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The changing epidemiology of resistance

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Antibiotic resistance is now a linked global problem. Dispersion of successful clones of multidrug resistant (MDR) bacteria is common, often via the movement of people. Local evolution of MDR bacteria is also important under the pressure of excessive antibiotic use, with horizontal gene transfer providing the means by which genes such as *bla*$_{CTX-M}$ spread amongst different bacterial species and strains. β-Lactamase production is a common resistance mechanism in Gram-negative bacteria, and the rapid dissemination of novel genes reflects their evolution under the selective pressure of antibiotic usage. Many Enterobacteriaceae now carry broad-spectrum β-lactamases such as CTX-M, with particular genotypes associated with different geographical regions. The spread of these enzymes has compromised the clinical utility of a number of β-lactam classes and with the spread of genes such as *bla*$_{KPC}$, carbapenems may be increasingly compromised in the future. High-level fluoroquinolone resistance (mainly caused by *gyrA* mutations) has also been shown to be associated with CTX-M and CMY-type enzymes, commonly due to co-carriage on conjugative plasmids of the gene for the aminoglycoside-inactivating enzyme AAC-6[1]-Ib-cr and *qnr* genes (which confer low-level resistance), allowing the easy selection of *gyrA* mutants in the host strain. Resistance in Gram-positive bacteria is also widely distributed and increasing, with the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) blurring the distinction between hospital and community strains. Antibiotic use and environmental factors all have a role in the emergence and spread of resistance. This article reviews some of the new mechanisms and recent trends in the global spread of MDR bacteria.

Keywords: ESBLs, carbapenemases, Gram-positive bacteria, Gram-negative bacteria

Introduction

Antimicrobial resistance increases the morbidity, mortality and costs of treating infectious diseases. The threat from resistance (particularly multiple resistance in bacterial strains that have disseminated widely) has never been so great. The key factors driving this threat are increased antibiotic usage (in both human and animal medicine), greater movement of people and increased industrialization. The emergence and global spread of the international clone 1 of penicillin-resistant *Streptococcus pneumoniae* (PRSP) is a good example of how multiresistant bacteria spread by the movement of people.[1] Furthermore, if total outpatient antibiotic consumption in different countries is correlated with the rate of PRSP, then a direct correlation is seen (Spearman coefficient $r=0.75$; $P<0.001$).[2] Horizontal gene transfer provides the single most important mechanism to accelerate the dispersal of antibiotic resistance genes. Although in the case of PRSP this occurs via transformation of DNA from penicillin-resistant commensal streptococci, in most bacteria (particularly Gram-negative species) plasmids are the major vector. The importance of plasmids carrying multiple drug resistance (MDR) markers in *Shigella* spp. and *Escherichia coli* was first described in the seminal work of Watanabe in Japan over 40 years ago.[3] Plasmids are capable of self transfer (conjugation) between strains and species and have a mosaic structure that has arisen by recombination and transposition, which is responsible for the capture of different resistance genes, giving rise to the MDR phenotype.[4] This mosaic structure poses problems when classifying and studying the evolutionary relationships of plasmids.[5] The application of multilocus sequence typing (MLST) to Inc HI1 plasmids of *Salmonella enterica* serovar Typhi has circumvented these problems, as evolution by acquisition of single nucleotide polymorphisms in core genes is not subject to the exogenously driven variation seen in restriction fragment length polymorphism (RFLP) studies of plasmids.[6] The study found that resistance plasmids distributed throughout

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the world after 1993 were very different from those occurring before that time. This suggests that although antibiotic selection maintains the resistance genes, there is competition between plasmids of the same incompatibility group encoding the same phenotype. The interaction of plasmids with the host bacterium is poorly studied but it is crucial to understanding the rapid emergence and spread of MDR bacteria. MDR bacteria are usually thought of as being disadvantaged by carriage of antibiotic resistance genes. However, recent work using a pig gut model shows that no disadvantage (indeed sometimes even an advantage) is seen in MDR strains in the absence of antibiotic selection.[7]

Resistance in Gram-negative bacteria

Extended-spectrum β-lactamases

The most important mechanism of resistance to β-lactam antibiotics among Gram-negative bacilli involves the production of β-lactamases. Extended-spectrum β-lactamases (ESBLs) are generally acquired by horizontal gene transfer and confer resistance to oxyimino-cephalosporins, some being mutant derivatives of established plasmid-mediated β-lactamases (e.g. TEM/SHV) or mobilized from environmental bacteria (e.g. CTX-M). The frequency with which novel enzymes have been described in the literature reflects not only the pace of discovery and the ability to differentiate these enzymes[2] but also their rapid emergence and evolution under the selective pressure of antibiotic usage. During the 1990s most reports of ESBLs concerned TEM/SHV types with the exception of the specific genotype CTX-M-2 from South America (five major families of CTX-M genotypes are recognized representing acquisition of β-lactamases genes from different species of *Kluyvera*).

Surveillance data reported high levels of ESBL-producing strains of *Klebsiella pneumoniae* and *E. coli* in Australasia, ranging from <10% in Australia and Japan to >30% in Singapore and China for *K. pneumoniae* and from ~11% in Singapore to 25% in China for *E. coli*.[8] Subsequent analysis of strains from China identified CTX-M-14 as the dominant genotype, which has been found particularly in the Far East but has also spread worldwide (Figure 1).[9,10] Particular CTX-M genotypes are associated with geographical regions (see above and Figure 1). CTX-M-15 is the only genotype reported from India[11] and is also very widely distributed across the world possibly because it is frequently carried by *E. coli* of sequence type (ST) 131, a very successful uropathogenic clone.[12] Very recently an outbreak of *Klebsiella* and *E. coli* producing CTX-M-15 has been reported from Southern China, where this genotype was extremely rare before, signifying potential extensive spread and displacement of the dominant CTX-M-14 and CTX-M-3 ESBLs.[13]

Since the turn of the century there have been dramatic shifts reported in both the prevalence and types of ESBLs reported in Europe, with strains producing CTX-M becoming dominant, particularly CTX-M-15 (Figure 1).[14] In some countries, reports of isolates producing CTX-M remain sporadic, while in Asia, much of Europe and South America, endemic prevalence has been reached.[15] In the USA, thought to have been spared a visitation from CTX-M, a survey undertaken in 2007 shows the globally dominant genotypes CTX-M-15 and -14 to be appearing.[16] More recent data from the global Study for Monitoring Antimicrobial Resistance Trends (SMART) showed that in the Asia-Pacific region and in Latin America, 40% and 30% of *E. coli* and *Klebsiella* spp. respectively, from patients with intra-abdominal infections, were ESBL positive.[17]

AmpC enzymes

AmpC cephalosporinases are species-specific chromosomally encoded β-lactams, common but not ubiquitous in Enterobacteriaceae and Pseudomonaceae, which have also become mobilized onto transmissible plasmids.[18] Consequently they can now appear in bacteria lacking or poorly expressing a chromosomal bla_{AmpC} gene, such as *E. coli*, *K. pneumoniae* and *Proteus mirabilis*. Between 2005 and 2006, plasmid-mediated AmpC β-lactamases were identified in 10% of *Klebsiella* spp. and 2% of *E. coli* from five children's hospitals in China, with DHA-1-type AmpC enzymes having the highest prevalence rate.[19] This finding reflects the relentless spread of these β-lactamases, the most frequently reported type worldwide being CMY-2.[18]

Metallo-β-lactamases

The emergence of metallo-β-lactamases (MBLs) with activity against carbapenems (e.g. the VIM and IMP families of enzymes) has compromised the clinical utility of this class of antibiotics.[20,21] Resistance to carbapenems may also be induced as a result of increased production of either AmpC or ESBL, coupled with a decrease in porin production or increased efflux.[21,22] Among 33 European countries participating in the European Antimicrobial Resistance Surveillance System (EARSS) in 2007, six countries reported carbapenem resistance rates of >25% among *Pseudomonas aeruginosa* isolates, the highest rate being reported from Greece (51%).[23] Greece also had the highest resistance rates among *K. pneumoniae* (46% to carbapenems, 58% to fluoroquinolones and 63% to third-generation cephalosporins). A recent review of the literature confirmed that VIM-2 is the most dominant MBL in *P. aeruginosa* and confers the greatest clinical threat.[21] VIM-2 has been reported from 37 countries across five continents (Figure 2). MBLs can hydrolyse all clinical β-lactam substrates, with the exception of aztreonam. The other major phylogenetic arm of the VIM MBLs, represented by VIM-1 and related genotypes, is now commonly found in Enterobacteriaceae (usually VIM-1), particularly from countries around the Mediterranean.[21] The gene for another mobile carbapenemase, $bla_{\text{SPM-1}}$, has been found in 70% of isolates of *P. aeruginosa* from Brazil,[21] but to date has not been reported from other countries. Other currently rare carbapenemases include GIM-1, which has only been reported from a few *P. aeruginosa* isolates in Germany, SIM-1, which appears confined to *Acinetobacter baumannii* isolates in Korea, and NDM-1 in *K. pneumoniae* described in New Delhi.[24] Some countries with high rates of ESBL producers, such as India, have recently increased their usage of carbapenem antibiotics, which may provide a selective pressure for the spread of strains producing carbapenemases.

IMP carbapenemases were first reported in 1991 from Japan.[21] Other countries in which early molecular variants appeared were China, Taiwan, Italy, Portugal, Australia and Canada (Table 1). To date 24 bla_{IMP} genes have been identified

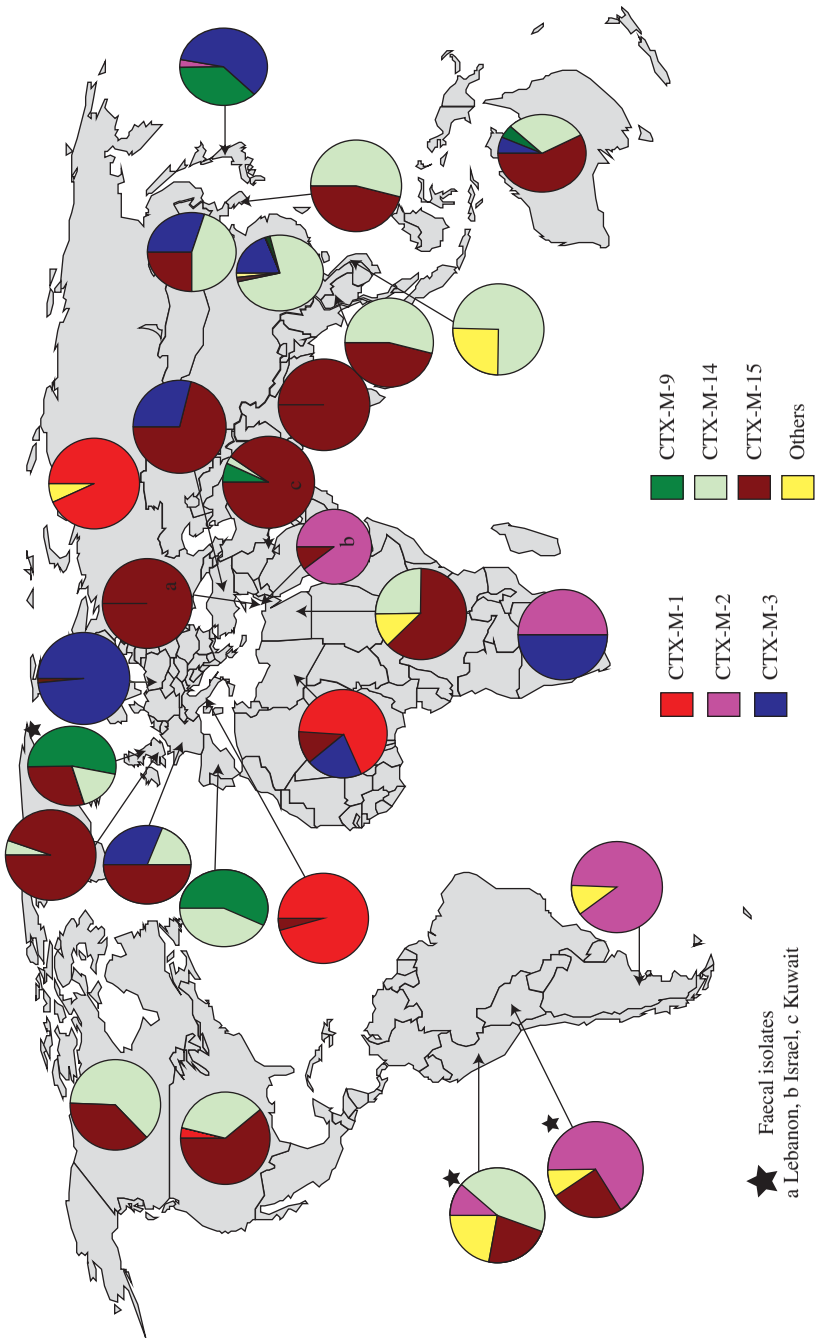

Figure 1. Global distribution of CTX-M genotypes.[11,13,16,62–84]

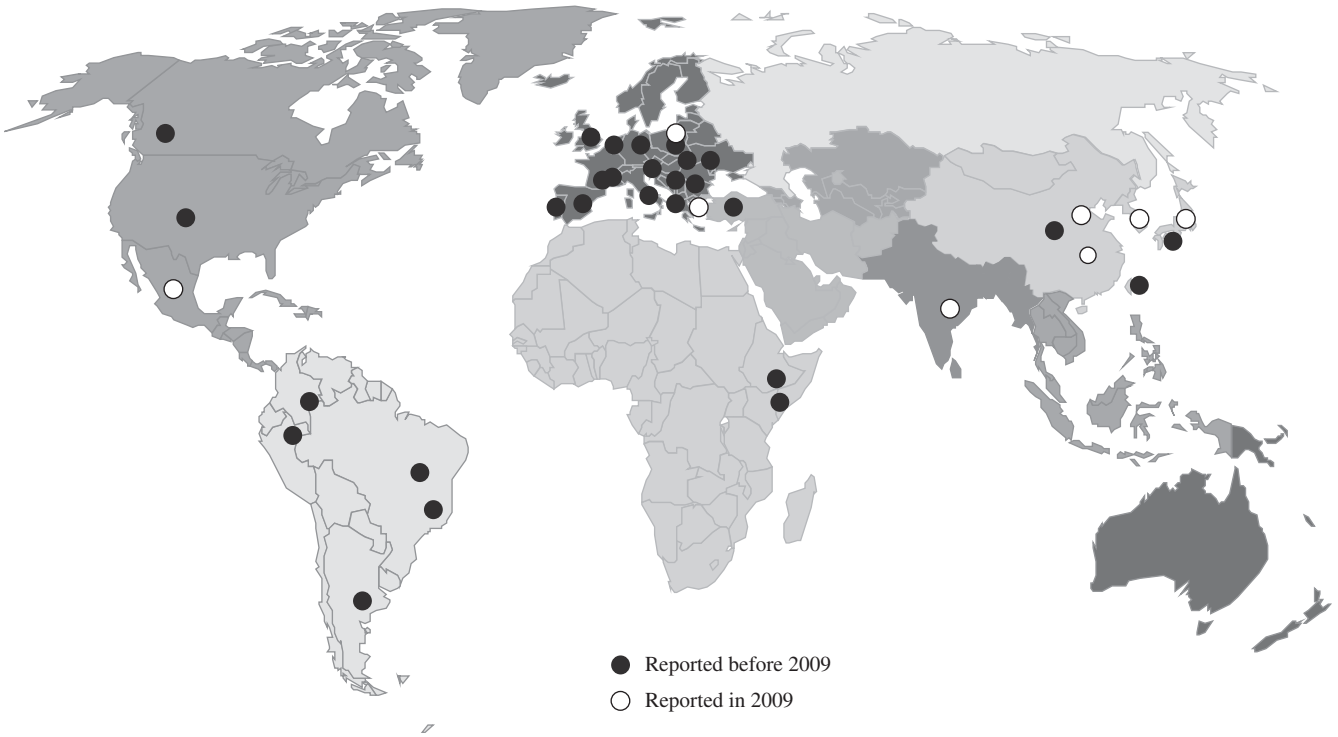

Figure 2. Global distribution of VIM-2.

Table 1. First observation and location of the earliest disseminated bla_{IMP} carbapenemases

Reported	IMP type	Species	Country	Reference
~1991	1	>12 species	Japan	52
2000	2	*Acinetobacter*	Italy	53
2000	3	*Shigella flexneri*	Japan	54
2000	4	*Citrobacter youngae*	Guangzhou, PRC	55
2001	5	*A. baumannii*	Portugal	56
2000	6	*Serratia marcescens*	Japan	57
2001	7	*P. aeruginosa*	Canada	58
2001	8	*K. pneumoniae*	Taiwan	59
2001	9	*P. aeruginosa*	Guangzhou, PRC	60
2002	10	*P. aeruginosa*	Japan	61

(http://www.lahey.org/Studies/other.asptable1). In a recent outbreak of infection at an Australian hospital, which was part of a wider interstate outbreak, MBL-producing Gram-negative bacilli belonging to eight different genera carrying bla_{IMP-4} were reported.[25] Although the spread of MBL-producing organisms is often attributed to the use of broad-spectrum cephalosporins and carbapenems, in that report only 30% of the patients had intensive care unit (ICU)-related acquisition and only 10% of patients with non-ICU related acquisition had received carbapenems within the 2 weeks prior to the first positive sample. These authors postulated that an undetected environmental reservoir or significant number of colonized patients contributed to the outbreak.

Molecular class A carbapenem-hydrolysing enzymes

The appearance and rapid spread in the USA and Israel of KPC-type β-lactamases is the most recent development in the epidemiology of carbapenem resistance. In 2001, a carbapenem-resistant strain of *K. pneumoniae* was reported in North Carolina,[26] while in 2004, 19 isolates of carbapenem-resistant *Klebsiella* spp. possessing the carbapenem-hydrolysing class A β-lactamase KPC-2, were recovered from seven hospitals in New York City,[27] with another genotype, KPC-3, also being reported.[28] Since then, there have been increasing numbers of reports of KPC-containing organisms from different states in the USA (mainly confined to the Eastern seaboard but recently more widely),[29,30] KPC is endemic in Israel[31] and sporadic isolates have been reported in China[32] and Europe.

The rapid dissemination of different β-lactamases has severely complicated and limited antibiotic choices. Local knowledge of the epidemiology and characterization of resistance has become even more important when considering empirical therapy. Table 2 summarizes the susceptibilities of bacteria producing different β-lactamases.

Fluoroquinolone resistance

Fluoroquinolones interact with DNA gyrase and topoisomerase IV, the enzymes that regulate conformational changes in bacterial DNA during replication and transcription. Resistance to fluoroquinolones arises through stepwise mutations in the coding regions of the gyrase subunits (*gyrA* and *gyrB*) and DNA topoisomerase IV (*parC*). Accumulation of mutations in several of these genes increases the MIC in a stepwise manner.[33] In recent years, the plasmid-mediated QNR mechanism, which protects DNA from quinolone binding, has become a concern because of

Table 2. Susceptibility patterns usually observed in bacteria producing different β-lactamases

Examples	AMP	TZP	RAD	CXM	CTX	FEP	ATM	IPM
Class A								
TEM1/SHV1	R	S	R	S	S	S	S	S
TEM3/CTX-M	R	S	R	R	R	R	R	S
KPC	R	S	R	R	R	R	R	R
Class B								
VIM/IMP	R	R	R	R	R	R	S	R
Class C								
chromosomal[a]	R	R	R	R	R	S	R	S
CMY/FOX	R	R	R	R	R	S	R	S
Class D								
OXA	R	R	R	R	S	S	S	S
OXA carbapenemase[b]	R	R	R	R	S	S	S	R

[a]Derepressed mutant.
[b]e.g. OXA-23, OXA-51.
AMP, ampicillin; TZP, piperacillin/tazobactam; RAD, cefradine; CXM, cefuroxime; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; R, resistant; S, susceptible.

its frequent association with CTX-M and CMY-type enzymes that inactivate third-generation cephalosporins.[34] In addition, the widely distributed plasmid-encoded aminoglycoside-modifying enzyme AAC-6^{I}-Ib-cr has been found to degrade fluoroquinolones with a piperazinyl moiety (e.g. ciprofloxacin, norfloxacin).[35] There is also a plasmid-encoded target protection mechanism enabled by the *qnr* genes,[35] with both genes being found on plasmids carrying $bla_{\text{CTX-M}}$. It is possible that the low-level plasmid-encoded fluoroquinolone resistance has provided a selective advantage for bacteria exposed to fluoroquinolones to allow the easier selection of high-level resistance mutations in *gyrA*, thus explaining the association of high-level chromosomal quinolone resistance with plasmid-encoded ESBL genes. Recent EARSS data show that fluoroquinolone resistance has increased significantly across the whole of Europe since 2001, with levels ranging from 7% (Estonia and Norway) to 53% (Turkey) in 2007.[36] Overall, only 47% of *E. coli* were susceptible to four classes of antibiotics in Europe in 2007, with the loss of susceptibility occurring more rapidly to fluoroquinolones than to any other antibiotic class included in the EARSS surveillance database.[36]

Resistance in Gram-positive bacteria

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in the early 1960s coincident with the introduction of methicillin. Following a fall in incidence in the 1970s, a steady rise was noted in many countries. Analysis of clones from various parts of the world using MLST reveals a limited number of clones, many of which are also represented by identical clonal methicillin-susceptible *S. aureus* (MSSA) isolates, suggesting that successful MSSA lineages have acquired the mobile resistance determinant SCC*mec*.[37] A recent study using single nucleotide polymorphism analysis of sequence type 5 MRSA suggests that contrary to dogma, SCC*mec* acquisition is at least 10-fold more common than thought and geographical dispersal is very restricted.[38]

In the late 1990s, levels of MRSA reached ~30% in many countries, and the first reports of community-associated MRSA (CA-MRSA) began to be appear in the literature.[39–41] Cases of CA-MRSA usually present in younger patients without underlying risk factors, typically cause skin and soft tissue infections (SSTIs), are usually susceptible to ciprofloxacin, clindamycin, gentamicin and trimethoprim/sulfamethoxazole, with exotoxin genes (e.g. Panton–Valentine leukocidin genes), significantly more likely to be found than in hospital-acquired isolates.[42] These strains are genetically unrelated to hospital-acquired strains of MRSA and in some centres have emerged as the predominant cause of SSTIs, with the USA300 clone being the most frequently isolated in North America.[43]

CA-MRSA has diverse lineages due to new acquisitions of SCC*mec* IV. Epidemic strains have emerged in the South-West Pacific, North America, Europe and elsewhere.[44,45] Initially thought to be distinct from hospital-acquired strains, recent reports have indicated that these strains may now be causing hospital cross-infection and also may have reduced susceptibility to vancomycin.[46]

Antibiotic resistance in the environment and animals

It is being increasingly recognized that MDR commensal bacteria in the gut of animals and humans are an important source of bacteria causing opportunistic infections or act as resistance gene reservoirs forming a source of spread to bacteria infecting humans. CTX-M-2 ESBL genes in *E. coli* in chicken meat imported into the UK were found in 50% of chicken breasts from Brazil this genotype is the most frequent in that country in humans.[47] Whilst an environmental origin for many antibiotic resistance genes seems likely (e.g. CTX-M from *Kluyvera*), release of antibiotics and other antibacterials into the

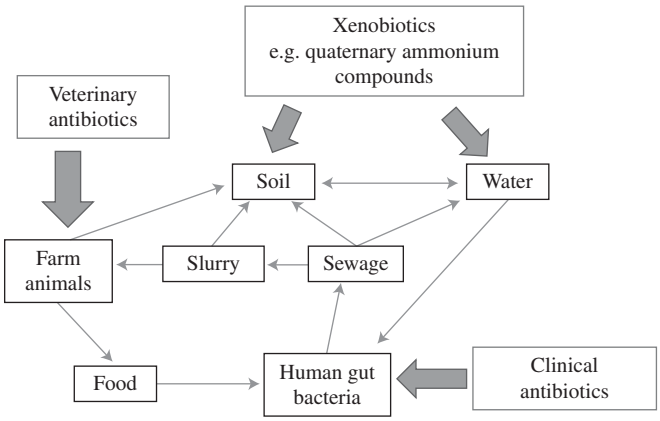

Figure 3. Flow of antibiotic resistance genes in *E. coli* in the biosphere. Thick arrows show major selective pressures on antibiotic resistance genes, thin arrows show the significant directions of gene flow.

environment is going to enrich both MDR and the vectors (plasmids and integrons) in that milieu. Release of fabric-conditioning chemicals (largely quaternary ammonium compounds) into a reed bed system has recently been shown to strongly select for Class I integron carriage, which is a key molecular mechanism for the spread of antibiotic resistance genes by horizontal gene transfer.[48] A model for the complex interlocking relationships of the sources and routes for MDR strain spread and horizontal gene transfer is shown in Figure 3. The MRSA clone ST398 and methicillin-susceptible ST9, which have their main reservoir in pigs, are increasingly causing infections in humans,[49] and ESBL genes of animal origin are being described in *E. coli* and *Salmonella* spp.[50]

Conclusions

Although some antibiotic resistances have remained rare (for example intermediate vancomycin resistance and linezolid resistance in MRSA and also some resistance genes, e.g. VIM and IMP in the UK), the limited surveillance currently undertaken indicates a generalized rise in antibiotic resistance with some specific genes (for example those encoding CTX-M ESBLs) reaching pandemic proportions. Pressures, both clinical and commercial, to use antibiotics in both humans and animals, the global mobility of populations and food products, ensure that the spread of MDR bacterial clones and resistance genes will be a continuing phenomenon. Increased use of older agents offers little hope as has been seen with the emergence of colistin resistance in *Klebsiella* spp. in Greece.[51] A number of initiatives have been established to encourage the prudent use of antimicrobials. Even with the development of these programmes and the heightened awareness of the interplay between resistance, geography, treatment and transmission, it is likely that antibiotic resistance will continue to develop more rapidly than new agents to treat infections become available, and that at best we can only hope to slow the spread of these infections.

Transparency declarations

None to declare.

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