# **Understanding the mechanisms and the drivers of antimicrobial resistance**

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## **Abstract:**

Combating the threat to human health and biosecurity from antimicrobial resistance (AMR) requires an understanding of its mechanisms and drivers. Emergence of AMR among micro-organisms is a natural phenomenon, yet AMR selection has been driven by antimicrobial exposure in healthcare, 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 AMR by removing antimicrobial selective pressure alone rely upon resistance imparting a ‘fitness cost’; an effect not always apparent. Minimising resistance must therefore be considered comprehensively; by resistance mechanism, by micro-organism, by antimicrobial drug, host and context. Parallel to new drug discovery there must be broad ranging, multidisciplinary research across these levels, interlinked across sectors. Intelligent, integrated approaches, mindful of potential unintended consequences, are required to ensure sustained, global access to effective antimicrobials.

## **Key messages:**

* The emergence of AMR is a natural evolutionary response to antimicrobial exposure. At a societal level, complex and interlinking drivers are increasing prevalence of AMR microbes, predominantly arising from use in humans and agriculture and the pollution of the environment.
* Acquisition of AMR 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 AMR following withdrawal of antimicrobial selective pressure is consequently not clear cut; minimising emergence of resistance to new and future agents is therefore essential.
* Gaining insight into the mechanisms of AMR, 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 AMR 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 impact on access to effective antimicrobial treatment.
* There is no single solution and multiple, synergistic, overlapping and complementing approaches will be needed, with a strong overarching shared goal to ensure and sustain access to effective antimicrobial therapies.

## **Introduction**

The increasing challenge to health care attributable to antimicrobial resistance (AMR), and the subsequent lack of access to effective antimicrobials, is of global concern. There is a real threat that the public health gains from improved access to antimicrobials, including the gains in childhood survival, could be undermined.1 Understanding the scientific basis of AMR is essential to combatting this public health threat. An understanding that must cover the resistance mechanisms, enabling novel approaches to diagnostics and therapeutics, through to the drivers of AMR in society and the environment; essential for the development of appropriate interventional policies.2–4 The numerous factors contributing to the current global status of AMR are reviewed in this article, 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 AMR are considered and assessed in the context of the community (including the environment and agriculture) and in healthcare systems (for an extended list of references see web appendix www.XXXXX). From this, stakeholders can engage with issues specific to their area of practice, yet also be mindful of cross-sectorial interconnectivity and the need for a “One Health” approach to AMR.

## **Emergence of resistance**

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

Through a Darwinian selection process micro-organisms have developed robust mechanisms to evade destruction from many toxic substances. Most antimicrobial agents are naturally produced by micro-organisms, including environmental fungi and saprophytic bacteria, or are synthetic modifications of them, with only a few agents (e.g. sulphonamides and fluoroquinolones) being wholly synthetic. The protective mechanisms that have evolved include preventing entry or exporting the agent, producing enzymes that destroy or modify the antimicrobial, or making changes to the antimicrobial target.Therefore, AMR could be considered to simply represent the Darwinian competition from natural micro-organism derived antimicrobial molecules. Recent functional meta-genomic studies of soil microorganisms have shown an extensive 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 impact on human health is the resistance developed against β-lactam antimicrobial drugs, where the enzymes (β-lactamases) that inactivate these antimicrobial molecules, have existed for millions of years.6

It was originally thought that the production of antimicrobial molecules by saprophytic organisms inhibited the growth of neighbouring organisms, providing a competitive advantage in the local environment; recent findings suggest a more complex interaction. First, the concentration of antimicrobial molecules in the soil appears to be too low to inhibit growth of other bacteria.7 Second, recent evidence suggests even sub-lethal levels of antimicrobials have significant 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), where the genes involved in the synthesis of carbapenems may also have a role in the quorum sensing apparatus (the mechanism through which a colony of micro-organisms coordinates growth and gene expression) or formation of biofilms.6 This leads to further questions about the unintended consequences of “anti”-microbial agents, with our understanding of their potential impact on micro-organisms, beyond their inhibitory action, remaining incomplete.9

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

Even though many micro-organisms in the environment, and higher organisms including plants and animals, naturally produce antimicrobial substances, there is little evidence to indicate that this contributes significantly to the selection of antimicrobial resistant micro-organisms in their native environment.7 Therefore the numerous and varied human, animal and agriculture uses of antimicrobials must be considered to be key global drivers of AMR.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 recent study in India observed that in a cohort of breast fed babies 14·3% harboured Enterobacteriaceae containing an enzyme that inactivates β-lactam drugs, an extended spectrum β-lactamase (ESβL), on day 1 and 41·5% at day 60.14 The environment, drinking water, and food are probably the most important vehicles for establishing the normal gut microflora. Antimicrobial resistant bacteria have been found in every environment examined to date including Antarctica, the sea, soil, drinking water,15 and various food products.16 This poly-microbial, variably antimicrobial resistant, commensal microbiome (micro-organisms that are currently not causing infection at that body site e.g. the gastrointestinal tract or skin) is established at an early age.

In a pristine (i.e. ‘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 micro-organism populations in humans include species that are naturally resistant to some antimicrobials. Selective pressure is exerted by any condition (e.g. 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 agents. *In vivo* development of *de novo* resistance within a human individual has been observed 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 i.e. they are hypermutable. Some antimicrobials exacerbate this hypermutability, promoting selection of resistance.19

### *3) Why does resistance emerge at the population level among 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 and misuse/overuse of antimicrobial agents is considered to be a major driving force towards resistance.21,22

In humans, the concentration of antibiotic prescribing may be highest in inpatient settings, with 30-40% on antibiotics in European hospitals.23 However, the overall incidence of antimicrobial prescribing is highest in the community from primary care.24 Even though guidelines recommend prudent use, needless prescriptions are seen even in countries with low prescribing.25 However an overall reduction in prescriptions for antimicrobials has been observed over the past decade, with a modest reduction in AMR seen in some settings.26 The role of educating prescribers is a key in overcoming antimicrobial misuse/overuse and has been seen to be effective in primary care27 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 appears clear cut, this relationship is complex.30 Confounding factors mean a uniform approach to understanding resistance cannot be taken. These include; pathogen-drug interactions, pathogen-host interactions, mutation rates of the pathogen, emergence of successful antimicrobial resistant clones, the transmission rates of pathogens between humans, 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

There is also evidence that more antimicrobials are used in food production than in humans,22 with marked national differences in the amount of antimicrobial agents used in food producing animals, varying a hundred fold from 4-400 mg antimicrobial per kilogram of meat produced among European countries.32 Various studies have demonstrated that AMR has, at least in part, emerged as a consequence 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 micro-organism, as is the impact of changes in this use. This necessitates that policies are 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 healthcare structures.

## **Transmission of resistance**

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

In addition to selection of AMR through mutations in genes encoded on microbe’s chromosomes, new genetic material can also be exchanges between organisms. This can provide the host cell and its progeny with new genetic material encoding AMR 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 AMR, but also by inducing transfer of resistance determinants between micro-organisms.41

### *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 AMR.21,22 In the community, faecal-oral transmission, often through failures in sanitation, plays an important role particularly for resistant Enterobacteriaceae.42 Transmission can also occur through sexual encounters; for *N.* *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 healthcare associated infections. Using MRSA as an example, modelling indicates duration of patient stay and contamination of health-care workers’ hands both contribute to ongoing 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 healthcare institutions. Moreover, the inability to rapidly identify resistant micro-organisms with appropriate and efficient diagnostic tests is likely to further contribute to ongoing transmission in healthcare settings.

During the last ten years the human microbiota has acquired AMR Enterobacteriaceae on an unprecedented scale. In some parts of the world the carrier rate of ESβL-positive Enterobacteriaceae in the gut is over 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 travelers were colonised with ESβL-producing Enterobacteriaceae before travel, but 30·5% acquired gut colonization 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 globe, and between organisms, with NDM,15 KPC50 and OXA-4851 enzymes being the greatest concern (**Figure 2**52–54). Travel related human-human spread has also been evident for Gram-positive organisms, notably in the spread of the antimicrobial resistant *S. pneumoniae* from Spain to Iceland.55

Population based strategies to interrupt human-human transmission, including through interventions such as mass drug administration and vaccination, may alter the transmission dynamics of pathogens and resistance determinants. Evidence for this is strongest for vaccines; use can result in reduction or elimination of the target infection reducing the need for antimicrobial treatment and selection of AMR.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 may be an effective tool in controlling AMR.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 AMR microbes from animals to humans, and the association between use of antimicrobial growth promoters in farm animals and transmission of resistant bacteria, were recognised in the 1960s.59 AMR that arises in animal husbandry is now well established and affects zoonotic pathogens such as *Salmonella* serovars60 and *Campylobacter* spp;61 the mechanisms of resistance are indistinguishable in bacteria isolated from animals or humans. Bacteria, and mobile genetic elements conferring resistance, linger on animal skin and in faeces and by a variety of means can make their way to humans, and between bacteria, respectively.62 Despite the subsequent accumulation of this and other evidence, and the publication of the seminal Swann report on this issue over 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 EU such use still occurs widely including in the USA. The impact on patterns of resistance from changing use of antimicrobials in animal healthcare 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 humans 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 *E. 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 categorised by their putative sources, such as health care- or community- associated. However, human MRSA cases associated with exposure to pigs (livestock-associated MRSA [LA-MRSA]) have recently been described,66 as has MRSA skin and soft-tissue infections associated with proximity to crop field pig manure and livestock operations.67

The contribution of the environment to AMR is also concerning. Use of metals in agriculture (for example when copper is applied directly as a bactericide/fungicide),68 and even natural occurrence of metals in certain geographical areas,69 can select for resistance; of more concern many metals co-select for AMR.7,70 Even the commonly used nitrogen fertilizers may influence the soil content of antibiotic resistance genes, causing shifts in the relative abundance of micro-organisms.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. In consequence numerous potentially pathogenic AMR microbes have been isolated from pre- and (importantly) post-treatment sewage systems.42,71 The subsequent detection of antimicrobials and AMR microbes in surface- and ground-water,35,42 reinforces the environmental need for preventive action to be taken.

In summary, the global acquisition, persistence, and transmission of AMR microbes by people, animals, and the environment is hugely influenced by: lack of access to clean water, open rather than closed sewage systems, variation in healthcare infection control practices, inadequate provision of antimicrobials and diagnostics, farming systems with sub-optimal regulation of antimicrobials, and high population densities (**Figure 3** and **Supplementary Table 1**). Whilst some of these issues exist in high resource settings, they are likely to represent particularly important drivers of AMR in low and middle income countries.

## **The complex issues of fitness and reversibility of antimicrobial resistance**

### *1) Does antimicrobial resistance impact the fitness of micro-organisms?*

There is a perception that AMR microbes may be less fit (i.e. less able to grow or cause an infection) than their antimicrobial susceptible counterparts. This would mean that reducing the burden of resistance might simply be achieved through removing the selective pressure of antimicrobials, leading to AMR microbes losing out in Darwinian competition with the susceptible strains. Unfortunately, this is frequently not the case and can be demonstrated 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 global high prevalence of resistance among several bacteria. This is exemplified by whole genome sequencing of an epidemic MRSA strain (EMRSA-15; ST22)73 and *Clostridium* *difficile* O27.74 Furthermore, there is widespread dissemination of fluoroquinolone-resistant clones of *E. coli* ST131,75 whilst fluoroquinolone-resistant *Salmonella* *enterica* serovar Typhi72 and *N. gonorrhoeae*76are not uncommon.

This development and global 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. However, recent data indicates that compensatory mutations that restore fitness to wild type levels explains, in part, why clinical isolates resistant to this class of drugs have proliferated and spread. For example, mutations detected in a fluoroquinolone-resistant strain of *E. coli* were experimentally re-constructed. 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 AMR genes, has also been proposed to reduce bacterial fitness. However, carriage of natural plasmids with no AMR genes is common, and may confer a benefit to hosts, thereby promoting expansion of plasmid carrying strains.78 For example, a plasmid, pCT, which carries one AMR gene (encoding the ESβL CTX-M-14) has no detectable fitness impact when introduced into new host strains and has spread globally in diverse *E. coli* strains from animals, humans and the environment.79 As such, absence of antimicrobials may not lead to a reduction in the prevalence of this third generation cephalosporin-resistant strain.80

### *2) Is emergence of resistance reversible?*

One strategy to reduce the development and spread of AMR is to lower the selective pressure by limiting or suspending the use of antimicrobials. This is based upon the assumption that resistant micro-organisms 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 AMR.82 These models support antimicrobial exposure as central to resistance emergence and spread, but importantly do find correlations between reduction in antimicrobial use and falls in AMR, at both the individual patient83 and human population levels.84

Complete eradication of AMR in populations of microbes following reduced selective pressure from antimicrobials is however not straightforward. Resistance determinants are easy for microbes to acquire and may persist at low, but detectable, levels for many years in the absence of particular antimicrobials,85 and in turn AMR microbes can persist for many years on human and animal skin and as faecal flora without any further exposure or selection pressure.86 The lack of a clear correlation between reduced use of antimicrobials and decreased AMR can be explained by the interplay of several factors. One such is that of context, such as whether the system is open (i.e. continuous in- and out-flow, where the incoming population has a differing frequency of AMR) or closed (i.e. a community where migration is limited). 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, as well as cross- and co-selection mechanisms.77 The latter is highly pertinent, as many bacteria are multidrug resistant due to the presence of multiple AMR genes; therefore only by reducing use of all drugs to which resistance is encoded will the prevalence of that multi-drug resistant microbe fall – and only then if this is beneficial to the bacterium.

## **Approaches to optimising antimicrobial use.**

### *1) How should antimicrobials be used to preserve effectiveness/delay resistance in humans?*

The literature on AMR across many different diseases converges on a remarkably consistent set of recommendations for prevention and containment.10,20–22,87,88 These principles focus on: (1) Improving diagnosis and prescription practices; (2) Reducing antimicrobial use in animal husbandry, fish farming, agriculture and environmental exposure in general; (3) Developing new antimicrobials; (4) Ensuring access to essential medicines of assured quality, and (5) Improving surveillance. Despite these “generic” principles being acknowledged, implementation has been slow.89 However, some disease specific approaches have been tried, and the evidence suggests several could be beneficial if tested and validated on a wider 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 healthcare settings, is an area of vigorous academic pursuit, and whilst many lessons have clearly been learnt,28 gaps in understanding still exist.90 The human and economic costs of overcoming these challenges, and filling these gaps, are likely to be small compared with unchecked resistance meaning drugs must be ‘retired’ and replaced with newly developed alternatives, or worse the inability to treat at all.91 Furthermore, beyond optimisation of antimicrobial use, development and implementation of robust infection prevention and control initiatives at national and local levels must be established to curtail onwards transmission of AMR microbes.22

### *2) How should antimicrobials be used to preserve effectiveness/delay resistance in animals?*

The evidence around reversibility of AMR 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. Finally, use of specific classes of antimicrobials should be restricted to either humans or animals.4

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

## **What are gaps in understanding that need addressing?**

The need to address the research gaps in AMR has never been more keenly felt. However, identifying priorities, increasing the research funding, and targeting research activity should be coordinated and cohesively addressed. The construction of a global database of previous and current AMR projects has been advocated,92 but irrespective several areas have particular priority.

First, understanding how to minimise the selection of AMR is fundamental, including understanding how to optimize antimicrobial use. This 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).93,94 The lack of basic knowledge regarding ideal prescribing regimens represents a significant gap. Furthermore, as noted, some pragmatic currently advocated options to address prescribing in AMR need further evaluation including investigating antimicrobial prescribing combinations. Many of these areas can only be investigated through translational research, including into novel educational tools,95 and through implementing international networking and collaborations. A re-organisation of AMR funding to support such translational work is needed.92

Second, in support of optimising antimicrobial use, improved targeting through rapid infection diagnostics must be enabled. Whilst this has been widely advocated,20,21,87 it has been slow to be implemented, due partly to technical and financial barriers,22 but also issues around innovation adoption.96 An example is next generation sequencing which is likely to radically change microbiological diagnostics, yet costs, data pipelines and clinical confidence in interpreting results have yet to be resolved.97 An alternative technological development already widely in use, namely matrix-assisted laser desorption/ionization time-of-flight, allows rapid micro-organism identification, and potentially also antimicrobial susceptibility testing.98 These avenues are suitable for secondary care, but for primary care optimising antimicrobial use through near patient inflammatory marker assays has growing evidence.99 For low- and middle-income countries (LMICs), diagnostic options several fold cheaper, with less need of logistic infrastructure, are needed and a focus on chromogenic tests may provide avenues for exploration.100

Third, whilst new drug discovery is essential, and through use of novel laboratory methods has recently taken a leap forward,101 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 must be an essential component of addressing AMR.

Fourth, understanding how to effectively reduce the prevalence of resistant organisms and their transmission underpins much of the research required. In the context of human-human transmission, delineating not just effective, but cost effective interventions to optimise antimicrobial use, minimise transmission, and prevent environmental contamination with AMR and antimicrobials is essential. Addressing this balance through minimising antimicrobial use in agri- and aquaculture, whilst meeting the ever increasing global 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 improving fine resolution surveillance to identify associations between antimicrobial use and abuse, and to clearly identify successful interventions. However current 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 limited use. Making primary data available internationally, and standardising which markers of resistance and species are tracked across international boundaries, may be one avenue to harmonise these efforts, 22,87 and is potentially possible to a fine resolution102 using automated methods.103 Such information can provide early warning of emerging resistance and allow prompt implementation of efforts to preserve and maximize the useful therapeutic life of current antimicrobials. This coordinated approach has been successfully implemented for malaria (www.wwarn.org) and could be productively applied to other pathogens.

Finally, at the level of the micro-organism 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 of host-pathogen interactions, and their contribution to AMR104 and AMR reversal. Pursuing areas such as these may uncover new targets for improved therapeutics and diagnostics. Areas of particular therapeutic interest87,105 include; small molecules to attenuate bacterial virulence and disrupt biofilm formation,106 bacteriophage therapy,107 the potential for eco-biological approaches,108 identification of drug targets that select for reduced bacterial fitness during development of AMR,8 and enhancement of host-immune responses56–58 including host-directed therapy.109

## **Conclusion**

Many of the drivers of AMR have a common origin in inappropriate use of antimicrobials in human and animal healthcare or in agriculture, or from environmental contamination. Whilst our understanding of AMR 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 minimise AMR must consider the role and influence of many factors, including the resistance mechanisms, species of micro-organism, the particular antimicrobial, as well as the setting and context. So far, all evidence indicates that there is no single solution and multiple, overlapping and synergistic approaches will be needed. Furthermore these must be coordinated at national and international levels, whilst engaging local stakeholders to ensure widespread implementation. The approaches should be mindful of any potential unintended consequences and should share a strong overarching goal to ensure access to effective antimicrobial therapies for this generation and for the future.

## **Word Count**

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Panel 1: 447

Panel 2: 469

## **Conflict of Interest**

A.H.H & L.S.P.M have consulted for bioMérieux (2013 and 2014 respectively). All other authors no conflict of interests to declare.

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## **Contributions**

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.

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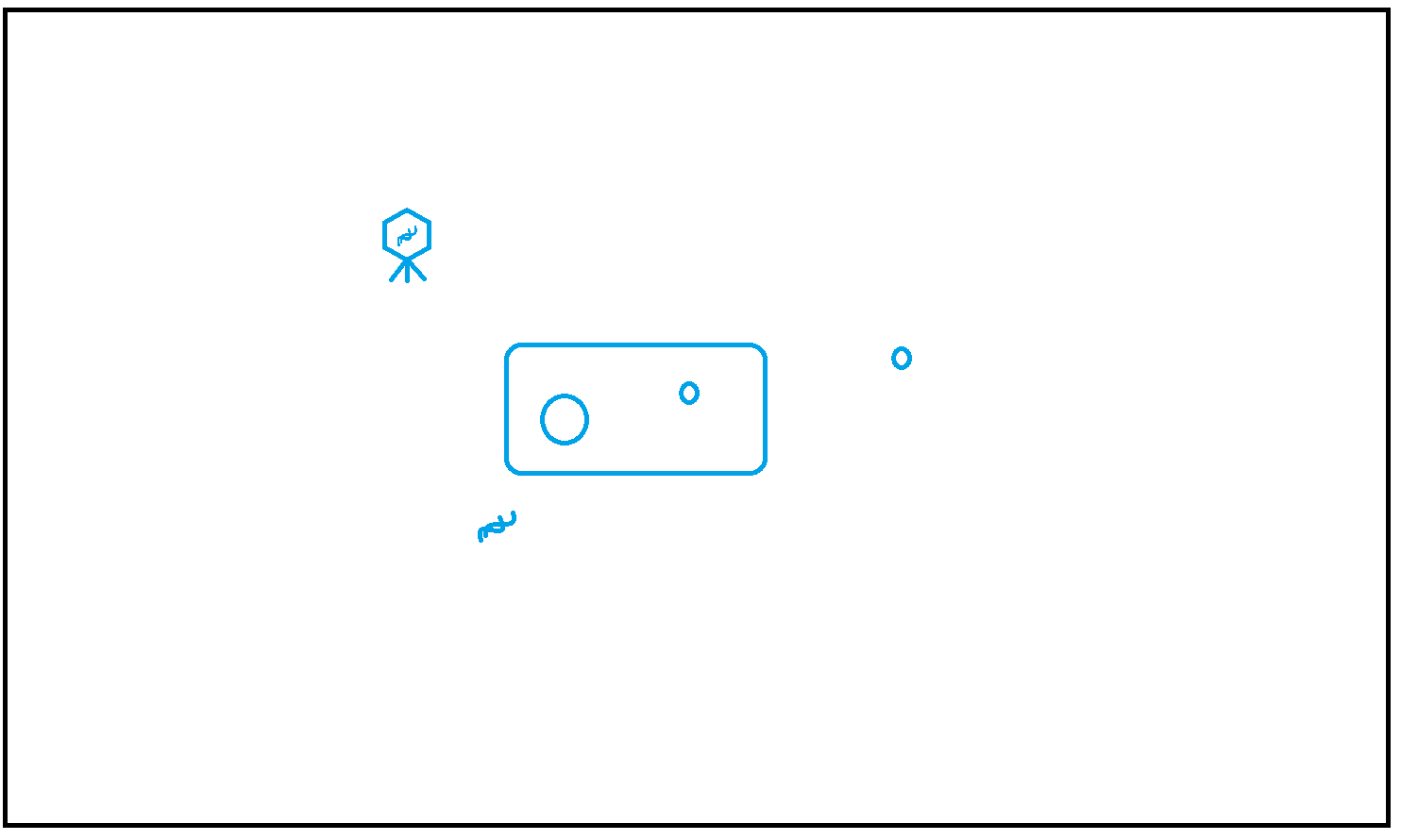
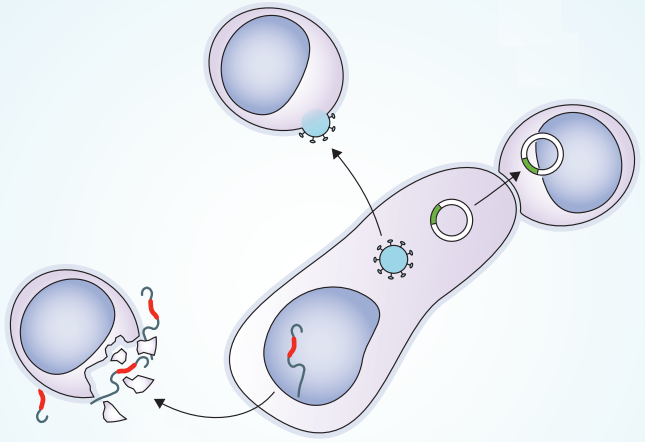
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**Figure 1**.

**Transmission of genetic material between micro-organisms.** *(NB. Figure graphics to be further developed with Elsevier illustrator/designer)*



**Transformation:**

Some bacteria area able to take up free DNA from the environment and incorporate it into their chromosome.

**Transduction:**

Bacteriophages (viruses that infect bacteria) mediate transfer of DNA between bacteria via transduction, whereby DNA from a donor bacterium is packaged into a virus particle and transferred into a recipient bacterium during infection.

**Conjugation:**

The mechanism of gene transfer responsible for the most concerning aspects of AMR; a sex pilus (small tube) forms between two bacterial cells through which a plasmid is transferred from one to the other.

**Clinical examples:**

Bacteria residing in host mucosa such as the human respiratory tract (e.g. *Streptococcus pneumoniae* and *Haemophilus influenzae*) or genital tract (e.g. *Neisseria gonorrhoeae*) are naturally transformable. Recombination of foreign DNA acquired from closely related species, such as *S. mitis* with that of *S. pneumoniae,* confers penicillin resistance via the formation of mosaic genes (i.e. genes that can contain DNA from more than one species of highly related micro-organism). The mosaic penicillin-binding protein (*pbp)* gene gives rise to a new target protein which is resistant to penicillin. Likewise, in *N. gonorrhoeae,* a mosaic *penA* gene is associated with ceftriaxone resistance.38

**Clinical examples:**

Responsible for the global dissemination of genes encoding carbapenemases (resistance mechanisms of particular clinical concern) including New Delhi Metallo-β-lactamase (NDM) and *Klebsiella pneumoniae* carbapenemase (KPC) enzymes, as well as ESβLs.15 Whilst many AMR genes can be carried by many different plasmids some, such as those of the IncFII group, are responsible for much of the dissemination of particular resistances such as the ESβL CTX-M-15.39 Some plasmids also appear restricted and have a narrow host range; this includes those that have spread OXA-48-like carbapenemases among Enterobacteriaceae. There are also elements that can transfer genes between plasmids and the bacterial host chromosome and these integrative chromosomal elements (ICEs) can carry genes conferring resistance to drugs including ampicillin, chloramphenicol, erythromycin, kanamycin, tetracycline and sulfamethoxazole in a range of Gram-negative species and Streptococci.40

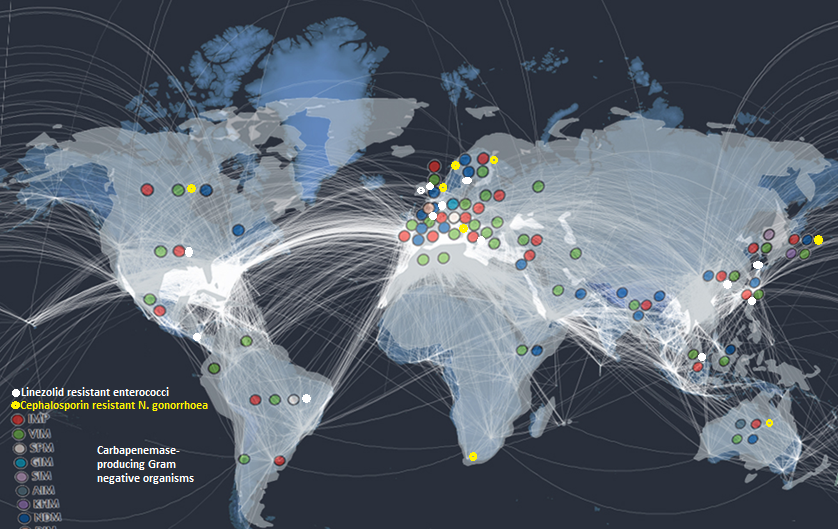
**Clinical examples:**

Transduction is common in staphylococci, but beyond this specific example, bacteriophages are common, and it has been proposed that they represent a reservoir of AMR genes. For instance, such AMR genetic material has been identified in phage DNA isolated from activated sludge from wastewater treatment,36 and specifically ESβL genes and *mecA* genes (the latter responsible for methicillin resistance in *Staphylococcus aureus*) have been found in bacteriophage extracted from faecal samples at farms and abattoirs in Spain.37

**Legend:** Genetic material is transferred between micro-organisms through three main routes: transformation, transduction and conjugation. Within this exchanged genetic material can lie resistance mechanisms, and selective pressure can drive resistance potentiation. ESBL=Extended spectrum β-lactamase; DNA=deoxyribonucleic acid. *Draft graphic courtesy of Adrian Roots, Elsevier, 2014*.

**Figure 2.**

**Global travel routes and emergence of AMR** *(NB. Figure graphics to be further developed with Elsevier illustrator/designer)*

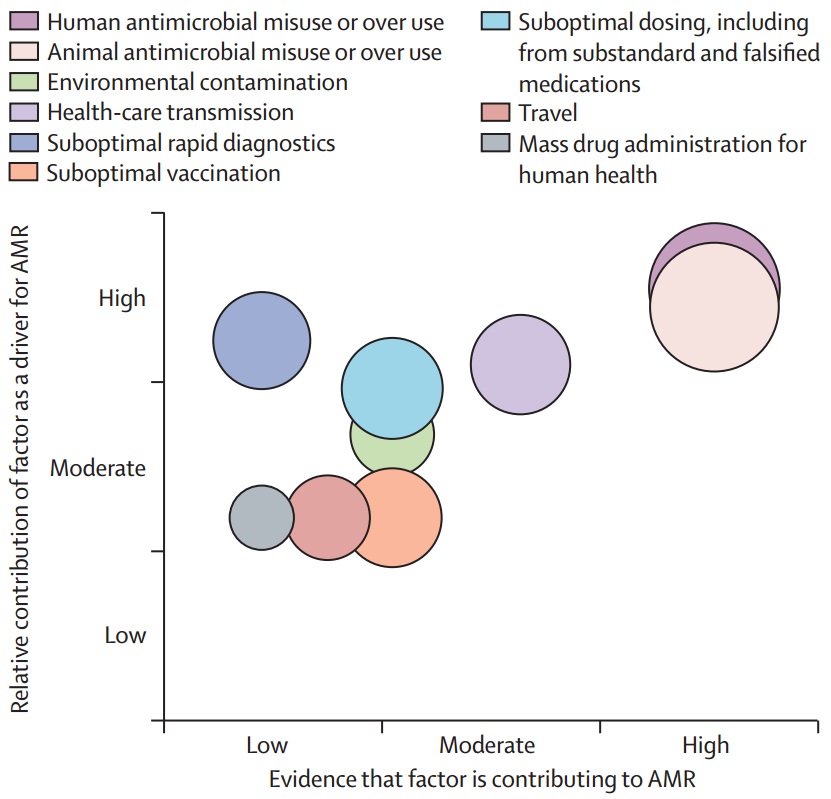


**Legend:** Whilst ESBL producing Enterobacteriaceae and MRSA are now nearly ubiquitous, certain novel types of resistance, amongst both Gram negative and Gram positive organisms, are of particular concern. Reports of these resistant organisms are widespread, and whilst the mechanisms of human-to-human transmission are likely to be complex, an association with travel might be suspected. Data shown includes metallo-carbapenemase producing Gram negative organisms,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 may be higher).

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**Figure 3.**

**Role of modifiable drivers towards antimicrobial resistance: a conceptual framework**

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**Legend:** A hypothetical info-graphic to illustrate the potential contribution of each factor as a driver for AMR, the supporting evidence for each, and potential population affected (diameter of bubble). Created from a two round Delphi method of contributing authors. Factors were identified from review of the national and international AMR policy documents. The GRADE approach was used to identify the quality of the evidence (English language scientific publications (1990-2014) identified from search of “factor” AND “antimicrobial resistance”; where multiple articles on the same topic were identified, the study with the highest GRADE estimate was cited) supporting each driver as being contributory to the rise in antimicrobial resistance (**Supplementary Table 2**). Relative contribution and potentially affected population based upon consensus expert opinion.

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

**Human Health:**

Evidence of correlation between reducing antimicrobial use and falls in AMR is complex, yet among Gram-positive organisms success have been seen. In a French prospective trial a reduction in antimicrobial use corresponded to significantly decreased pharyngeal colonization penicillin-non-susceptible *S. pneumonia.*110 In Finland reductions in macrolide use led to falls in erythromycin resistance among *S. pyogenes,*111 although this was possibly due to clonal replacement rather than reduced selection pressure. Correlation has also been seen among Gram-negative organisms. In bloodstream infections in the UK, non-susceptibility to cephalosporins and quinolones among Enterobacteriaceae has shown a modest decline over the last decade, likely reflecting prescribing shifts.26 However, the relative contribution of reductions in antimicrobial use and concomitant infection control interventions is difficult to quantify.

In contrast, evidence also exists where reduced antimicrobial use has not equated to falls in AMR. In a prospective trial in Sweden an 85% reduction in trimethoprim consumption resulted in only a marginal slowing of the rise in trimethoprim resistance among uropathogenic *E. coli*.112 In the UK, a 98% decrease in cotrimoxazole consumption did not result in AMR reduction, instead inexplicably was followed by a 6% increase in sulphonamide resistance in clinical isolates of *E. coli*.113

This lack of clear correlation between reduced use of antimicrobials and decreased AMR urgently necessitates 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 falls in AMR. In Denmark and Norway, following 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.114 However, whilst post-ban studies suggested that the amount of faecal GRE in broilers may have decreased significantly, the prevalence of animals colonised with GRE was still high several years after the ban.115 A similar situation is 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.116

Yet importantly reducing animal antimicrobial use may not alter production volumes. The avoparcin ban for antimicrobial growth-promotion noted above had no deleterious effect on production volume.117 Similarly among Danish pigs, a 60% reduction in pig antimicrobial consumption had no negative effect on productivity.118 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.119

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

**Panel 2. Potential approaches to prolong the useful therapeutic life of currently available antimicrobials.**

**Maintain heterogeneity of antimicrobial agents;** excessively homogenous antimicrobial use may contribute to selective pressure.Maintaining prescribing diversity can be achieved through several methods. One such 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 may 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), maintaining 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**; sub-therapeutic 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, over weight adults, infrequent dosing), inadequate drug absorption (malnutrition, drug interactions), unusual large apparent volume of distribution (pregnancy), or particularly rapid clearance.2 Taken individually, populations exposed to sub-therapeutic concentrations may appear small, but they represent a high proportion of the patients receiving antimicrobials in LMICs. Optimising dosage and ensuring drug quality could reduce sub-therapeutic drug exposure and reduce this modifiable driver of resistance.

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

**Combination therapy;** use of multiple antimicrobials to which the targeted organisms do not show cross-resistance. This relies on microbial populations containing singly resistant mutants, but none resistant simultaneously to multiple drugs. However the increasing prevalence of multidrug resistant strains necessitates careful evaluation 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 to date, often because of the increased cost, but also from fear of incremental, unwanted, disturbance of the microbiome. Furthermore, the differentials in half-life of agents used in combination must be carefully considered, or unintentional monotherapy may ensue. In conclusion, the risk-benefit of combination therapy is unclear and further work is urgently needed to clarify these issues.