

Assessing risks for a pre-emergent pathogen: virginiamycin use and the emergence of streptogramin resistance in *Enterococcus faecium*

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Vancomycin-resistant enterococci (VRE) are an important cause of hospital-acquired infections and an emerging infectious disease. VRE infections were resistant to standard antibiotics until quinupristin/dalfopristin (QD), a streptogramin antibiotic, was approved in 1999 for the treatment of vancomycin-resistant *Enterococcus faecium* infections in people. After that decision, the practice of using virginiamycin in agriculture for animal growth promotion came under intense scrutiny. Virginiamycin, another streptogramin, threatens the efficacy of QD in medicine because streptogramin resistance in enterococci associated with food animals may be transferred to *E faecium* in hospitalised patients. Policy makers face an unavoidable conundrum when assessing risks for pre-emergent pathogens; good policies that prevent or delay adverse outcomes may leave little evidence that they had an effect. To provide a sound basis for policy, we have reviewed the epidemiology of *E faecium* and streptogramin resistance and present qualitative results from mathematical models. These models are based on simple assumptions consistent with evidence, and they establish reasonable expectations about the population-genetic and population-dynamic processes underlying the emergence of streptogramin-resistant *E faecium* (SREF). Using the model, we have identified critical aspects of SREF emergence. We conclude that the emergence of SREF is likely to be the result of an interaction between QD use in medicine and the long-term use of virginiamycin for animal growth promotion. Virginiamycin use has created a credible threat to the efficacy of QD by increasing the mobility and frequency of high-level resistance genes. The potential effects are greatest for intermediate rates of human-to-human transmission ($R_0 \approx 1$).

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The emergence of bacteria with resistance to new drugs represents a readily identifiable threat to public health. Vancomycin-resistant enterococci (VRE) were first reported in 1986 and spread epidemically through hospitals in the USA leading to classification of VRE as an emerging infectious disease.¹ Now VRE are a leading cause of hospital-acquired bloodstream, urinary tract, and wound infections, especially in intensive care units.² Such infections were not treatable by any standard antibiotics until the streptogramin antibiotic quinupristin/dalfopristin (QD) was approved in

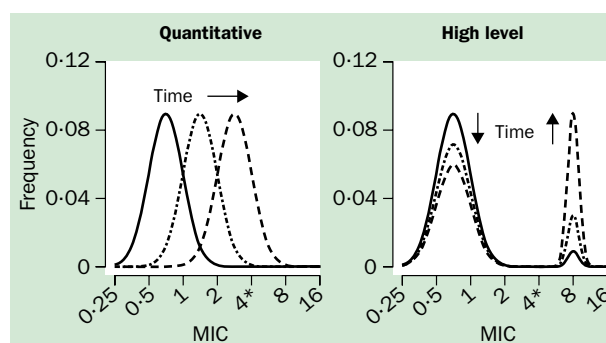


Figure 1. Quantitative resistance (left) involves the accumulation of point mutations conferring slightly increased resistance to streptogramins. The distribution represents the most resistant strain across a population of human beings. Over time, the distribution of resistant types may shift due to selection by QD. By contrast, high-level resistance (right) involves the epidemic spread of a highly resistant type or acquisition of a gene that confers high-level resistance followed by clonal expansion. Such a process results in a bimodal distribution of resistance; patients are either colonised by the highly resistant strain or not. The fraction of people colonised by the strain with high-level resistance due to clonal expansion is driven mainly by medical antibiotic use. *Breakpoint.

1999 for the treatment of people infected with vancomycin-resistant *Enterococcus faecium* (VREF). The approval of QD focused public attention and scrutiny on the practice of using virginiamycin, another streptogramin, in agriculture as a nutritional supplement (growth promoter) for poultry and other food animals. QD and virginiamycin each contain a pair of chemicals that inhibit protein synthesis and have a synergistic effect when combined.³ More importantly, resistance to QD confers some cross-resistance to virginiamycin, and vice versa.⁴ Concerns about virginiamycin use are based on the theory that its use in animals has established a reservoir of streptogramin-resistant bacteria in poultry and other food animals leading

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to contamination of animal food products with streptogramin-resistant *E faecium* (SREF), colonisation of people by novel strains of SREF, and the emergence of SREF or multidrug-resistant *E faecium* (MREF) in hospitals. The use of antibiotics for growth promotion was recently banned by the European Union, and the USA and Australia are considering some regulations on virginiamycin. The scientific basis for regulating animal growth promoters is under intense scrutiny by the drug industry, poultry producers, advocacy groups, consumers, scientists, and public-health officials.

The potential emergence of streptogramin resistance in *E faecium*, like vancomycin resistance in *Staphylococcus aureus*, represents a special kind of threat to public health.⁵ These new forms are pre-emergent pathogens or, in other words, currently rare pathogens that have a high potential for epidemic spread. Three aspects of SREF would lead to

classification as pre-emergent: first, VRE are emerging infectious diseases; second, poultry and pork in supermarkets are frequently contaminated by SREF, including some with high-level resistance genes; and third, some genes conferring high-level resistance to streptogramins are highly mobile. Pre-emergent pathogens present unique problems for policy makers. First-case prevention generally requires less effort than management of the subsequent spread.^{6,7} On the other hand, uncertainty about policies to prevent emergence is inevitable because direct observations cannot be made for pathogens that have not emerged. It seems likely that SREF will eventually evolve, yet SREF showing high-level resistance to streptogramins are rarely seen in human beings despite three decades of virginiamycin use.

To make science-based decisions about medical consequences of virginiamycin use and SREF emergence, we developed a simple mathematical model to describe the qualitative dynamics.⁸ The model we used is similar to other models for the population biology of antibiotic-resistant bacteria, especially VRE.^{9–15} Based on the qualitative conclusions of this model, we identified critical parameters and reviewed the published material to assess our current understanding of those parameters. Thus, we are not specifically assessing the risk of infection and treatment failure so much as narrowing the debate about the current scientific basis for concluding whether virginiamycin use would lead to increases in the fraction of patients colonised by SREF. Increased prevalence is closely related to the frequency of treatment failure and doctors' decisions to treat patients with QD. Qualitative analysis of this sort may be a useful way to assess other pre-emergent pathogens.

Ecology, epidemiology, and genetics of SREF

The emergence of high-level QD resistance in VRE would substantially undermine doctors' ability to treat the infection. VRE represent a unique sort of emerging pathogen; enterococci are generally considered to be commensal—ie, harmless or beneficial bacteria ordinarily found in healthy people. Exposure to enterococci is common and normally of little epidemiological significance. However, enterococci cause opportunistic infections that arise endogenously from populations in the patient's gut, or infection may be initiated nosocomially after transmission on the hands of a hospital worker. Colonisation by VRE threatens other patients by providing additional sources of exposure that may lead to colonisation or infection.¹⁶ VRE infections are more difficult to treat than infections with susceptible enterococci, leading to increased morbidity, mortality, and hospitalisation costs,^{17,18} and streptogramin resistance would further complicate treatment.

Based on experience with other antibiotics and bacteria, it seems inevitable that SREF will eventually evolve and spread. Until such an event happens, it will be impossible to provide direct quantitative estimates of some critical parameters or a more concrete assessment of the risks. On the other hand, resistance to streptogramins and the ecology and epidemiology of *E faecium* are fairly well-understood because of the recent emergence of VRE.^{1,19,20} Moreover, the

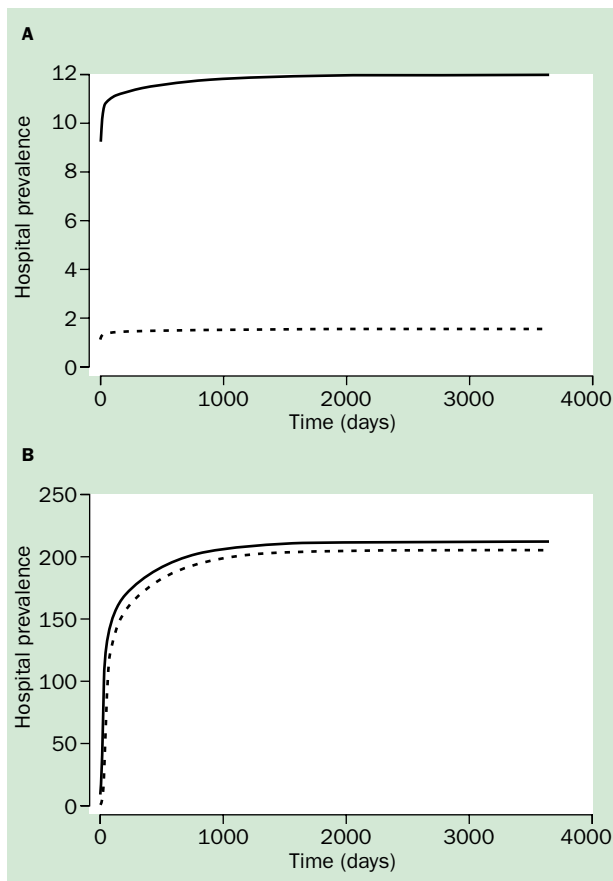


Figure 2. The potential effects of virginiamycin use for non-epidemics (A) and epidemics (B) shown as projected hospital prevalence of SREF over time using a deterministic mathematical model. Solid lines and dashed lines represent projections with and without virginiamycin use, respectively, assuming virginiamycin use is the main reason new SREF strains arise in human beings. Both panels represent different assumptions about R_0 and about the rate of exposure to novel strains of SREF (note that the scale of the y-axis changes). The initial conditions for each projection represent the equilibrium prevalence of SREF without exposure due to virginiamycin use and without medical use of QD. With low epidemic potential (A), person-to-person transmission is rare. With high epidemic potential (B), epidemic transmission due to medical antibiotic use is a major factor.

potential transfer of resistance genes from animals has been studied because avoparcin (a glycopeptide similar to vancomycin) was used for growth promotion throughout Europe. VRE is more commonly isolated from the stools of individuals without a recent history of hospitalisation in Europe compared with the USA where avoparcin has never been used.^{21–26} By contrast, VRE are a serious problem in US hospitals, but a minor problem in European hospitals, sparking an active debate.^{1,7,27–34}

Our analysis is based on the ecology and epidemiology of VRE and specific genetic aspects of resistance to streptogramins in *E faecium*. Our goal was to identify parameters that are critical for the emergence of SREF and direct future research on parameters that may be most informative. We have identified three important aspects.⁷ First, what are the likely genetic mechanisms of resistance to streptogramin resistance in *E faecium*, and how frequently do these move from animals to human beings? Second, what fraction of new SREF strains are attributable to virginiamycin use? Finally, what are reasonable scenarios for the spread of SREF among hospitalised patients? Antibiotic use favours resistance, and high transmission rates of antibiotic-resistant bacteria are associated with high rates of antibiotic use,^{1,12} but the rate of QD use in humans is currently low and may remain low for some time. How should existing evidence be interpreted? We address these issues in the following sections.

Quantitative resistance

Resistance may arise through the accumulation of mutations or the acquisition of extrachromosomal DNA. Naturally occurring quantitative resistance should not be confused with the potential threats from high-level resistance. Variation in resistance to streptogramins exists in *E faecium* populations and provides the basic material for selection. QD resistance is defined by a minimum inhibitory concentration (MIC) at QD concentrations of 2 µg/mL.³⁵ The distribution of MICs in *E faecium* isolated from people^{36–43} is typically below the breakpoint for QD resistance. Some QD resistance above the breakpoint may represent the tail end of a distribution; such strains are not necessarily associated with the high-level resistance that has been seen in *E faecium* isolated from poultry or other food animals. Moreover, treatment failure may occur with or without high-level resistance genes; some strains with increased MICs post treatment have been associated with treatment failure.⁴⁴

Based on experience with other antibiotics and the basic principles of population genetics, it is reasonable to expect shifts in the distribution of resistance to streptogramins over time; QD use selects for QD resistance. Resistant strains are expected to increase in frequency and evolve greater resistance by accumulating mutations. As the average rate of resistance to streptogramins in *E faecium* increases, the fraction of the population showing resistance that exceeds a breakpoint is also expected to increase (figure 1).

High-level resistance

The emergence of high-level resistance may represent a serious threat to the efficacy of QD. *E faecium* from poultry

and pork frequently show high-level resistance to streptogramins. QD and virginiamycin are a combination of chemicals from two groups called streptogramin B (quinupristin, virginiamycin S, or pristinamycin IIA), and streptogramin A (dalfopristin, virginiamycin M, pristinamycin IA). *E faecium* isolates resistant to one component or the other show increased resistance, but strains resistant to both components are highly resistant. Genes conferring high-level resistance to each component of the drug have been identified and reviewed elsewhere.³ Of concern are the *vatD* (or *satA*) and *vatE* (or *satG*) genes that confer resistance to streptogramin A,⁴⁵ and the *vgbB* and *ermB* genes that confer high-level resistance to streptogramin B.³

SREF are relatively common among *E faecium* isolated from farm animals⁴⁶ or animal food products sold in grocery stores.⁴ SREF are present on 50–70% of poultry products purchased in supermarkets.^{43,47–49} Novel SREF strains may also spread indirectly from farms through the environment in raw manure or through surface or ground water.^{50,51} The *vat* genes were seen in 25% (22/89)⁵² of isolates from pigs and chickens, or 29% (41/140) of isolates from animals and farm workers.⁵³ QD resistance genes isolated from poultry were genetically diverse.⁵⁴

A critical question is how often high-level resistance genes are transferred into *E faecium* strains already circulating among human beings. Enterococci are considered to be promiscuous.⁵⁵ These genes are mobile, and are transferred at high rates.⁵² Transfer rates are generally higher in vitro than in vivo,⁵⁶ but in-vivo transfer of the *vatD* has been reported experimentally.⁵⁷

The presence of high-level resistance genes is sometimes sought in isolates with MICs above the breakpoint.⁵⁸ High-level resistance genes are rare, but they have been found in *E faecium* isolated from people.^{42,53,59} SREF containing high-level resistance genes were isolated from several farm workers who may be at greater risk of colonisation by SREF, possibly from frequent contact with chickens, chicken faecal matter, or the dust from medicated animal food.^{53,60–62} Although farm workers may not represent a high portion of the population, they may play an important role by introducing SREF into hospitals where other factors affect transmission.

Unlike the *vat* genes, *ermB* is relatively common in enterococci colonising humans as well as poultry.³ Strains with resistance to one component may acquire resistance to the other, but it seems increasingly likely that these resistance genes may be transmitted together. Genes conferring high-level resistance to both streptogramin chemicals might be transmitted on a plasmid or other mobile genetic element from SREF (or some other species) in animals to *E faecium* in humans. Evidence that *vatE* and *ermB* are linked suggests that the resistance genes were transmitted from a common source.^{63,64}

Ecology of SREF

E faecium are genetically diverse—closely related subgroups are apparently specialised to particular hosts or habitats. SREF from poultry may be well-adapted to cloaca and have

difficulty surviving the gastric barrier and colonising the gut. SREF from animals are able to establish transient populations in the gut after experimental ingestion,⁵⁶ but the prevalence of SREF with high-level resistance remains low despite the high rate of exposure on contaminated meat.⁴³ Different genetic profiles of VRE isolated from human beings, poultry, and pork suggest that strains of SREF may be highly host specific.⁶⁵ Poultry strains of SREF rarely establish in human beings, but strains from pork may colonise more often.^{65,66} For example, analysis by pulsed-field gel electrophoresis (PFGE) showed one large cluster from poultry and another cluster from human beings. However, a few strains were seen in smaller clusters that contained isolates from poultry and human beings.⁶⁷ Other studies reported similar genetic patterns in poultry and human isolates.⁶⁸ Moreover, clinical strains and epidemic strains of SREF form distinct genetic clusters, consistent with the hypothesis that the strains have adapted to a clinical environment.^{69,70}

These data are consistent with a hypothesis of strong, but not absolute, host specificity—ie, most host-to-host transmission occurs among hosts of the same species. Based on these data, colonisation of human beings by SREF of animal origin seems unlikely. If it does happen, pork is a more likely source than poultry. However, *E faecium* from animals may establish transient populations that contribute genes to resident strains. The bacteria themselves may never establish; nevertheless, they may contribute to the spread of resistance by donating high-level resistance genes.

Attributing new exposure to virginiamycin use

The impact of virginiamycin use must be assessed by comparison with a counter-factual world in which virginiamycin was never used. Genes conferring high-level resistance to streptogramins may have been present in the environment for reasons that are unrelated to the use of virginiamycin for animal growth promotion. It is probably not possible to estimate the fraction of new strains introduced into human beings as a consequence of virginiamycin use without knowing how resistance will eventually evolve, and whether animals are the primary source of those genes. However, after nearly three decades of virginiamycin use in agriculture, it may be impossible to estimate what fraction of new SREF strains introduced into the human population are not directly attributable to virginiamycin use. Estimates of SREF prevalence in most environments are not available from before the approval of virginiamycin for growth promotion.

Genes conferring resistance to both streptogramin chemicals may have been extremely rare without three decades of heavy virginiamycin use, and limited to communities with bacteria that naturally produce streptogramins. Moreover, it is plausible that the long-term use of virginiamycin is responsible for high mobility and linkage of the high-level resistance genes in SREF isolated from poultry; such evolutionary events are difficult to reverse once they have occurred. Thus, virginiamycin use may have had an effect that is similar to an evolutionary “ratchet”.

Person-to-person transmission

Enterococci differ from campylobacter and other zoonotic microbes in their propensity to spread from person to person. A single SREF could, in theory, initiate an epidemic of colonisation that becomes clinically significant in individuals far removed from the person initially colonised. Subsequent transfer to human beings may be important even if the genes are transmitted to *E faecium* associated with humans only a handful of times.⁷

To understand the impact of virginiamycin use, we must understand the spread of SREF among people. The emergence of SREF may be an interaction between virginiamycin use and QD use in medicine. The propensity to cause epidemics or epidemigenicity, and the rate of person-to-person transmission are summarised by the notion of the basic reproductive number, R_0 . The basic reproductive number for SREF is a specific instance of a general concept, the maximum lifetime reproductive output of an organism. Although R_0 is useful, it is difficult to compute for a specific pathogen at a particular time or place. Calculation of R_0 for SREF is based on our understanding of microparasites,⁷¹ pathogens that reproduce within a host making it sensible to assume that a host is either infected or not. The maximum lifetime reproductive output for a microparasite is not based on the growth rate within a host, but the number of hosts that would be infected by a single infected individual introduced into an otherwise naive population. Commensal microbes are unlike microparasites in some ways because microbial loads may vary by several orders of magnitude, they are rarely pathogenic, and the host does not form long-lasting immune response. For SREF, we define R_0 for a hospital population as the number of patients who would be colonised by a single colonised patient before being discharged or losing the SREF population by normal processes.

Estimates of R_0 for SREF must be based on the population biology of enterococci and VRE. Clearly, the rate of transmission varies from hospital to hospital, depending on standard procedures, the size of the hospital,¹⁰ the rate of antibiotic use,¹⁵ and compliance with good sanitation and hygiene guidelines. In addition, R_0 for VRE within a hospital varies substantially over time due to fluctuations in the patient population, antibiotic prescription rates, and compliance with hospital infection control.^{10,72} The emergence of VRE and estimates of R_0 for VRE indicate a potential for rapid epidemic spread.^{11,14} R_0 has been estimated for VRE in a few different hospital populations. Estimates range from $R_0 \approx 3$ –4 down to $R_0 \approx 0.6$ after hospital infection control,¹⁴ or perhaps R_0 is lower and most cases indicate colonisation on admission.⁷³

Antibiotic use and R_0

Calls for prudent use of antibiotics in medicine are based on the notion that selective pressure and R_0 for antibiotic-resistant bacteria are related to the rate of antibiotic use^{1,2,15} but few studies have analysed how multiple antibiotics will affect R_0 specifically for SREF. Observational and prospective studies of VRE and other antibiotic-resistant bacteria in people and direct manipulation of VRE in people and in animal models suggest that many antibiotics affect transmission rates of SREF.²⁰ Antibiotic use has four distinct effects on the

prevalence of SREF—it may increase the likelihood of colonisation and so reduce competition from other bacteria, increase transmission rates from colonised patients by increasing the population densities of SREF, increase persistence times by eliminating competitors, or eliminate SREF. These effects can often lead to counterintuitive associations in populations.⁹

QD is perhaps the most important drug that affects the prevalence of SREF, although its role may be limited. Streptogramins eliminate sensitive *E faecium* and select SREF as well as the naturally resistant and more virulent species, *E faecalis*.⁷⁴ Without QD, SREF has few advantages over other enterococci. The pharmacokinetics of streptogramins may mitigate this effect; QD is given intravenously and a significant portion of dalfopristin is metabolised into pristinamycin IIA (similar to quinupristin) before being excreted into the gut.⁷⁵

Once QD begins to spread in a hospital, antibiotics targeted at other bacteria may amplify SREF densities or increase colonisation rates. One critical difference between SREF and VRE may be the role of macrolide antibiotics; the *ermB* gene confers resistance to dalfopristin as well as macrolides. Drugs that eliminate competitors of enterococci may increase population density and colonisation rates of VRE, especially cephalosporins.²⁰ Antibiotics may eliminate bacteria that inhibit enterococci, providing an opportunity for SREF to invade—these drugs include clindamycin, piperacillin-tazobactam, ticarcillin-clavulanic acid, metronidazole, cefotetan, ampicillin, and ampicillin-sulbactam.^{74,76–78}

Linezolid eliminates SREF from patients and reduces secondary transmission. Linezolid may change the prescription patterns of QD and affect SREF dynamics in other ways. Linezolid may be preferred to QD because it is less expensive and may be taken orally, whereas QD must be given intravenously. Linezolid is perceived by some doctors to have fewer side-effects than QD. Finally, QD is only effective against *E faecium*, but linezolid is also effective against *E faecalis*. These two species of enterococci account for most nosocomial enterococci infections. Treatment with QD requires a clinical test to speciate enterococci, an added expense and delay that might favour linezolid for the treatment of SREF. However, resistance to linezolid has already been reported.⁷⁹

The role of vancomycin is also likely to change over time. Initially, vancomycin use will eliminate SREF, high prevalence of VRE, and, hence, a high need for QD or linezolid. The effect of these drugs on the densities and transmission of SREF is uncertain. Once a strain resistant to both vancomycin and streptogramin evolves it may spread as fast as VRE. Concurrent use of QD and vancomycin has occurred only recently. Thus far, MREF has been isolated from a few patients but has not been associated with epidemic spread in clinical settings.⁶¹ Once epidemics begin, vancomycin may change roles from selecting against SREF to selecting for multi-resistant VRE. Some insights into the future epidemiology of SREF in the USA may be gained by studying the epidemiology of *E faecium* in France where pristinamycin has been prescribed for upper respiratory infections, but we are not aware of studies that have examined QD resistance in *E faecium* there.⁸⁰

Quantitative aspects of SREF emergence

Changes in the frequency of SREF over time are quantitative events involving multiple, non-linear interacting factors. Mathematical models describing those changes are quantitative descriptions of the biological process based on a simple, biologically justifiable description of the process. The mathematical model we have used is a compartment model, a set of coupled ordinary differential equations. A similar model⁷ shares many features with this one, but we have explicitly coupled community exposure and colonisation by SREF to nosocomial transmission (a diagram of the model is provided, and the full model is available at <http://medschool.umaryland.edu/Epidemiology/dsmith/sref.htm>).

The critical features of the model are as follows: (1) human beings are unexposed, exposed, colonised. Colonised patients may be highly contagious or not. (2) Unexposed people are exposed to new strains of SREF. The origin of these strains is not specified; many potential routes from animals to people exist, and new strains of SREF may arise from sources other than animals. A fraction of the new strains are a direct result of virginiamycin use. The rate of exposure and the fraction due to virginiamycin use are critical parameters in the model. (3) After exposure, a population of SREF in the human gut is transient, unless the bacteria colonise. Transient populations last a few days, but a fraction colonise and persist for a few months. (4) SREF spreads from person to person and hospital populations are well-mixed. (5) Antibiotic use disturbs the natural flora of the human gut, increasing the probability of colonising the population density of SREF. High-density populations of SREF are expected to persist longer than transient populations, and colonisation is more likely a result of amplification. People who have recently used antibiotics shed SREF at a higher rate and are more contagious. QD is expected to play an important part, as are several antibiotics that have been implicated in the transmission of VRE. (6) Patients are discharged at random from the hospital into the community where antibiotic use and transmission rates are extremely low. The community from which patients are drawn is much larger than the hospital population, and the length of stay in hospitals is relatively short.

The model is deliberately simple, and it omits many aspects of SREF transmission and persistence that affect the dynamic behaviour of the model. For example, transmission of SREF in the intensive care units of hospitals may occur at higher rates than other units within a hospital.² The hospital environment may be contaminated by SREF, or SREF may colonise hospital workers.⁸¹ Many patients are discharged to long-term care facilities that may have transmission rates similar to hospitals, but the patients of long-term care facilities are permanent residents.⁸² Recently discharged patients may transmit SREF to their family members at high rates, but with much lower rates of transmission outside of family members.⁸³ Based on observations, farm workers admitted to hospitals may be much more likely to be colonised with SREF.^{53,62–64} We have to assume that people are either exposed, colonised, or amplified, but the quantitative dynamics of SREF in the gut are probably much more complicated. Despite the caveats, the qualitative behaviour of the models and the general conclusions are fairly robust.

Estimating the impact of virginiamycin use involves an understanding of the interplay between medical antibiotic use and the history of virginiamycin use. The issue is more complicated than simply estimating current exposure rates on contaminated food and forces examination of all the effects of virginiamycin use.⁷ We have used this mathematical model to synthesise existing data and establish reasonable expectations across a range of parameter values.

Assessing impact

The basic reproductive number is an extremely useful concept because it establishes a simple criterion for determining whether an epidemic will occur. If $R_0 > 1$, each host exposed to SREF leads to exposure of at least one other infected host, so an epidemic ensues. If $R_0 < 1$, each infected host tends to infect less than one host, and the pathogen eventually dies out. With the introduction from external sources, the dynamics of SREF are a complicated interaction between exposure, which may be due to virginiamycin use, and R_0 , which is probably not affected by virginiamycin use.^{7,73} Because of external exposure we also describe the dynamics of “quasi-epidemics”, the nearly epidemic spread of SREF in a population, or in other words, for R_0 close to one. In this case, the introduction of SREF from external sources has a more critical role in the dynamics. To illustrate qualitative aspects of virginiamycin use, we treat epidemics ($R_0 > 1$), quasi-epidemics ($R_0 \approx 1$), and non-epidemic ($R_0 \approx 0$) as separate cases.

Non-epidemic dynamics, $R_0 \approx 0$

If $R_0 \approx 0$ all new strains introduced into a population die out fairly rapidly; most strains that are isolated from human beings are the direct result of recent exposure. The effect of virginiamycin use in this case is directly related to the fraction of exposure that is attributable to virginiamycin use (figure 2A).

Epidemics, $R_0 > 1$

If $R_0 > 1$ epidemics of SREF occur (figure 2B). The transmission of SREF is probably affected by hospital

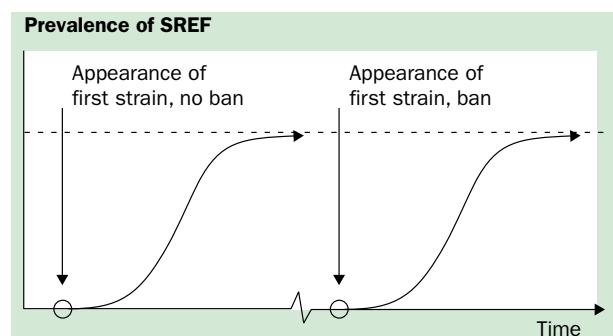


Figure 3. A schematic diagram for the emergence of epidemic multidrug-resistant bacteria. A “honeymoon” period follows the introduction of the drug, followed by the isolation of the first resistant strain. Stochastic events probably have a critical role in determining the time to appearance. The subsequent epidemic asymptotically approaches an endemic prevalence. The critical question to be answered for streptogramin resistance is whether the first medical strain would emerge much later if virginiamycin is banned. The medical cost of using virginiamycin is the elapsed time between the two events (at least one of them hypothetical) multiplied by the asymptotic prevalence.

infection control and hygiene, and by the total pattern of antibiotic use within a hospital, but not by virginiamycin use. In SREF epidemics, the prevalence of SREF over time follows a sigmoidal curve. Initially, SREF prevalence increases exponentially. As the prevalence of SREF increases colonisation pressure increases, leading to increased risks of infection. On the other hand there are fewer individuals who were not already exposed. The prevalence of SREF reaches an equilibrium that balances new exposure and the loss of existing SREF populations from natural turnover.

Exposure to new strains of SREF may have a large effect when SREF is initially rare. During the exponentially increasing phase, the rate of exposure to new strains of SREF may be substantially higher than the rate of spread from person to person. Exposure when SREF is rare may lead to the early emergence. The effect of virginiamycin is assessed by calculating SREF prevalence over time with and without virginiamycin use;⁷ this amounts to subtracting two logistic curves from one another. In general, the magnitude of the delay is felt more acutely for values of R_0 close to one. Critically, spread is initially slow in the deterministic model, but long delays before emergence may be due to stochastic effects when SREF is very rare. The simple deterministic models we have used to illustrate general principles are probably not well suited to predict the magnitude of the delay (figure 3).

Quasi-epidemic dynamics, $R_0 \approx 1$

The potential effects of virginiamycin use are most severe for quasi-epidemics (figure 4). The prevalence of any particular strain fluctuates stochastically over time in a random way, but changes in the prevalence of SREF in the population over time are complicated interactions between the rate at which new strains enter the population and subsequent transmission at rates that are too low to cause an epidemic.⁷³ Quasi-epidemic transmission may allow a strain to persist for long periods of time. Thus, low-level transmission amplifies new exposure leading to excess prevalence of SREF that is sustained indefinitely. If most new strains are the result of virginiamycin use its impact would be considered unacceptable by most reasonable criteria. Quasi-epidemic events have a greater and more prolonged effect than do epidemic events, because the persistence of each strain is extended by low-level transmission. With intermediate values of R_0 the equilibrium prevalence and the prevalence over time are more strongly impacted by the rate of exposure, and medical antibiotic use plays a less important part.

The emergence of multidrug resistance

Increased prevalence of SREF is a concern primarily because of the potential emergence of *E faecium* resistant to both vancomycin and streptogramins. Increased prevalence of SREF increases the likelihood that streptogramin resistance will be acquired by VRE, or that vancomycin resistance genes will be acquired by SREF. Once MREF emerges it may be reasonable to expect MREF to spread epidemically ($R_0 > 1$) especially in places where VRE has become endemic. Since we expect $R_0 > 1$ for MREF, the questions to be answered are when will MREF spread epidemically, when would such

events have happened if virginiamycin had never been used, and whether such events may be delayed by banning virginiamycin use.

MREF may remain rare in human populations for several years before becoming a problem in hospitals. Such a lag would not be unusual, and it may provide the opportunity for policy to have a positive public-health effect. In the meantime, the withdrawal of virginiamycin might substantially delay the emergence of MREF if SREF in poultry declines rapidly enough to delay the emergence of clinically significant strains of MREF. The use of linezolid may reduce the use of QD, opening a window of time for the ban to work and increasing the chances that a ban would be effective. However, high-level resistance to SREF may persist in poultry for years after the withdrawal.

Discussion

The emergence of SREF or MREF will probably involve an interaction between the use of QD and other antibiotics in medicine and the long history of virginiamycin use in agriculture. The effect involves complicated population-dynamic and population-genetic processes that are non-linear, and which may be difficult to measure directly. Ordinary notions involving reasonable burdens and standards for scientific proof usually applied to specific experiments or hypotheses may not be as useful as a synthesis of existing data. We have used mathematical models in an a-priori approach to the problem, to describe quantitative aspects of emergence in qualitative terms based on reasonable assumptions about the underlying biology, and to develop reasonable expectations about the potential for SREF emergence. These qualitative descriptions may be regarded as a basis for understanding more complicated models. By focusing on qualitative aspects, we have narrowed the debate about quantitative aspects of emergence to a few critical questions. Such an approach may be useful for assessing the specific issues associated with other pre-emergent pathogens.

Three critical issues should decide whether virginiamycin should be regulated. How frequently does SREF emerge in human beings because of virginiamycin use? How frequently does SREF emerge in human beings from other sources? What is R_0 for SREF in hospitals? Based on previous experience with other antibiotics, it is reasonable to expect that quantitative resistance will tend to increase because of QD use in hospitals, but high-level resistance to streptogramins presents a more important threat to the efficacy of QD. Evidence suggests that SREF is highly host and habitat specific. Thus, the likelihood that a poultry strain will colonise people seems remote. More importantly, it is plausible that pork or poultry strains may transfer genes conferring high-level resistance to streptogramins to *E faecium* in people and generate new and clinically significant strains of SREF. High-level resistance genes have been seen in SREF isolated from human beings, but they may remain rare. For emerging infectious diseases such rare events may be very important.

The subsequent spread of these newly introduced strains of SREF, described by the basic reproductive number R_0 , depends on many unknown aspects of the ecology of SREF. Fundamental questions about antibiotic use and the ecology of

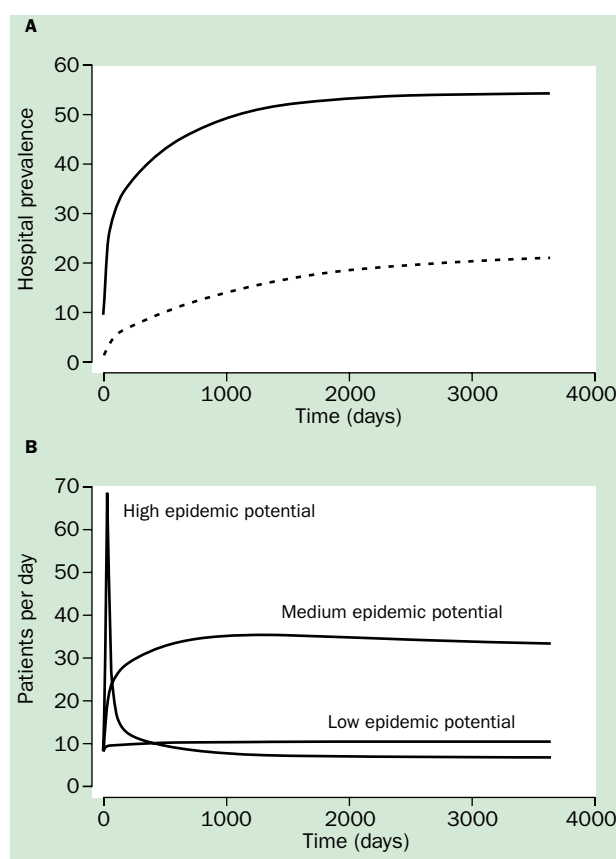


Figure 4. The potential effects of virginiamycin use for quasi-epidemics shown as projected hospital prevalence of SREF over time using a deterministic mathematical model. Solid lines and dashed lines represent projections with and without virginiamycin use, respectively, assuming virginiamycin use is the main reason new SREF strains arise in human beings. Quasi-epidemic transmission may allow a strain to persist for long periods of time. Thus, low-level transmission amplifies new exposure leading to excess prevalence of SREF that is sustained indefinitely. The top panel should be compared with figure 2 (note that the scale of the y-axis changes). With intermediate transmission ($R_0 \approx 1$) (A), the effect is greatest. The effect of virginiamycin use (B) is measured by subtracting the projections of SREF prevalence without virginiamycin use from the prevalence with it.

SREF in the human gut are involved, as are common hospital practices and hospital infection control. Currently, QD is not heavily prescribed. It is given intravenously and one component is metabolised before being absorbed into the gut; thus, QD may provide only weak selection for SREF in the gut. On the other hand, QD amplifies the density of enterococci and may lead to increased frequency of *E faecalis*. Typically, patients are not given QD unless other options are not available and an infection is caused by *E faecium*, but resistance to other antibiotics may increase the demands on QD in the future. Specifically, the availability of linezolid would seem to decrease demands on QD and make regulation of virginiamycin seem less urgent. However, linezolid might provide a window of opportunity for a ban on virginiamycin to have a positive benefit.

High-level resistance genes to both streptogramin components are frequent contaminants of food and may be

Search strategy and selection criteria

The authors have closely followed publications on mathematical models for antibiotic resistant bacteria. For the genetics of streptogramin resistance, we searched the PubMed database. The search was for “(streptogramin OR virginiamycin OR synercid OR quinupristin) AND (enterococci OR enterococcus OR faecium)”, yielding 208 unique references. We screened these references to find articles that might provide useful data using the title and abstract to obtain 63 primary publications. We obtained additional references from the bibliographies of these articles. For the epidemiology of VRE, we searched PubMed for “(VRE OR enterococcus OR enterococci) AND (epidemiology OR farm)”. This general search was to be used for several articles focusing on the population dynamics of VRE in hospitalised populations. We obtained a list of 1071 articles. We screened the titles to select primary research articles in the English language that were relevant to streptogramin resistance. We also used the bibliographies of articles to find additional articles about the epidemiology of VRE.

transmitted together. Such genes may be irreversible consequences of 30 years of virginiamycin use for growth promotion. The rate of QD use in hospitals may never compare with the long and sustained high rate of virginiamycin use in agriculture. Now that the high-level resistance genes exist and may be transmitted together, QD use may trigger the emergence of MREF. Without

virginiamycin use, such events may have taken much longer to occur in hospital patients. Based on the epidemiology of VRE, it is plausible that R_0 for SREF will have a potential for epidemic or quasi-epidemic spread in hospitals. Thus, the emergence of SREF is likely to be the result of an interaction between QD use in medicine and the long-term use of virginiamycin for animal growth promotion. It may be meaningless to assign a probability to these events without more data, but we conclude that long-term virginiamycin use for growth promotion is likely to reduce the future efficacy of QD.

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Conflicts of interest

Pfizer Corporation originally owned the rights to produce virginiamycin, but recently sold those rights. The authors have all received some form of funding from Pfizer Corporation within the last 3 years. DLS, JAJ, and JGM were funded by the grant entitled “Risk assessment of use of quinupristin/dalfopristin in animals/animal feed (preliminary studies/data evaluation)” in collaboration with Veterinary Laboratories Association, Weybridge, UK. JAJ was supported by a grant entitled, “Quinupristin/dalfopristin-resistant enterococci in poultry” from Pfizer Corporation. ADH received the Pfizer Scholars Grant for Faculty Development in Clinical Epidemiology, entitled “Study design importance in analyzing emerging pathogens”. ENP, JPF, and ADH received a grant, “In vitro susceptibility testing and clinical outcomes: an ecologic analysis”. All editorial, content, and publication decisions were retained by the authors.

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Useful websites

Further information is available on the web from the following sources:

A longer report with details of the mathematical model is available at:

<http://medschool.umaryland.edu/Epidemiology/dsmith/sref.html>

US Food and Drug Administration, Center for Veterinary Medicine, Antimicrobial Resistance

<http://www.fda.gov/cvm/antimicrobial/antimicrobial.html>

Alliance for the Prudent Use of Antibiotics <http://www.tufts.edu/med/apua/>

Keep Antibiotics Working <http://www.keepantibioticsworking.com/pages/home.cfm>

Animal Health Institute <http://www.ahi.org/>

Institute for Animal Health <http://www.iah.bbsrc.ac.uk/>