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Antimicrobial resistance: its emergence and transmission

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Abstract

New concepts have emerged in the past few years that help us to better understand the emergence and spread of antimicrobial resistance (AMR). These include, among others, the discovery of the mutator state and the concept of mutant selection window for resistances emerging primarily through mutations in existing genes. Our understanding of horizontal gene transfer has also evolved significantly in the past few years, and important new mechanisms of AMR transfer have been discovered, including, among others, integrative conjugative elements and IS*CR* (insertion sequences with common regions) elements. Simultaneously, large-scale studies have helped us to start comprehending the immense and yet untapped reservoir of both AMR genes and mobile genetic elements present in the environment. Finally, new PCR- and DNA sequencing-based techniques are being developed that will allow us to better understand the epidemiology of classical vectors of AMR genes, such as plasmids, and to monitor them in a more global and systematic way.

Keywords: mutation, horizontal gene transfer, mobile genetic elements, selection

Introduction

Because of the considerable use of antimicrobial agents in human and veterinary medicine and animal husbandry, antimicrobial resistance (AMR) has developed into a prime illustration of how bacterial populations can readily adapt and react to selective pressure. We have witnessed, during the past decades, not only the emergence of a multitude of new resistance mechanisms, but also their spread across entire bacterial populations and ecological niches. This article will review some recent insights into the mechanisms used by bacteria to develop resistance to antimicrobials, and how AMR can spread or emerge repeatedly in entire populations.

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The molecular mechanisms of emergence and transmission

Resistance to antimicrobials can be acquired in two ways: (i) mutations in pre-existing or previously acquired genes, and (ii) horizontal gene transfer (HGT), the acquisition of new genes from other bacteria. Depending on the antimicrobial, both mechanisms can play important roles in the development of the dramatic AMR situation that we face today.

Emergence of AMR through mutations

It is the interplay between the occurrence of random mutations and selective antimicrobial pressure that drives specific resistant mutants through evolutionary bottlenecks and ultimately to multiply and emerge out of the

overall anonymity. Excellent reviews have been dedicated to the resistance mechanisms for specific antimicrobials (see for instance Schwarz *et al*., 2006), and these topics will not be reviewed here. Instead, the following section of this article will focus on two important notions that have emerged recently in the context of AMR development and spread. The first is the 'mutator state' and the second is the concept of 'selection windows' and 'mutant preventing concentration'.

Mutators

Mutations occur on a regular basis in every living organism as a consequence of either alterations in existing DNA or errors during DNA replication. Since mutations are more likely to have deleterious than advantageous effects on the survival of an individual cell, bacteria have evolved a range of proofreading and DNA repair mechanisms (for a review, see for instance Chopra *et al*., 2003 or Horst *et al*., 1999). However, the genes encoding these control mechanisms may themselves undergo mutations, thus resulting in bacteria with increased mutation rates – the so-called 'mutators' (Chopra *et al*., 2003). Recent studies have demonstrated surprisingly high frequencies of mutators in natural populations (LeClerc *et al*., 1996; Matic *et al*., 1997), suggesting that they may play an important role in the evolution and adaptation of bacteria to changing environments (Travis and Travis, 2002; Tanaka *et al*., 2003). Bacterial populations may undergo bursts of mutations when encountering new selection pressures (Giraud *et al*., 2001). Such bursts are caused by the selection of bacteria with new advantageous characteristics that, like deleterious mutations, are more prone to emerge in mutator than in non-mutator strains. There is therefore a co-selection of bacteria in the mutator state together with the selection of advantageous mutations. This is of particular advantage to a population in a highly variable environment or when multiple successive mutations are needed to attain an optimally adapted phenotype (Tenaillon *et al*., 1999; Denamur and Matic, 2006). This mechanism is thought to play a role in the emergence of resistance to antimicrobial agents arising through mutations (Blazquez, 2003; Macia *et al*., 2005), such as for fluoroquinolones (Komp Lindgren *et al*., 2003; Levy *et al*., 2004; Trong *et al*., 2005). It may also play a role in the acquisition of resistance genes through HGT, because the most frequent mutations leading to the mutator state (i.e. mutations in methyl-directed mismatch repair genes such as *mutS* and *mutL* in *Enterobacteriaceae*) also significantly increase the efficiency of HGT and of homologous recombination (Rayssiguier *et al*., 1989; Townsend *et al*., 2003). Furthermore, the mere stress and DNA alteration provided by some antimicrobials may induce the SOS system of bacteria and, consequently, the activity of error-prone DNA polymerases, which results in a transient mutator state (Foster, 2007). Thus, exposure to fluoroquinolones increases the frequency of mutants resistant to this class of antimicrobials in an exposed bacterial population (Cirz *et al*., 2005).

The mutator state may also be involved in the generation and spread of β-lactamase variants, particularly of extended-spectrum β-lactamases (ESBLs) (Woodford and Ellington, 2007). Two different studies have made use of *in vitro* models to mimic the evolution of bla_{TEM} in mutator strains and have shown that variants of TEM-1 similar to those observed in Nature can be obtained under such circumstances (Stepanova *et al*., 2008), including the otherwise unlikely accumulation of multiple mutations leading to the $bla_{TEM\text{-}52}$ variant (Orencia *et al*., 2001). Although there is no formal proof that this phenomenon is occurring in Nature, the higher prevalence of mutators among clinical *Escherichia coli* isolates that produce ESBL than among non-ESBL producers (Baquero *et al*., 2005) strongly supports the hypothesis. Thus, ways to avoid mutators and their effects on the emergence of resistant isolates may become part of the strategies that we will have to envision in our fight against resistance.

The mutant selection window (MSW)

Common knowledge suggests that minimal inhibitory concentrations (MICs) should drive treatment dosage for bacterial infections (Drlica, 2003). However, there is a range of antimicrobial concentrations just above the MIC which would kill or inhibit susceptible bacteria, but at which the few spontaneous mutants in the infecting bacterial population would be able to survive or multiply. This range of concentrations is called MSW (Zhao and Drlica, 2001). The lower limit of the MSW is usually considered to be between the MIC and MIC_{99} of the organism under scrutiny (Zhao and Drlica, 2001; Drlica and Zhao, 2007). The upper MSW limit, or mutant prevention concentration (MPC), has been defined as the concentration at which no resistant mutant can grow when testing 10^{10} cells *in vitro* (Drlica, 2003). The width of the MSW is a function of the microorganism, the antimicrobial agent, and the spectrum and type of MIC changes provided by mutations. In some cases, the MPC may be so high that it cannot be reached realistically in clinical settings and the principles of mutant selection prevention discussed here may not apply. This is, for instance, the case for many AMR genes, whose acquisition results in a major MIC shift to levels not achievable by any treatment. Also, under *in vivo* conditions, and depending on the mode of activity of the antimicrobial (i.e. bacteriostatic or bactericidal) and the organisms, the MPC may need to be adjusted further (Drlica and Zhao, 2007).

The concept of MSW may have many practical implications in the case of stepwise mutations or of the acquisition of resistance genes that provide only small changes in MIC, and when multiple mutations are needed to reach clinically significant resistance levels. Prime examples of such situations are found in human medicine in relation to treatment of mycobacterial diseases

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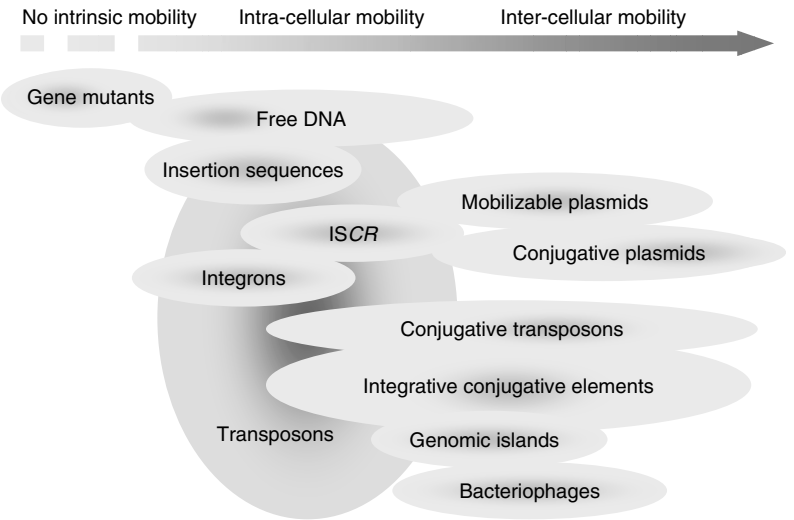

Fig. 1. Molecular mechanisms and elements involved in the spread of AMR. For details of the involvement of each mechanism and element in the spread of AMR, please refer to the respective sections in the text. Overlapping mechanisms/elements indicate functional or physical linkage. Specific elements may be involved to variable degrees in both intra- and inter-cellular mobility and the width of each element is only an approximation of its respective involvement in these two levels of mobility. Because of the multidimensional nature of interactions, the relationships between elements may in fact be more direct than possibly represented in this figure. IS*CR*: the atypical class of insertion sequences with common regions.

(Almeida *et al*., 2007). Fluoroquinolones, which are more relevant to veterinary medicine, represent another example of the potential implications of the MSW concept. In most bacteria, high-level fluoroquinolone resistance is essentially the result of multiple cumulative mutations (Hopkins *et al*., 2005). *In vitro* as well as *in vivo* experiments show, for instance, that *Staphylococcus aureus* mutants with elevated fluoroquinolone MICs can be selected by levofloxacin concentrations within the MSW, but neither below nor above it (Firsov *et al*., 2003; Cui *et al*., 2006). The phenomenon is bound to be the same and of general relevance for many other combinations of organism and fluoroquinolone (see for instance Croisier *et al*., 2004; Ferran *et al*., 2007; Olofsson *et al*., 2007), as well as for other antimicrobial agents (Goessens *et al*., 2007; Zinner *et al*., 2008). When using antimicrobials for which the concept of MSW is applicable, special care may have to be applied, including, if possible, the determination of MPCs. In particular, some treatment modalities, which leave local *in vivo* antimicrobial concentrations too close to the MICs of the organism (i.e. within the MSW) for extended periods of time may be prone to select resistant mutants. Long acting formulations can potentially lead to such unwanted situations (Drlica and Zhao, 2007). It is also possible that this approach should be applied to the use of new generation β-lactams in the treatment of infections by organisms that already possess a resistance mechanism to β-lactams of earlier generations. Such organisms may present a slightly elevated MIC and a higher MPC for new generation β-lactams when compared with fully susceptible isolates. This may facilitate the selection of ESBL mutants through the resulting elevated MSW.

HGT and AMR

For the majority of antimicrobials, resistance is mainly caused by the acquisition of new resistance genes rather than by spontaneous mutations. The exact origin of these genes is frequently unknown, but most of them apparently originate from environmental organisms. Some AMR genes seem to originate from natural antibiotic producers, where they protect the bacterium against its own weapons (Webb and Davies, 1993; Davies, 1994; Lu *et al*., 2004). Others have been suggested to play different roles in their original host, including detoxification of components other than antimicrobials, and a variety of other metabolic (Martinez, 2008) and signaling functions (Davies *et al*., 2006; Linares *et al*., 2006; Fajardo and Martinez, 2008). A vast but broadly unknown reservoir of such genes is still lurking in natural environments (D'Costa *et al*., 2007), thus providing transferrable resources for other bacteria for many years to come.

The genetic elements involved in the spread of resistance genes

The movement of AMR genes can take place at two distinct levels (Fig. 1), and different elements are involved at each level. At the intracellular level, AMR genes can move within the genome, including between chromosome and replicons such as plasmids and phages. Transposons and integrons are the major elements involved in these movements; they rely on both homologous and non-homologous recombination. In the case of inter-cellular movement (horizontal spread) of AMR genes, three major mechanisms are potentially

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involved: transformation (uptake of naked DNA), transduction (transfer by bacteriophages) and conjugation (transfer by plasmids and other conjugative elements). Numerous reviews have been written on these mechanisms and their role in the transfer of AMR (see for instance Aarestrup, 2006; Schwarz *et al.*, 2006). Therefore, this article will focus essentially on recent developments in that field.

Evolving integrons

Resistance integrons of classes 1 and 2 (Fluit and Schmitz, 2004) are widespread and well established among pathogens and commensal bacteria, resulting in a plethora of publications on their diversity and distribution in bacteria from animals and humans. Through their gene capture ability and association with widespread transposons such as Tn*21* (Liebert *et al.*, 1999) and Tn*7* (Hansson *et al.*, 2002), they play a major role in the development and spread of multiresistance. New AMR genes keep showing up in classical and modified integrons. This is, for instance, the case with the newly identified *qnr* genes for quinolone resistance (Wang *et al.*, 2003), as well as with more classical genes such as the sulfonamide resistance gene *sul3* (Bischoff *et al.*, 2005; Antunes *et al.*, 2007). Thus, known integrons are continuously evolving in order to provide bacteria with the tools to resist newer antimicrobials. In addition, recent findings on integron diversity and evolution suggest that they are much more diverse than originally thought (Boucher and Corey, 2008). They probably originated and evolved in environmental bacteria (Gillings *et al.*, 2008) and spread across a very broad range of microorganisms, by both vertical and horizontal transfer (Nemergut *et al.*, 2008). Some integrons, such as those of class 1, have evolved from their original host to become vectors of resistance, and to spread in commensal and pathogenic bacteria from animals and humans through their associations with transposons (see for instance the model of evolution for class 1 integrons proposed by Gillings and collaborators in Gillings *et al.*, 2008). However, a wealth of other integrons exists that may surface as additional AMR gene carriers in the future (Gillings *et al.*, 2008).

*Transposons, insertion sequences and IS*CR *elements*

Insertion sequences (ISs) and composite or complex transposons have long been known to play a major role in the mobilization of AMR genes (for an introductory review, see for instance Bennett, 2008). Recently, a new class of mobile genetic elements with characteristics similar to IS*91* has emerged. These are designated as IS*CR*s to stress their relationships with insertion sequence elements and the presence of conserved recombinase sequences (refered to as common regions) that facilitated their identification (Toleman *et al.*, 2006b; Toleman and Walsh, 2008). IS*CR*s are characterized by a transposase-like gene but lack the typical repeats found at the ends of classical ISs. They are flanked and delimited by sequences called *ori*IS and *ter*IS (for origin and termination of replication), and they typically transpose using a rolling circle replication mechanism, which makes them very different from classical ISs (Mendiola *et al.*, 1994; Toleman *et al.*, 2006b). They seem to insert relatively randomly in any DNA molecule and, very importantly, the termination mechanism for the replication of IS*CR*s is not very accurate (Tavakoli *et al.*, 2000; Toleman *et al.*, 2006a). Termination frequently occurs beyond the limit marked by the *ter*IS site, thus allowing for the mobilization of sequences adjacent to the IS*CR* elements, including AMR genes. IS*CR*s appear to have played a major role in the emergence and spread of a variety of recently identified AMR genes, including a number of ESBLs, and in the evolution of complex integrons. These complex integrons have resulted in combinations and clusters of AMR genes that would not have been possible through classical integrons alone (see for instance Toleman *et al.*, 2006a, 2006b). The *qnr* genes for quinolone resistance are typical examples of such complex integrons in which IS*CR*s have played a major role in bringing together AMR gene combinations, including fluoroquinolone and extended-spectrum β-lactam resistance genes, on single conjugative plasmids (Wang *et al.*, 2003; Nordmann and Poirel, 2005; Garnier *et al.*, 2006; Quiroga *et al.*, 2007).

AMR plasmids

Conjugative or mobilizable plasmids are the most common transmission vectors for AMR genes. Many of them carry multiple resistance genes leading to what can be termed 'infectious multiresistance'. This topic will not be developed further here (for more information, see for instance Bennett, 2008). Despite the major relevance of plasmids in AMR, it is surprising how little has been attempted in the study of their diversity and epidemiology in relation to AMR in a broad and systematic way. The exponential growth of DNA sequencing capabilities and of informatics for sequence analysis and annotation have opened new avenues for a more comprehensive understanding of the molecular epidemiology of AMR plasmids. The bla_{CMY-2} plasmids, encoding resistance to extended-spectrum cephalosporins, provide a good illustration of this evolution. Restriction analysis of plasmids has been the workhorse of microbiologists in assessing global relationships between plasmids; four to five groups of bla_{CMY-2} plasmids were originally identified using this approach (Carattoli *et al.*, 2002; Giles *et al.*, 2004). Recently, Carattoli and collaborators have developed new tools for plasmid characterization and molecular epidemiological analysis. They first developed an accessible PCR-based replicon-typing system that classifies plasmids by incompatibility group (Novick, 1987; Carattoli *et al.*, 2005). This system is significantly less tedious than the classical incompatibility grouping technique (Couturier *et al.*, 1988). Although some plasmids remained untypable by this method, these researchers showed that bla_{CMY-2} plasmids belong mainly to the I1 and A/C

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incompatibility groups, which have spread between continents (Carattoli *et al.*, 2006; Hopkins *et al.*, 2006). AMR plasmids within specific incompatibility groups present a relatively conserved backbone structure on top of which variable accessory regions, such as AMR genes, insert (Schluter *et al.*, 2007). This conserved backbone can be used to compare the evolutionary relationships among plasmids in order to track plasmid movement across bacterial populations, and can consequently help us to better understand how mobile accessory elements move in and out of plasmids. Plasmid multilocus sequence typing (pMLST) (Garcia-Fernandez *et al.*, 2008) is one approach that makes use of the conserved backbone mentioned above to classify plasmids. This method may replace restriction analysis of plasmids by providing similar but more reproducible results (Garcia-Fernandez *et al.*, 2008). However, it may be less discriminatory than restriction analysis, and will not replace entirely the notoriously tedious and difficult sequencing of entire AMR plasmids.

Bacteriophages and AMR

Although they represent important mechanisms in the long-term evolution of pathogens, transformation and transduction have been considered to be not very significant in HGT of AMR genes. Recent reports on the transduction of multiple AMR genes from the *Salmonella* genomic island 1 (SGI1) of *Salmonella* Typhimurium DT104 (Schmieger and Schicklmaier, 1999) and of the extended-spectrum cephalosporin resistance gene $bla_{\text{CMY-2}}$ of *Salmonella* Heidelberg (Zhang and LeJeune, 2008) suggest that bacteriophages may play a more important role than originally thought in the transfer of AMR genes. Further studies are certainly warranted on this subject.

Integrative conjugative elements (ICEs) and genomic islands

Besides plasmids and bacteriophages, a number of other mobile elements involved in transfer of AMR have emerged in recent years, tentatively grouped under the concept of ICE (Burrus *et al.*, 2002). Such elements, in contrast with plasmids, are not self-replicating but integrate into the chromosome (with a variable site-specificity, dependent on the element and the host bacterium) to be stably passed from one generation to the next. They encode both excision and integration mechanisms, as well as transfer between bacteria by modes of conjugation (Burrus and Waldor, 2004). Conjugative transposons, such as the archetypal tetracycline-resistance transposon Tn*916*, are the best known examples of ICEs of importance for AMR in human and veterinary medicine (Franke and Clewell, 1981; Rice, 1998). They are widespread among Gram-positive organisms such as streptococci and enterococci but are also frequently found in *Bacteroides* spp. and other anaerobes. Since their discovery (Franke and Clewell, 1981), the list of host species for these elements has broadened continuously, and we now know that they can even be found among *Enterobacteriaceae* (Murphy and Pembroke, 1995; Hochhut *et al.*, 1997).

Although genomic islands were first identified in relation to virulence (for a review, see for instance Schmidt and Hensel, 2004), they are also important in the spread of AMR genes. Their mode of transmission is not entirely clear but genomic islands are thought to be transferred by bacteriophages, and in ways similar to ICEs (Burrus *et al.*, 2004). Two genomic islands have become famous for their role in the spread of AMR in the past decades – the SGI1 and the chromosomal elements responsible for methicillin resistance in coagulase-positive staphylococci.

SGI1 was first discovered in relation to the intercontinental spread of the pentaresistant (ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline or ACSSuT) *Salmonella* Typhimurium phage type DT104 (Poppe *et al.*, 1998; Threlfall, 2000). Molecular investigations later showed that the AMR genes responsible for this resistance profile are all clustered together (Briggs and Fratamico, 1999), and are part of a larger genomic island of approximately 43 kilobase pairs (Boyd *et al.*, 2001). Several of these AMR genes ($bla_{\text{PSE-1}}$ for ampicillin, *floR* for chloramphenicol, *aadA2* for streptomycin and *tetG* for tetracycline) were not the most common ones usually encoding resistance to these antimicrobials in *Enterobacteriaceae* and their origin still remains uncertain. The AMR genes of SGI1 are part of a complex integron in which two type 1 integrons have come together with tetracycline and florfenicol–chloramphenicol resistance genes of plasmid origin (Boyd *et al.*, 2001). SGI1 shares many features with ICEs, including the presence of recombinase genes for excision and integration, but seem to lack the self-transfer components of these elements. Nevertheless, *in vitro* experiments have demonstrated that helper plasmids can provide the necessary apparatus for an effective transfer of SGI1 between bacteria (Doublet *et al.*, 2005). As a proof that this potential mobility is not just a laboratory curiosity, numerous reports show that SGI1 has now spread to many *Salmonella* serovars other than Typhimurium, integrating relatively consistently at identical sites in the chromosome of these bacteria (Doublet *et al.*, 2005; Mulvey *et al.*, 2006). In addition, numerous variants of SGI1 have emerged through homologous recombination and transposition events (Mulvey *et al.*, 2006).

The first methicillin-resistant *Staphylococcus aureus* (MRSA) emerged very shortly after the introduction of this antimicrobial in clinical practice (Jevons *et al.*, 1963). Methicilllin resistance in MRSA is caused by the presence of an alternative β-lactam-insensitive penicillin-binding protein (PBP2a) encoded by the *mecA* gene (Matsuhashi *et al.*, 1986). This gene is located within staphylococcal cassette chromosome (SCC*mec*) elements, which also

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encode recombinases allowing for the excision and integration of the cassettes downstream of a specific locus called *orfX* (Katayama *et al.*, 2000). At least seven major types of SCC cassettes have been identified to date in MRSAs (Deurenberg and Stobberingh, 2008). Molecular investigations on methicillin-resistant and -susceptible strains have demonstrated that these cassettes have been acquired repeatedly by a variety of *S. aureus* strains as well as by the same clonal lineages (Enright *et al.*, 2002). After this acquisition, the resulting MRSA clones spread internationally. The exact origin of the *mec* genes found in MRSA is not entirely clear, but the high homology between a PBP of *Staphylococcus sciuri* and PBP2a suggests that they may have originated in this organism (Wu *et al.*, 2001). Other studies suggest that the assembling of the SCC, including the amalgamation of the *mec* genes with the *crr* recombinase genes, may have taken place in coagulase-negative staphylococci (Hanssen and Ericson Sollid, 2006). SCC*mec* similar to those of MRSA have also been demonstrated in coagulase-negative staphylococci such as *Staphylococcus epidermidis*, and these organisms are considered by some as a reservoir of SCCs (Wisplinghoff *et al.*, 2003; Hanssen and Ericson Sollid, 2006). After the first emergence of hospital-associated MRSA, community-acquired MRSA emerged (for a review, see for instance Boucher *et al.*, 2008), and we now face the emergence of MRSA in animals. They were first detected in horses (Weese, 2004) and companion animals (Weese, 2005), but recent findings suggest that they may be even more widespread in swine (de Neeling *et al.*, 2007). Interestingly, whereas MRSA strains found in pets are similar to strains prevalent in humans, those from horses seem to be less frequent in humans. The emerging strains from swine seem to belong to a new clone previously absent in humans, although it is now found in populations at risk such as veterinarians and pig farmers (Huijsdens *et al.*, 2006; van Loo *et al.*, 2007; Khanna *et al.*, 2008). SCC*mec* have spread to coagulase-positive staphylococci other than *S. aureus*, and can now also be found in the mainly animal-associated *Staphylococcus pseudintermedius* (formerly called *Staphylococcus intermedius*) (Bannoehr *et al.*, 2007; Loeffler *et al.*, 2007; Zubeir *et al.*, 2007; Griffeth *et al.*, 2008).

The global picture

The interplay of mutations and HGT

Whether originally by mutation or HGT, the impact of resistance on human and animal health is, at least in part, a function of the spread of that resistance across bacterial populations. If selection pressure is sustained and a mutation event is simple, *de novo* mutation in pathogens can occur many times over, as is the case in the response of *Campylobacter* to fluoroquinolone selection pressure (Zhang *et al.*, 2003). If selection pressure is sustained, and the mutation or assembly of resistance elements complex, the key to the human or animal health impact may lie mainly in clonal spread, as was the case for the global dissemination of the ACSSuT penta-resistance cassette, originally associated primarily with *Salmonella* Typhimurium DT104 (Threlfall, 2000). In other scenarios, the key to global dissemination may be the ease of HGT between bacterial strains or species, e.g. the spread of bla_{CMY-2} in *Salmonella* and other *Enterobacteriacea*. The recent example of the withdrawal of ceftiofur use in broiler hatching eggs in Québec Canada and subsequent changes in the prevalence of extended-spectrum cephalosporin resistance in human and chicken *Salmonella*, and chicken *E. coli* (Government of Canada, 2007; Irwin *et al.*, 2008) provides population evidence of both clonal and horizontal spread, i.e. the gene is present in different species of *Enterobacteriacea* (likely horizontal spread) but also widespread in *Salmonella* Heidelberg recovered from chickens and humans (likely clonal spread). The bla_{CMY-2} gene was first identified in *Klebsiella* in Greece before spreading to other pathogens and commensals (Bauernfeind *et al.*, 1996). There is similar evidence of both clonal and horizontal dissemination in the spread of the bla_{CTX-M} gene around the world (Cantón and Coque, 2006; Liu *et al.*, 2007; Machado *et al.*, 2008).

The relevance of the distinction between clonal spread and HGT

From a public/animal health perspective, and since both mechanisms are ultimately involved in the spread of AMR, it is unclear if there is relevance to the distinction between clonal spread and HGT in the spread of AMR. Although this distinction helps to refine our understanding of the epidemiology or resistance in specific situations, for many purposes, AMR epidemiology could be regarded on a global scale without any regard to its mode of transfer and with more attention to the potential routes of spread (Fig. 2). Clonal spread may be more rapid and driven by specific selection pressures, many unclear and not always related to antimicrobial use only. Clonal extinctions and replacements may also take place. These could possibly be driven by interventions directed at specific serotypes, including, in the case of resistant clones, modification to antimicrobial selection pressure, or the vagaries of clonal biology (e.g. the subsidence of *Salmonella* Typhimurium DTs 29 and 204, the apparent current subsidence of DT104, the reasons for which remain unclear (Threlfall, 2000, 2008)). HGT is potentially more sustained and overall less erratic, as genes or genetic elements shift on an apparent regular basis between bacteria occupying overlapping but different ecological niches. The transfer of individual genes and genetic elements becomes particularly important if the traffic between non-pathogen resistance gene reservoir (e.g. commensal *E. coli*) and

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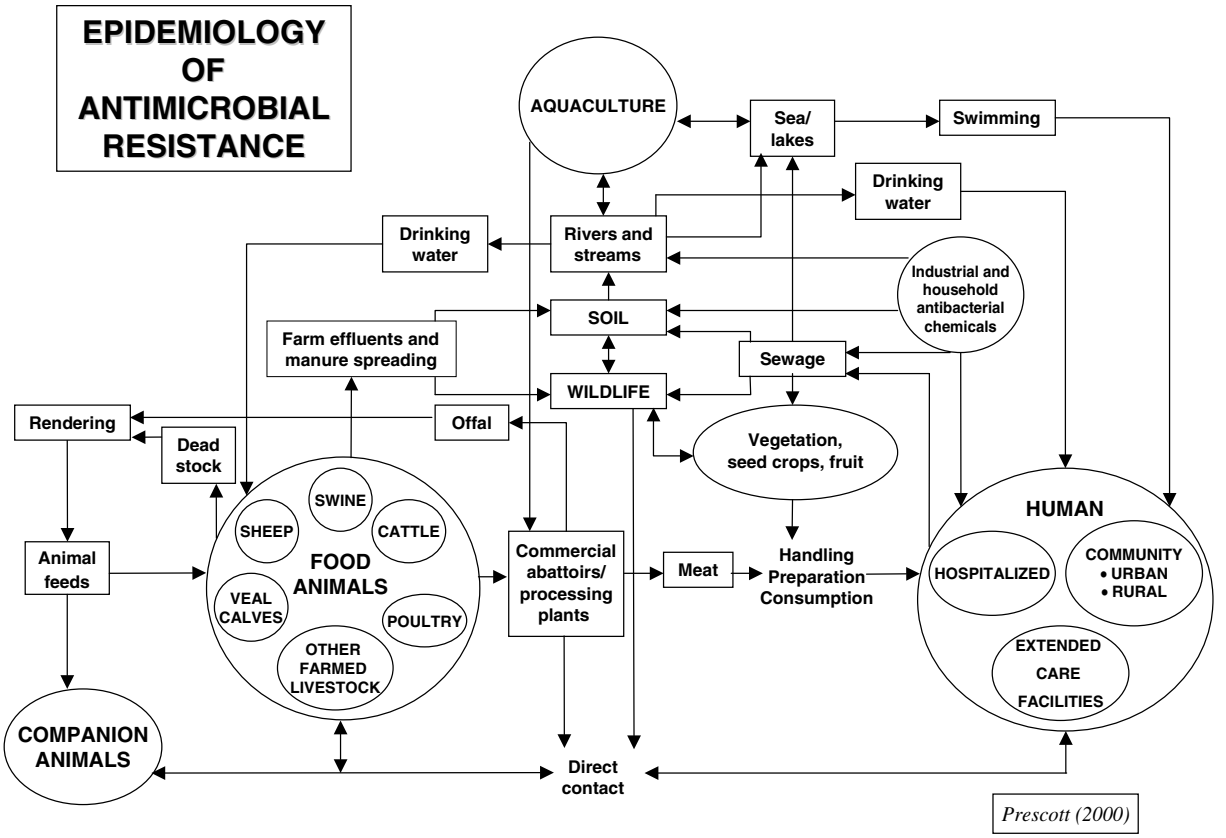

Fig. 2. The pathways of resistance transmission. This diagram (the 'Confusogram') has been used in various incarnations to depict the epidemiology of antimicrobial resistance and plausible pathways of spread between various environments. This version was adapted from Prescott (2000). The circles represent potential anthropogenic antimicrobial selection pressure. Some pathways are well described, while others are plausible but lack substantive evidence. Not all plausible pathways are depicted. Please see the text for example references. Reproduced with permission of John Wiley and Sons, Inc.; *Antimicrobial Therapy in Veterinary Medicine,* by J. F. Prescott *et al.,* pp. 39, ©1988.

pathogens (e.g. *Salmonella*, *Shigella* and *Klebsiella*) is happening on anything more than an occasional basis.

Commensals as a reservoir

The reservoirs of AMR genes may be thought of in two categories – one being commensal microflora, primarily of the gastrointestinal tract of humans and animals (Salyers *et al.*, 2004), but including other non-sterile body systems. There are well-documented examples of *in vitro* and *in vivo* transfer between commensals and pathogens in gastrointestinal tracts and food matrices (Zhao *et al.*, 2001; Poppe *et al.*, 2005; Walsh *et al.*, 2008). This reservoir, the development and maintenance of resistance in it, and subsequent transfer to pathogens are thought by many to be a more global threat to health than direct selection pressure on the pathogens themselves, i.e. the occasional *de novo* development of resistance in a pathogen may be less frequent and less impactful than the constant traffic from the vast commensal reservoir into the relatively small pathogen pool. Understanding the human and animal health impact of gene traffic between this reservoir and pathogens is, however, blurred and complicated by the fact that the distinction between commensal and pathogen is artificial in several regards: what is a commensal to one host species can be a pathogen to another (e.g. *Campylobacter* in pigs and humans); what is commensal to an individual host can be an opportunistic pathogen to another (e.g. *S. aureus*, *Enterococcus*; Top *et al.*, 2008); and what is commensal in one body system may be pathogenic in another (e.g. extraintestinal pathogenic *E. coli* (ExPEC) are commensal in the gastrointestinal tract and pathogenic in the urinary tract or bloodstream; they may also be an example of the spread among food animals, companion animals, and humans (see Smith *et al.*, 2007, for a review of ExPEC).

Environmental reservoir

The other major reservoir of AMR determinants is the environmental and soil microbiota; most, if not all, transferrable AMR genes in pathogens and commensals arose in, and were transferred from, environmental

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bacteria (D'Costa *et al.*, 2007). The extent to which these transfers happen on a regular basis, as opposed to being isolated events in microbial evolution, is unclear. However, in the modern era of antimicrobial use, there may be an artificial selection pressure for this transfer, not to mention disruption of natural microbial ecosystems, under the influence of environmental antimicrobial residues in the breeding grounds represented by sewage, and farm and aquacultural effluent (Kostich and Lazorchak, 2008).

The pathways of resistance transmission ('The Confusogram')

The traffic of genes and genetic elements, and of resistant commensal and pathogenic bacteria between different hosts and ecological niches is complex (Fig. 2). Evidence for these potential pathways is global, and evidentiary examples for most pathways can be found in data from many countries. However, the evidence for each pathway is often incomplete, demonstrating plausibility for a portion of the pathway rather than fully illuminating the entire 'farm to fork' pathway for example. The primary focus in the study of the movement, or potential for movement, of bacteria and resistance genes between different ecological niches has been on the direct routes of transmission of human health concern: between human populations directly in the community or in health care settings, and indirectly through food, the environment, particularly in health care settings, and shared fomites; and from food animals to humans, directly or through the food chain via meat, milk and dairy products, eggs, and sea food (see, for example, Smith *et al.*, 1999; White *et al.*, 2001; Huijsdens *et al.*, 2006; Adesiyun *et al.*, 2007; Klevens *et al.*, 2007; Khanna *et al.*, 2008; Machado *et al.*, 2008). The global nature of the evidence for these pathways is particularly important in the context of international travel, animal movement and food trade. There is also evidence for the potential spread through other routes: between food animals directly or via human intermediaries such as veterinarians and farmers; between companion animals and people; between horses and humans; from food animals to companion animals directly or via the food chain; via wildlife, zoo animals, insects, pet rodents and aquarium fish; from food animals to water and soil; from humans to water and soil; from aquaculture to water; via contaminated fruits and vegetables; and via animal feed (see, for example, Österblad *et al.*, 2001; Petersen *et al.*, 2002; Sengeløv *et al.*, 2003; Weese, 2004; Dargatz *et al.*, 2005; Anderson *et al.*, 2008; Lefebvre *et al.*, 2008; Macovei *et al.*, 2008; Yoke-Kqueen *et al.*, 2008). Because the epidemiology of AMR is so complex, it is unlikely that a comprehensive integrative understanding is possible, i.e. our understanding may always be limited to specific segments of the epidemiology of AMR – the role of a certain genetic element, the effect of particular risk factors, the likelihood of infection with resistant organism given exposure to a given food, etc. The issue at the global level has been likened by some to climate change because of this complexity, the cumulative and synergistic effects of various causative elements, the separation of cause and effect by intervening variables, and the interplay between effects of natural and human origin. As with climate change, interventions (e.g. antimicrobial use bans, restricted labeling, voluntary cessation of specific antimicrobial uses, clinical practice guidelines, infection control and biosecurity practices, development of vaccines and alternatives to antimicrobials, education and behavior modification programs, etc.) targeted at identifiable issues or through specific mechanisms may have an important and, in some cases, measurable impact (e.g. the withdrawal of fluoroquinolones from use in poultry in the US (Nelson *et al.*, 2007), the growth promoter ban in Sweden, Denmark and then the European Union (Aaerstrup *et al.*, 2001), the voluntary cessation of ceftiofur use in broiler hatching eggs in Québec Canada (Irwin *et al.*, 2008)), but, ultimately, the greater value of such interventions may be in effecting an incremental long-term shift in the attitude of physicians, veterinarians and other health care providers, food animal producers and the general public toward the prudent use and conservation of antimicrobials.

Conclusion

Refinements in our understanding of AMR emergence and transfer may help develop strategies to better control AMR. Simultaneously, new molecular tools for tracing genes and their carriers will also support both control strategies and monitoring. However, given the complexity of the transmission routes, the unexploited reservoirs of new AMR genes and of mobile elements present in the environment, it is unlikely that a magic bullet will ever solve our fight against AMR. Prudent use of antimicrobials can be refined continuously using the new information and tools, and will always remain the cornerstone of our strategy to protect the efficacy of both old and new antimicrobials.

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