ling LL, Schneider T, Peoples AJ, et al. A new antibiotic kills pathogens without detectable resistance. *Nature* 2015; **517:** 455–59.

112 Moore LS, Freeman R, Gilchrist MJ, et al. Homogeneity of antimicrobial policy, yet heterogeneity of antimicrobial resistance: antimicrobial non-susceptibility among 108,717 clinical isolates from primary, secondary and tertiary care patients in London. *J Antimicrob Chemother* 2014; **69:** 3409–22.

113 Freeman R, Charlett A, Hopkins S, et al. Evaluation of a national microbiological surveillance system to inform automated outbreak detection. *J Infect* 2013; **67:** 378–84.

114 Balaban NQ, Gerdes K, Lewis K, McKinney JD. A problem of persistence: still more questions than answers? *Nat Rev Microbiol* 2013; **11:** 587–91.

115 NIAID's Antibacteria Resistance Program: Current Status and Future Directions. Bathesda, MD, 2014.

116 Wang Y, Ma S. Small molecules modulating AHL-based quorum sensing to attenuate bacteria virulence and biofilms as promising antimicrobial drugs. *Curr Med Chem* 2014; **21:** 296–311.

117 Soothill J. Use of bacteriophages in the treatment of *Pseudomonas aeruginosa* infections. *Expert Rev Anti Infect Ther* 2013; **11:** 909–15.

118 van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med* 2013; **368:** 407–15.

119 Czyż DM, Potluri L-P, Jain-Gupta N, et al. Host-directed antimicrobial drugs with broad-spectrum efficacy against intracellular bacterial pathogens. *MBio* 2014; **5:** e01534–14.