Factors affecting the reversal of antimicrobial-drug resistance

Pål J Johnsen, Jeffrey P Townsend, Thomas Bøhn, Gunnar S Simonsen, Arnfinn Sundsfjord, Kaare M Nielsen

The persistence or loss of acquired antimicrobial-drug resistance in bacterial populations previously exposed to drug-selective pressure depends on several biological processes. We review mechanisms promoting or preventing the loss of resistance, including rates of reacquisition, effects of resistance traits on bacterial fitness, linked selection, and segregational stability of resistance determinants. As a case study, we discuss the persistence of glycopeptide-resistant enterococci in Norwegian and Danish poultry farms 12 years after the ban of the animal growth promoter avoparcin. We conclude that complete eradication of antimicrobial resistance in bacterial populations following relaxed drug-selective pressures is not straightforward. Resistance determinants may persist at low, but detectable, levels for many years in the absence of the corresponding drugs.

Lancet Infect Dis 2009; 9: 357–64

Department of Pharmacy (P J Johnsen PhD, K M Nielsen PhD) **and Department of Medical Biology** (G S Simonsen MD, A Sundsfjord MD)**, Faculty of Medicine, University of Tromsø, Tromsø, Norway; Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA** (J P Townsend PhD)**; GenØk—Centre for Biosafety, Tromsø** (T Bøhn PhD, K M Nielsen)**; Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø** (G S Simonsen, A Sundsfjord)**; and Norwegian Institute of Public Health, Oslo, Norway** (G S Simonsen)

Correspondence to:
Pål J Johnsen, Department of Pharmacy, Faculty of Medicine, University of Tromsø, 9037 Tromsø, Norway
paal.johnsen@uit.no

Introduction

Over the past 6 decades, bacterial populations have responded to the selective pressure of antimicrobial drugs by evolving resistance to all commercially available agents.[1] Decreased discovery rates of new classes of antimicrobial agents have substantiated a notion that, for some bacterial species, we might face clinical infections for which there are no treatment options.[2,3] The frequency of acquired resistance in the targeted bacterial population is positively associated with the consumption of antimicrobial drugs. France, Spain, and Portugal were the European countries with the highest per head, non-hospital antimicrobial-drug consumption levels in 1997.[4] These countries have also reported the highest prevalence of penicillin non-susceptible *Streptococcus pneumoniae* (PNSP) and β-lactamase producing *Haemophilus influenzae*.[5] Correspondingly, countries with low levels of antimicrobial consumption report low frequencies of resistance.[6–9] The levels of resistance from country to country are consistent with empirical and theoretical studies that have identified the volume of drug use as the major cause of increased frequency of resistance.[10,11]

Despite the well-described correlation and strong theoretical basis for the prediction of resistance development, few epidemiological studies have recorded temporal changes in the frequency of resistance to a specific drug when the volume of drug consumption in the community is deliberately reduced. One highly cited study[12] reported a 50% reduction in the proportion of macrolide-resistant group A streptococci in Finland following reduced consumption of macrolides. Several attempts have also been made to reduce the frequency of PNSP through decreased usage of antimicrobial agents. A successful intervention was reported in Iceland, where reduced consumption levels of antimicrobials were followed by a decrease in the frequency of PNSP from 20% to 12%.[11] A reduction in PNSP-colonisation rates from 52·5% to 34·5% was also obtained in France after antimicrobial prescriptions were reduced by 19%.[13] Recently, Dagan and colleagues[14] reported on seasonal variations in antimicrobial prescription patterns: higher antimicrobial consumption during winter was associated with higher proportions of resistant *S pneumoniae*. When prescription rates declined during the summer, a substantial reduction in antimicrobial-drug-resistant *S pneumoniae* was reported among Jewish children. Notably, the same level of seasonality could not be observed among Bedouin children in the same period, probably because they had a higher year-round level of antimicrobial use. However, other studies have not been able to associate overall reduced consumption levels of antimicrobials in the community with reduced levels of resistance in *S pneumoniae*.[15,16] Perhaps most disturbing was a study on sulfonamide-resistant *Escherichia coli* in the UK, where a 98% decrease in sulfonamide prescriptions during the 1990s was followed by a 6·2% increase in the frequency of sulfonamide resistance.[17] A follow-up study showed that sulfonamide resistance persisted undiminished in *E coli* 5 years later.[18]

These contrasting results clearly indicate that the fate of antimicrobial-resistance determinants, following a significant reduction in the selective pressure, depends on factors other than drug consumption alone. Several processes reduce the reversal of acquired antimicrobial-drug resistance in the absence of the corresponding drug. These processes include rates of reacquisition (facilitated by ongoing horizontal gene transfer [HGT] and spontaneous mutation events), mutation-based alterations in microbial physiology to reduce the fitness costs of acquired-resistance determinants, directional selection of genetically linked traits, and the presence of systems regulating segregational stability of extra-chromosomal elements carrying resistance determinants. These processes may act alone or in concert to determine the fate of resistance determinants in the larger bacterial population, and determine whether the acquired-resistant phenotypes will subsequently be replaced with susceptible phenotypes (ie, reversal of resistance; see figure 1) within relevant time scales. In the following sections, each process will be discussed.

Rates of reacquisition of antimicrobial resistance

Bacteria acquire antibiotic resistance by spontaneous mutations or by HGT through the processes of

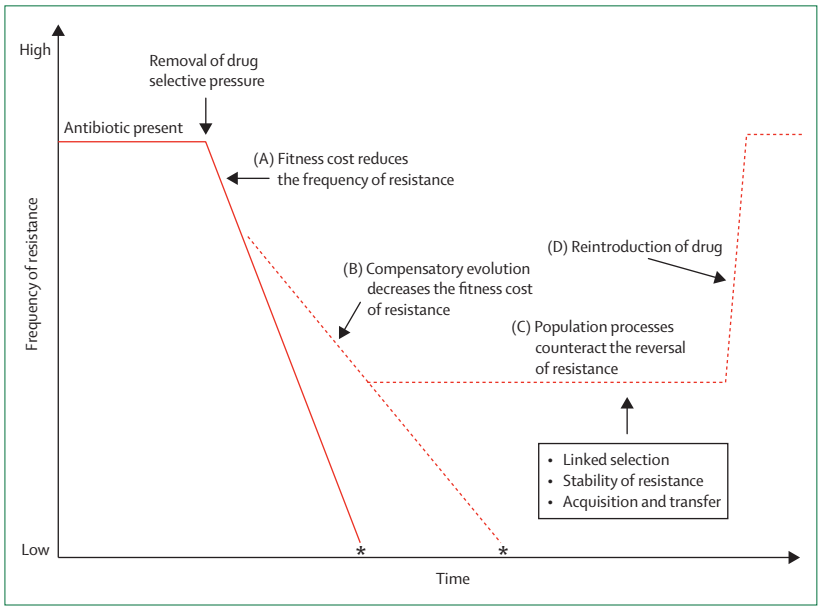

Figure 1: **The processes governing reversal of antimicrobial-drug resistance in bacterial populations**
The presence of an antimicrobial drug selects for resistant strains rendering an increasing fraction of the population resistant. Removal of the selective pressure (eg, by suspending drug usage) can lead to various outcomes. If a biological cost is associated with carriage of antimicrobial-drug resistance (A) the frequency of resistance will decline to a level where resistance is regenerated by mutations or by HGT (marked with an asterisk). The biological cost of resistance may be decreased over time through compensatory mutations (B) that reduce the rate at which resistant clones are outcompeted by susceptible counterparts. Other population processes (C), alone or in concert, might further undermine the reversal of resistance. These processes include linked selection of resistance determinants with other beneficial traits, reacquisition of resistance determinants through frequent HGT, and postsegregational killing systems that maintain plasmid-carrying cells in the bacterial population (ie, stability of resistance). A reintroduction of the antimicrobial agent (D) will lead to a rapid increase in the frequency of resistance.

conjugation, transformation, and transduction. The rate at which bacteria evolve or reacquire resistance by these means is a crucial component of any model developed to enhance control of the reversal of resistance. Spontaneous mutation to a resistant phenotype is often the first means of reacquisition considered. However, rates of spontaneous mutation are generally too low to undermine the reversal of resistance in drug-free environments through the recurrent generation of resistant mutants—although bacteria with elevated mutation rates (mutators) have been suggested to play a part in pathogenicity and resistance development.[19,20] Mutator phenotypes that have elevated mutation rates, mainly due to defective DNA-repair mechanisms, have been shown to be common in both natural and pathogenic isolates of *E coli* and *Salmonella* spp.[19,21] On a cell-by-cell basis, increased mutation rates straightforwardly increase the frequency of resistant phenotypes. However, the population effects of mutators depend on the level of environmental adaptation of the bacterial population to its environment. Mutators are unsuccessful in a constant environment to which the bacterial population is well adapted.[22] Under these constant conditions, the negative effect of deleterious mutations on host fitness outweighs that of the less frequent beneficial mutations. The host fitness effect may however be reversed in new or fluctuating environments, such as in hospital settings, where bacteria face bottlenecks and periodic strong directional selection. A number of studies have pointed out that clinical isolates of *Pseudomonas aeruginosa*, *E coli*, and *Neisseria meningitides* have elevated mutation frequencies, suggesting selection of mutator phenotypes.[19,23–25] Population dynamic models have shown that mutators may transiently increase and persist at high frequencies when multiple mutations are needed for an adaptive character,[21] and even for single mutations if these beneficial mutations occur first in the mutator subpopulation.[26]

Bacterial acquisition of resistance determinants through HGT,[27,28] in the context of reversal of resistance, is important in at least two ways. First, a high rate of HGT may undermine reversal of resistance by directly supplying resistance determinants from resistant strains to susceptible strains within the same population. Little is known about actual horizontal transfer rates outside of the laboratory. However, population dynamic models supported by experimental studies show that the rate of conjugal plasmid transfer may match the rate at which plasmids are lost by segregation in *E coli* populations.[29,30] If plasmid transfer balances the effect of segregation, the fate of a plasmid as a genetic parasite is dependent on the fitness cost of the plasmid only. In this regard, it is important to note that conjugative plasmids may have very high transfer frequencies even though the donor ability varies in a heterogeneous population.[31,32] Dionisio and colleagues[31] showed in mixed cultures of *E coli* donor and recipient cells that plasmid R-1 transconjugants increased several orders of magnitudes in frequency after only 5 days in serial transfer cultures. These experiments support the notion that the influence of recurring HGT on reversal of resistance that has occurred via plasmid loss may be substantial. Second, conjugative elements harbouring antimicrobial-resistance determinants might escape negative selection of their host by rapid transfer to other host genetic backgrounds (ie, non-resistant bacterial strains and species) that may experience different selective conditions. This escape from negative selection may be of particular importance for broad host range extra-chromosomal elements such as Inc18 plasmids, and for conjugative transposons.[33,34]

Relative fitness costs of antimicrobial-resistance determinants

Both in-vitro and in-vivo studies have demonstrated that newly acquired antimicrobial-resistance traits impose a biological cost in terms of reduced relative fitness of the host bacterium when compared with their susceptible counterparts in drug-free environments.[35–40] For example, resistance to streptomycin due to point mutations in *rpsL* in *E coli* reduces the translation rate, and consequently the growth rate, when compared with *E coli* wild type cells.[39,40] The acquisition of a mobile genetic element such as a

plasmid that encodes proteins conferring antimicrobial resistance should generally reduce the growth rate due to the extra burden of replication and gene expression. From a population perspective, the difference in relative fitness between susceptible and resistant bacteria will lead to reduced growth rates or transmission efficacy, or both, and hence lower frequencies of resistant phenotypes. Eventually, these fitness costs will lead to elimination of newly resistant strains or reduction of their frequency to a minimal level representing the rate at which the resistance is regenerated by mutations or HGT.

Of course, a variety of mutations may confer resistance to the same antibiotic, and the fitness cost of each mutant may be distinct. Gagneux and colleagues[41] recently presented data from in-vitro generated rifampin-resistant *Mycobacterium tuberculosis* mutants showing fitness costs (ie, relative fitness compared with a rifampin-susceptible ancestor) across a range of 5–40%. Consistent results were also presented by Rozen and colleagues[42] on fluoroquinolone-resistance mutations in *S pneumoniae*. For large populations in an environment without selection for resistance, the expected reversal of resistance would be directly proportional to the magnitude of the biological cost of resistance determinants over time. Resistance would disappear, or at least decrease to the frequency of regeneration, in a predictable manner.

However, bacteria might acquire compensatory mutations that reduce the host-fitness cost of the resistance trait. Compensatory mutations have been shown in vitro, in animal experiments, and have been indicated in clinical situations.[35,38,40,41,43–46] In bottlenecked populations (such as serial transfer cultures or microbial host-to-host transmission models), resistant strains carrying fitness-compensatory mutations are unlikely to revert to susceptibility. This persistence occurs because more compensatory mutation loci exist than susceptibility reversions.[40] Moreover, new spontaneous mutations restoring susceptibility might lead to lower host fitness when present in a genetic background that is well adapted to resistance.[40,47] Thus, the original fitness-restoring compensatory mutations in the resistant population can result in an adaptive valley in the population genetic landscape, where susceptible revertants are less fit than their resistant counterparts. Consistent with this concept, some resistant strains show increased fitness when compared to their susceptible counterparts.[38,45,46] When resistant strains are more fit than their susceptible counterparts, the frequency of resistance will not decline simply by removing selective antimicrobial pressures, and an intervention-based reversal of antimicrobial resistance becomes an increasingly difficult challenge.

Linked selection and segregational stability of resistance determinants

Physical linkage of several resistance determinants on the same genetic element enables genetic hitch-hiking of the unselected determinant through positive selection of the other. The presence of several resistance genes on the same replication unit (plasmid or other horizontally mobile elements) is widespread in the bacterial world.[48–51] Physically linked beneficial host genes (eg, virulence genes or heavy metal resistance) can favour persistence of other resistance determinants that are not selected for. An example is the fusidic acid and cadmium resistance determinants that are present on pUB101 of *Staphylococcus aureus*.[52] Selection of bacteria in environments high in fusidic acid will simultaneously maintain cadmium resistance, even when there is no cadmium in the environment, and vice versa.

Antibiotic-resistance determinants can also be linked to beneficial genes in the chromosome. One example is the link between epidemicity and antibiotic resistance (for a comprehensive review see Martinez and Baquero).[53] For some nosocomial pathogens, a few clones (*S aureus*)[54,55] or clonal complexes (*Enterococcus faecium*)[56,57] have rapidly spread worldwide. It is likely that antibiotics have played a role in restricting diversity during their evolution and spread.[58,59] It is also evident that the antibiotic-resistance determinants in these epidemic clones are physically linked to genes that constitute a genetic landscape highly adapted for host-to-host transmission and colonisation. Thus, increased frequencies of resistance to a given antibiotic may not be causally linked to increased consumption of the correspondent drug, but rather through selection for another transmission or colonisation factor. A compelling example was provided in Iceland where, despite a substantial reduction in antibiotic prescriptions, increased frequencies of resistance to multiple antibiotics among PNSP were reported between 1993 and 1998. This surprising result was due to the efficient spread of the Spanish–Icelandic PNSP clone in the community, possibly due to little or no herd immunity in the human population.[60] Conversely, increased levels of herd immunity in the human population might lead to reduced frequency of resistance through negative selection of immunological markers of the bacterial strain that are genetically linked to the resistance determinants. This may occur as a consequence of specific clones spreading through the host population or vaccination strategies.

Physical linkage of resistance determinants to plasmid stability systems may also influence persistence in the absence of drug-selective pressures. Plasmid stability depends upon multimer resolution (*mrs*), active partitioning (*par*), and postsegregation killing (PSK) systems.[61] PSK systems promote plasmid maintenance through selective killing of plasmid-free cells. These systems have also been called plasmid addiction systems[62] or toxin–antitoxin loci.[63] A PSK system consists of a toxin and an antitoxin that are coexpressed. If the plasmid is lost, the bacterium is killed or impaired as a result of the higher cytoplasmic stability of the toxin compared with the antitoxin.[61–63] A PSK system that selectively kills or impedes the growth of plasmid-free daughter cells, restricts loss of resistance from the bacterial population

due to random plasmid loss, and would contribute substantially to the persistence of plasmid-encoded resistance in the absence of antimicrobial selection.

Effects of reduced consumption of antimicrobial drugs

Opposing results have been reported on the effects of reduced drug consumption levels in the community.[12,17] No study has reported the complete reversal of acquired drug resistance in bacterial populations that are no longer exposed to specific antimicrobial drugs. However, such analyses based on drug prescription and resistance surveillance data are often hampered by the fact that antimicrobials are rarely entirely removed from the environment, although they may no longer be used for certain human or veterinary purposes. Thus, sporadic exposure to antimicrobials (periodic selection) may subtly but forcefully maintain resistant populations. Long-term studies of the changes in antimicrobial-resistance patterns in bacterial populations from environments where usage of a specific antimicrobial drug has been completely stopped have rarely been possible, but such studies are highly desirable for analysis of the fate of resistance determinants in the absence of positive selection. In the following section, we present a case study of the persistence of glycopeptide-resistant enterococci (GRE) after the presumably complete removal of glycopeptide-selective pressures.

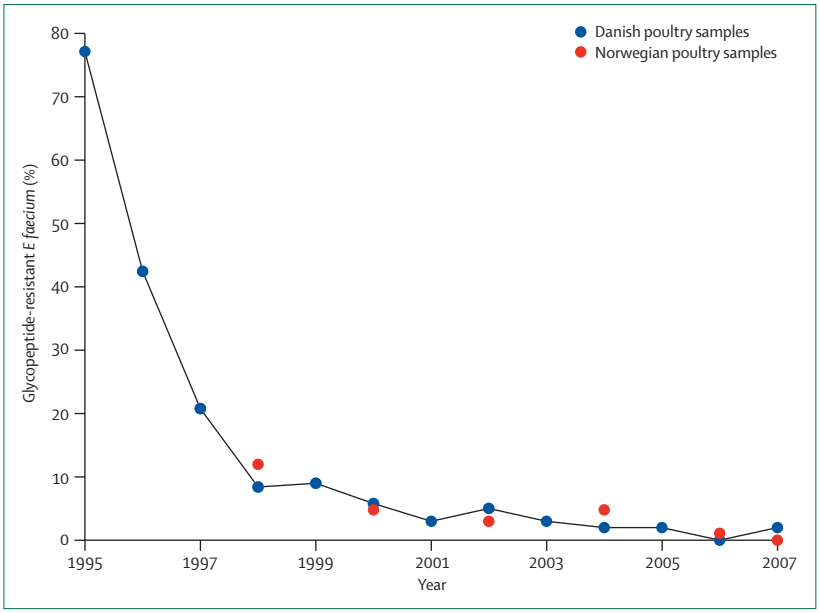

Figure 2: **Occurrence of glycopeptide-resistant *Enterococcus faecium* in Danish and Norwegian poultry samples**
Percentage of glycopeptide resistant *E faecium* (GREF) in Danish and Norwegian poultry samples following the 1995 ban on the glycopeptide growth promoter avoparcin. The plotted line is based on the complete Danish data sets where *E faecium* were isolated from cloacal-swab samples before susceptibility testing.[8] The Norwegian proportions of GREF among collected *E faecium* isolates were determined from both faecal and meat samples.[6] *E faecium* isolated from meat are of faecal origin and the GREF proportions in infected meat are most likely directly reflecting the proportions in faeces. No GREF were detected among Danish faecal *E faecium* samples in 2006.[8] However, GREF were isolated from Danish poultry meat samples in 2006, suggesting that GREF occurrence below 1–2% is close to the detection limit of the surveillance programme.[8]

A case study on the reversal of antimicrobial resistance

In 1993, researchers from the UK and Germany presented evidence for community reservoirs of GRE.[64,65] GRE were isolated from sewage (UK, Germany), farm animals (UK), and uncooked chicken (UK). During the late 1990s isolations of GRE from animals and non-hospitalised people were reported from several European countries.[66–71] Soon, a causal relation between avoparcin use in animal husbandry and the occurrence of GRE was established.[66,68,69,72] Avoparcin resistance confers cross-resistance to vancomycin, an important antimicrobial agent for the treatment of multiresistant Gram-positive infections in human beings.[73] As a consequence of the reported link between the use of avoparcin as an animal growth promoter and the occurrence of GRE, avoparcin was abandoned in Norway and Denmark in 1995, Germany 1996, and in remaining countries of the European Union in 1997.

After the use of avoparcin was discontinued, reports from Italy, Germany, the Netherlands, and Denmark showed an expected decline in the proportion of GRE in animals and humans, suggesting a successful intervention.[70,74–77] However, studies from Norway and Denmark showed GRE persistence on poultry farms up to 8 years after the avoparcin ban.[78–80] These studies indicate that, even though the frequencies of GRE had declined immediately after the ban on avoparcin, GRE persisted in the farm environments. Surveillance data from Danish poultry farms reveal that the steep decline in the proportion of glycopeptide-resistant *E faecium* (GREF) that occurred in the first 3 years after the ban on avoparcin was followed by more moderate declines between 1998 and 2007.[8] A plot of the available Norwegian *E faecium* surveillance data together with the complete Danish data set is consistent with a single pattern of resistance decline (figure 2). The data suggest that the proportions of GREF have tended toward stabilisation since 2001, with more than 1–2% of the enterococcal population being glycopeptide resistant.[6] These data show the persistence of glycopeptide-resistance determinants in avoparcin-free environments up to 12 years after the ban in Norway and Denmark, and provide a rare case study on the effect of abolished drug consumption (avoparcin) on corresponding resistance determinants (glycopeptide resistance) in bacterial populations. In the following sections key processes affecting the reversal of glycopeptide resistance are discussed.

Acquisition of glycopeptide-resistance determinants in enterococci

Acquired glycopeptide resistance in enterococci is encoded by six different genotypes: *vanA, vanB, vanD, vanE, vanG*, and *vanL*.[81,82] Resistance based on the *vanA* genotype is inducible, is only expressed in the presence of glycopeptides,[83] is encoded by Tn*1546* (also designated the *vanA* gene-cluster), and is the only genotype found in the Norwegian and Danish agricultural reservoirs. The

original Tn*1546* (10·8 kbp) contains nine genes responsible for regulation of the gene cluster, resistance to vancomycin and teicoplanin, and transposon movement.[84]

Several lines of evidence suggest substantial horizontal transfer of Tn*1546*. Identical Tn*1546* elements, predominantly plasmid associated, are present in a wide variety of different enterococcal strains and reservoirs.[85–87] Moreover, plasmid-associated Tn*1546* elements easily transfer under both in-vitro and in-vivo conditions.[88–92] In a recent study,[80] 94% of Norwegian GRE isolates harboured a common plasmid-mediated Tn*1546*-junction fragment, supporting the rapid spread of a specific extra-chromosomal Tn*1546* containing element. Although these data suggest repeated HGT of plasmid mediated Tn*1546*, it is neither clear to what extent such transfer might occur in typical farm environments nor to what extent repeated HGT may counteract reversal of glycopeptide resistance. In-vivo data indicate that diminishing plasmid frequency due to segregation instability and competition from plasmid-free cells might have been overwhelmed by repeated plasmid transfer.[90] These data suggest that frequent HGT not only counteract resistance loss due to inefficient plasmid segregation, but also the negative fitness effect caused by a decreased reproduction rate for plasmid carrying strains.

The fitness cost of carrying glycopeptide-resistance determinants

The relative fitness difference between resistant and susceptible strains is the key factor controlling the frequency of resistance in bacterial populations.[1,93] Implicit in the reasoning behind the European avoparcin bans was the hypothesis that glycopeptide-resistant enterococci are less fit than their susceptible counterparts, and that the glycopeptide-susceptible population would eventually outcompete and replace the resistant population.

The published work suggests that glycopeptide resistance in farm environments is frequently located on plasmids.[80,89,94] The only study that experimentally investigated the biological cost of a *vanA* plasmid suggested a 4% reduced fitness relative to the plasmid-free ancestor.[90] Recently, an in-vivo colonisation and transfer study revealed that a plasmid (pVEF1)[95] from a Norwegian poultry farm was stably maintained in the recipient for more than 20 days.[88] Interestingly, the ratios of exconjugants and plasmid-free recipient strains were stable throughout the experiments, suggesting little if any biological cost of the newly acquired glycopeptide-resistance plasmid.

The rapid decline in GREF occurrence following the ban on avoparcin (figure 2) is consistent with a fitness cost associated with glycopeptide resistance. A plausible explanation for the seemingly reduced rate of GREF decline between 1998 and 2007 is the reduction of putative fitness costs associated with glycopeptide-resistance determinants. However, it should be noted that a reduced rate of GREF decline could be due to other mechanisms, such as transient episodes of linked selection or clonal shifts in the GREF populations, or both.

Linked selection and segregation stability of glycopeptide-resistance determinants

A potential mechanism for persistence of plasmid-mediated *vanA* GREF is linked selection. Physical linkage between *vanA* glycopeptide resistance and both $CuSO_4$ and erythromycin-resistance determinants has been reported on GREF plasmids of animal origin.[48,94] For instance, Hasman and colleagues[94] reported that 36% (10/29) of *E faecium* isolated from Danish poultry harboured the *tcrB* gene, mediating reduced susceptibility to the growth promoting agent $CuSO_4$. However, only 10% (3/29) were also resistant to glycopeptides, and despite the lack of evidence for physical linkage in GREF of poultry origin, it is likely that $CuSO_4$ was coselected with glycopeptide resistance in Danish poultry. In Norway, no resistances to $CuSO_4$ or commonly used antimicrobials have been detected in transconjugants,[89] or in several sequenced plasmids[95,96] supporting a general absence of physically linked resistance determinants. Avoparcin was the only antibacterial feed additive used in substantial quantities in Norway from 1986 to 1995. Alternative growth promoters did not replace avoparcin.[6] However, ionophore coccidiostats are still used in Norwegian poultry production, and total sales have been stable at roughly the same level as before the abolishment of avoparcin.[6] Reduced susceptibility to narasin appears to be common in *E faecium* isolated from Norwegian poultry farms; however, it has not been associated with glycopeptide resistance.[6] The situation is different in Sweden, where an increased prevalence of *vanA* GREF in poultry faecal samples is probably due to clonal expansion of *E faecium* resistant to vancomycin, low levels of erythromycin (8–16 mg/L), and reduced susceptibility to narasin.[97] Thus, the continued use of either narasin or erythromycin, or both, probably allows for sustained but low proportions of GREF through genetic hitch-hiking.

Indirect stabilisation of plasmid-encoded glycopeptide resistance determinants through physical linkage to PSK systems might also influence long-term persistence in antibiotic-free environments. A ω-ε-ξ-like PSK system was linked to the *vanA* genes widespread on Norwegian poultry farms previously exposed to avoparcin.[80,89,95] One of the plasmids (pVEF1) harbouring this putative PSK system showed complete segregational stability in a serial transfer assay,[88] and the ω-ε-ξ-like operon was able to stabilise pAT18 in *Enterococcus faecalis*.[96] The ω-ε-ξ operon of a streptococcal plasmid (pSM19035) stabilised plamids in both *Bacillus subtilis* and *E coli*.[98] It is tempting to speculate on the possibility that Tn*1546* linkage to PSK systems on conjugative plasmids constitutes the basis for the persistent GREF core populations in the Norwegian farm environments, continuously addicting new genetic backgrounds to the plasmids through conjugal transfer.

Search strategy and selection criteria

Data for this Review were identified through searching PubMed. Search terms included "fitness cost of antibiotic resistance", "glycopeptide resistance and reservoirs", and "growth promoter and ban". Additionally, papers from the authors' own files and references in relevant papers were included. English language articles only were reviewed. No date restrictions were set on any literature searches.

Conclusions

The processes contributing to the reversal of antimicrobial resistance are complex and will vary with antimicrobial-consumption patterns, bacterial species, lifestyle, and environment. It is increasingly clear that antimicrobial resistance is opposite to the good things in life: it is easy to get, but hard to lose.[99] On the positive side, the abolition of a given antimicrobial drug can, when resistance frequencies are very high, relatively quickly reduce the frequency of resistance. Thus, human exposure rates to antimicrobial-resistant bacteria and their transferable genetic elements will be substantially reduced. On the downside, it is also clear that resistant phenotypes may persist at low but detectable frequencies for many years after removal of the selective pressures.

In conclusion, complete eradication of antimicrobial-resistance determinants once present in bacterial populations by simply removing drug-selective pressures appears not to be straightforward. A more detailed understanding is necessary of the species and strain specific biological cost of resistance with respect to both spontaneous mutations and transferable units, the contribution of in-vivo reacquisition rates of resistance determinants (by repeated HGT), and linked selection and segregational stability of extra-chromosomal elements, to more accurately predict the effect of various intervention strategies on the reversal of resistance in bacterial species and populations.

Conflicts of interest

We declare that we have no conflict of interests.

Acknowledgments

P J Johnsen and K M Nielsen were supported by the Norwegian Research Council (NFR) (172046). A Sundsfjord and G S Simonsen were supported by research grants from the NFR (165997, 183653/S10), the Northern Norway Regional Health Authority Medical Research Programme, and the European Commission (LSHE-CT-2007-03410 "ACE"). We thank Maria Sjolund and Omar Cornejo for reading the initial versions of this manuscript and providing valuable suggestions. We also thank the three anonymous reviewers for valuable comments and suggestions.

References

1 Levin BR. Minimizing potential resistance: a population dynamics view. *Clin Infect Dis* 2001; **33:** S161–69.

2 Projan SJ. Why is big Pharma getting out of antibacterial drug discovery? *Curr Opin Microbiol* 2003; **6:** 427–30.

3 Projan SJ, Bradford PA. Late stage antibacterial drugs in the clinical pipeline. *Curr Opin Microbiol* 2007; **10:** 441–46.

4 Cars O, Molstad S, Melander A. Variation in antibiotic use in the European Union. *Lancet* 2001; **9:** 1851–53.

5 Bronzwaer SL, Cars O, Buchholz U, et al. A European study on the relationship between antimicrobial use and antimicrobial resistance. *Emerg Infect Dis* 2002; **3:** 278–82.

6 NORM/NORMVET 2000–07. Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. http://www.unn.no/tidligere-rapporter/category21927.html (accessed April 22, 2009).

7 SWEDRES 2004. A report on Swedish antibiotic utilisation and resistance in human medicine. 2005. Solna: The Swedish Strategic Programme for the Rational Use of Antimicrobial Agents (STRAMA), and the Swedish Institute for Infectious Disease Control, 2004. http://www.smittskyddsinstitutet.se/upload/Publikationer/SWEDRES-2004.pdf (accessed April 22, 2009).

8 DANMAP 2001–07. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods, and humans in Denmark. http://www.danmap.org/ (accessed April 22, 2009).

9 NethMap 2004. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. Amsterdam: Stichting Werkgroep Antibiotica Beleid, 2005. http://www.swab.nl/swab/swabfu.nsf/uploads/4725079A0D5E0FDCC1256EBA003530EA/$file/nethmap_2004.pdf (accessed April 22, 2009).

10 Bergstrom CT, Lipsitch M, Levin BR. Natural selection, infectious transfer and the existence conditions for bacterial plasmids. *Genetics* 2000; **155:** 1505–19.

11 Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proc Natl Acad Sci USA* 1999; **96:** 1152–56.

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

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

14 Dagan R, Barkai G, Givon-Lavi N, et al. Seasonality of antibiotic-resistant *Streptococcus pneumoniae* that causes acute otitis media: a clue for an antibiotic-restriction policy? *J Infect Dis* 2008; **197:** 1094–102.

15 Hennessy TW, Petersen KM, Bruden D, et al. Changes in antibiotic-prescribing practices and carriage of penicillin-resistant *Streptococcus pneumoniae*: a controlled intervention trial in rural Alaska. *Clin Infect Dis* 2002; **34:** 1543–50.

16 Barkai G, Greenberg D, Givon-Lavi N, Dreifuss E, Vardy D, Dagan R. Community prescribing and resistant *Streptococcus pneumoniae*. *Emerg Infect Dis* 2005; **6:** 829–37.

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

18 Bean DC, Livermore DM, Papa I, Hall LM. Resistance among *Escherichia coli* to sulphonamides and other antimicrobials now little used in man. *J Antimicrob Chemother* 2005; **5:** 962–64.

19 LeClerc JE, Li B, Payne WL, Cebula TA. High mutation frequencies among *Escherichia coli* and salmonella pathogens. *Science* 1996; **274:** 1208–11.

20 Komp Lindgren P, Karlsson A, Hughes D. Mutation rate and evolution of fluoroquinolone resistance in *Escherichia coli* isolates from patients with urinary tract infections. *Antimicrob Agents Chemother* 2003; **10:** 3222–32.

21 Matic I, Radman M, Taddei F, et al. Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. *Science* 1997; **277:** 1833–34.

22 de Visser JA. The fate of microbial mutators. *Microbiology* 2002; **148:** 1247–52.

23 Baquero MR, Nilsson AI, Turrientes Mdel C, et al. Polymorphic mutation frequencies in *Escherichia coli*: emergence of weak mutators in clinical isolates. *J Bacteriol* 2004; **186:** 5538–42.

24 Oliver A, Canton R, Campo P, Baquero F, Blazquez J. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science*; **288:** 1251–54.

25 Richardson AR, Yu Z, Popovic T, Stojiljkovic I. Mutator clones of *Neisseria meningitidis* in epidemic serogroup A disease. *Proc Natl Acad Sci USA* 2002; **99**: 6103–07.

26 Tanaka MM, Bergstrom CT, Levin BR. The evolution of mutator genes in bacterial populations: the roles of environmental change and timing. *Genetics* 2003; **164:** 843–54.

27 Gogarten JP, Townsend JP. Horizontal gene transfer, genome innovation and evolution. *Nat Rev Microbiol* 2005; **3:** 679–87.

28 Nielsen KM, Townsend JP. Monitoring and modeling horizontal gene transfer. *Nat Biotechnol* 2004; **22:** 1110–14.

29 Lundquist PD, Levin BR. Transitory derepression and the maintenance of conjugative plasmids. *Genetics* 1986; **113:** 483–97.

30 Simonsen L, Gordon DM, Stewart FM, Levin BR. Estimating the rate of plasmid transfer: an end-point method. *J Gen Microbiol* 1990; **136:** 2319–25.

31 Dionisio F, Matic I, Radman M, Rodrigues OR, Taddei F. Plasmids spread very fast in heterogeneous bacterial communities. *Genetics* 2002; **162:** 1525–32.

32 Kao SM, Olmsted SB, Viksnins AS, Gallo JC, Dunny GM. Molecular and genetic analysis of a region of plasmid pCF10 containing positive control genes and structural genes encoding surface proteins involved in pheromone-inducible conjugation in *Enterococcus faecalis*. *J Bacteriol* 1991; **173:** 7650–64.

33 Kurenbach B, Bohn C, Prabhu J, Abudukerim M, Szewzyk U, Grohmann E. Intergeneric transfer of the *Enterococcus faecalis* plasmid pIP501 to *Escherichia coli* and *Streptomyces lividans* and sequence analysis of its tra region. *Plasmid* 2003; **50:** 86–93.

34 Salyers AA, Whittle G, Shoemaker N. Conjugative and mobelizable transposons. In: Miller RV, Day JM, eds. Microbial evolution: gene establishment, survival, and exchange. Washington, DC: ASM Press, 2004: 125–43.

35 Bjorkman J, Nagaev I, Berg OG, Hughes D, Andersson DI. Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. *Science* 2000; **287:** 1479–82.

36 Bouma JE, Lenski RE. Evolution of a bacteria/plasmid association. *Nature* 1988; **335:** 351–52.

37 Dahlberg C, Chao L. Amelioration of the cost of conjugative plasmid carriage in *Eschericha coli* K12. *Genetics* 2003; **165:** 1641–49.

38 Gustafsson I, Cars O, Andersson DI. Fitness of antibiotic resistant *Staphylococcus epidermidis* assessed by competition on the skin of human volunteers. *J Antimicrob Chemother* 2003; **52:** 258–63.

39 Schrag SJ, Perrot V. Reducing antibiotic resistance. *Nature* 1996; **381:** 120–21.

40 Schrag SJ, Perrot V, Levin BR. Adaptation to the fitness costs of antibiotic resistance in *Escherichia coli*. *Proc Biol Sci* 1997; **264:** 1287–91.

41 Gagneux S, Long CD, Small PM, Van T, Schoolnik GK, Bohannan BJ. The competitive cost of antibiotic resistance in *Mycobacterium tuberculosis*. *Science* 2006; **312:** 1944–46.

42 Rozen DE, McGee L, Levin BR, Klugman KP. Fitness costs of fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2007; **51:** 412–16.

43 Nagaev I, Bjorkman J, Andersson DI, Hughes D. Biological cost and compensatory evolution in fusidic acid-resistant *Staphylococcus aureus*. *Mol Microbiol* 2001; **40:** 433–39.

44 Bjorkholm B, Sjolund M, Falk PG, Berg OG, Engstrand L, Andersson DI. Mutation frequency and biological cost of antibiotic resistance in *Helicobacter pylori*. *Proc Nat Acad Sci USA* 2001; **98:** 14607–12.

45 Lenski RE, Simpson SC, Nguyen TT. Genetic analysis of a plasmid-encoded, host genotype-specific enhancement of bacterial fitness. *J Bacteriol* 1994; **176:** 3140–47.

46 Reynolds MG. Compensatory evolution in rifampin-resistant *Escherichia coli*. *Genetics* 2000; **156:** 1471–81.

47 Levin BR, Perrot V, Walker N. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics* 2000; **154:** 985–97.

48 Aarestrup FM. Characterization of glycopeptide-resistant *Enterococcus faecium* (GRE) from broilers and pigs in Denmark: genetic evidence that persistence of GRE in pig herds is associated with coselection by resistance to macrolides. *J Clin Microbiol* 2000; **38:** 2774–77.

49 Grady R, Hayes F. Axe-Txe, a broad-spectrum proteic toxin-antitoxin system specified by a multidrug-resistant, clinical isolate of *Enterococcus faecium*. *Mol Microbiol* 2003; **47:** 1419–32.

50 Jensen LB, Hammerum AM, Aarestrup FM. Linkage of vat(E) and erm(B) in streptogamin-resistant *Enterococcus faecium* isolates from Europe. *Antimicrob Agents Chemother* 2000; **44:** 2231–32.

51 Sidhu MS, Heir E, Sorum H, Holck A. Genetic linkage between resistance to quaternary ammonium compounds and β-lactam antibiotics in food-related *Staphylococcus* spp. *Microb Drug Res* 2001; **7:** 363–71.

52 O'Brien FG, Price C, Grubb WB, Gustafson JE. Genetic characterization of the fusidic acid and cadmium resistance determinants of *Staphylococcus aureus* plasmid pUB101. *J Antimicrob Chemother* 2002; **50:** 313–21.

53 Martinez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. *Clin Microbiol Rev* 2002; **15:** 647–79.

54 Oliveira DC, Tomasz A, de Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis* 2002; **2:** 180–89.

55 Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Nat Acad Sci USA* 2002; **99:** 7687–92.

56 Leavis HL, Willems RJ, Top J, et al. Epidemic and nonepidemic multidrug-resistant *Enterococcus faecium*. *Emerg Infect Dis* 2003; **9:** 1108–15.

57 Willems RJ, Top J, van Santen M, et al. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *Emerg Infect Dis* 2005; **11:** 821–28.

58 Willems RJ, Bonten MJ. Glycopeptide-resistant enterococci: deciphering virulence, resistance and epidemicity. *Curr Opin Infect Dis* 2007; **20:** 384–90.

59 de Lencastre H, Oliveira D, Tomasz A. Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power. *Curr Opin Microbiol* 2007; **10:** 428–35.

60 Arason VA, Gunnlaugsson A, Sigurdsson JA, Erlendsdottir H, Gudmundsson S, Kristinsson KG. Clonal spread of resistant pneumococci despite diminished antimicrobial use. *Microb Drug Res* 2002; **8:** 187–92.

61 Thomas CM. Paradigms of plasmid organization. *Molecular Microbiol* 2000; **37:** 485–91.

62 Engelberg-Kulka H, Glaser G. Addiction modules and programmed cell death and antideath in bacterial cultures. *Ann Rev Microbiol* 1999; **53:** 43–70.

63 Gerdes K, Christensen SK, Lobner-Olesen A. Prokaryotic toxin-antitoxin stress response loci. *Nat Rev Microbiol* 2005; **3:** 371–82.

64 Bates J, Jordens Z, Selkon JB. Evidence for an animal origin of vancomycin-resistant enterococci. *Lancet* 1993; **342:** 490–91.

65 Klare I, Heier H, Claus H, Witte W. Environmental strains of *Enterococcus faecium* with inducible high-level resistance to glycopeptides. *FEMS Microbiol Lett* 1993; **106:** 23–29.

66 Aarestrup FM. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. *Microb Drug Res* 1995; **1:** 255–57.

67 Endtz HP, van den Braak N, van Belkum A, et al. Fecal carriage of vancomycin-resistant enterococci in hospitalized patients and those living in the community in The Netherlands. *J Clin Microbiol* 1997; **35:** 3026–31.

68 Klare I, Heier H, Claus H, Reissbrodt R, Witte W. vanA-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. *FEMS Microbiology Lett* 1995; **125:** 165–71.

69 Kruse H, Johansen BK, Rorvik LM, Schaller G. The use of avoparcin as a growth promoter and the occurrence of vancomycin-resistant *Enterococcus* species in Norwegian poultry and swine production. *Microb Drug Res* 1999; **5:** 135–39.

70 Pantosti A, Del Grosso M, Tagliabue S, Macri A, Caprioli A. Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. *Lancet* 1999; **354:** 741–42.

71 Torres C, Reguera JA, Sanmartin MJ, Perez-Diaz JC, Baquero F. vanA-mediated vancomycin-resistant *Enterococcus* spp. in sewage. *J Antimicrob Chemother* 1994; **33:** 553–61.

72 Bager F, Madsen M, Christensen J, Aarestrup FM. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. *Prev Vet Med* 1997; **31:** 95–112.

73 Wegener HC, Aarestrup FM, Jensen LB, Hammerum AM, Bager F. Use of antimicrobial growth promoters in food animals and *Enterococcus faecium* resistance to therapeutic antimicrobial drugs in Europe. *Emerg Infect Dis* 1999; **3:** 329–35.

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

75 Bager F, Aarestrup FM, Madsen M, Wegener HC. Glycopeptide resistance in *Enterococcus faecium* from broilers and pigs following discontinued use of avoparcin. *Microb Drug Res* 1999; **5:** 53–56.

76 Klare I, Badstubner D, Konstabel C, Bohme G, Claus H, Witte W. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. *Microb Drug Res* 1999; **5:** 45–52.

77 van den Bogaard AE, Bruinsma N, Stobberingh EE. The effect of banning avoparcin on VRE carriage in The Netherlands. *J Antimicrob Chemother* 2000; **46:** 146–48.

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

79 Heuer OE, Pedersen K, Jensen LB, Madsen M, Olsen JE. Persistence of vancomycin-resistant enterococci (VRE) in broiler houses after the avoparcin ban. *Microb Drug Res* 2002; **8:** 355–61.

80 Sorum M, Johnsen PJ, Aasnes B, et al. Prevalence, persistence, and molecular characterization of glycopeptide-resistant enterococci in Norwegian poultry and poultry farmers 3 to 8 years after the ban on avoparcin. *Appl Environ Microbiol* 2006; **72:** 516–21.

81 Courvalin P. Vancomycin resistance in gram-positive cocci. *Clin Infect Dis* 2006; **42:** S25–34.

82 Boyd AB, Willey BM, Fawcet D, Gillani N, Muley MR. Molecular characterization of *Enterococcus faecalis* N06-0364 with low-level vancomycin resistance harboring a novel D-Ala-D-Ser gene cluster, *vanL*. *Antimicrob Agents Chemother* 2008; **52:** 2667–72.

83 Arthur M, Molinas C, Courvalin P. The VanS-VanR two-component regulatory system controls synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol* 1992; **174:** 2582–91.

84 Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol* 1993; **175:** 117–27.

85 Simonsen GS, Myhre MR, Dahl KH, Olsvik O, Sundsfjord A. Typeability of Tn1546-like elements in vancomycin-resistant enterococci using long-range PCRs and specific analysis of polymorphic regions. *Microb Drug Res* 2000; **6:** 49–57.

86 Willems RJ, Top J, van Den Braak N, et al. Host specificity of vancomycin-resistant *Enterococcus faecium*. *J Infect Dis* 2000; **182:** 816–23.

87 Willems RJ, Top J, van den Braak N, et al. Molecular diversity and evolutionary relationships of Tn1546-like elements in enterococci from humans and animals. *Antimicrob Agents Chemother* 1999; **43:** 483–91.

88 Dahl KH, Mater DD, Flores MJ, et al. Transfer of plasmid and chromosomal glycopeptide resistance determinants occurs more readily in the digestive tract of mice than in vitro and exconjugants can persist stably in vivo in the absence of glycopeptide selection. *J Antimicrob Chemother* 2007; **59:** 478–86.

89 Johnsen PJ, Osterhus JI, Sletvold H, et al. Persistence of animal and human glycopeptide-resistant enterococci on two Norwegian poultry farms formerly exposed to avoparcin is associated with a widespread plasmid-mediated vanA element within a polyclonal enterococcus faecium population. *Appl Environ Microbiol* 2005; **71:** 159–68.

90 Johnsen PJ, Simonsen GS, Olsvik O, Midtvedt T, Sundsfjord A. Stability, persistence, and evolution of plasmid-encoded VanA glycopeptide resistance in enterococci in the absence of antibiotic selection in vitro and in gnotobiotic mice. *Microb Drug Res* 2002; **8:** 161–70.

91 Lester CH, Frimodt-Moller N, Sorensen TL, Monnet DL, Hammerum AM. In vivo transfer of the vanA resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers. *Antimicrob Agents Chemother* 2006; **50:** 596–99.

92 Werner G, Klare I, Witte W. Large conjugative vanA plasmids in vancomycin-resistant *Enterococcus faecium*. *J Clin Microbiol* 1999; **37:** 2383–84.

93 Andersson DI. Persistence of antibiotic resistant bacteria. *Curr Opin Microbiol* 2003; **6:** 452–56.

94 Hasman H, Aarestrup FM. tcrB, a gene conferring transferable copper resistance in *Enterococcus faecium*: occurrence, transferability, and linkage to macrolide and glycopeptide resistance. *Antimicrob Agents Chemother* 2002; **46:** 1410–16.

95 Sletvold H, Johnsen PJ, Simonsen GS, Aasnaes B, Sundsfjord A, Nielsen KM. Comparative DNA analysis of two vanA plasmids from *Enterococcus faecium* strains isolated from poultry and a poultry farmer in Norway. *Antimicrob Agents Chemother* 2007; **51:** 736–39.

96 Sletvold H, Johnsen PJ, Hamre I, Simonsen GS, Sundsfjord A, Nielsen KM. Complete sequence of *Enterococcus faecium* pVEF3 and the detection of an ω-ε-ξ toxin-antitoxin module and an ABC transporter. *Plasmid* 2008; **60:** 75–85.

97 SVARM 2004. Swedish veterinary antimicrobial resistance monitoring. Uppsala: The National Veterinary Institute, 2005. http://www.sva.se/upload/pdf/Tj%C3%A4nster%20och%20produkter/Trycksaker/svarm2004.pdf (accessed April 22, 2009).

98 Zielenkiewicz U, Ceglowski P. The toxin-antitoxin system of the streptococcal plasmid pSM19035. *J Bacteriol* 2005; **187:** 6094–105.

99 Salyers AA, Amabile-Cuevas CF. Why are antibiotic resistance genes so resistant to elimination? *Antimicrob Agents Chemother* 1997; **41:** 2321–25.