FDA Center for Veterinary Medicine

Risk Assessment of Streptogramin Resistance in *Enterococcus faecium* Attributable to the Use of Streptogramins in Animals

"Virginiamycin Risk Assessment"

DRAFT FOR COMMENT

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EXECUTIVE SUMMARY

With the approval of the drug Synercid in 1999 for the treatment of *Enterococcus faecium* infections in humans, increased attention was focused on the related drug, virginiamycin, which has been used in food-producing animals for over 20 years. Because some scientists believe on-farm use of virginiamycin can cause the development of resistance to Synercid® in humans, the Center for Veterinary Medicine began the virginiamycin risk assessment in 2000. The Center is now reporting the first draft results of the risk assessment.

The draft risk assessment specifically addresses the risk of humans failing Synercid therapy for *E. faecium* infections due to the acquisition of resistance as a result of the ingestion of resistant strains of *E. faecium* present on food commodities. Virginiamycin, which is approved for use in chickens, turkeys, swine, and cattle, and Synercid are both members of the streptogramin class of antimicrobial drugs. Streptogramin-resistant *E. faecium* are found in isolates obtained from poultry and swine sources in both the US and Europe. The prevalence of resistance in these isolates appears to be related to the usage pattern of virginiamycin on farms. Further, streptogramin-resistant *E. faecium* can be recovered from food animal products purchased from retail sources. Therefore, humans can be exposed to streptogramin-resistant *E. faecium* via the foodborne pathway.

It is difficult to assess the extent of transfer of streptogramin resistance from virginiamycin-exposed *E. faecium* to *E. faecium* found in human infections based on the available data. Literature reports demonstrate that there are differences in the characteristics of resistant *E. faecium* isolated from animal and human sources, with respect to minimum inhibitory concentration (MIC) distributions and the presence of known resistance genes. These two findings, along with the current incomplete knowledge of the genetic basis of streptogramin resistance, prevents the risk assessment from making firm conclusions as to whether, and, if so, how much, the use of streptogramins in food animals contributes to the occurrence of streptogramin-resistant *E. faecium* infections in humans via a foodborne pathway.

To address this foodborne-pathway attribution factor, the risk assessment provides two scenarios to estimate the risk of a random member of the "at risk" population having a streptogramin-resistant *E. faecium* infection that may result in impaired Synercid therapy attributable to food animal use of virginiamycin. The first scenario assumes a food pathway attribution factor of 10%; *i.e.*, that 10% of the risk of acquiring resistant streptogramin-resistant *E. faecium* in the hospital is due to a food pathway. Under this scenario, the risk assessment estimates that the average risk to a random hospitalized member of the US population ranges from 6 to 120 chances in 100 million in one year. For a random member of the general US population, the risk estimates range from 0.7 to 14 chances in 100 million in one year.

The risk assessment provides a second scenario that assumes a food pathway attribution factor of 100%; *i.e.*, that all existing resistance to streptogramins among the human population originated from food animal uses of virginiamycin. Using this scenario, the risk estimates are 10-fold greater than under the previous scenario, or 60 to 1,200 chances in 100 million per person per year among the hospitalized population and 7 to 140 chances in 100 million per person per year for the general US population.

Table of Contents

List of Tables

List of Figures

1 INTRODUCTION

Enterococcus bacteria include commensal bacteria—organisms normally present in the intestines of animals and man. Some strains of enterococci are commonly found in cheeses, sour dough bread cultures and on other foods, generally without adverse impacts on public health. It is almost always in cases in which a person's immune system is compromised, or an individual is severely ill, that there is an opportunity for enterococci to cause infection and possibly life-threatening illness. Enterococcal infections comprise more than 20 percent of approximately 2 million hospital-acquired infections each year in the United States[1]. In such cases, antimicrobial drugs are sometimes necessary to treat the infection.

Vancomycin is a glycopeptide antibiotic that was approved for human use in 1987; since then, it has been the antimicrobial drug of choice in fighting hospital-acquired *Enterococcus faecium* infections. According to the Centers for Disease Control and Prevention (CDC), however, resistance to vancomycin rose rapidly from less than 1% in 1989 to its contemporary prevalence of about 25% of hospital-acquired enterococcal infections in intensive care units (ICUs). There may be as many as 70,000 vancomycin resistant *E. faecium* (VREF) infections in the US each year[2]. The prevalence of VREF infections varies widely from hospital to hospital according to a hospital's vancomycin use, whether the hospital is a teaching or non-teaching hospital, and the hospital size (number of beds). While a portion of these infections may be intrinsically resistant to vancomycin, it is likely that most would have acquired resistance to vancomycin from vancomycin use in hospitals or other settings.

The dramatic rise in VREF infections fueled a search for a new antibiotic that could control or cure these serious infections. In September 1999, the Center for Drug Evaluation and Research of the FDA approved Synercid® to treat VREF bloodstream infections in addition to *Staphylococcus aureus* and *Streptococcus pyogenes* skin and soft

[1] Federal Register 65 (76): 20992-20995, April 19, 2000.
[2] Federal Register 65 (76): 20992-20995, April 19, 2000.

tissue infections. At the time of its approval, Synercid was considered to be a therapy of last resort for VREF infections.

Synercid is a mixture of two compounds, quinupristin and dalfopristin, that are members of the streptogramin class of antimicrobials. Virginiamycin, also a mixture of two streptogramin compounds, is an animal drug approved for use since 1975 in feed for food-producing animals. Virginiamycin is currently approved for use in turkeys, swine, cattle, but mainly in chickens, for growth promotion and prevention or control of certain diseases.

The approval of Synercid focused increased attention on the use of virginiamycin; specifically, whether on-farm use of the chemically-related drug caused the development of streptogramin resistance in bacteria that could result in impaired Synercid therapy in humans. This concern compelled the Center for Veterinary Medicine (CVM) of the FDA to develop this risk assessment to evaluate the potential impact of virginiamycin use in food animals on streptogramin resistance in *E. faecium* infections in humans. The risk assessment particularly addresses streptogramin resistance that originates via foodborne pathways.

The Concern for Transfer of Streptogramin Resistance

E. faecium that develop resistance due to virginiamycin exposure have been shown in laboratory settings to have reduced susceptibility to Synercid. It has been proposed by the FDA and others that streptogramin-resistant strains of *E. faecium* from food animal sources contaminate meat or poultry products and thereby expose humans through the food pathway. Ingestion of resistant strains of *E. faecium* present on food commodities would therefore place humans at risk of acquiring streptogramin resistance and at risk of failing antimicrobial drug therapy with Synercid. Clinical antimicrobial resistance as a result of opportunistic infection is possible from two different pathways: first, animal-derived, resistant *E. faecium* might colonize the human coincidently with streptogramin resistance; and, second, animal-derived *E. faecium* might transfer

Ingestion of resistant strains of E. faecium *present on food commodities might place humans at risk of acquiring streptogramin resistance and at risk of failing antimicrobial drug therapy.*

resistance genes to the human *E. faecium* prior to or coincidently with antimicrobial treatment. Although it is generally accepted that the intestinal microflora of healthy humans inhibits colonization by bacteria from exogenous (*e.g.*, animal or "zoonotic") sources, it is also accepted that disturbances in the microflora occur from illness or when the immune system is compromised by drug therapies. This situation is particularly relevant in illnesses requiring oral antibiotic therapy that might select for rare, drug-resistant organisms normally not abundant among the microflora of healthy individuals. This scenario might result in the intestinal colonization and proliferation of antibiotic-resistant bacteria from the external environment and, in particular, the food chain.

The fact that an exposure pathway connecting sources of resistant food animal bacteria to humans is possible raises concern for streptogramin resistance in the human food chain. *E. faecium* are widely distributed among animals and humans, suggesting that, if a proportion of the bacteria population become resistant to an antimicrobial drug, then, in the presence of selection pressure, the resistant population might eventually distribute among both animals and humans by mechanisms of colonization or genetic transfer of resistance. Additionally, the existence of such a pathway is supported by general evidence that:

- *E. faecium* is commonly recovered from food-producing animals;
- *E. faecium is* frequently recovered from retail meat and poultry samples;
- bacterial species commonly associated with food-producing animals can also be frequently recovered from human stool samples; and
- transfer of genetic determinants that confer certain types of antibiotic resistance has been demonstrated to occur readily among enterococci in controlled studies.

FDA sought information through the risk assessment process to affirm or refute potential pathways connecting the food animal uses of virginiamycin and resistance to streptogramins in human medicine.

Risk Analysis

Risk analysis is the principal process by which science-based public health decisions are made. Generally, risk analysis includes the identification of health hazards and their adverse consequences; assessment of the likelihood of the adverse consequences in exposed populations; management of health risks using programs designed to mitigate, control or eliminate risks; and communication of risks and risk management goals among all interested parties and stakeholders. These risk analytical processes are typically described under four major headings: hazard identification, risk assessment, risk management and risk communication (Figure 1).

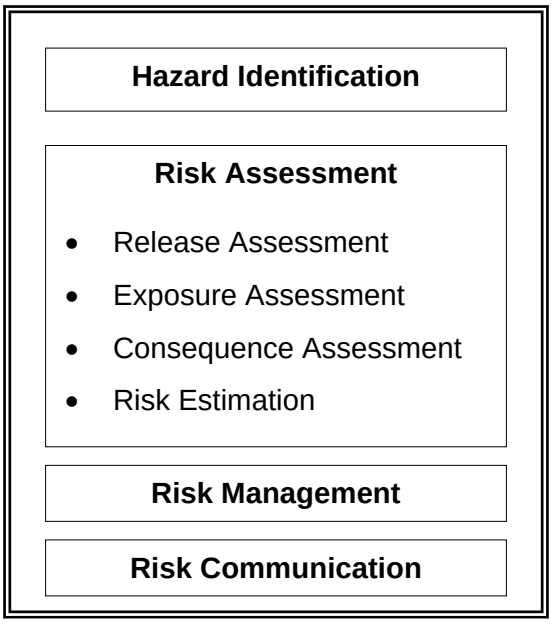

Figure 1. Risk Analysis. The four major activities of risk analysis, Hazard Identification, Risk Assessment, Risk Management and Risk Communication are shown in the Figure. This report focuses only on the Risk Assessment activity. Regulatory decision making is done under a separate, Risk Management process.

Risk assessment is the risk analytical process in which the chance of a defined adverse human health effect is estimated given exposures to the hazardous agent. Risk assessments can take many forms, from qualitative discussions of potential hazards, exposures to the hazards and the attendant risk of adverse health effects, to highly

Risk assessment is a process in which the chance of a specific adverse human health effect is estimated, given that exposure to the hazardous agent occurs.

sophisticated and quantitatively rigorous estimates of probabilities of occurrence for each step in the process, from hazard through exposure and finally to risk. Whether a risk assessment is qualitative or quantitative in nature, it should include a thorough discussion of the sources and magnitude of uncertainty in the risk assessment, and identification of significant gaps in information available for the risk assessment.

Antimicrobial resistance risk assessment (ARRA) is an emerging area of human health risk assessment. ARRA is closely related to microbiological risk assessment (MRA), which is the analytic process used to assess the risks of illness from foodborne microbial pathogens. Recently, two international organizations recommended risk analysis models for assessing the risks from either microbiological agents or veterinary antimicrobial drugs in food animal uses: Codex Alimentarius Commission (Codex) and the Office International des Epizooties (OIE). The two methods are based on the classical National Academy of Sciences (NAS) paradigm that calls for hazard identification, dose-response assessment, exposure assessment and risk characterization. For the purposes of the present risk assessment, CVM used a revision of the "four-step" model proposed by the OIE. Similar to the OIE process, the CVM approach recognizes that the hazard identification is a separate process in risk analysis; and hazard identification is the process described in the Federal Register of April 2000 leading to the stated need for the virginiamycin risk assessment.

ARRA differs somewhat from the related process, MRA, used elsewhere in the FDA. The first difference between MRA and ARRA is that MRA generally concerns pathogenic microorganisms, including bacteria, whereas ARRA focuses on the determinants of antimicrobial resistance carried by bacteria strains of interest. "Determinants" refers to a gene or group of genes that ultimately confers biochemical resistance to the antimicrobial drug of interest in the risk assessment. While MRAs generally focus on pathogenic bacteria, the bacteria of interest in an ARRA might be commensal organisms which are normally non-pathogenic and thus are benign carriers of the resistance determinant(s). A second feature distinguishing ARRA from MRA is that, although antimicrobial resistance is carried by bacteria associated with humans or animals, the adverse health effect of concern (impaired treatment of illness) may not be observed until treatment is attempted with the antimicrobial drug of interest.

The CVM SREF Risk Assessment

In general, the purpose of a risk assessment is to provide a systematic organization of scientific evidence about:

- the identified health hazard and the hazardous agents that can elicit the health hazard;
- the magnitude, extent and duration of human or animal exposures to the hazardous agents;
- the estimation of the likelihood of adverse consequence in human or animal populations as a result of exposures to the hazardous agents;
- the remaining gaps in both data and scientific knowledge about the risk in question; and
- the overall uncertainty in the risk assessment.

The risk assessment process does not provide a decision to manage a given risk; rather, the information from the risk assessment provides only one component of input into decision-making by risk management. Other inputs to decision-making include information about the availability and effectiveness of various risk management options, benefit-cost analyses and risk trade-off analyses. Discussions about risk management options and decision-making are not a part of this report.

The risk assessment is presented in draft form in anticipation of additional data to come from CVM-supported research and from comments on this draft (See "Future of the SREF Risk Assessment", below). The Risk Analysis Team of CVM believes, however, that sufficient information is available with which to inform risk management of the human health risks from food animal applications of virginiamycin.

The CVM SREF risk assessment is organized under main headings of hazard identification, release assessment, exposure assessment, consequence assessment and risk estimation. The hazard identification provides an update of material presented in the original Federal Register publication (April 2000) in addition to details about the hazard which either were not available in 2000 or otherwise were beyond the scope of a Federal Register notice. The release assessment and exposure assessment discuss the evidence

for animal shedding of streptogramin-resistant *E. faecium* and for foodborne pathways of human exposure to resistant bacteria. The consequence assessment and risk estimation, taken together, estimate the potential number of people who might incur streptogramin-resistant infections in which Synercid resistance is potentially due to animal applications of virginiamycin.

Individual versus Population Risk Estimates

The risk assessment process estimates risks among members of defined populations, not individuals. The CVM risk assessment cannot be used to predict the risk of an SREF infection in an individual; rather, the risk assessment can estimate the risk of the adverse event in a population of individuals who share similar exposures to the antimicrobial resistance determinants. Thus, no one reading the results of the SREF risk assessment should assume that the results are predictive of his/her personal risk of a streptogramin resistant VREF infection. Although the risk estimates cannot be used to predict individual risk, an individual who has the similar attributes of the at risk population including the particular pathway of exposure is, *on average*, at a greater risk of the adverse health outcomes than a person who does not have those attributes.

The numerical risk estimated from a risk assessment can be applied to populations of individuals and are not predictive of a specific individual's risk of disease.

It is recognized that estimates of risk from a risk assessment process cannot be precisely known. Indeed, risk is not precisely knowable until after the events have occurred. Hence, risk is correctly presented as a range of credible values rather than a single number. A risk assessment report often devotes much effort to describing the remaining uncertainties in the risk estimation and the degree of uncertainty in the hypothesized exposure pathway. The present report includes a discussion of the remaining data gaps and the relative uncertainty these gaps create in the risk estimation.

The Future of the SREF Risk Assessment

Risk assessment is one component of risk analysis and often feeds into broader agency processes designed to identify public health problems for purposes of risk

management. In general, public health agencies periodically review health risk assessments for the health hazards that they regulate, in order to assess the effectiveness of risk management programs in mitigating risks.

The present risk assessment is the first iteration of the risk estimation process for the potential of streptogramin resistance in food animals to increase the risk of adverse health consequences in human populations. The risk assessment includes data and literature reviews collected up to and during the writing of the draft document. As a part of its public health mission, CVM monitors trends in food animal antimicrobial use and human bacteria antimicrobial resistance (e.g., the National Antimicrobial Resistance Monitoring System, or NARMS) that might indicate a change in the status of human health risks. Clearly, if new data or information become available that can narrow an information gap, the risk estimation can be improved by incorporating the new data into the assessment. Once that new information is incorporated, the revised risk estimation may increase, decrease or stay essentially equivalent within a range of statistical uncertainty. In general, new information brought to an existing risk assessment reduces the uncertainty about the risk estimates. At present, there is no defined schedule for updating the streptogramin-Enterococcus risk assessment.

2 HAZARD IDENTIFICATION

2.1 Purpose

The purpose of the hazard identification is to identify hazardous agents and the conditions under which they might produce adverse human health consequences. In a regulatory setting, hazard identification is often performed as a separate process from risk assessments, particularly because agencies need to identify and prioritize health hazards as part of planning their risk management portfolios. The preliminary hazard identification might be qualitative and informal; nevertheless, hazard identification process provides risk managers with compelling evidence either for or against the initiation of a formal risk assessment for the identified hazard.

The preliminary hazard identification for the streptogramin-resistant *Enterococcus faecium* (SREF) risk assessment was published as a *Federal Register* announcement on April 19, 2000 (65 FR 20992). The Federal Register publication identified the potential hazards and potential human health consequences from the acquisition of streptogramin-resistant *Enterococcus faecium* attributable to food animal applications of virginiamycin. The present chapter on hazard identification updates the original hazard assessment by elaborating on the nature of the hazard and the potential adverse human health effects.

2.2 Introduction

Synercid®, a mixture of the two streptogramin antibiotics quinupristin and dalfopristin (QD), was approved in September 1999 by the US FDA for treatment of bacteremias in humans. In particular the drug was approved for therapy against vancomycin-resistant *Enterococcus faecium* (VREF) and for the treatment of skin and soft tissue infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. At the time of approval, Synercid was considered to be a last resort of therapy for potentially life-threatening bloodstream infections (BSIs) caused by VREF. Strains of *Enterococcus* resistant to vancomycin had become common in the human clinical environment and were becoming a serious cause of morbidity and mortality in intensive care units (ICUs).

In fact, enterococci have been recognized as the second to third most common cause of nosocomial infections in the United States (Murray, 1997).

Virginiamycin is a streptogramin antibiotic mixture chemically similar to Synercid, suggesting that bacterial resistance to virginiamycin might confer resistance to Synercid. Thus, the use of virginiamycin in animal agriculture for over a quarter of a century might create a potential health risk for humans who need Synercid for the treatment of serious enterococcal infections. The concern for a potential link between animal and human resistance to streptogramins is further supported by the ecological distribution of the enterococci and their physiological attributes: enterococci are extremely hardy and widely distributed in the environment, especially in the gastrointestinal tracts of animals and man. They are common contaminants on unprepared foods, particularly animal-derived food commodities. In addition, enterococci have the ability to acquire and transfer resistance determinants from other bacteria making infections caused by them potentially more difficult to treat (see below). Because of these characteristics it was considered prudent to evaluate the risk associated with continued use of virginiamycin in animal agriculture and the potential for the transfer of resistance determinants to humans through food pathways that, in turn, might compromise the therapeutic efficacy of Synercid.

2.3 Identification of the Potential Human Health Impact

2.3.1 Impact Scenario

Enterococci are gram-positive bacteria normally resident in the human large bowel. Although enterococci are thought to account for less than one percent of intestinal microflora, *Enterococcus* species are nevertheless considered to be medically significant bacteria causing various infections (Tannock and Cook, 2002). Based on microbiological surveys, it is reasonable to assume that a proportion of bowel enterococci at any given time is *E. faecium,* and furthermore, that any member of the human population is potentially at risk of acquiring streptogramin-resistance. Note that the acquisition of resistance is not likely to occur through single or multiple mutations in a bacterium, but through horizontal gene transfer (de la Cruz and Davies, 2000). Thus, exposure to exogenous sources of bacteria already carrying resistance genes for

streptogramin resistance must occur in order to colonize with resistant bacteria or to transfer resistance to existing bacteria in the human bowel. For the purposes of risk assessment, the resistant bacterium/bacteria might be of either animal or human origin; however, it is thought that colonization by zoonotic bacteria is transient, perhaps lasting only a few weeks at most.

The principal pathway of interest is through food. The exposure pathway is described qualitatively as one in which food animals fed virginiamycin develop SREF. During the slaughter process, a proportion of the animal carcasses may become contaminated with SREF. Although cooking and other food processing techniques should destroy most Enterococcus, abundant food safety experience in the US and elsewhere suggests that there is a finite probability of finding contaminating bacteria surviving food preparation that might, in turn, cause foodborne illness. In the present scenario, neither drug-resistant nor drug-susceptible *E. faecium* are expected to cause foodborne illness in the healthy human population. Rather, the health consequence of interest is an opportunistic infection, generally speaking, in a subpopulation of individuals who are already under medical care. Therefore, in the impact scenario, the food animal pathway primarily serves to create a reservoir of resistance among the ill and not as a direct exposure pathway for defined adverse health consequences.

One among many possible conceptual models of an impact scenario is shown in Figure **2.** The conceptual model shows that acquisition of resistant bacteria might occur from exposures to either animal or human enterococci. Current evidence suggests that the association of animal-derived *E. faecium* with humans is transient. A second pathway is shown to account for the conjugational transfer of resistance from animal to human *E. faecium*. The starting assumption in this impact scenario is that colonization by SREF can occur once the resistance genes are transferred to a human-adapted *E. faecium* strain. It is further assumed that, while a resistant subpopulation of the commensal *E. faecium* might survive long term in the human bowel, the adverse consequence of a streptogramin (e.g., Synercid) resistant infection occurs upon treatment failure of an infection with streptogramin mixtures. In classical theory of antimicrobial drug resistance, the growth of the susceptible fraction of the colonizing *E. faecium* would be attenuated by the presence

of the drug while the resistant fraction of the bacterial population would continue to expand.

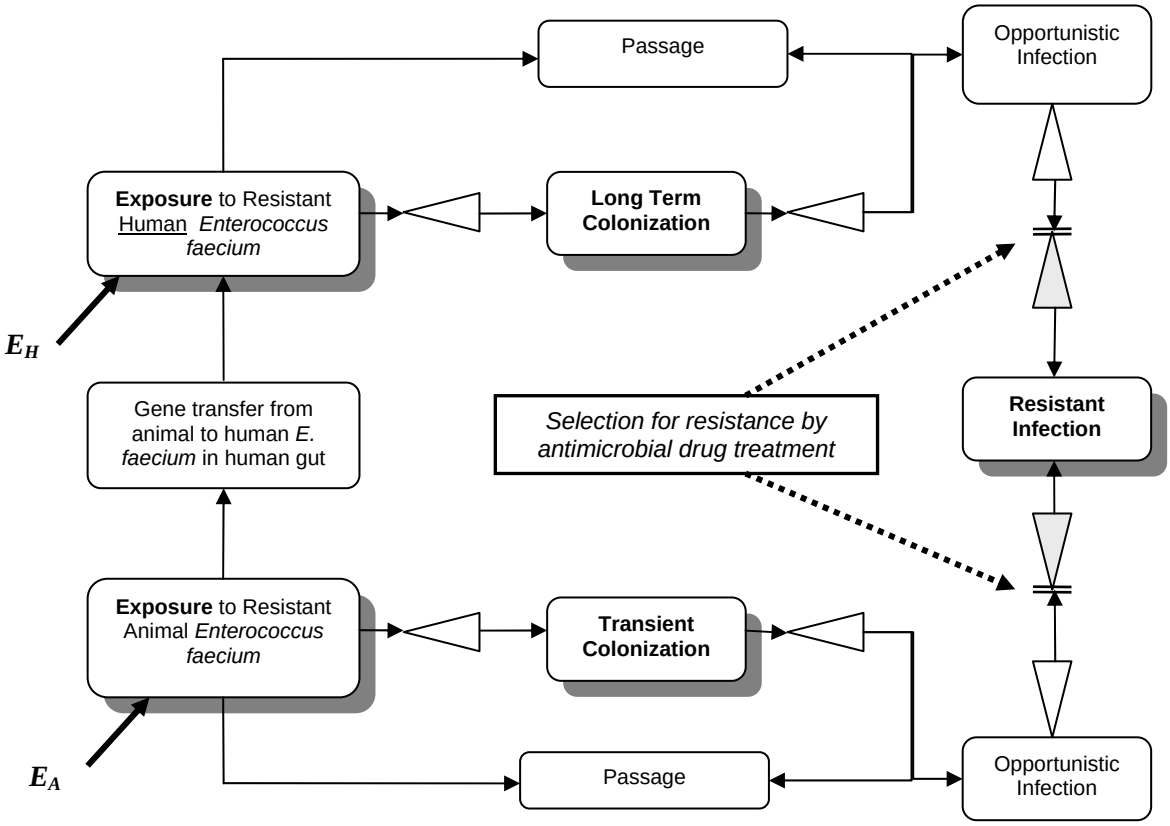

Figure 2. **Proposed pathway for drug resistant infection caused by *E. faecium*.** Colonization might be either transient, ending in loss or "passage" of the bacteria, or long-term. Exposures are shown as originating from food animal sources (E_A) or human sources (E_H). Because animal-adapted enterococci are believed to colonize humans only transiently, long-term colonization by drug resistant bacteria would require transfer of resistance genes to the human-adapted strains of enterococci. The triangles indicate growth of bacteria and the shaded triangles indicate growth of bacteria dominated by antimicrobial resistant strains.

The impact scenario shown in Figure **2** is for one antimicrobial drug. The present risk assessment concerns impacts of virginiamycin uses on the ability to use Synercid to treat VREF infections. Thus, the SREF must have concurrent drug resistance to vancomycin, or the infection must be from a clonally mixed population of VREF and

SREF bacteria.[3] This scenario does not obviate the potential for dual resistance to vancomycin and QD in the same bacterium.

2.3.2 Populations at Risk of SREF Infection

In contrast to the ubiquitous colonization of humans by *E. faecium*, the prevalence of *E. faecium* in bloodstream, urinary tract or other infections is generally limited to a subpopulation of hospitalized individuals. In fact, a major reason for the risk assessment is that *Enterococcus* species account for as many as 800,000 infections and $500M in medical costs each year (Tannock and Cook, 2002). The species of particular interest in this risk assessment, *E. faecium*, is frequently associated with nosocomial bloodstream infections (Garbutt et al., 2000). Nosocomial bloodstream infections, in turn, are associated with medical procedures in ICUs, most likely due to the use of invasive medical devices in ICUs (Witte, 2001; Jacoby, 1996; Linden, 1998; Malathum and Murray, 1999). The proportion of the population at risk of resistant, opportunistic *E. faecium* infections is represented by the proportion of the population who are at risk of nosocomial infections. The purpose of the following discussion is to identify in general terms the human populations at risk of streptogramin-resistant *E. faecium* infections.

Nosocomial infections are a vexing problem encountered during the management of seriously ill patients. Patients in intensive care units, particularly those who have central venous catheters, urinary catheters, respiratory ventilators or other invasive medical devices, are at significant risk of nosocomial infection from a variety of gram-negative and gram-positive bacteria (Pittet et al., 1999; Witte, 2001; Linden, 1998; Malathum and Murray, 1999). For a number of years, the proportion of infections due to gram-positive bacteria has been increasing (Hellinger, 2000).

The trend in US healthcare over the past two decades is for fewer and smaller acute care facilities and greater numbers of long-term and home-based care delivery systems (Jarvis, 2001). The changing demographics of health care means that the inpatient population is in worse condition on average and of an older average age than

[3] Although Synercid approval is for VREF infections, in the interest of treating patients quickly and efficiently, ICUs having a high endemic frequency of VREF infections might use a *presumptive* VREF status to begin using Synercid. A portion of treated cases in such ICUs is likely to be vancomycin-susceptible.

was the case in the recent past (Jarvis, 2001; Popovic and Hall, 2001). Additionally, there are an estimated 1.5 million residents of nursing homes who are older than age 65 (Gabrel, 2000). Many nursing home residents have recurring infections (Nicolle et al., 1996; Strausbaugh et al., 2001) and can be colonized by *Enterococcus spp*. (e.g., Mylotte et al., 2001). When residents of long-term care facilities are admitted to acute care hospitals, recent estimates are that 20% are already colonized by antimicrobial resistant bacteria (Mylotte et al., 2001).

Since the 1980s, the relative proportion of bloodstream infections caused by gram-positive organisms increased dramatically (Linden, 1998; Bannerjee et al., 1991; Pittet & Wenzel, 1995; Jones et al., 1994; Jones, 1996a). Populations at risk of nosocomial infections have been characterized in a number of reports, including those by Moellering, 1999; Moellering et al., 1999; Gaynes et al., 2001; Richards et al., 2000; Richards et al., 1999a; Richards et al., 1999b; Richards et al., 1998; and Jones et al., 1999. The numbers at risk can be deduced using data from the National Nosocomial Infection Surveillance (NNIS) system of the Centers for Disease Control (Annual Report) (NNIS 2002). For example, the numbers of nosocomial infections in a given year can be estimated from the rates of nosocomial infections. The surveillance network reports the infection rates for bloodstream infections (BSI), urinary tract infections (UTI), and ventilator associated pneumonias. *E. faecium* is implicated in primarily BSI and UTI.

2.3.3 The Nature of the Hazardous Agent

The hazardous agent in this risk assessment is the biological entity that confers antimicrobial resistance to Synercid. The hazardous agent includes bacterial genes that code for proteins affecting streptogramins' ability to inhibit cell growth or kill cells. In general, antimicrobial resistance can result from a variety of different mechanisms related to gene mutations and/or combinations of mutated genes. Thus, a more general and conceptually relevant term, "resistance determinant," is sometimes used to describe the hazardous agent. Unless discussion in a given section of the document is focused on a particular component of streptogramin resistance, this risk assessment will use "resistance determinant" to denote the gene, genes or biochemical pathways that confer antimicrobial resistance in the bacterial species of interest.

Although the fundamental hazardous agent is a resistance gene or resistance genes, the hazard cannot factor into human exposure and risk without a carrying bacterium. The reason that the drug-resistant bacterium is not identified as the hazard *per se* is that, in general, resistance determinants can be transmitted among a variety of bacterial subspecies and species. In the present risk assessment, the bacterium of principal concern for the carriage of streptogramin resistance is the human commensal bacterium, *Enterococcus faecium* (*E. faecium*).

2.3.4 Identification of the Hazard from Food or Human Samples

It is well-known that enterococci are commonly found on food commodities. A growing body of literature is increasing our knowledge of the proportion of Enterococcus on food commodities that is *E. faecium*, and the proportion of contaminating *E. faecium* that are also streptogramin resistant. Although the lack of direct observations at some steps in the exposure pathway complicates the validation of causal relationships, it is known that zoonotic Enterococcus bacteria can inhabit humans, at least temporarily, and may transfer resistance determinants to human communal Enterococcus bacteria.

The proper identification of bacteria and antimicrobial resistances carried by the bacteria is not a trivial task for microbiologists. There are numerous approaches used to enrich a sample in the bacteria of interest, select for specific antimicrobial drug resistance, and to define the breakpoint between "susceptible" and "resistant." Controversy about the prevalence of specific bacteria often arises due to differences among laboratory methods. Due to these considerations, the interpretation and application of microbiology data on resistance is associated with a level of uncertainty that needs to be considered in the risk assessment process.

2.3.5 Exposure Pathways

The principal exposure pathway of interest in this risk assessment is consumption of food commodities that are contaminated with streptogramin-resistant *E. faecium.* In particular, CVM's interest is resistant *E. faecium* in which the resistance gene originated in food animal *E. faecium*.[4] Although it is recognized that both animal and human strains

[4] Bacterial contamination pathways are known to be bi-directional: humans can contaminate food or other animals with bacteria.

of *E. faecium* can be transported in non-food pathways, the regulatory focus of CVM is on food pathways directly related to uses of animal drugs. Other regulatory agencies, including the US Environmental Protection Agency and the US Department of Agriculture, have within their regulatory scope other pertinent pathways for animal drug and bacterial hazards. The exposure assessment phase of the risk assessment will focus on characterization of foodborne pathways.

Exposure to streptogramin-resistant *E. faecium* can occur through food or, in the institutional care setting, by secondary transmission (e.g., "indirectly") from hospital staff. The secondary transmission of resistant bacteria is well-known in nosocomial epidemiology (Arthur and Courvalin, 1993; Nichols, 1998; Huycke et al., 1998; Harbarth et al., 2002); however, self-contamination with commensal bacteria occurs in which fecal bacteria are found on the hands and then are transferred to surgical wounds or medical devices, such as venous catheters.

The probability of human exposure to streptogramin-resistant *E. faecium* originating from a foodborne pathway can be estimated from the prevalence of resistant *E. faecium* in the community at the time of the intensive care incident. Unfortunately, data on community resistance are scarce and subject to methodological uncertainties with respect to microbiology practices and ascertainment of case histories (i.e., prior clinical exposures to streptogramins). Additionally, community resistance varies considerably from location to location, and even the relative colonization of *E. faecalis* and *E. faecium* has been shown to vary depending on geographic locale (Rice et al., 1995). In the absence of specific information about a particular locale, exposure can be estimated only using prevalence and incidence from surveillance databases.

Sources of information with which to estimate the relative exposures of nosocomial-relevant bacteria are the scientific literature and the NNIS. Although based on a nonrandom sample of hospitals, the NNIS database is designed to cover a spectrum of short-stay hospital types and geographical locations. Periodic reports enable trends in nosocomial infection rates to be monitored, particularly by device utilization and the number of days that patients are on invasive devices. The NNIS system, while characterizing sentinel increases in infections, unfortunately does not include surveillance

of long term care facilities (LTCF). These health care facilities might include a significant proportion of the population at risk of serious infections.

Other pertinent information for estimating exposures includes the rates of hospitalization and rates of intensive care unit procedures. Similar to the NNIS, these data are also acquired and maintained by the CDC in its National Center for Health Statistics (NCHS). Relevant data from the NCHS are the rates of hospitalizations for members of the population. The base measure in the hospital survey is the "discharges," defined as the numbers of releases from hospital care, including a proportion of the patients who are deceased at the time of release. It is important to note that "discharges" is not equal to the number of individuals that are hospitalized in a given year. Data for the multiple hospitalizations of individuals are needed to adjust to the number of *individuals* discharged from the hospital each year.

2.4 Streptogramins – Properties

Antimicrobial drugs are the agents causing selection pressure for resistant *Enterococcus*. The assumption of causal pathway between the use of veterinary antimicrobial drugs and the potential acquisition of resistance to a related human use antimicrobial drug requires chemical similarity between the veterinary and human drugs. The following sections discuss the chemistry, regulatory approvals, and antimicrobial activity of the veterinary and human use drugs that are the focus of concern in the SREF risk assessment, Virginiamycin and Synercid.

Virginiamycin and Synercid are members of the streptogramin class of antibiotics that are naturally occurring compounds produced predominantly by members of the genus *Streptomyces*. The streptogramins are of two types, A and B, based on their primary structure. Type A compounds are polyunsaturated cyclic peptidolide compounds (also known as polyunsaturated cyclic macrolactones), and include virginiamycin M and pristinamycin IIA. Type B compounds are cyclic hexadepsipeptides such as virginiamycin S and pristinamyicn IA.

Naturally produced streptogramin mixtures, such as pristinamycin and virginiamycin, have been used orally and topically to treat human bacterial infections, primarily staphylococcal infections. However, the insolubility of these preparations

limited their intravenous use, leading to the development of Synercid, a water soluble, semisynthetic injectable product approved for use in the US in 1997.

2.4.1 Streptogramin Chemistry

Virginiamycin – CAS Registry number [11006-76-1]. Virginiamycin is a streptogramin antibiotic mixture produced by a *Streptomyces* related to *S. virginiae*. An antibiotic complex composed of virginiamycin M_1 **(**fraction M_1**)** and virginiamycin S_1 (fraction S_1) is found in the commercial product containing approximately 75% fraction M_1 and approximately 5% fraction S_1. Each fraction is composed of up to three similar and related subfractions. The commercial product is distributed under the trade name Stafac.

Virginiamycin S_1 - CAS Registry number [23152-29-6]. The compound has the molecular formula $C_{43}H_{49}N_7O_{10}$, molecular weight of 823.90 and the following structural formula Figure 3. It also has the additional name staphylomycin S according to the Merck Index.

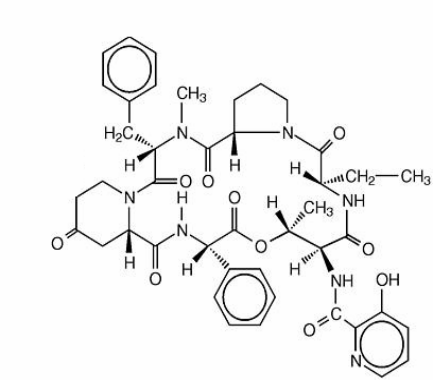

Figure 3. The chemical structure of Virginiamycin S_1

Virginiamycin M_1 – CAS Registry number [21411-53-0]. The compound has the molecular formula $C_{28}H_{35}N_3O_7$, molecular weight of 525.60 and structural formula given in Figure 4. Virginiamycin M_1 is the most abundant factor in the commercial product and is also known by the following names: mikamycin A, ostreogrycin A, pristinomycin IIA, staphylomycin M_1, vernamycin A, and streptogramin A according to the Merck Index.

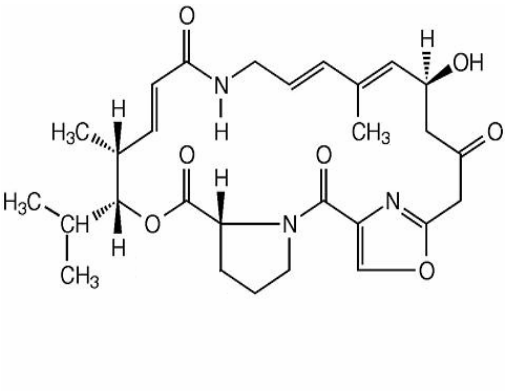

Figure 4. The chemical structure of Virginiamycin M_1

Synercid (quinupristin and dalfopristin powder for injection) I.V., a streptogramin antibacterial agent for intravenous administration, is a sterile lyophilized formulation of two semisynthetic pristinamycin derivatives, quinupristin (derived from pristinamycin I) and dalfopristin (derived from pristinamycin IIA) in the ratio of 30:70 (w/w) according to the Physicians Desk Reference.

Quinupristin has a molecular formula of $C_{53}H_{67}N_9O_{10}S$, a molecular weight of 1022.24 and the structural formula given in :

Dalfopristin has a molecular formula of $C_{34}H_{50}N_4O_9S$, a molecular weight of 690.85 and the following structural formula shown in Figure 6.

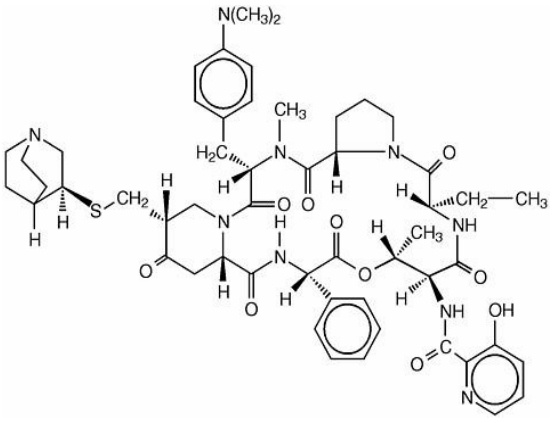

Figure 5 The chemical structure of quinupristin

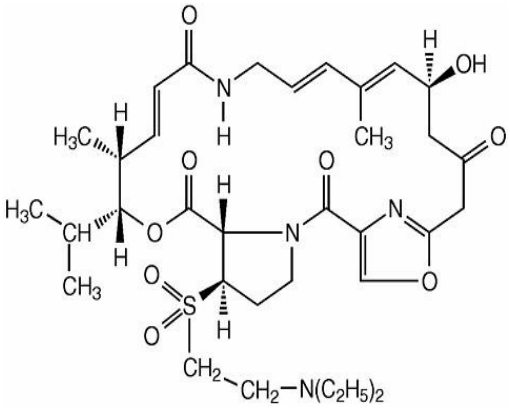

Figure 6. The chemical structure of dalfopristin

Examination of the molecular structures of Synercid and Virginiamycin shows a high degree of similarity between the two highest percentage components in these two drugs, namely dalfopristin and virginiamycin M_1. They differ only by the absence in virginiamycin M_1 of [2-(diethylamino)ethyl]sulfonyl observed at position 26 in the dalfopristin molecule leaving a double bond in its place. Not unexpectedly, there is also a great deal of similarity between quinupristin and virginiamycin S. Again in this case, the virginiamycin S component has the greater simplicity. In the quinupristin molecule, the benzyl moiety has a p-dimethylamino group and the 3-quinuclidinylthiomethyl moiety is attached to the piperidone structure. The large similarity between these major structures undoubtedly relates to the similar bactericidal activity and high degree of cross-resistance between the drugs.

2.4.2 Approved Regulatory Uses

Virginiamycin

The following information on the approved regulatory uses of virginiamycin was adapted from the Code of Federal Regulations, Title 21 – Food and Drugs; Part 558 – New Animal Drugs for Use in Animal Feeds.

Section 558.635 Virginiamycin

(a) *Approvals*. Type A medicated articles.

(1) 1.1 percent activity (5 grams per pound), 2.2 percent activity (10 grams per pound), 4.4 percent activity (20 grams per pound), 11 percent activity (50 grams per pound), and 50 percent activity (227 grams per pound) used as in paragraph (d) of this section; and 30 percent activity (136.2 grams per pound) for the manufacture of Type C medicated feed for cattle used as in paragraph (d)(3); to 066104 in Sec. 510.600(c) of this chapter.

(2) 2.2 percent activity (10 grams per pound) to 046573, 016968, and 017790 in Sec. 510.600(c) of this chapter for use as in paragraphs (d)(1)(iv) and (d)(1)(v) of this section.

(b) *Related tolerances*. See Sec. 556.750 of this chapter.

(c) Special considerations.

(1) Not for use in breeding swine over 120 pounds.

(2) Dilute Type A article with at least 10 pounds of a feed ingredient prior to final mixing in 1 ton of Type C feed.

(d) Conditions of use—

(1) *Swine*. It is used as follows:

(i) 100 grams per ton for 2 weeks, for treatment of swine dysentery in nonbreeding swine over 120 pounds.

(ii) 100 grams per ton for 2 weeks, 50 grams per ton thereafter, for treatment and control of swine dysentery in swine up to 120 pounds.

(iii) 25 grams per ton, as an aid in control of dysentery in swine up to 120 pounds. For use in animals or on premises with a history of swine dysentery but where symptoms have not yet occurred.

(iv) 10 grams per ton from weaning up to 120 pounds for increased rate of weight gain and improved feed efficiency, followed by 5 grams per ton to market weight for increased rate of weight gain and improved feed efficiency. For continuous use from weaning to market weight.

(v) 10 grams per ton from weaning up to 120 pounds for increased rate of weight gain and improved feed efficiency, followed by 5 to 10 grams per ton to market weight for increased rate of weight gain. For continuous use from weaning to market weight.

(2) *Poultry*. It is used as follows:

(i) 5 to 15 grams per ton for increased rate of weight gain, for use in broiler chickens, not for use in layers.

(ii) 5 grams per ton for increased rate of weight gain and improved feed efficiency in broiler chickens, not for use in layers.

(iii) 20 grams per ton for prevention of necrotic enteritis caused by Clostridium perfringens susceptible to virginiamycin in broiler chickens; not for use in layers.

(iv) 10 to 20 grams per ton for increased rate of weight gain and improved feed efficiency in growing turkeys.

(3) *Cattle*. It is used as follows:

(i) 16.0 to 22.5 grams per ton to provide 100 to 340 milligrams per head per day for increased rate of weight gain.

(ii) 13.5 to 16.0 grams per ton to provide 85 to 240 milligrams per head per day for reduction of incidence of liver abscesses.

(iii) 11.0 to 16.0 grams per ton to provide 70 to 240 milligrams per head per day for improved feed efficiency.

(iv) Feed continuously as sole ration to cattle fed in confinement for slaughter. Not for use in animals intended for breeding.

(4) Virginiamycin may be used in combination with:

(i) Amprolium and ethopabate as in Sec. 558.58.

(ii) Diclazuril as in Sec. 558.198.

(iii) Halofuginone as in Sec. 558.265.

(iv) Lasalocid as in Sec. 558.311.

(v) Monensin alone or with roxarsone as in Sec. 558.355.

(vi) Salinomycin alone or with roxarsone as in Sec. 558.550.

(vii) Semduramicin as in Sec. 558.555.

Synercid

The following insert is adapted from the FDA Center for Drug Evaluation Research public information website (http://www.fda.gov/consumerinfo/druginfo/SYNERCID.htm). Additional details about Synercid are available through the FDA website.

Synercid	
Brand Name:	Synercid
Active Ingredient:	quinupristin/dalfopristin
Strength(s):	500 mg (150 mg of quinupristin and 350 mg of dalfopristin)
Dosage Form(s):	Powder for injection
Company Name:	Rhone-Poulenc Rorer
Availability:	Prescription only

*Date Approved by the FDA:	September 21, 1999
**Approval by FDA does not mean that the drug is available for consumers at this time.*	

What is Synercid used for?
Synercid is used to treat adult patients with serious or life-threatening infections and certain skin infections caused by specific types of bacteria.

Synercid was approved based on its ability to clear bacteria from the bloodstream. At this time, it is not known if Synercid will cure the underlying infection. Clinical studies to determine Synercid's ability to cure underlying infection are presently under way.

Special Warnings with Synercid:

- Synercid can interact with many medications. Review ALL medications that you are taking with your health care provider, including those that you take without a prescription.
- Tell your health care provider if you are taking cyclosporine, midazolam, or nifedipine.

General Precautions with Synercid:

Tell your health care provider right away if you develop diarrhea while taking Synercid.

- Tell your health care provider if you are trying to become pregnant, are already pregnant, or are breast-feeding.
- Irritation of the vein can occur when Synercid is given. Therefore, it is recommended to flush the vein with 5% Dextrose in Water following completion of the infusion.
- Do not dilute Synercid with Saline solution.

How is Synercid given?

Synercid is given by IV in a hospital setting.

What are some possible side effects of Synercid? *(This is **NOT** a complete list of side effects reported with Synercid. Your health care provider can discuss with you a more complete list of side effects.)*

Pain, swelling, and irritation at the infusion site

- Muscle and joint pain
- Nausea
- Vomiting
- Rash
- Diarrhea

- Headache
- Itching

For more detailed information about Synercid, ask your health care provider.
5/23/00

2.4.3 Mechanism of Antimicrobial Activity

Streptogramins act by binding to the 50S ribosomal subunit, interfering with peptidyltransferase activity, and, consequently, inhibiting bacterial protein synthesis Type A and type B streptogramins share separate but overlapping binding regions, but the binding of a type A streptogramin causes a conformational change which increases the affinity of a type B streptogramin for its target. Thus, the synergistic effect observed in mixtures of streptogramins A and B is due to synergistic binding to the ribosomal target site. Because type A and type B streptogramins are chemically unrelated and have different binding sites, mechanisms of resistance to the two types of streptogramins are different.

Two other families of antibiotics, the macrolides and the lincosamides, are functionally related to the streptogramins in that their antimicrobial activity also involves interactions with the 50S ribosomal subunit, resulting in inhibition of protein synthesis. The macrolides, lincosamides, and streptogramins antibiotics constitute what is known as the MLS superfamily. The macrolides and lincosamides share over-lapping binding sites with type B streptogramins, enabling the development of the cross-resistance among these three families of antibiotics known as MLS_B resistance. This is a significant consideration because both macrolides and lincosamides are widely used in animal production and this combined usage might select for a population of enterococci resistant to streptogramin B compounds.

2.5 Microbiological Background

Information in this section on the background microbiology was obtained from several recent reviews (Butaye et al., 2003; Werner et al., 2002; Johnston et al., 2002; Gilmore 2002; Roberts et al., 1999; Cocito et al., 1997)

2.5.1 Prevalence and Distribution of *Enterococcus* spp.

The physiological characteristics of *Enterococcus* spp., which allow them to grow and survive in harsh environments, also permit them to persist almost everywhere. Enterococci are gram-positive cocci and are facultative anaerobes with a growth range from 10 to 45°C. Enterococci can be found in soil, water, and food, and in most if not all mammals, including humans, as well as birds, insects, and reptiles. They occur naturally in soil and can be readily isolated from the roots of most plants. In water, they are generally considered as fecal contaminants. In foods, the presence of enterococci can result from the direct addition of the bacteria as a food processing aid (e.g., they have been used as starter cultures for food fermentation and for making hard cheese) or from a contaminant pathway (e.g., vegetation, processing equipment, processing environments, and/or fecal contamination). Enterococci are routinely recovered from seafood, cheese, dried whole egg powder, raw and pasteurized milk, frozen fruits, fruit juices, and vegetables. In most mammals and birds, enterococci are a natural part of the intestinal flora; in humans, enterococci comprise no more than 1% of the intestinal microflora of an adult, a number that does not reflect the medical importance of enterococci.

More than 20 enterococcal species are recognized, although the most commonly detected species of enterococci isolated from human feces are *E. faecalis* and *E. faecium*. Other species found in humans includes *E. durans*, and *E. avium*. With respect to human infections, enterococci are opportunistic pathogens and the incidence of each species found in human infections probably reflects the distribution of the different *Enterococcus* species in the human gastrointestinal tract; *E. faecalis* accounts for 80 to 90% of clinical isolates whereas *E. faecium* is detected in less that 10% of these isolates. Other enterococci that cause infections in humans include *E. durans*, *E. avium*, *E. gallinarium*, *E. casssliflavus*, *E. hirae*, *E. muntdii*, and *E. raffinosus*.

The distribution of enterococcal species in animals varies with different host species and even age of the host species. The most commonly found enterococcal species in the intestines of farm animals are *E. faecalis*, *E. faecium*, *E. hirae*, and *E. durans*. In chickens, a high prevalence of *E. faecalis* is found in day-old chicks, which is replaced by *E. faecium*, *E. hirae*, and *E. durans* as the chicken ages, and these latter strains are replaced by *E. cecorum* in chickens about 12 weeks old. Other species present

in chickens include *E. casseliflavus*, *E. gallinarium*, and *E. mundtii*. In cattle, age-dependent colonization has also been reported, in which *E. faecalis*, *E. faecium*, and *E. avium* are gradually replaced by *E. cecorum*. However, cattle exhibit a general diminishment of enterococcal species in adults. In swine, *E. faecalis* is found in the intestines and *E. faecium* in the feces in low numbers. Recent data on the distribution of enterococcal species in various animal feed commodities suggests that *E. faecalis* occurs infrequently outside human and animal environments and that *E. faecium* is, by far, the species most prevalent in these sources.

In plants, the distribution of enterococcal species appears to be limited to *E. casseliflavus*, *E. mundtii*, and *E. sulfurous*, although additional species may be found on plants due to environmental contamination from human and animal wastes. Similarly, *E. faecalis* and *E. faecium* are found in water and many foods as contaminants. The hardiness of enterococci presents a concern for their presence in foods, as they may survive some types of food processing.

2.5.2 Mechanisms of Resistance in *Enterococcus faecium*

Enterococci exhibit both intrinsic and acquired resistance to antimicrobial drugs. Genes conferring intrinsic resistance reside on the bacterial chromosome and are characteristic of the species. Specific enterococcal species exhibit resistance to the glycopeptides, as seen with *E. gallinarum* and *E. casseliflavus*, and the streptogramins, as seen with *E. faecalis*. Unlike *E. faecalis*, *E. faecium* does not exhibit intrinsic resistance to the streptogramins. Acquired resistance encompasses resistance patterns that are derived from either genetic mutations, mutational resistance, or more commonly by the acquisition of foreign DNA (transmissible resistance). Acquired resistance in enterococci is phenotypically exemplified by high-level aminoglycoside resistance, β-lactamase production, tetracycline resistance, high-level glycopeptide resistance and most recently streptogramin resistance and linezolid resistance. Acquired resistance due to the transmission of resistance genes is the primary concern in enterococci. The direct transfer of genetic material from one bacterial cell to another, via conjugation involving plasmids and transposons, has the potential to convey resistance to classes of antibiotics

and can lead to a relatively broad dissemination of the resistance among susceptible bacteria.

The known mechanisms of resistance to streptogramins in gram-positive organisms are enzymatic inactivation, active efflux, and ribosomal target site modification. Table 2-1 summarizes the mechanisms of resistance associated with the two types of streptogramin compounds, the genes associated with the mechanisms, and whether the resistance genes have been found in *Enterococcus*. Other novel mechanisms are likely to exist that will account for observed resistance in *E. faecium* isolates that were not found to contain any of these known resistance genes.

Table 2-1. Resistance Mechanisms Against Streptogramins

Compound Type	Mechanism	Genes	Occurrence
Streptogramin A - Dalfopristin - Pristinamycin IIA - Virginiamycin M	Inactivation mediated by acetyltransferase	*vat(*D*)* *vat(*E*)*	Enterococcus
		*vat(*A*)* *vat(*B*)* *vat(*C*)*	Staphylococcus
	Active efflux mediated by ATP-binding proteins	*vga(*A*)* *vga(*B*)*	Staphylococcus
Streptogramin B - Quinupristin - Pristinamycin IB - Virginiamycin S	Target site alteration mediated by methylases	*erm* genes	*erm(*B*)* found in enterococci, *erm(*A*)* and *erm(*C*)* more common in staphylococci
	Inactivation mediated by a hydrolase (lactonase)	*vgb(*A*)* *vgb(*B*)*	*vgb(*A*)* gene not found in *E. faecium* with the exception of two human isolates
	Active efflux mediated by ABC-binding proteins	*msr(*A*)* *msr(*B*)* *msr(*C*)*	*msr(*A*)* and *msr(*B*)* found in staphylococci; *msr(*C*)* found in enterococci

Streptogramin A resistance

The most common known mechanism of inactivation of streptogramin A compounds is due to O-acetylation by acetyltransferases designated vat for virginiamycin acetyltransferase. The genes that code for the enzymes are plasmid-borne in staphylococci (*vat(A)*, *vat(B)*, and *vat(C)*) and enterococci (*vat(*D*)* and *vat(*E*)*). The acetyltransferase proteins VatA – VatE are highly related, with 50.4 to 60.1% identical amino acids (Haroche et al., 2000). Variations in the *vat(*E*)* allele due to single base substitutions were reported in streptogramin-resistant *E. faecium* isolates, suggesting regional variation among isolates, although there was no correlation between the number or position of the base changes in each allele and the minimum inhibitory concentration (MIC) of resistance (Soltani et al., 2001, Simjee et al., 2001).

A second mechanism of resistance to streptogramin A compounds is active efflux via ATP-binding cassette proteins (or ABC porters) encoded by plasmid-borne *vga(A)* and *vga(B)* genes. These genes have been found in Staphylococcus isolates but generally not those of Enterococcus.

Streptogramin B resistance

The most common type of streptogramin resistance is through modification of ribosomal RNA and proteins; in particular, methylation of rRNA by methylases encoded by the *erm* (erythromycin-ribosome methylase) gene family. This methylation results in a conformational change in the ribosome and subsequent reduced binding of the antimicrobial compound. Macrolides, lincosamides, and type B streptogramins share over-lapping binding sites in the region of methylation, resulting in cross-resistance to all three classes of antimicrobials and the common MLS_B phenotype. A large number of *erm* genes have been isolated, and differences are observed among them in the regulation of expression of the phenotype. Some of the enzymes are inducible (e.g., by erythromycin) and some are constitutively expressed. *erm* genes are found on chromosomes, associated with conjugative or nonconjugative transposons, but also can be found in plasmids.

In enterococci, *ermB*-mediated resistance against streptogramin B compounds is widespread. It is important to note that type A streptogramins are not affected by *ermB*

gene expression and that the synergistic effect of type A and B mixtures is retained in the presence of the *erm* gene, although reduced susceptibility has often been observed.

Resistance to type B streptogramins may also be due to hydrolysis of the ring molecule via lactonases VgbA and VgbB. The *vgb* genes were initially reported in staphylococcus, although there are recent reports that they have been found in human clinical isolates of *E. faecium.*

Recently, a gene encoding an efflux pump, *msrC*, was described in *E. faecium* that may account for streptogramin B resistance. Initially, it was thought that this efflux pump was intrinsic to all *E. faecium* and may confer low levels of streptogramin resistance. However, recent studies have indicated that *msrC* is not an intrinsic efflux pump in *E. faecium*.

It is important to note that the relationships between these known mechanisms of resistance, and others not yet defined, and observed resistance to streptogramin-combination drugs, such as virginiamycin and Synercid, is an unfinished story. Resistance to both type A and B streptogramins is thought to be required for high-level resistance to the combination drugs in *E. faecium*. The presence of a resistance mechanism to a type A or type B streptogramin alone may lead to an increased MIC, but one that may remain within the clinically manageable range. Studies of resistant isolates obtained from farm animals, retail meats, and humans have found isolates in which no known resistance mechanisms are present, or a larger number of isolates in which only one resistance mechanism is present. Further, as noted above, the presence of a type A resistance mechanism and *ermB* mediated resistance, a type B mechanism, is not sufficient to cause resistance to combination streptogramin drugs. These observations suggest a role for other unknown mechanisms conferring resistance to streptogramins in enterococci.

2.5.3 Flow of Resistance Determinants

A critical step in the assessment of hazard and, ultimately, the assessment of risk is evidence for the ability of (e.g.) a chicken-derived *E. faecium* either to colonize humans or to transfer antimicrobial resistance from chicken *E. faecium* to human *E. faecium*. In general, bacteria can become host specific in which the subspecies best

suited for the conditions of the host becomes the dominant subspecies. Although a chicken-derived *E. faecium* might grow in the human intestine for a period of time, colonization is generally transient in nature. This leads to two different antimicrobial resistance scenarios in the human (Figure 2, above). First, if the colonization is transient and the resistance determinants transfer to the resident human strain, the potential for long-term carriage of the resistance determinants exists. Second, if the resistance determinants do not readily transfer to the human *E. faecium*, then it is likely that the antimicrobial resistance will also be transient.

For the first scenario, it is plausible that transfer of streptogramin-resistant genes from animal *E. faecium* to human *E. faecium* could occur during the transient phase of colonization, possibly leading to a streptogramin-resistant infection caused by human commensal *E. faecium*. Studies have shown the *vat(D)* gene to be transferable both *in vitro* and *in vivo*, and the *vat(E)* gene to be transferable *in vitro*.

In the second scenario, there is no transfer of resistance determinants, requiring the streptogramin-resistant infection to be caused by *E. faecium* of animal origin within the transient carriage time. In this case, the occurrence of a streptogramin-resistant infection would be partially dependent on the time from last exposure to a contaminated food product. The extent of the transient carriage time may be dependent on the animal species and/or specific genotypic characteristics of the bacterial strain.

Finally, as noted in the discussion of the impact scenario, this risk assessment concerns the impact of streptogramin resistance arising from the use of virginiamycin in food animals on the ability to use Synercid to treat *vancomycin*-resistant *E. faecium* infections. For this impact to occur, the SREF must have concurrent drug resistance to vancomycin, or the infection must be from a clonally mixed population of VREF and SREF bacteria. Concurrent drug resistance to both streptogramins and vancomycin requires an additional transfer of resistant determinants, either vancomycin-resistance genes from a VREF to an existing streptogramin-resistant *E. faecium* or streptogramin-resistance genes from an SREF of animal origin to a vancomycin-resistant *E. faecium*. Given that the predominant source of VREF in the US is in hospitals, the additional transfer of resistance determinants would likely occur in hospitals and would require co-

mingling of SREF and VREF. Although this sequence of events is possible, we believe that it is of a lower likelihood than the possibility of an infection from a clonally mixed population of VREF and SREF.

3 RELEASE ASSESSMENT

3.1 Purpose

The purpose of release assessment is to assess the likelihood that the hazardous agent will be released from food animals. In this risk assessment, the release assessment focuses on the likelihood virginiamycin-resistant *E. faecium* are released in food animals. This biological hazard may subsequently be released to the human food chain, potentially causing human exposures.

3.2 Introduction

The SREF risk assessment is concerned with human antimicrobial drug resistance that might arise from food animal uses of the streptogramin antimicrobial drug, virginiamycin. The release assessment is intended to determine the probability of release of streptogramin-resistant *E. faecium* that might contaminate meat at slaughter and subsequently enter the exposure pathway to humans. Given the complicated exposure pathway, it is useful to compartmentalize exposure assessment into a consideration of factors that determine the rate of release, "source-specific terms," versus those factors that influence the presentation of the hazard to consumers and the physiological factors that govern whether or not an uptake or intake of bacteria occurs at the human-food interface, "receptor-specific terms." Because the compelling reason for partitioning exposure assessment into "release" and "exposure" components is to more clearly organize the complex undertaking of exposure assessment, the selection of a boundary between "release" and "exposure" may be based on other factors than the biological, physical and chemical properties of a complexity of the exposure pathway. For the purposes of this risk assessment, CVM followed the recently published Guidance for Industry #152 that considers the release-exposure boundary to be at the point that animals are presented for slaughter. At that point, the further transport of resistant bacteria and the factors contributing to human exposure are primarily human controlled factors.

3.3 Prevalence of Resistance in Farm Animals

The kinds of studies that contribute data pertinent to release assessment include microbiological surveys for streptogramin resistance in food animals (anywhere from on

the farm to the point of slaughter) or *in vivo* laboratory research on the acquisition of resistant bacteria due to feeding QD or virginiamycin in feed or water. CVM sought information from all types of studies, resulting in a combination of peer-reviewed reports, in-house research and contracted studies at academic or hospital research centers. Brief descriptions and tabulations of pertinent information from the studies are summarized in Table 3-1 for *E. faecium* isolates of animal origin.

Table 3-1 Streptogramin Resistance in Enterococcus faecium Isolates of Animal Origin

Animal	Isolate Source	Streptogramin Tested[1]	Number of Isolates	Resistance (%)[2]	Comments (MIC in µg/ml)	Reference
Broilers	Denmark 1997	Vir	211	70	MIC range: 0.5 to >128	Aarestrup et al., 2000a
	Finland 1996		42	21	MIC range: <0.25 to 64	
	Norway 1995-1997		55	0	MIC range: <0.25 to 2	
Broilers	Denmark 1998	QD	122	79	MIC range: 0.5 to 32	Aarestrup et al., 2000b
		Vir		75	MIC range: 1 to 128	
Broilers	Belgium 1998-1999	Vir	31	NR	MIC90 = 64 MIC range: 0.5 – 64	Butaye et al., 2001
Broilers	Denmark 2002	QD	102	28.5	MIC range: 0.5 to >32	DANMAP, 2002
Chickens	Sweden 2000	Vir	151	29	MIC range: 0.5 to 32	SVARM, 2000
	Sweden 2001	Vir	204	30		SVARM, 2001
	Sweden 2002	Vir	189	25		SVARM, 2002
Chickens	US	QD	101	62	MIC range: 0.5 to > 32 10% of resistant isolates had MIC ≥ 32	Hayes et al., 2001
Chickens	US	QD	57	98		Zervos et al., 2003
Turkeys	US	QD	142	52		Zervos et al., 2003
Turkeys	US 1995-1996	QD	86	29		Welton et al., 1998
	Denmark 1997		55	49	MIC range: <0.25 to 128	

Animal	Isolate Source	Streptogramin Tested[1]	Number of Isolates	Resistance (%)[2]	Comments (MIC in µg/ml)	Reference
	Finland 1996		43	2.3	MIC range: <0.25 to 8	
	Norway 1995-1997		4	0	MIC range: <0.25 to 2	
Pigs	Denmark 1998	QD	88	52		Aarestrup et al., 2002
	Spain 1998-1999		124	71		
	Sweden 2000		18	6		
Pigs	Denmark 1998	QD	88	60	MIC range: 0.25 to 16	Aarestrup et al., 2000b
		Vir		85	MIC range: 1 to 128	
Pigs	Sweden 2000	Vir	48	38	MIC range: 0.5 to 16; 91% of resistant isolates had MIC = 4 to 8	SVARM, 2000
	Sweden 2001	Vir	106	33		SVARM, 2001
Pigs	Denmark 2002	QD	194	12.9	MIC range: 0.5 to 8 96% of resistant isolates had MIC = 4	DANMAP, 2002
Swine	Belgium 1998-1999	Vir	33	NR	MIC90 = 8 MIC range: 0.5 – 32	Butaye et al., 2001
Swine	US	QD	269	20		Zervos et al., 2003
Wild boars	Sweden 2001	Vir	35	14	MIC range: 0.5 to 8	SVARM, 2001
Cattle	Denmark 2002	QD	15	0	MIC range: 0.5 to 2	DANMAP, 2002
Cattle	Norway 2001	Vir	26	NR	No isolates had MIC ≥ 8	NORM-VET, 2001
Cattle	Sweden 2000	Vir	71	24	MIC range: 0.5 to 64; 94% of resistant isolates had MIC = 4 to 8	SVARM, 2000

Animal	Isolate Source	Streptogramin Tested[1]	Number of Isolates	Resistance (%)[2]	Comments (MIC in µg/ml)	Reference
Cattle (Beef)	US	QD	107	3		Zervos et al., 2003
Cattle (Dairy)	US	QD	534	8		Zervos et al., 2003
Sheep	Norway 2001	Vir	9	NR	No isolates had MIC ≥ 8	NORM-VET, 2001
Ruminants	Belgium 1998-1999	Vir	10	NR	MIC90 = 1 MIC range: 0.25 – 8	Butaye et al., 2001
Avian Pets	Belgium 1998-1999	Vir	42	NR	MIC90 = 1 MIC range: 0.25 – 4	Butaye et al., 2001
Mammalian Pets	Belgium 1998-1999	Vir	30	NR	MIC90 = 2 MIC range: 0.25 - 16	Butaye et al., 2001

[1] QD = Quinupristin/Dalfopristin; Vir = Virginiamycin

[2] NR = Not Reported

Table 3-2. Prevalence of Quinupristin/Dalfopristin Resistant Enterococcus faecium in Animal Populations

Source	Population	Number of Samples	Resistance (%)[1]	Comments	Reference
US 1998-1999	Retail chicken carcasses	407	58	Resistant isolates detected using selective medium containing antibiotics	McDonald et al., 2001
			3	Resistant isolates detected using non-selective medium	
The Netherlands 1997	Broilers	50	92	Prevalence based on growth in selective medium containing antibiotics	van den Bogaard et al., 2002
	Laying Hens	25	12		
US 1995-1966	Turkeys	95	18	Resistant isolates detected using non-selective medium	Welton et al., 1998

[1] Percent of population with QD-resistant *E. faecium*.

A review of the data in Table 3-1 shows a wide range of streptogramin resistance in *E. faecium* isolates, with levels of resistance *E. faecium* in poultry as high as 98%, slightly lower levels in pigs, and relatively low levels in cattle and other animal species. An important consideration in interpreting this data is the date and source of isolate collection relative to the use of virginiamycin as a growth promoter in food-producing animals. In Finland, Norway, and Sweden, virginiamycin was either banned or little used in the 1990s, and the isolate data confirms that resistance is reduced in those countries compared to isolates obtained from countries where virginiamycin was still in use. For example, Norway officially prohibited the use of virginiamycin in 1998, but sales figures show little use as far back as 1995 (NORM-VET 2001). Thus, the low levels of resistance in broilers, pigs, and cattle from Norway are in agreement with that usage pattern. Denmark banned virginiamycin use in 1999 and resistance has dramatically dropped, as shown in Figures 7 and 8 (taken from DANMAP 2002).

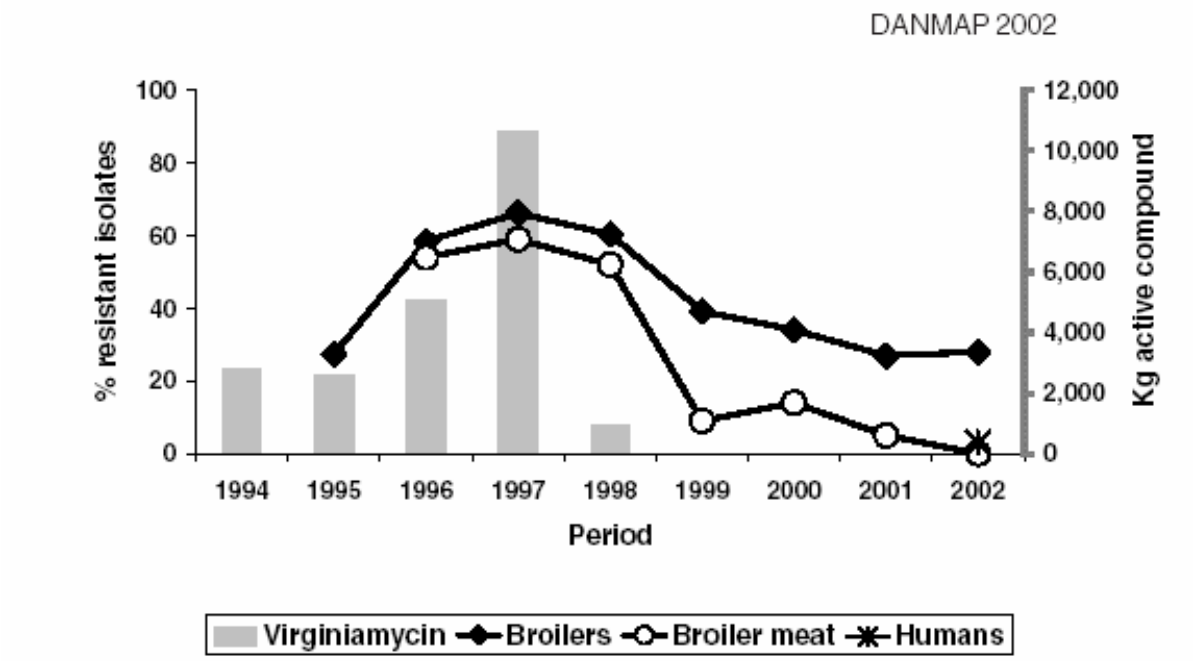

Figure 7. Trends in streptogramin resistance among *E. faecium* from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark.

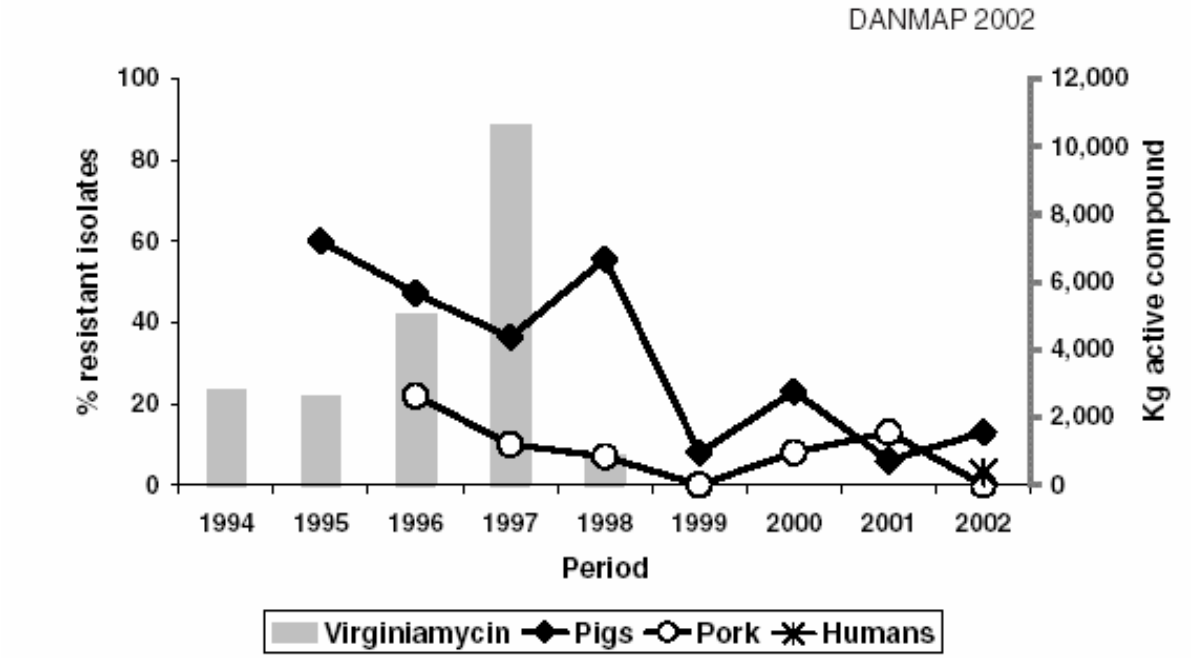

Figure 8. Trends in streptogramin resistance among *E. faecium* from pigs, pork and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark

There is some speculation as to the source of the remaining low levels of streptogramin resistance observed in *E. faecium* in those countries where virginiamycin is not used in animal production. This resistance may be a remnant of past use or the result of co-selection, in which the use of one antimicrobial might select for resistance to others. The macrolide-lincosamide-streptogramin B (MLS_B) phenotype may be an example of this co-selection, as the use of macrolides on the farm may result in streptogramin resistance. However, data suggests that MLS_B associated streptogramin resistance is unlikely to cause the high levels of resistance (MIC $\geq$ 32 µg/mL) observed in the isolates summarized in Table 3-1 (see discussion in Section 2.5.2).

The presence of relatively low levels of streptogramin resistance in *E. faecium* isolates from cattle may also be related to co-selection from other antimicrobials used on the farms. In the study by Zervos et al., no virginiamycin was used in the beef, swine, and dairy farms sampled, yet low levels of resistance were observed. The observed resistance in avian and mammalian pets is somewhat unexplained, as virginiamycin is only used therapeutically as a topical preparation in mammalian pet animals and not used

in pet birds (Butaye et al., 2001). One other alternative to explain findings of streptogramin resistance without direct use of virginiamycin in the sampled species may be that the resistant *E. faecium* are widespread and persistent in the farm environment as the result of past usage, or current usage in other species. Ongoing studies are examining this possibility in the context of organic farms, where antibiotic use is limited.

Beyond the levels or prevalence of resistance, it is also informative to examine the MIC distribution in these animal isolates. The upper range of MICs in poultry in all of the studies in which resistance is reported is greater than or equal to 32 µg/mL. In pigs, MICs ≥ 32 µg/mL are reached less frequently and only in those studies with high prevalence of resistance. These observations are in marked contrast to the findings in humans where few studies reported isolates with an upper range of MICs ≥ 32 µg/mL (see Table 4-2). The different distribution of MICs between the animals and human isolates may be due to different mechanisms of resistance, or the presence of different resistance genes, as discussed in Section 4.6

It is also worth noting that the poultry data from European countries (those that permit use of virginiamycin) is comparable to the data from US farms, where virginiamycin is in use. A similar comparison cannot be made in pigs, due to a lack of data from US farms, or in cattle, where the data is sparse from both the US and Europe.

Table 3-2 presents a summary of studies for which carriage rates (the percent of the population with QD-resistant *E. faecium*, as opposed to the percent of *E. faecium* isolates that are QD resistant) could be estimated. Estimates of the carriage rate differ widely, which may be a function of the microbiological methods used in these studies, but generally confirm the high levels of streptogramin-resistant *E. faecium* present in poultry. The data also highlight some of the methodological issues that influence the interpretation of results from susceptibility testing, particularly in the effects of using selective medium containing antibiotics in detecting *E. faecium* isolates. For example, a large difference in results was observed (58% vs. 3%) in McDonald et al. (2001) when comparing the results from using selective medium containing antibiotics vs. non-selective medium. The data in Table 3-1 were generated from studies using non-selective medium.

3.4 Data Gaps in Release Assessment

The fact that enterococci are widely-distributed commensal bacteria simplifies the starting point in a release assessment because it is reasonable to assume that all food animals can shed enterococci. The question of release in the risk assessment then focuses on the proportions of *Enterococcus* that are the specific strain of interest, *E. faecium*, and the proportion of *E. faecium* that are resistant to virginiamycin or QD.

There are several factors in this dataset that may lead to uncertainty in the results as discussed in the release assessment. Microbiological methods are not consistent throughout these studies, although the reporting of resistance prevalence was standardized on the same MIC breakpoint (MIC $\geq$ 4 µg/mL). MIC distributions were not available for several of these studies, which prevented further analysis for the presence of high-level resistance, which was defined in this document as an MIC $\geq$ 32 µg/mL. Additional data on isolates from pigs in the US, and from cattle in general may have permitted further analysis of the relationship between virginiamycin use and the prevalence of resistance across different species. Nevertheless, the available data is sufficient to draw certain conclusions concerning the prevalence of streptogramin resistance determinants in *E. faecium* isolated from food-producing animals.

3.5 Conclusions

The available data demonstrates that streptogramin-resistant *E. faecium* are highly prevalent in isolates obtained from poultry and swine sources in both the US and Europe. The prevalence of resistance appears to be related to the usage pattern of virginiamycin on the farms. Further, high level resistance, based on a MIC distribution range that includes MICs $\geq$ 32 µg/mL, is present in all of the poultry studies in which resistance was observed. Similar high-level resistance was observed to a lesser extent in the swine studies. The data on cattle are limited; the available studies reported relatively low prevalence rates for resistance, consistent with the use of virginiamycin in the species.

4 EXPOSURE ASSESSMENT

4.1 Purpose

The purpose of exposure assessment is to describe the possible pathways of human exposure to the antimicrobial resistance determinants of interest and to estimate the likelihood of human exposure, given the specified pathway.

4.2 Introduction

Human exposure to antimicrobial resistance determinants from food animal sources can be represented by multiple potential exposure pathways that are direct or indirect in nature. Further complicating the understanding of human exposure is that "direct" and "indirect" are terms that can apply to both microscopic and macroscopic events in the exposure pathway. By "microscopic," we refer to parameters of exposure governing the behavior of the microbe in question in a given exposure pathway. These factors include the type and rate of transfer of resistance determinants between animal and human-adapted *E. faecium* in the human gastrointestinal (GI) tract, the microenvironment of GI tract, micro-environmental properties of contaminated surfaces, including retail meats and food preparation surfaces, and others. In contrast, "macroscopic" factors in exposure include the population and "human-level" properties of exposure including human-to-human and human-surface-human contact rates, the sizes of the infected population, etc.

The modifying terms, direct and indirect, also refer to both micro- and macroscopic elements of exposure analysis. As discussed earlier in this report, the acquisition of resistant bacteria in humans, at a microscopic level, can be hypothesized as due to direct mechanisms, in which the resistant animal bacteria adapts to the human host, or indirect mechanisms in which the animal bacterium transfers resistance determinants to human bacteria already colonizing the GI tract. On a macroscopic level, direct exposure pathways are those in which the individual experiencing an increased risk of adverse health consequences is also the individual who consumed the antimicrobial resistant bacteria on a food commodity. Alternatively, indirect exposure refers to individuals who are exposed to resistant bacteria by human contact with infected

individuals or contaminated surfaces. The directly and indirectly colonized human populations, as used here, are analogous to the index and secondarily infected populations in infectious disease transmission (e.g., Eisenberg et al., 2002).

As discussed in the release assessment, the boundary between release and exposure assessment is considered to be the slaughter process. The pathways that comprise the exposure assessment commence with the presence of resistance determinants on retail meats for human consumption. A discussion of the factors that may influence the possibility of spread and/or transmission of resistance between slaughter and processing of meats for retail purchase are beyond the scope CVM has defined for this risk assessment. Nevertheless, the available data suggests that the prevalence of resistance is similar between that observed at the boundary of the release assessment and the presentation of poultry products at the retail level. The exposure assessment continues with events that influence human exposure, and the approach follows the flow of resistance determinants as the hazardous agents of interest. This approach is consistent with the microscopic events pathway linking the "ends" of exposure assessment. The events in the macroscopic exposure pathway—human activities causing secondary or indirect transmission of resistant *E. faecium* are relevant to the overall risk assessment process, but there are little available data on individual transmission rates of resistance in the community or in food preparation environments that might inform this risk assessment.

Although the understanding of the key elements necessary for a detailed model of exposure for this risk assessment has developed over the course of study, a clear picture of quantitative human exposures has yet to emerge from the scientific literature or from studies commissioned by CVM. Therefore, the exposure assessment, similar to the release assessment, remains principally qualitative in nature. Additional data on the molecular genetics of streptogramin resistance will provide needed insight on the nature of the exposure pathways of concern and the relationship between antibiotic use in animals and antibiotic resistance observed in humans.

4.3 Prevalence of Resistance in Retail Meats

The primary pathway of interest for human exposure to streptogramin-resistant *E. faecium*, and streptogramin resistance determinants, is through the consumption of *E. faecium* contaminated meat and poultry products. Table 4-1 provides a summary of studies on QD resistance in *E. faecium* isolates from retail animal meat samples. The prevalence of resistance is of the same magnitude as that observed in the isolates of animal origin (Table 3-1). The poultry isolates had the highest prevalence of streptogramin-resistant *E. faecium*, the pork meat isolates were slightly lower but still had appreciable levels of resistance, and low levels of resistance was observed from limited data on isolates from cattle sources. Other foods tested, preparations of cheese alone or a mixture of cheese and raw pork, were found to have resistant *E. faecium*, but it was a low-level resistance (MIC = 4 μg/mL, the breakpoint for QD resistance testing).

Also as found in the farm isolates, the range of MICs included high-level resistance (MIC ≥ 32 μg/mL) for most of the poultry isolates. The MIC distribution for the pork isolates did not include an upper range of high-level resistance as observed in the poultry isolates.

The study by Butaye et al. (2000) reported differences in the results from susceptibility testing between virginiamycin and quinupristin/dalfopristin, a finding that the authors described as difficult to interpret, given the widely-accepted assumption that cross-resistance between these two antimicrobials is near 100%. Butaye et al. (2000) suggest a possible explanation may be that separate or simultaneous resistance mechanisms may be present in certain strains, with virginiamycin being less susceptible to one of these mechanisms. The likelihood of occurrence of such a scenario is difficult to assess given the incomplete picture of streptogramin resistance mechanisms currently available.

Table 4-1. Streptogramin Resistance in Enterococcus faecium Isolates from Retail Meat

Food Item	Isolate Source	Streptogramin Tested	Number of Isolates	Resistance (%)[1]	Comments (MIC in µg/ml)	Reference
Raw poultry meat	Belgium	QD	24	79	MIC range: 0.25 to > 32	Butaye et al., 2000
		Vir		58		
Retail chicken, raw meat	US 2001-2002	QD	245	27	MIC range: ≤ 1 to 32 MIC90 = 16	Hayes et al., 2003
Retail chicken carcasses	U.K.	QD	60	NR	MIC range: 0.25 to 64; 4 isolates had MIC = 8 to 32	Chen et al., 2002
		Vir		NR	MIC range: 0.5 to 32; 4 isolates had MIC = 8 to 64	
Retail chicken carcasses	US 1998-1999	QD	202	55	MIC range: ≤ 0.25 to 16	McDonald et al., 2001
			2542	93	MIC range: 0.5 to 32	
Retail chicken carcasses and turkey breasts	US 1999-2000	QD	33	82	resistant isolate MIC range: 4 to 16	Simjee et al., 2002
Retail turkey, raw meat	US 2001-2002	QD	213	54	MIC range: ≤ 1 to 32 MIC90 = 32	Hayes et al., 2003
Retail Pork, raw meat	US 2001-2002	QD	114	8.8	MIC range: ≤ 1 to 8 MIC90 = 2	Hayes et al., 2003
Raw pork	Belgium	QD	17	35	all resistant isolates had MIC = 4	Butaye et al., 2000
		Vir		0		
Retail Beef, raw meat	US 2001-2002	QD	254	18	MIC range: ≤ 1 to 16 MIC90 = 4	Hayes et al., 2003

Food Item	Isolate Source	Streptogramin Tested	Number of Isolates	Resistance (%)[1]	Comments (MIC in µg/ml)	Reference
Preparations of cheese and raw pork	Belgium	QD	23	70	94% of resistant isolates had MIC = 4	Butaye et al., 2000
		Vir		0		
Cheese	Belgium	QD	12	33	all resistant isolates had MIC = 4	Butaye et al., 2000
		Vir		0		

1 NR = Not Reported

2 The top number are the results from using a nonselective medium for screening colonies; the bottom number are for isolates screened in a selective medium containing antibiotics

Table 4-2. Quinupristin/Dalfopristin Resistance in Enterococcus faecium Isolates of Human Origin

Isolate Source and Collection Dates	Species[1]	Number of Isolates	QD Resistance (%)[2]	Comments (MIC in µg/mL)	Reference
Western Pacific 1999-2000	EF	149	0		Bell et al., 2003
US and Italy 1991-1996	VREF	82	0	MIC range: 0.06 – 2	Bonilla et al., 1996
UK 1992-1996	VREF	31	0	MIC range: 0.5 – 1	Chen et al., 2002
	VSEF	23	0	MIC range: 0.5 – 1	
US 2000-2001	VREF	114	0	MIC_{90} = 1; MIC range: 0.25 – 2	Critchley et al., 2003a
	VSEF	333	NR	MIC_{90} = 2; MIC range: ≤ 0.12 – 8; 14.3% of isolates had MIC ≥ 2	
US and Canada; 1996-1997	EF	1,011	0.2	no high level resistance observed (MIC = 4 or zone diameter = 15 mm)	Jones et al., 1998
US 1996	EF	281	0.4	Resistant isolate had MIC = 4	CVM, 2003
US 1991-1995	EF	298	0.7	Resistant isolates had MIC = 4; 94% of isolates were VREF	CVM, 2003
Sweden 1996-1998	EF	74	1.4	MIC range: 0.5 – 4	Hallgren et al., 2001

Isolate Source and Collection Dates	Species[1]	Number of Isolates	QD Resistance (%)[2]	Comments (MIC in µg/mL)	Reference
US, UK and Germany	VREF	291	1.4		Moellering et al., 1999
Latin America	VREF	21	NR	Results confounded by clonal dissemination of a single resistant strain	Sader et al., 2001
	VSEF	94	2	MIC_{90} = 2	
Denmark 2002	EF	40	2.5	Resistant isolate had MIC = 4	DANMAP, 2002
UK 1996-1997	EF	31	3.2	Single resistant isolate has MIC = 4	Bell et al., 2003
US 1994-1996	VREF	875	4.9	All isolates collected: MIC_{90} = 2; MIC range: 0.25 - 32 81% of resistant isolates had MIC = 4	Eliopoulos et al., 1998
		352	1.1	Collection of first isolates with duplicate strains excluded: MIC_{90} = 1; MIC range: 0.25 – 8	
EU 2000-2001	VREF	114	5.3	MIC_{90} = 2; MIC range: 0.25 – 32	Critchley et al., 2003b
	VSEF	333	3	MIC_{90} = 2; MIC range: ≤ 0.12 – 16	
US 1998-1999	EF	58	5	all resistant isolates had MIC = 4	McDonald et al., 2001
Worldwide 1997-1999	EF	820	5	MIC ≥ 8: 1.8% 63% of resistant isolates had MIC = 4	Low et al., 2001
US and Canada 1999-2000	VREF	598	3.8	MIC_{90} = 1	Ballow et al., 2002

Isolate Source and Collection Dates	Species[1]	Number of Isolates	QD Resistance (%)[2]	Comments (MIC in µg/mL)	Reference
	VSEF	310	13.2	MIC_{90} = 4	
Europe 1997	VREF	22	9	MIC_{90} = 2; MIC range: 0.25 – 8	Schouten et al., 1999
	EF	552	8	MIC_{90} = 2; MIC range: 0.25 – 32	
US	VREF	130	5.7		Jones et al., 2001b
	VSEF	39	12.9		
Denmark 1998	EF	65	11	MIC range: 0.25 – 4	Aarestrup et al., 2000b
South Africa 1996-1997	EF	47	15	MIC_{90} = 4; MIC range: 0.25 – 8	Struwig et al., 1998
Worldwide	VREF	107	17.8	MIC_{90} = 8	Jones et al., 2001a
	VSEF	157	23.6	MIC_{90} = 8	
Spain 2000	EF	29	41.4	Human volunteers; MIC range: ≤ 0.5 – 8; 92% of resistant isolates had MIC = 4	Del Campo et al., 2003
		45	26.7	Food handlers; MIC range: ≤ 0.5 – 64;	
Worldwide 1989-1996	VREF	422	NR	MIC_{90} = 1; MIC range: ≤ 0.06 – 8	Dowzicky et al., 1998

Isolate Source and Collection Dates	Species[1]	Number of Isolates	QD Resistance (%)[2]	Comments (MIC in µg/mL)	Reference
	EF	1667	NR	MIC_{90} = 1; 5% of isolates had MIC ≥ 2	
US, Canada, and Latin America 1997	EF	170	NR	MIC range: ≤ 0.06 – 4	Pfaller et al., 1999
Europe 1997-1998	EF	90	NR	MIC_{90} = 4; no isolates had MIC > 4	Schmitz et al., 1999
Taiwan 1996-1999	VREF	100	NR	MIC range: 0.5 – 128 51% of isolates had MIC ≥ 2	Luh et al., 2000

[1] EF indicates isolates were *Enterococcus faecium*; VREF indicates vancomycin-resistant *Enterococcus faecium* isolates; VSEF indicates vancomycin-sensitive *Enterococcus faecium* isolates.

[2] NR = Not Reported

Table 4-3. Prevalence of Quinupristin/Dalfopristin Resistant Enterococcus faecium in Human Populations

Population	Source	Number of Samples	Prevalence (%)[1]	Comments	Reference
Humans with a history of diarrhea	Denmark 1998	254	3	Resistant isolates detected using non-selective medium, all had MIC =4	Aarestrup et al., 2000
Nonhospitalized Humans	Germany 1998-1999	200	14	23 of 28 resistant isolates were *E. faecium* (four *E. hirae*, one *E. durans*); isolates detected using selective medium containing antibiotics and pre-enrichment	Werner et al., 2000a
Outpatients	US 1998-1999	334	1	Resistant isolates detected using non-selective medium (all had MIC = 4);	McDonald et al., 2001
			0	no isolates detected using selective medium containing antibiotics	
Broiler farmers	The Netherlands 1997	51	37	Prevalence based on growth in selective medium containing antibiotics; no statistical differences between worker populations	van den Bogaard et al., 2002
Laying-hen farmers		25	8		
Poultry slaughters		46	15		

[1] Percent of population with QD-resistant *E. faecium*.

4.4 Other Exposures to Resistance Determinants

Given the ubiquitous nature of enterococci, pathways other than the direct consumption of contaminated animal meat products (or clinical/therapeutic exposures) may contribute to human exposures to streptogramin-resistant determinants. Pathways involving occupational exposures (e.g., abattoir workers) or drinking water exposures (in which *E. faecium* may be a fecal contaminant) are beyond the scope of this risk assessment, which is focused primarily on foodborne pathways. One foodborne pathway that may be a source of antibiotic-resistant bacteria involves produce grown in fields using untreated irrigation water or manure slurries.

In a study on produce samples (a variety of leafy greens, herbs, and cantaloupe) collected throughout production and processing from a site in the Southwestern US, at least one *Enterococcus* strain was isolated from over half the produce samples; and most of these isolates were *E. faecium* (Johnston and Jaykus, 2003). Of the *E. faecium* isolates, 13% were resistant to quinupristin/dalfopristin. The extent of the contribution of this pathway to human exposures is unclear, although the authors of the study noted that, unlike most animal products, fresh produce can be consumed directly, obviating the heat inactivation and killing of bacteria from cooking.

4.5 Prevalence of Resistance in Humans

As noted in the Hazard Identification, the probability of human exposure to streptogramin-resistant *E. faecium* originating from a foodborne pathway can be estimated from the prevalence of resistant *E. faecium* in the community. Surveillance databases can be used to estimate human community prevalence levels, although conclusions drawn from these databases must be viewed in relation to the uncertainty associated with the methods of data collection common to such surveillance efforts.

Table 4-2 presents a summary of available data on the occurrence of QD resistance in *E. faecium* isolates from human sources. The reported resistance levels provide an approximate estimate of the prevalence of QD resistant *E. faecium* in the human community. The data show a wide range of reported resistance to QD in *E.*

faecium; the majority of studies provide estimates of QD resistance in the 0 to 4% range. Of those studies that report higher resistance, the potential misidentification of *E. faecium* may be a substantial factor and is cited as relevant in two of these studies (Jones et al., 2001a; 2001b) (see section 4.5.1 for further discussion).

Ideally, to estimate the true background rate of resistance, this estimate should be based on samples obtained from populations that have not been previously exposed to the drug, as treatment with QD results in emerging resistance in recovered isolates (Chow et al., 1997; Dowzicky et al., 2000). For example, Eliopoulis et al. (1998) found increased resistance in isolates collected after the start of treatment compared to a collection of first isolates. Thus, the QD exposure status of the sampled population could affect estimates of community prevalence of resistance. Synercid was approved for use in the US and Europe in 1999, which suggests that samples collected prior to 1999 could be assumed to originate from a QD-unexposed population. Low et al. (2001) noted that an increasing trend of resistance to QD with time that may be due to therapeutic use of Synercid. However, this trend is not evident from the data in Table 4-2, particularly considering that two of the studies reporting higher levels of resistance, Aarestrup et al. (2000b) and Del Campo et al. (2003) specifically sampled populations that had no recent history of QD treatment or hospital stays.

The available data on MIC distribution indicates that most of the resistant isolates in the human surveillance studies have an MIC = 4 µg/mL, a concentration of QD that may still be transiently achievable in serum (Eliopoulis et al., 1998), and the range of MICs generally does not extend beyond 8 µg/mL. It is uncertain whether intermediate resistance (MIC = 4 to 16) should be regarded as acquired resistance (Butaye et al., 2003). The observed range of MICs from the human studies is in contrast to the results from the animal and retail meat studies, in which reported MICs are generally higher ((8 to 64 µg/ml) than those from human studies (see Table 3-1 and Table 4-2). Interestingly, the large majority of those studies that report high-level QD resistance in humans (MIC > 16) occur in studies outside of the US. The different MIC distribution between animal and human isolates is inconsistent with the postulated attribution of human streptogramin resistance to animal sources. Available data from studies on the molecular genetics of

streptogramin resistance appear to provide some rationale for the different MIC distributions (see Section 4.6) but the data are insufficient to draw strong conclusions.

The data in Table 4-2 also suggest that *E. faecium* isolates that are vancomycin-resistant may be less resistant than vancomycin sensitive *E. faecium* isolates. The strength of this observation is not clear, nor is the mechanistic basis for such a difference. However, as the primary use of QD is for patients with vancomycin-resistant *E. faecium* infections, it would be important to determine whether the presence of vancomycin resistance affect resistance to QD.

Table 4-3 is a compilation of those studies for which carriage rate (the percent of the population with QD-resistant *E. faecium*, as opposed to the percent of *E. faecium* isolates that are QD resistant) could be estimated. Estimates of the carriage rate differ widely, which may be a function of the microbiological methods used in these studies. The two studies that used methods most similar to the standards established by the NCCLS (2004), Aarestrup et al. (2000) and McDonald et al. (2001), estimate the carriage rate in the human population as 1 to 3%. Werner et al. (2000), using selective medium containing antibiotics and enrichment methods, places the rate at 14%. van den Bogaard et al. (2002) using a greatly different method that does not include susceptibility testing of individual isolates, provides carriage rates in populations of poultry workers varying from 8 to 37%. However, the van den Bogaard et al. (2002) study was not addressing resistance in the human community population, but in occupationally-exposed workers, although their finding of elevated resistance in workers is evidence of transmission of streptogramin-resistance determinants from animals to humans.

4.5.1 Data Uncertainties Associated with Surveillance Studies

The interpretation of data in Table 4-2 (and other surveillance-based datasets) should be made while recognizing several limitations common to epidemiological surveillance studies (Kahlmeter and Brown 2002), including bias and error related to populations sampled or differences in susceptibility test methods and breakpoints. Nevertheless, risk assessments often rely on surveillance studies due to their availability and designs that focus on the consequence(s) of interest in the risk assessment. For microbial populations with very low prevalence, limitations in sampling methodology

and sensitivity of the assay may result in under-estimation of the true prevalence rate. Specifically for *Enterococcus*, the use of selective vs. non-selective media or various enrichment procedures in growing the isolates may affect the level of resistance reported by investigators (Butaye et al., 1999; Del Campo et al., 2003). Further, different investigators used different breakpoints in determining resistance. Updated guidelines have been published (NCCLS 2004) describing methods and interpretive criteria for susceptibility testing in enterococci, but these are a recent development and may not have been implemented in the studies presented in Table 4-2. To limit the influence of such factors, the studies presented in Table 4-2 were limited to those that most conformed to the recent standards in that they used non-selective media, and QD resistance is only reported for those studies in which sufficient data was provided in the report to allow the calculation of resistance using the NCCLS breakpoint of MIC = 4 μg/mL.

An additional uncertainty that should be considered in reviewing Table 4-2 is the problem of misidentification of *E. faecium* (Willey et al., 1999; Jones et al., 1998). Because *E. faecalis* is intrinsically resistant to streptogramins, the misidentification of *E. faecalis* as *E. faecium* could result in an overestimation of the level of streptogramin resistance in the samples under study. Jones et al. (1998), in a large surveillance study of over 28,000 isolates, reported that retesting of "QD resistant" *E. faecium* strains resulted in misidentification of *E. faecalis* as *E. faecium* in 57.9% of the retested isolates. The retesting also gave susceptible results in 26.3% of the retested isolates and 10.5% of the isolates were shown to be mixed cultures, usually containing an *E. faecalis* strain, resulting in an error rate of 94.7% in identifying resistant isolates. In Jones et al. (2001a), the pattern of susceptibility rates for *E. faecium* revealed potential misidentification of *E. faecalis* as *E. faecium* in approximately 20% of cases. Notably, the reported resistance values from Jones et al., 2001 were not adjusted for the misidentification factor. Many of the studies in Table 4-2 did not include confirmation of resistance isolates as *E. faecium*, and it is therefore likely that many of the resistance values in Table 4-2 may be overestimates of true acquired resistance.

One additional consideration in reviewing the data in Table 4-2 is the inconsistent reporting of results within these reports. If levels of resistance discussed in the text of the

paper differed from those in the data tables, resistance values were reported in Table 4-2 based on the data tables in the published paper.

It also should be noted that the detection of resistance genes may be a more reliable method of determining acquired resistance. Section 4.6 of this report adopts this approach. However, as will be discussed, the incomplete picture of streptogramin resistance genes limits such a genotypic approach, resulting in a continued reliance on data derived from surveillance studies using a phenotypic approach to determining the prevalence of streptogramin resistance in *E. faecium*.

4.6 Flow of Resistance Determinants

There are two scenarios by which streptogramin-resistant *E. faecium* in the affected human might arise from the consumption of contaminated meat products. The first is from the adaptation of resistant zoonotic *E. faecium* to the human host, i.e., streptogramin-resistant *E. faecium* from the meat product colonize the human intestinal tract. The second scenario entails consumption of the zoonotic SREF and subsequent transfer of resistance determinants to human bacteria already residing in the intestine. It should be noted that in both scenarios, the presence of SREF, either human or zoonotic, in the human intestinal tract does not necessarily result in the adverse human health effect—impaired therapeutic efficacy of Synercid—that is the endpoint of concern in this risk assessment. Additionally, enterococci are not normally pathogenic in the GI tract; the infections of primary concern are bloodstream and urinary tract infections. Further, Synercid is prescribed primarily for the treatment of vancomycin-resistant infections. Thus, for impaired Synercid therapy to be observed, the streptogramin-resistant *E. faecium* must be involved in an infection remote from the intestinal tract, and must be associated with vancomycin resistance determinants, either through transfer of resistant determinants resulting in a doubly-resistant bacteria or through co-populations of VREF and SREF at the infection site that result in a vancomycin-resistant determination.

4.6.1 Colonization

Both scenarios that describe the emergence of streptogramin resistant *E. faecium* in humans from the consumption of animal meat products require at least transient colonization of the human intestine by zoonotic *E. faecium*. Sorensen et al. (2001) fed

streptogramin-resistant *E. faecium* isolated from pig carcasses immediately after slaughter to six healthy volunteers in concentrations similar to that present in meat sold in grocery stores. Detectable levels of the resistant strain in stools were found in five of the six volunteers for up to 6 days, and in the remaining volunteer for up to 14 days after ingestion. No streptogramin-resistant enterococci were isolated after 35 days. In the same study, resistant strains were recovered for up to 6 days from volunteers who ingested vancomycin-resistant *E. faecium* isolated from a retail chicken product. A study with an ingested probiotic containing *E. faecium* isolated from a human source reported that the ingested *E. faecium* could be detected in feces from human volunteers on day 10 but not on day 31 (Lund et al., 2002). In a mouse model, a single oral administration of vancomycin-resistant *E. faecium* isolated from a human source resulted in colonization on day 7, but by day 14 the resistant strain was no longer detected in any of the animals (Whitman et al., 1996).

These data suggest that ingestion of *E. faecium*, either from an animal or human source, results in transient colonization for a period of time of approximately 6 to 14 days. The implication for the first scenario, in which transient colonization with a zoonotic streptogramin-resistant *E. faecium* causes a streptogramin-resistant infection, is that the infection is likely to occur within 14 days following consumption of the contaminated meat product. Similarly, for the second scenario, the transfer of the streptogramin resistance determinants to a commensal human *E. faecium* should occur within the 14 day transient colonization time. However, in both scenarios, continued consumption of contaminated meat and poultry products, on a population basis, may serve to maintain a "constant" transient colonization via repeated introduction of streptogramin-resistant *E. faecium* to the intestinal tract.

As described previously, the first scenario for a streptogramin-resistant *E. faecium* infection in humans requires exposure to and subsequent infection by a zoonotic streptogramin-resistant *E. faecium*. The ability of zoonotic enterococci to cause the types of infection of concern to this risk assessment, bloodstream and urinary tract infections, is not well understood. Studies on the genetic relationships among vancomycin-resistant and vancomycin-susceptible *E. faecium* (Bruinsma et al., 2002) and the host specificity of vancomycin-resistant *E. faecium* (Willems et al., 2000) are beginning to provide more

insight into the transmission routes and persistence of *E. faecium* strains from animals in the human gut. The results of a recent study suggest that the spread of *E. faecium* from pigs to humans was the cause of an outbreak of sepsis in humans and pigs in China (Lu et al., 2002). It therefore appears that a scenario in which ingested zoonotic streptogramin-resistant *E. faecium* results in a bloodstream or urinary tract infection in humans is plausible, although there is clearly a need for additional studies into the factors that might influence such an event.

4.6.2 Transfer of Streptogramin Resistance Determinants from Animal to Human *E. faecium*

The second scenario by which the emergence of streptogramin-resistant *E. faecium* in the affected human might arise from the consumption of contaminated meat products involves transient colonization and subsequent transfer of streptogramin-resistant determinants from zoonotic *E. faecium* to human commensal *E. faecium*. The transfer of streptogramin resistance among *E. faecium* strains has been studied using *in vitro* methods, *in vivo* animal models, and studies using molecular genetic tools to study human and animal isolates.

Hammerum et al. (1998) were able to transfer the *vat(D)* gene and other unidentified genes encoding streptogramin resistance between isogenic strains using *in vitro* filter mating procedures, and reported transfer frequencies ranging from 2.3×10^{-4} to 2.2×10^{-3} transconjugants per donor. Interestingly, the authors found no relationship between the presence or absence of *vat(D)* and the levels of resistance to virginiamycin. Further, the majority of virginiamycin-resistant isolates did not contain *vat(D)* but were able to transfer resistance to sensitive *E. faecium* strains. The authors concluded that there must be another streptogramin A resistance gene present in the donor *E. faecium*; it is likely this other gene was *vat(E)*, which was not identified at the time of this study. The data also suggested that the streptogramin resistance genes were presumably located on a plasmid that was transferred to the recipient strains.

Werner et al. (2000a) investigated the conjugative transferability of the streptogramin resistance determinants in 32 isolates from different animal (poultry meat, pig manure, and pork) and sewage sources. Fourteen isolates (5 with the *vat(D)* gene and

9 with the *vat(E)* gene) transferred their streptogramin determinant, whereas 18 isolates (5 with *vat(D)* and 13 with *vat(E)*) failed to transfer their resistance determinants in *in vitro* filter mating experiments. The *vat(E)* gene was plasmid-determined in resistant isolates from animal, sewage, and hospitalized patients. The authors also performed macrorestriction analysis on the QD-resistant *Enterococcus* isolates and found no related patterns among isolates from different origins, leading the authors to conclude that a clonal spread of resistance determinants in human is unlikely, and that horizontal gene transfer is the likely mechanism for spread of streptogramin resistance in enterococci.

Jacobsen et al. (1999) studied the horizontal transfer of the *vat(D)* gene between isogenic strains of *E. faecium* in the gastrointestinal tract of gnotobiotic rats. High numbers of transconjugants were observed throughout the experimental period of approximately 18 days, indicating horizontal transfer of the *vat(D)* gene. The rate and/or extent of transfer could not be determined as the experimental design did not permit a differentiation between a single horizontal transfer followed by growth of the transconjugant in the gastrointestinal tract and multiple consistent transfers between donors and recipients.

In a study in gnotobiotic mice, Moubareck et al. (2003) used *E. faecium* isolates of porcine origin harboring several different antibiotic resistance genes (conferring resistance to vancomycin, erythromycin, tetracycline, and streptomycin) to study horizontal gene transfer to a human fecal isolate *E. faecium*. As in the gnotobiotic rat study, horizontal gene transfer of resistance determinants was found to occur readily in gnotobiotic mice.

Jensen et al. (1998) examined streptogramin-resistant *E. faecium* isolates from healthy suburban residents, farmers, poultry, and pigs for the presence of the *vat(*D*)* and *vgb(*A*)* genes. In addition, the authors compared genotypes between these isolates as determined by pulsed-field gel electrophoresis (PFGE). PFGE-identical isolates with *vat(*D*)* genes were found in a farmer and his animals, leading the authors to conclude that transfer of streptogramin-resistant *E. faecium* between animals and humans occurs. Werner et al. (2000b), studying linkages between the *vat(*E*)* and *erm(*B*)* genes, found identical gene clusters in *E. faecium* isolates from animals and humans, again suggesting

the possible spread of the resistance genes via the food chain to humans. It is not known whether these findings represent the scenario in which resistance in human commensal *E. faecium* is the product of horizontal gene flow of the *vat(*D*)* gene from an animal streptogramin-resistant *E. faecium*, or the scenario in which there is transient colonization of the animal SREF in the human intestinal tract at the time samples were obtained for this study.

The most abundant evidence cited to support the transfer of streptogramin-resistant determinants in *E. faecium* from animals to humans is the observation that streptogramin resistance was found in humans prior to the introduction of the human therapeutic streptogramin, Synercid (see Table 4-2). Virginiamycin has been used in animals for more than 25 years, whereas Synercid use generally began in 1999, which suggests that streptogramin resistance observed in humans prior to 1999 was likely due to transfer of resistance determinants from animals, and that this transfer is a continuing process. There are several potentially confounding factors in assessing the extent that the prevalence of streptogramin resistance in human *E. faecium* can be attributed to animal agriculture via a foodborne pathway. For example, the reported differences in prevalence of SREF between European and US populations may be due to the use of the streptogramin drug pristinamycin in France well before the onset of Synercid use. Cross-resistance to other antimicrobials that may select for streptogramin resistance may be an important influence on SREF prevalence rates, but the extent of this influence is difficult to assess without additional data on mechanisms of resistance. Also, the methodological problems in obtaining accurate surveillance data result in increased uncertainty in comparing prevalence rates between animal and human populations. Thus, it is difficult to use prevalence rates to determine the significance and extent of transfer of resistance determinants from animals to humans.

An important consideration in assessing the relationship between streptogramin resistance in animals and humans is the different MIC distributions observed between animal and human isolates. Enterococci recovered from the poultry production environment and from poultry products at retail were significantly less sensitive to quinupristin/dalfopristin than human isolates. Isolates from poultry frequently display MICs to QD >16 μg/mL and many have MICs of 32 μg/ml. Few isolates with MICs of

this magnitude have been recovered from human sources, where most of the resistant isolates in the US have MICs equal to 4 µg/mL. These results are not consistent with a direct flow of resistance determinants from animal to human *E. faecium* and suggest that our understanding of the acquisition of streptogramin resistance by human-associated enterococci is less than complete.

4.6.3 Prevalence of Resistance Genes in Streptogramin-Resistant *E. faecium* Isolates

To further characterize the relationship between streptogramin use in food animals and the development of streptogramin resistance in *E. faecium* isolates from both animal and human sources, recent studies have examined resistant isolates for the presence of selected genes encoding resistance to the streptogramins. A summary of the available data on the prevalence of streptogramin-resistance genes in *E. faecium* isolates from farms, retail food products, and humans is presented in Table 4-4.

One striking finding is the distribution of the *vat(*D*)* and *vat(*E*)* genes on a geographical basis. Results from the poultry farm and retail food isolates from Europe generally show that *vat(*D*)* or *vat(*E*)* can be found in close to 100% of the examined resistant isolates, with *vat(*E*)* being more prevalent than *vat(*D*)*. However, no *vat(*D*)* was found in any isolates from similar sources in the US; nor was *vat(*D*)* found in any of US isolates, animal or human. Also, the prevalence of *vat(*E*)* in the US poultry isolates was somewhat less than that reported in the corresponding European isolates. Given the lack of *vat(*D*)* genes and lower prevalence of *vat(*E*)* genes, a large proportion of the streptogramin-resistant US isolates did not possess any known genes encoding resistance to streptogramin A compounds. As discussed previously, resistance to streptogramin A compounds is believed to be necessary for resistance to the quinupristin/dalfopristin combination drug. A further unexplained set of observations is that Soltani et al. (2000) reported that isolates with either *vat(*D*)* or *vat(*E*)* had high level QD resistance (MIC ≥ 32 µg/mL) and isolates with lower MICs did not have either of the two genes, yet Simjee et al. (2002) reported finding *vat(*E*)* in resistant isolates with MICs ranging from 4 to 16 µg/mL. These findings suggest both the existence of undefined mechanisms of streptogramin resistance and the need for additional study on the mechanisms of streptogramin resistance.

The data on the pig and human isolates is difficult to interpret; in some studies *vat(*D*)* or *vat(*E*)* was found in a large majority of resistant isolates, whereas in other studies *vat(*D*)* or *vat(*E*)* was found in no more than 14% of the examined isolates. The lone study from the US found no *vat(*D*)* or *vat(*E*)* in any of the swine isolates, and a lone *vat(*E*)* and no *vat(*D*)* among the human isolates. As for the poultry samples, it appears that mechanisms of resistant yet to be characterized are involved in the streptogramin resistance observed in the pig and human isolates.

One finding that was similar between the European and US isolates from all sources was the high prevalence of the *erm(*B*)* gene in the streptogramin-resistant isolates, although the available US data on *erm(*B*)* prevalence is limited to a single study on retail poultry products. Interestingly, only one other streptogramin-resistance gene was found: one isolate from a farmer in The Netherlands had the *vgb(*A*)* gene in addition to *vat(*E*)*. The lack of presence of the *vgb(*A*)* gene is inconsistent with the observations from *in vitro* studies that both a gene encoding resistance to streptogramin A compounds (e.g., *vat(*D*)* or *vat(*E*)*) and a gene encoding resistance to streptogramin B compounds (e.g., *vgb(*A*)* but not *erm(*B*)*) is required for QD resistance (Bozdogan and Leclercq 1999). However, a number of studies have reported finding a link between *erm(*B*)* and *vat(*D*)* or between *erm(*B*)* and *vat(*E*)* (Hammerum et al., 2001; Jensen et al., 2000, 2002; Werner et al., 2000b). Werner et al. (2000b) hypothesize that the *in vitro* constructs of *E. faecium* may not reflect the situation in natural isolates where *erm(*B*)* and *vat(D)/(E)* are sufficient for streptogramin resistance. The authors also suggest that the recently identified *msr(*C*)* gene, which is thought to encode an efflux pump that mediates MLS_B resistance (Portillo et al., 2000), may contribute to the streptogramin B resistance needed for the expression of QD resistance. Werner et al. (2000b) found the *msr(*C*)* gene in 59% of the resistant isolates examined in their study.

Table 4-4. Prevalence of Streptogramin Resistance Genes in Resistant Isolates from Farms, Retail Meats, and Humans

Animal	Isolate Source	Number of Resistant Isolates	Prevalence of resistance gene (%) among resistant isolates										Reference
			vat(D)	*vat*(E)	*erm*(B)	*vat*(A)	*vat*(B)	*vat*(C)	*vga*(A)	*vga*(B)	*vgb*(A)	*vgb*(B)	
Farm Isolates													
Chickens	US	56	0	25							0		Zervos et al., 2003
Turkey	US	74	0	18							0		Zervos et al., 2003
Poultry	The Netherlands	22	18/14[1]	86		0	0	0	0	0	0	0	Jensen et al., 1998 and Haroche et al., 2000
Poultry	Denmark	140	10[2]	89[2]	84						0		Jensen et al., 2002
Poultry manure	Germany 1998-1999	17	0	100									Werner et al., 2000a
Broilers	Denmark	146	11	72	88[3]								Aarestrup et al., 2000
	Finland	9	0	100	88[3]								
Broilers	Denmark; 1995-1996	48	35			0[4]	0[4]		0[4]	0[4]	0[4]		Hammerum et al., 1998
Pigs		41	12										
Pigs and chickens	UK, EU	18	33	39	≥ 75	0	0	0	0	0	0	0	Soltani et al., 2000

Animal	Isolate Source	Number of Resistant Isolates	Prevalence of resistance gene (%) among resistant isolates										Reference
			vat(D)	*vat*(E)	*erm*(B)	*vat*(A)	*vat*(B)	*vat*(C)	*vga*(A)	*vga*(B)	*vgb*(A)	*vgb*(B)	
Pigs	Denmark	28	7	7	86						0		Jensen et al., 2002
Pigs	The Netherlands	5	20/60[1]	40		0	0	0	0	0	0	0	Jensen et al., 1998 and Haroche et al., 2000
Pigs	Denmark	27	7	7	88[3]								Aarestrup et al., 2000
	Finland	1	0	0	88[3]								
Pigs	Denmark	46	2	4									Aarestrup et al., 2002a
	Spain	88	6	6									
	Sweden	1	0	0									
Swine	US	59	0	0									Zervos et al., 2003
Pig manure	Germany 1998-1999	21	48	52									Werner et al., 2000a
Cattle (Beef)	US	3	0	0							0		Zervos et al., 2003
Cattle (Dairy)	US	51	0	0							0		Zervos et al., 2003
Retail Food Isolates													

Animal	Isolate Source	Number of Resistant Isolates	Prevalence of resistance gene (%) among resistant isolates										Reference
			vat(D)	*vat*(E)	*erm*(B)	*vat*(A)	*vat*(B)	*vat*(C)	*vga*(A)	*vga*(B)	*vgb*(A)	*vgb*(B)	
Retail poultry (chicken carcasses and turkey breasts)	US 1999-2000	27	0	44	41	0	0	0	0	0	0	0	Simjee et al., 2002
Broiler carcasses	Germany 1998-1999	20	35	65									Werner et al., 2000a
Pork	Germany 1998-1999	1	0	100									Werner et al., 2000a
Raw meat	UK, EU	4	0	75	≥ 75	0	0	0	0	0	0	0	Soltani et al., 2000
Isolates From Human Sources													
Human	US	27	0	3.7							0		Zervos et al., 2003
Human/ Food Handlers	Spain	12	25	0									Del Campo et al., 2003
Human/ Volunteers	Spain	12	0	0									Del Campo et al., 2003
Human/ Community	The Netherlands	5	80	20		0	0	0	0	0	0	0	Jensen et al., 1998 and Haroche et al., 2000
Farmers	The Netherlands	19	53/47[1]	53		5[5]	0	0	0	0	5[5]	0	Jensen et al., 1998 and Haroche et al., 2000
Human/ Hospital patients	UK, EU	4	0	100	≥ 75	0	0	0	0	0	0	0	Soltani et al., 2000

Animal	Isolate Source	Number of Resistant Isolates	Prevalence of resistance gene (%) among resistant isolates										Reference
			vat(D)	*vat*(E)	*erm(*B)	*vat*(A)	*vat*(B)	*vat*(C)	*vga(*A*)*	*vga*(B)	*vgb(*A*)*	*vgb*(B)	
Stool samples from outpatients	Germany 1998-1999	30	40	50									Werner et al., 2000a
Hospitalized patients	Germany 1998-1999	36	64	25									Werner et al., 2000a
Sewage	UK, EU	2	0	0	≥ 75	0	0	0	0	0	0	0	Soltani et al., 2000
Sewage	Germany 1998-1999	23	52	48									Werner et al., 2000a

[1] The same isolates were used in the two studies; the second number is from Haroche et al. 2000

2 Three isolates contained both vat(D) and vat(E)

[3] The *erm(*B) gene was observed in 88% of all the isolates in the study

4 Testing performed on 6 isolates that were vat(D) negative and in which resistance was transferred in vitro

5 vat(A) and vgb(A) detected in same strain and were contiguous, vat(E) was detected in same isolate, but data suggests not carried by same plasmid

4.7 Data Gaps in Exposure Assessment

The exposure assessment is qualitative and it is often argued that qualitative approaches contribute relatively more uncertainty in risk assessment than do quantitative methods. On the other hand, quantitative methods that are employed in the face of significant uncertainties can lead to perceptions of unwarranted *certainty* in the data and models. Other sections of the risk assessment, such as the risk estimation, suggest that the conclusions based in part on qualitative exposure assessment are likely to be overly conservative. Thus, the improvement of exposure assessment with quantitative information is likely to lead to downward characterizations of the likelihood of human exposures.

The type of information that would be useful in adopting a more quantitative approach to the exposure assessment is molecular genetics data that would allow the tracking of resistance determinants from retail meat products to human bacteria populations. This type of data is not currently available for streptogramin resistance in *E. faecium*, leaving the exposure assessment to draw conclusions based on the biological plausibility of occurrence of certain events in the flow of resistance determinants, and on a summary of surveillance studies, which may have high levels of uncertainty in their reported data (see Section 4.5.1). However, even these conclusions could be strengthened by an improved understanding of the mechanisms of streptogramin resistance.

4.8 Conclusions

Data from surveillance studies provides ample evidence that streptogramin resistance determinants in *E. faecium* are present on retail meats and may contribute to direct human exposures. Data from *in vitro*, *in vivo* animal models, and animal/human molecular genetics studies suggest that streptogramin resistance is transferable among *E. faecium* from different sources, and that zoonotic streptogramin-resistant *E. faecium* may serve as a reservoir of resistance genes for human *E. faecium*. The majority of surveillance studies on human isolates suggest a background incidence of streptogramin resistance in *E. faecium* isolates in the range of 0 to 4%, which may be due to transfer of resistance from animal sources. However, due to our incomplete understanding of the

mechanisms of streptogramin resistance, it is difficult to assess the likelihood and extent that such transfer occurs and to quantify the impact of zoonotic-based streptogramin resistance on the occurrence of resistance in humans.

5 CONSEQUENCE ASSESSMENT

5.1 Purpose

Consequence assessment is a description of the relationship between specified exposures to hazardous agents and the consequences of those exposures. It is also known as "hazard characterization" in the Codex Alimentarius model and "dose-response assessment" in the National Academy of Sciences model for risk assessment (NRC 1996). Consequence assessments are intended to estimate the numerical probability of a given adverse health consequence, given the dose of hazardous agent. For this purpose, a causal process is assumed to exist between exposures to hazardous agents and increased risks of adverse health effects among the exposed populations. Although consequence assessment has its origins in classical dose-response assessment, the contemporary meaning of consequence assessment includes qualitative analyses of an array of potentially adverse consequences from exposure to a presumptive or known hazardous agent.

5.2 Introduction

Discussed previously is the fact that the streptogramin resistant *Enterococcus* risk assessment is between qualitative and quantitative risk assessments in execution. Although it is generally accepted that the colonization of individuals by antimicrobial-resistant bacteria leads to an increased risk of resistant microbial infection, there is a dearth of data that can be used to derive quantitative dose-response relationships for a consequence assessment. The missing information includes the numbers (infective dose) of resistant bacteria needed to colonize members of the population, the rates of transfer of various resistant determinants among bacteria resident in the human intestines, and the persistence of resistance determinants in the absence of selective pressure from antimicrobials. Indeed, microbiological risk assessment (MRA), in general, suffers from significant model and data uncertainties that often obviate the use of mechanistic dose-response relationships in favor of prevalence models. For example, at least six different quantitative dose-response relationships have been cited as potential candidates for use in

MRAs (Holcomb et al., 1999; Buchanan et al., 2000), yet seldom are there sufficient data to rigorously support a particular model as more likely than the alternatives.

There are several potential conceptual models for dose-response relationships between antimicrobial-resistant cells and the occurrence or likelihood of disease. For microbes that have acquired resistance to antimicrobial drugs, the resistant cells are seldom represented by the greater proportion of the particular species. In theory, the courses of infection that end in resistance to antibiotic treatment begin as a mixture of susceptible and resistant cells. Generally speaking, susceptible cells greatly outnumber resistant cells because there is a net metabolic cost to the cell to maintain resistance to a drug. In the face of an antimicrobial drug, however, the resistant portion of the infective population has a selective advantage and might overtake in abundance the susceptible portion of the population (Figure 9). At some point, the infection becomes clinically resistant to therapy with the drug leading to a need for alternative therapies. The key influences on the overgrowth of the resistant population will depend on the host factors, the rate of antimicrobial drug usage with cells and the proportion of resistant cells in the original infection. The endpoints that are of most utility in the estimation of consequences and, subsequently, of risk, are those endpoints associated with the population acquisition of resistant infections.

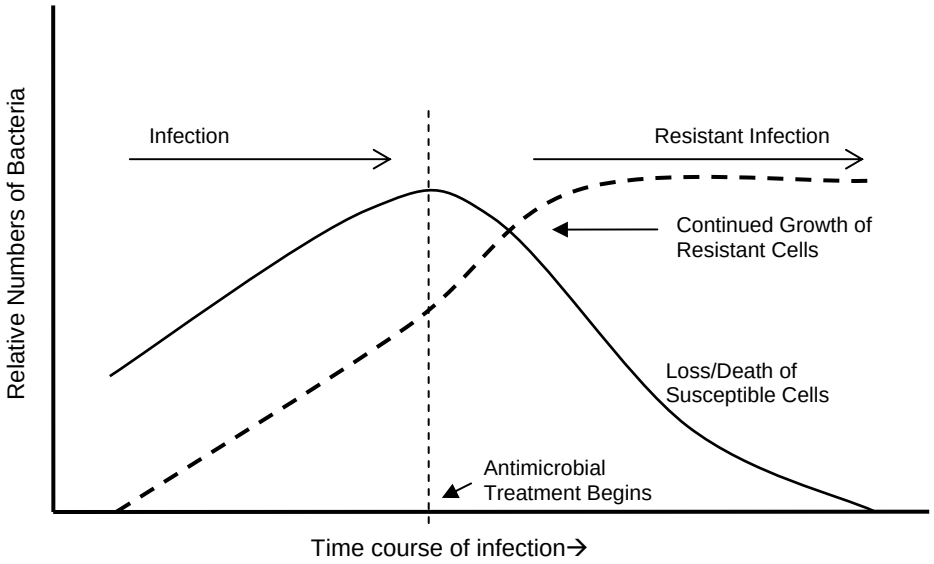

Figure 9. Schematic of mixed infection: susceptible and resistant Cells. Generally, drug resistant cells are initially a small proportion of an infecting population. The exposures to antimicrobial drug selects for resistant cells while population growth of susceptible cells is inhibited.

The potential health endpoints for consequence assessment are shown in Table 5-1. For reasons discussed at length in the preceding chapters, all of the endpoints are ambiguous in terms of identifying causal pathways except the final endpoint in the table, "Transfer of Resistance to Human Bacteria." Unequivocal molecular genetic evidence for animal bacteria origins of streptogramin resistance among human-adapted *E. faecium* has yet to emerge. The consequence pathway is established primarily by a surrogate argument using the avoparcin and vancomycin literature for the European experience. However, extrapolation from the avoparcin-vancomycin experience should be made with caution, given the relative genetic simplicity of vancomycin resistance in face of the known genetic complexity of streptogramin resistance.

Given the myriad of supportive and contravening evidence for the causal pathway in this risk assessment, and the total absence of dose-response relationships for antimicrobial-resistant infections generally and specifically for Synercid resistance, it is unlikely that a quantitative dose-response relationship between the titer of QD resistant cells and a consequence will be of any value in deriving a quantitative model.

Table 5-1. Potential Endpoints for Consequence Assessment

Agent	Consequence	Estimated By
Antimicrobial Drug	Drug Resistance among treated animals	Prevalence among treated herd
Antimicrobial drug-resistant animal or human bacteria	Colonization of the gut by antimicrobial drug-resistant bacteria	Prevalence of carriers of resistant bacteria
	Drug-resistant, subclinical infection	Inferred by resistance in isolates
	Drug-resistant, clinical infection	Resistant isolates
	Mortality from infection	Identification of first cause of mortality
	Chronic disease	Unknown
Antimicrobial drug-resistant animal bacteria	Transfer of resistance to human bacteria	Molecular genetic observations of animal resistance marker in human host-specific bacteria

The likelihood of successful infection in an individual or in a group is determined by host, pathogen and environmental factors. This "infectious disease triad" is a fundamental concept for understanding dose-response relationships in infectious disease. Factors under each category work in combination to determine the likelihood that an individual will experience a given consequence, such as transient colonization, subclinical infection, frank clinical infection or mortality. The factors that are important to consider in the foodborne pathway are described in Table 5-2. These factors can influence both susceptibility and severity of an infection.

Table 5-2. Host, Pathogen and Environmental Factors Affecting Consequences of Microbial Pathogen Exposure

Host	Pathogen	Environment[1]
Age (Very young and the elderly)	Generation time (microbial)	Salt and water content
Chronic Diseases	Pathogenic or toxigenic	pH
Immune-suppressed or Immuno-compromised	Spore-forming or not	Fat content
Nutritional state	Virulence factors	Buffering capacity
Alcoholism	Microbial adaptation and tolerance	Matrix characteristics: solid, liquid, emulsion…
Multiple infections	Genetic transfer	
Cell receptors	Pathogenic patterns: latency, infectivity, etc.	

[1] Particularly focused on properties of the food as environmental medium.

5.3 Consequence Assessment for Opportunistic Pathogens

Consequence assessment for adverse health consequences that might occur from commensal bacteria presents special problems in analysis. By definition, human commensal bacteria are resident among human intestinal flora and are *not* pathogenic except when presented with the opportunity for infection. Humans carrying a subpopulation of antimicrobial resistant cells represent a reservoir for self infection as well as potential transmission vectors for spread to other humans. A classical dose-response relationship (e.g.) between the ingested bacteria quantity and the probability of illness is not meaningful in this context as it is in the usual application in risk assessment. Rather, a steady state prevalence of commensal bacteria is anticipated whose numerical value will depend on host, pathogen and environmental factors such as those presented in Table 5-2. Given the commensal property of enterococci, it can be hypothesized that the proportion of individuals "exposed" to human *E. faecium* is up to 100% of the population. The proportion of the population who are transiently colonized by food animal *E. faecium* will depend on exposure factors (Exposure Assessment) and the

factors identified in Table 5-2. Given the potential ubiquity of both animal and human *Enterococcus* among the human population, it is likely that neither of these proportions can adequately inform a consequence assessment.

The most logical and informative dose metric for consequence assessment would be the number of resistant bacteria necessary to elicit a resistant infection. Since this number is seldom sought or indirectly measured in the nosocomial disease literature, a quantitative consequence assessment cannot be accomplished at this time. Rather, this risk assessment uses a population-based event model as opposed to a mechanistic dose-response relationship in which disease outcomes are predicted as a function of the dose or concentration of bacteria in the body. A population-based event model is ecological in design, leading to usual caveats about the likelihood of causal pathways. Additionally, event probability models lead to blurring of the border between consequence and risk assessment because the estimate of a particular consequence is closely related to the desired risk estimate. Therefore, Risk Estimation (Chapter 6) will discuss both consequence and risk.

6 RISK ESTIMATION

6.1 Purpose

The risk estimation integrates the results from the release assessment, exposure assessment, and consequence assessment to produce an overall estimate of the risk. All three elements of the risk assessment process are important contributing factors and should be integrated and considered as a whole when assessing the risk.

6.2 Introduction

Given the epidemiological nature of data available for this risk assessment and the lack of established, mechanistically-derived quantitative dose-response relationships, this risk assessment relies on probability calculations for the purpose of risk estimation. Probability calculations underlying epidemiological methods are used throughout public health risk assessments in situations where exposures to hazardous agents are recognized and the risks of adverse health effects are statistically associated with "membership" in the exposed group(s). These methods are appropriate for estimating population risks associated with previous exposures to the hazard in question. The predictive nature of risk-based decision making in public health requires that risk managers draw from epidemiological analyses and address the "what if?" scenarios about future adverse health risks given the potential for future exposures.

The novelty of antimicrobial resistance risk analysis (ARRA) and the uncertainties in the data and models for ARRA, lead to a milieu where any number of models might be proposed for risk estimation. At this early stage in ARRA, the judgment of the quality of alternative risk estimation models will depend on the quality of available data, the plausibility of the logical pathway defined in the model and the model's ability to match known empirical results at intermediate stages of the risk estimation. For the purposes of the present risk assessment, three models have been derived, the first of which relies on epidemiological surveillance of ICUs and data from the nosocomial infection literature; a second model that derives risk estimates from the usage rates of Synercid to treat resistant infections; and a third model that estimates risk beginning with septicemia cases reported in National Center for Health Statistics (NCHS) databases.

The formal derivation of all three models is explained as are the bases of initial parameterization. Uncertainty estimates are made using simple Monte Carlo methods.

This risk assessment seeks an estimate of the number of cases of streptogramin-resistant *Enterococcus faecium* (SREF) bacteremias where the streptogramin resistance is potentially linked to food animal uses of related streptogramin antimicrobial drugs. To date, the only quinupristin-dalfopristin (QD) streptogramin mixture approved for use in humans in the US is Synercid®. For reasons discussed previously (Chapter 2, Hazard Identification), this risk assessment focuses on *E. faecium* bacteremias (bloodstream infections, or "BSIs") as opposed to complicated skin and skin structure infections involving other gram-positive strains of bacteria.

The human health risk of failing streptogramin treatment, as an adverse health impact from streptogramins used in animal agriculture, includes a "gate keeping" step of vancomycin resistance because Synercid drug approval is for VREF bloodstream infections. Individuals having VREF represent the subset of the US population who are at risk of the adverse health consequence defined for this risk assessment—the loss of efficacy of streptogramin antimicrobials (Synercid) against VREF infections. In addition to its role as a necessary intermediate step in the estimation of the population of individuals who might receive streptogramin therapy, the number of VREF cases in a given time period also represents a logical upper limit on the number of cases that are "at risk" of streptogramin therapy. This upper limit, in turn, is an upper limit on the number of individuals who might receive streptogramins *and* suffer a loss of efficacy of streptogramin antimicrobials. Models 1 and 3, discussed in the following sections, rely on estimates of VREF cases as intermediate steps in the risk assessment.

6.3 Model 1: ICU Bloodstream Infections for Risk Estimation

As previously discussed, VRE and particularly VREF infections occur almost exclusively in hospital settings in the US. In particular, VREF bloodstream infections are associated with pre-existing serious illnesses and time spent in hospital intensive care units (ICUs). A quantitative risk assessment for the risk of VREF bloodstream infections would be derived from information on the quantity of hazardous agent, the duration of exposure to the agents, the estimate of dose given the exposure quantity and its duration,

and the estimate of the likelihood of the response (infection) given the dose. In the present study, the kinds of data that can inform a classical dose-response relationship (i.e., consequence assessment) are either non-existent or are too sparse to be of value in quantitative model. Therefore, surrogate measures of exposure and dose have been sought in order to estimate chances of various adverse outcomes.

There are several ways that publicly available data sources can be used to estimate the number of VREF cases in the US during a given year. Model 1 is an epidemiological approach that relies on data from the NNIS system, the NCHS and epidemiological data from the peer-reviewed scientific literature. Ultimately, this information could be supplemented by results from intramural and extramural research sponsored by the FDA and other agencies.

One of the fundamental relationships in the risk estimation is that the number of nosocomial infections is proportional to the days at risk of infection in the hospital. Studies of nosocomial infections have identified *time* in critical care as a risk factor for nosocomial infections (Moellering 1999); however, it is also recognized that nosocomial infections can prolong hospital stays.[5] The number of infections, in turn, is related to the "*Time in ICU*" variable by a proportionality constant, λ (lambda), by the following equation:

$$Expected\ Infections = \lambda_{inf} \times Time\ in\ ICU$$
$$Expected\ Infections = \lambda_{inf} \times t_{ICU} \qquad \textbf{Eq. 1}$$

where λ_{inf} is the rate of infection in a specific hospital unit expressed in units of "infections per patient per day," and *Time in ICU* (t_{ICU}) is the number of days that the patient is in the specific ICU. In order to use this model in risk estimation, estimates of both the rate of infection and the numbers of days at risk are needed from epidemiologic surveillance databases.

[5] Some studies provide evidence that longer hospital stays with serious medical treatments increase risk of nosocomial infections. A reverse causal pathway is also possible, since nosocomial infections are serious medical conditions that, in turn, also might prolong hospital stays.

6.3.1 Estimation of the rate of bloodstream infection, λ_{inf}

The NNIS system of the CDC surveys hospital-based infections from more than 200 member hospitals (Richards et al., 2001). The hospitals joining the NNIS must have 100 beds or greater and at least one full time equivalent (FTE) infection control professional for the first 100 beds (and 1 FTE professional for each additional 250 beds). Since the database is constructed using voluntary hospital data submissions, as opposed to a random sample of all hospitals, it is by definition a potentially biased sample of ICU use and infection rates with respect to the entire population of US hospitals. In fact, in a recent characterization of the NNIS database, Richards et al. (2001) reported that NNIS hospitals were larger in both total hospital beds and in the average daily census (Table 6-1).

Table 6-1. Total hospital beds and average daily census (comparison of National Nosocomial Infections Surveillance [NNIS] hospitals with all US hospitals with 100 or more beds).[1]

Characteristic	N	Median	Interquartile range
Total hospital beds[2]			
NNIS system	227	360	250-500
US hospitals[3]	3321	210	141-333
Average Daily Census[2]			
NNIS system	221	239	150-218
US hospitals[3]	3321	133	82-350

[1] From Richards et al., (2001).

[2] P value ≤ 0.0001

[3] US Hospitals data for hospitals with 100 or more beds from the American Hospital Association Annual Survey, 1997.

Within specified categories of data, useful estimates of infection rates and other data can be obtained from the NNIS database. For example, NNIS reports infection rates for each type of ICU and for procedures used in the ICUs (NNIS, 2002). Device

infection (*IR*) rates are reported as the number of infections divided by the number of device days during which the patient is at risk of exposure from the resistant bacteria. Device utilization ratios are the ratios of the total number of days that patients are on the particular device divided by the total number of days that the patients are in the ICU. These formulae are

$$Device\ infection\ rate = IR = \left[\frac{number\ of\ infections}{1{,}000\ device \cdot days}\right]_{ICU,Device} \quad \textbf{Eq. 2}$$

$$Device\ \ utilizatio\ n\ ratio = DU = \left[\frac{number\ \ of\ \ device\ \ days}{patient\ \cdot days}\right]_{ICU,Device} \quad \textbf{Eq. 3}$$

In these equations (Eqs. 2-3), the subscript variables are indexes for the type of intensive care unit (*ICU*) and the type of medical invasive device (*Device*), such as urinary catheters, ventilator tubes, or venous catheters. It is important to note that "patient days" in Eq. 2 refers to days in the ICU, not the total days that the patient is hospitalized in either ICUs or other inpatient areas. Presently, the NNIS database indexes three major types of invasive medical devices: central (venous) lines, urinary catheters and ventilators. In its highest resolution, ICU type is one of 10 types of ICU: coronary, cardiothoracic, medical, medical-surgical, neurosurgical, pediatric, surgical, trauma, burn, and respiratory (NNIS, 2002). Although the database indexes and surveys all 10 kinds of ICUs, the corresponding data for infections, in terms of the strain of bacterium causing the infection, are less well-resolved. In order to make linked calculations for this risk assessment, it is necessary to restrict the index to the general term of "ICU" rather than the specific categories of medical, surgical, pediatric, etc., defined in the NNIS. Using the database estimates of IR and DU, we can obtain estimates of lambda for the ICU and device in question:

$$\lambda_{ICU,Device} = IR_{ICU,Device} \times DU_{ICU,Device} \quad \textbf{Eq. 4}$$

6.3.2 Estimation of the days at risk of SREF bloodstream infections

The major information missing for risk estimation in this and related methods is a direct estimate of the number of patients in the ICUs. In other words, given that a person is hospitalized, what is the probability that the person is in the ICU? This is clearly an

age-dependent probability, most likely decreasing soon after birth and eventually increasing with age of the patient later in life. Although the NCHS compiles age-categorized data for the number of hospital visits in a given year, the data are not specific enough to sub-classify the type of hospitalization by time spent in ICU or non-ICU wards. Other approaches are needed to estimate the number of persons in the ICU in a given year including using bed numbers or average stay as surrogate estimates of persons hospitalized in ICUs.

The NNIS database reports only the total (integral) number of ICU patient-days recorded in the database, from 1992 through June 2002. In order to generate annualized estimates for the time spent in ICUs, an intuitive approach is to simply divide the total ICU patient-days divided by the number of years; however, this approach is expected to produce a non-representative estimate of the time due to factors in the design and implementation of the NNIS system. For example, the NNIS database was not designed as a research database, but as an epidemiological surveillance tool.[6] Because the total sampling frame was not established at the outset and the number of ICUs in the survey was not constant during the period from 1992 to the present, the derivation of the ICU patient-days in one year from the ratio of the total ICU patient-days divided by the number of years is likely to introduce a significant source of uncertainty in the estimate of the annual rates of infection. Alternatively, estimates of the total hospital beds and ICU beds are relatively current and contemporary with the recent NNIS summary reports.

The estimated proportion of time in the ICU might be derived from the nationally estimated number of hospital-days and the proportion of beds devoted to the ICU,

[6] The comments on the NNIS system in no instances are intended as criticisms of the surveillance system. Rather, this discussion highlights the difficulties faced in using a surveillance tool for risk analysis. The NNIS was designed essentially for hazard identification—a health *surveillance* purpose. The optimal design of a database for risk analysis would be to randomly sample ICUs and hospitals for the incidence of infection classified by ICU, bacteria species and type of invasive device.

$$\text{Prob(ICU)} \quad \propto \quad \frac{(\text{Number of ICU beds})}{(\text{Total Hospital beds})} \qquad \textbf{Eq. 5}$$

$$p_{ICU} \quad \approx \quad F_{ICU}$$

where the symbol ∝ means "is proportional to." For the present risk assessment, p_{ICU}, the expected probability that the patient is in the ICU (during a fixed period of time), is estimated from F_{ICU}, the proportion of hospital beds devoted to the ICU. F_{ICU} can be corrected for the proportions of *patient-occupied* beds in the ICU using the occupancy rates for the beds (Eq. 6). The probability, during a fixed period of time, a patient is in the ICU might be approximated from the ratio of bed types using the hospital survey databases. For example, the chance that a patient is in the ICU might be derived from the ratio of beds:

$$F_{ICU} \approx \frac{(ICU\ beds) \times (Occupancy\ rate,\ ICU)}{(Non\text{-}ICU\ beds) \times (Occupancy\ rate,\ Non\text{-}ICU) + (ICU\ beds) \times (Occupancy\ rate,\ ICU)} \qquad \textbf{Eq. 6}$$

Again, the NNIS data inform part of this relationship—the median number of ICU and Non-ICU beds. The occupancy rate for non-ICU beds is estimated from data in Table 6-1, 239/360 = 66%. Based on literature reports that show demand for ICU beds often exceeds supply (Lantos et al., 1997; Sprung et al., 1999), we might *assume* that the ICU bed occupancy rate approaches 100%. The median estimate of the proportion of occupied beds that are in the ICU, is 25.6% based on the NNIS data reported in 1999.

The estimate of hospital time (t_{hosp}) is derived from data reported in the National Hospital Discharge Survey (NHDS) from the NCHS of CDC (Hall and DeFrances 2003). The NHDS reported that the number of hospital discharges (n_{dis}) during the year 2001 was 32.7 million (32.7×10^6) discharges. The average hospital stay per discharge (t_{hosp}) was reported to be 4.9 days according to the same NHDS data. Taken together, the estimated number of infections per year related to ICUs is given by

$$n_{Inf} = \left(IR \times DU\right)_{ICU,\,Device} \times t_{hosp} \times n_{dis} \times P_{ICU} \qquad \textbf{Eq. 7}$$

where n_{Inf} = number of ICU infections per year; and
IR = device infection rate, in infections per 1,000 line-days;
DU = device utilization ratio (device days per patient day in the ICU);
t_{hosp} = average number of hospital days per hospital discharge;

n_{dis} = total number of discharges in a year; and
P_{ICU} = proportion of hospital discharges associated with the ICU.

The first group of terms within the parentheses is equivalent to the estimate of the rate, λ_{Inf}, and the second set of terms estimate the time spent in the ICU, t_{ICU} (Eq. 1).

6.3.3 Estimation of Streptogramin-Resistant *Enterococcus faecium* Cases

The specific population at risk of failing streptogramin therapy for VREF infections is the population of VREF cases which is itself a subset of VRE cases. The number of VREF cases per year can be estimated by the following equation:[7]

$$N_{VREF} = N_{Inf} \times P_{Ent,Inf} \times P_{EF,Ent} \times P_{VR,EF} \quad \textbf{Eq. 8}$$

where N_{VREF} = number of VREF infections in one year;
N_{Inf} = total bacterial infections in one year;
$P_{Ent,Inf}$ = probability that the bacteria are enterococci, given that there is an infection;
$P_{EF,Ent}$ = probability that the infection is E. faecium, given that it is an Enterococcus infection; and
$P_{VR,EF}$ = probability that the E. faecium infection is vancomycin-resistant, given that it is an E. faecium infection.

In a detailed model, the terms in Eq. 8 might be indexed by the type of ICU, the type of medical device and other relevant index variables. The probabilities in Eq. 8 are estimated from prevalence data reported in the scientific literature or various public health databases. The same data sources used for the estimation of VREF cases can provide information useful for the estimation of the number of SREF cases in a year. Again, continuing the basic probability calculations, we have:

$$N_{DREF} = P_{SR,VREF} \times N_{VREF} \quad \textbf{Eq. 9}$$

where N_{DREF} = the estimated number of cases of doubly-resistant *E. faecium* (DREF) infections in one year, and

[7] By convention, the symbol for variable definition is in upper case (e.g., "N_{VREF}") while an estimate of the value is written in lower case (e.g., n_{VREF}). The exceptions, for clarity purposes are *IR* and *DU*.

$P_{SR,VREF}$ = the probability of streptogramin resistance, given that the *E. faecium* infection is vancomycin resistant.

Low et al. (2001) reported that about 82% of the vancomycin-resistant *Enterococcus* isolates were also susceptible to quinupristin-dalfopristin (QD) streptogramins. A crude estimate of the percent of the VRE that have some level of QD resistance (or "SRE") is 100-82 = 18%.[8] Intrinsically QD-resistant *E. faecalis* strains comprise about 73% and *E. faecium* about 25% of Enterococcus strains in isolates that have been have been identified in the SENTRY sample. The balance (≈ 2%) of the identified clinical isolates in the SENTRY sample included the uncommon strains*, E. avium, E. casseliflavus, E. durans, E. flavescens, E. gallinarum,* and *E. raffinosus*.

The final probability estimate needed to attribute SREF to food animal sources is $P_{SR,EF}$, the probability of Synercid resistance among the population of individuals carrying *E. faecium*. The expected value of this variable would optimally be obtained from an epidemiological study *designed* to attribute SREF to prior exposures to food and other sources of streptogramin resistance. Since no such studies are available, two assumptions are necessary to parameterize this variable for the simulations. The first assumption is that all humans carry *E. faecium*. The second assumption made for this study is that *all* streptogramin resistance in the non-hospitalized community is due to food animal uses of virginiamycin. Thus, the community resistance between 0 and 4% is used as a first estimate of probability in the attribution pathway. This model distribution is further narrowed to between 0.4 and 4% based on the measurements of community resistance prior to Synercid (~ 0.4 %).

6.3.4 Attribution of Risk to Food Animal Sources of Streptogramin Resistance

Perhaps the greatest uncertainty in any of the models proposed for this risk assessment is the fraction of cases that might be attributed to streptogramin resistance originating in food animal *E. faecium*. As discussed in previous chapters of this report, resistance determinant transfer has been identified in test tube studies using

[8] Because determinations of "resistance" and "susceptibility" among bacterial isolates are often made under differing experimental conditions and theoretical assumptions, the equation, (Proportion of susceptible isolates) = 1- (Proportion of resistant isolates), is not necessarily true.

Enterococcus. On the other hand, results of studies on human volunteers showed that vancomycin resistant *E. faecium* from animal sources fails to colonize humans (Sorensen et al., 2001). Additionally, virulence factors are less frequent in *E. faecium* from animal isolates than those from human isolates (2% versus 35-42%; Hammerum et al., 2002), suggesting that host specificity is important. Finally, Willems et al. (2000), studies on VREF isolates in Europe estimated that the upper bound on transfer from food animals to hospitalized groups is 11.5%.

For the purposes of informing risk management decisions, a central estimate of 10% is used for the probability of origination in food pathways. This estimate was derived from Willems et al. (2000), in which 255 VREF strains were examined in a study designed to identify sources of VREF. Assuming that antimicrobial resistance, per se, does not affect the probability of occurrence of enterococcus in a given food pathway, the VREF study was used as a surrogate estimate for SREF in the models. In order to reflect uncertainty about this estimate, a triangular distribution between 0 and 20% and peaking at 10% is used in the simulations (Table 6-2). The parameter distributions are for illustration purposes only, and other estimates can be proposed and analyzed to provide alternative risk scenarios.

6.3.5 Summary Parameter Estimates for Model 1

Model 1 implements a Monte Carlo simulation in order to propagate uncertainty through the calculations. Each iteration in the simulation performs a calculation using single point estimates of the variables drawn from the model probability density functions (distributions). Thousands of iterations are typically used in order to develop a distribution for the expected numbers of cases (or, subsequently, the risk) from the input parameters. The initial estimates of the parameters and the sources of the estimates are given in Table 6-2.

Table 6-2. Starting Parameter Estimates for Model 1

Variable[1]	Description	Mean Estimate	Distribution and Parameters (θ_1, θ_2)	Source
IR	Device Infection Rate per 1000 patient-days	3.50	Normal(3.50, 1.00)	Weighted average of ICU data reported in NNIS (2002).
DU	Device Utilization Rate	0.727	Normal(0.727, 0.057)	Weighted average of ICU data reported in NNIS (2002).
$n_{hospbed}$	No. of Hospital Beds	237	Normal(237,72), Truncated at <100	Richards et al., 2001
n_{ICUbed}	No. of ICU Beds	39.5	Normal(39.5,15), Truncated at <1	Richards et al., 2001
n_{dis}	Hospital Discharges in one year	32.7×10^6	Normal(32.7M, 1.05M)	Hall and DeFrances 2003
t_{hosp}	Length of Stay in Hospital	4.9	Normal(4.9, 0.1)	Hall and DeFrances 2003
p_{ent}	Probability ICU infection is *Enterococcus spp*.	0.0993	Beta(1723, 15680)	Low et al., 2001
$p_{EF,ent}$	Probability Enterococcus infection is *E. faecium*	0.250	Beta(480, 1444)	Low et al., 2001
$p_{VR,EF}$	Probability *E. faecium* infection is vancomycin-resistant	0.5	Beta(8,8)	Low et al., 2001
$p_{SR,EF}$	Community prevalence of SREF	0.022	Uniform(0.004,0.04)	Range of values discussed in this report.
p_{trans}	Food-Attributable Fraction	0.1	Triangular(0, 0.1, 0.2)	Range of values discussed in this report.

[1] In general, upper case is used for the theoretical variable, and a lower case symbol represents a sample of the variable for the calculation.

6.4 Parameter (Data) Uncertainties Using Model 1

Risk assessments, by necessity, often rely on the application of data and results of studies for purposes other than the original purpose of the study. For example, some of the health statistics databases were assembled to survey health trends and utilization, not to estimate rates, odds ratios or other "risk" indices. As it is often practiced in regulatory and industry settings, risk assessment is a meta-analytic science, as opposed to a basic or

fundamental science. The total effect of such an approach introduces significant sources of uncertainty in the risk estimates. The following discussion highlights some of the sources of uncertainty identified during risk estimation using Model 1.

6.4.1 Use of Hospital Discharge Rates to Estimate Hospital Time

The use of the hospital discharge rate as an estimate of the number of patients in a given year informs the risk of infection *per hospital discharge* (hospital release) but this number does not estimate the risk to a member of the population. Uncertainty in the risk estimate is introduced by the assumption that "1 discharge = 1 patient." In fact, it is expected that some individuals are readmitted to the hospital multiple times during a given year. Thus, discharges are expected to be comprised of patients who have been discharged *0, 1, 2, 3,..., N* times prior to the current discharge during the period of interest. Additionally, there is a proportion of the hospital discharges represented as "deceased" population at the time of discharge. The nature of the uncertainty introduced by the assumption that 1 patient = 1 discharge is to dilute the risk by a greater denominator than the actual numbers of patients in the discharge pool. This will tend to underestimate risk by a factor that can be estimated depending on the median number of discharges per patient per year.

Although the repeated rate of hospitalization is not readily accessed within publicly available databases, medical care statistics might illustrate the nature of the uncertainty. The NCHS, in *Health, United States,* surveys the numbers of visits to acute care, physician offices or home visits within the past twelve month period. The questionnaire brackets 0, 1-3, 4-9 and "10 or more" visits recalled during the past year. The data for the entire survey sample shows that 17.5% of the study population recalled no visits to doctor's offices, emergency departments or home visits within the preceding 12 months (Figure 10). The distribution of hospital visits is also strongly age-dependent, again suggesting that the elderly and very young are those entering the nosocomial environment (hospitals) and are at relatively increased risk of nosocomial infections.

Finally, based on the epidemiology studies of vancomycin resistant enterococci, it is anticipated that the risk of VRE is over-represented in the proportion of the patient population at risk of extended stays in the hospital and/or repeated hospitalizations.

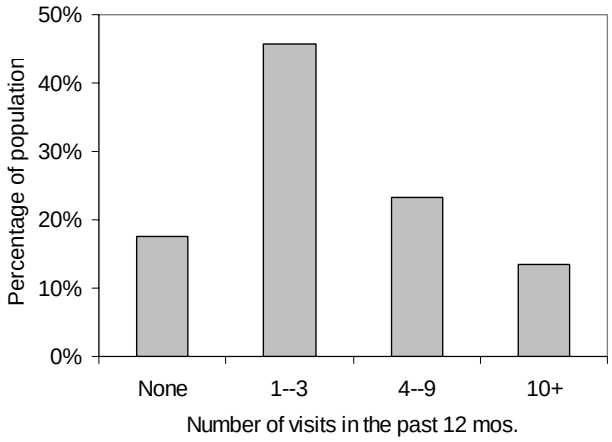

Figure 10. Health care visits to doctors' offices, emergency department, and home visits within the past 12 months (Popovic, 2001).

These observations have been reported by Moellering et al. (1998) who reported that two of the principal risk factors for vancomycin resistant infections are repeat hospitalizations and length of hospital stay.

6.4.2 Use of the Bed Ratio to Estimate ICU Time

A second important source of uncertainty in the estimates is the use of the *bed ratio* as a frequency of ICU use. Using the bed ratio for this estimate treats the entry into an ICU as a random event, independent of age, nature of disease and other factors linked with expected ICU occupancy. An improved estimate of risk would include age-dependent rates of ICU use and, optimally, the rate of ICU use as a function of the particular illness.

6.4.3 Probabilities from Isolate Data

Most of the data reported for the "prevalence of resistance" in the infectious disease literature pertinent to this risk assessment is, in fact, prevalence of resistance among isolates of bacteria. Literature reports or public health databases that define the number of isolates per patient are very rare. Thus, uncertainty in the risk estimation is introduced by the assumption that "1 isolate = 1 patient." The probabilities estimated from the SENTRY and NNIS databases are based on isolate prevalence given in those databases.

6.4.4 ICU Based Infection Rates

The assumptions involved in the use of the ICU device-based infection rates contribute to the uncertainty in risk estimation. The use of the device-based rates instead of human disease endpoints is an ecological design in epidemiology and, thus, suffers from the fact that devices do not get the infections of interest, but humans do. There is no assurance that the infection rates and time in the ICU can directly convert to the numbers of human infections as is assumed by this approach. Additionally, it is possible that ICU-based data under represents the total pool of bacteremias. For examples, bacteremias might occur within general hospital treatment areas and in long-term care facilities (LTCFs). In the former case, Model 3 (Section 6.6) is expected to capture greater numbers of potential bacteremias. The LTCF issue is clouded by the fact that there is relatively little information other than for urinary tract infections among elderly LTCF residents.

6.5 Model 2: Risk Estimation from Synercid Usage Rates

An alternative approach to estimating the number of VRE cases in the US in a given year that may be treated with Synercid is to use actual prescriptions rates. Such information is known by drug manufacturers or estimated by private market research firms; however, drug use rates and sales volumes are not routinely available to the FDA. At one time, an intermediate level of information was available in the form of sales volumes of Synercid that Aventis Pharmaceuticals published in their annual reports. After the sale of Synercid to Monarch (King) Pharmaceuticals, however, Synercid-specific sales volumes have not been available except by private communication.

Data provided to the FDA indicate that 356,800 counting units of Synercid (U_{Syn}) were sold in 2001.[9] Given that the recommended therapy for VREF is 7.5 mg/kg q8h, approximately 3 counting units might be used per day of treatment for a roughly 70 kg patient. Given the assumption in the rate of Synercid delivery, the number of treatment days of Synercid is given by

$$\lambda_{Synercid} = n_{CU} \times \lambda_{Rx} \qquad \text{Eq. 10}$$

[9] IMS Health, IMS National Sales Perspectives™, Annual Jan. 2000 – Dec. 2003 inclusive. Data extracted February 2004. The IMS Health data.data are used for FDA custom analyses.

where

$\lambda_{Synercid}$ = the number of Synercid treatment days per year,

λ_{Rx} = the rate of Synercid treatment in counting units per day, and

n_{CU} = the number counting units in one year.

At the mean estimate, approximately 356,800 ÷ 3 ≈ 119,000 Synercid treatment days were available in 2001. Note that this estimate does not account for losses due to expiration or to other adverse events nor does it account for the fraction of Synercid that might be used to treat staphylococcal or streptococcal infections.

The conversion of Synercid treatment days to an estimated number of patients treated requires the average length of treatment per patient. This is seldom reported in the ICU and nosocomial literature for specific antimicrobial drugs. Rather, the length of time in the ICU or the total length of stay (t_{hosp}) for a hospital is sometimes reported in the nosocomial infection literature. The average length of stay of hospitalized populations typically doubles or triples when the patients are in an ICU during part of the hospital stay. Thus, in order to initialize the parameter for t_{hosp}, the NCHS average of 4.9 ± 0.1 days per hospital discharge could be doubled as a first estimate of t_{hosp} for a bacteremic patient. Severe sepsis patients have significantly longer t_{hosp}, perhaps 18 days as an average (e.g., Angus et al., 2001). The other uncertain feature about these estimates is the proportion of the length of stay for which the patients are on antibiotic therapies. Given inherent uncertainty, a point estimate for an average duration of treatment might be about 10 days in accordance with the severity of VREF disease. For the purpose of conservative risk estimation, a smaller duration of treatment is used (7 days) as a median in a lognormal distribution. The use of a lognormal distribution to approximate the LOS enables a capture of very long stays in the tail of the distribution, while also favoring a lower most likely value for conservative risk estimation. Using a point estimate of the median = 7, then the 113,000 days of Synercid therapy represents, on average, 113,000 ÷ 7 = 16,100 infections.

The final step in this chain is to calculate the proportion of these cases that might be Synercid resistant stemming from community as opposed to ICU sources of resistance.

Unlike the Model 1 approach, a chain of probabilities through vancomycin resistance is unnecessary because the number of cases is estimated at the final, Synercid-resistant step in the probability chain. For example, if the community rate of resistance is, as discussed above, 2.2% (uniform mean) or less, and it is given that the infections are nearly all *E. faecium*, then the expected number of SREF infections is 16,100 × 0.022 = 354 SREF infections in 1 year. Again for the purpose of conservative estimation, greater community prevalence might be assumed as a starting estimate. The starting parameter estimates are given in Table 6-3.

Table 6-3. Initial Parameter Estimates for Model 2

Variable[1]	Description	Mean Estimate	Distribution and Parameters (θ1, θ2)	Source
U_{Syn}	Counting Units	356,800	Deterministic	IMS Health, IMS National Sales Perspectives™; data uncertainties not available.[2]
λ_{Rx}	Treatment Rate (in Counting Units per day)	3.0	Normal(3.0, 0.15)	Recommended rate for treatment of SREF plus uncertainty in body weights
t_{Rx}	Treatment duration (days)	7.6	Lognormal(7,1.5)	Approximated from ICU and nosocomial disease literature.
$p_{SR,EF}$	Probability of SREF	0.022	Uniform(0.004, 0.04)	Community prevalence: see discussion above.
p_{trans}	Food-Attributable Fraction	0.1	Triangular(0, 0.1, 0.2)	Range of values discussed in this report, and based on Willems et al. (2000).

[1] In general, upper case is used for the theoretical variable, and a lower case symbol represents a sample of the variable for the calculation.

[2] IMS Health data are used in a custom analysis by the FDA.

6.5.1 Uncertainties Using Model 2

All parameter estimates used in Model 2 are uncertain due to unknown properties of the input data. The uncertainties identified include

- uncertainty in the industry-furnished annual sales volume;
- the distribution of treatment rates (number of counting units per patient);

- the duration of treatment;
- the rate of loss of Synercid due to expiration or other causes;
- the community prevalence of SREF; and
- the proportion of Synercid destined for SREF bacteremias and not staphylococcal or streptococcal skin and skin structure infections.

Similarly to Model 1, Model 2 is uncertain in terms of estimating numbers of persons from a surrogate variable. In particular, the number of patients potentially receiving Synercid estimate from the number of units sold contributes to data uncertainty. Finally, Model 2 is a custom FDA analysis based on IMS Health data. The results of Model 2 are the expressed opinion of the FDA.

6.6 Model 3: Risk Estimation Using Septicemia Statistics

The National Hospital Discharge Survey publishes the first-listed diagnosis for hospital discharges (patients) in the US The NHDS estimated the number of septicemias (ICD-9-CM Code 038) for 2001 to be 315,000 ± 38,000 (Kozak et al., 2004). Septicemia as a diagnostic syndrome includes bacteremias, but also might includes a sepsis syndrome involving single or multiple organ failures (CDC). Model 3 estimates the number of SREF cases along a similar path as Model 1 with the exception beginning with the number of septicemias instead of the derived number of BSI from NNIS data. The starting parameter estimates are given in Table 6-4.

6.7 Results of Simulations Using Models 1 – 3

All three models were implemented in Analytica® software. Although uncertainties in the models are tractable for analytical error propagation, Monte Carlo analyses were used for computational convenience. The uncertainty sampling used for the Monte Carlo analysis included a median Latin Hypercube sampling for uncertainty samples of 1,000 simulations. The pseudorandom number generator uses the Minimal Standard random number generator of Park and Miller with a Bays-Durham shuffle.[10] This generator is useful for simulations of less than 100,000,000 samples.

[10] Analytica® documentation.

Table 6-4. Model 3: Initial Parameter Estimates for Model 3

Variable	Description	Mean Estimate	Distribution and Parameters (θ1, θ2)	Source
Sep	Annual number of septicemias	315,000	Normal(315000, 38000)	Kozak et al., 2004
p_{ent}	Probability ICU infection is *Enterococcus* spp.	0.0993	Beta(1723, 15680)	Low et al., 2001
$p_{EF,ent}$	Probability Enterococcus infection is *E. faecium*	0.250	Beta(480, 1444)	Low et al., 2001
$p_{VR,EF}$	Probability *E. faecium* infection is vancomycin-resistant	0.5	Beta(8,8)	Low et al., 2001
$p_{SR,EF}$	Community prevalence of SREF	0.022	Uniform(0.004,0.04)	Range of values discussed in this report.
p_{trans}	Food-Attributable Fraction	0.1	Triangular(0, 0.1, 0.2)	Range of values discussed in this report.

The computation of risk yields a distribution of risk estimates. Since it is impractical to tabulate all of the individual values in the iterative calculations, the risk assessment reports the summary statistics for the outcome distributions. The risk estimates for the 5th, 25th, 50th, 75th, and 95th percentiles are show to illustrate the overall uncertainty in the simulations. A single run of the simulation, for which the number of random samples was 3,000, was used to estimate risk.

It is important to note that Model 1 estimates less than 1 case at the 5th and 25th percentiles of the simulated distributions. Because <1 human case is equivalent to 0, the results from Model 1 show that a risk estimate of zero cases cannot be excluded from application of Model 1. Although the single digit numbers at the 5th percentile in Model 2 and 3 risk estimates suggest that zero cases cannot be excluded from the left tail of the distribution, the likelihood of estimating zero in Models 2 and 3 is much less than that for Model 1.

When expressed as a risk estimate, the range at the mean estimate is 7 in 1 billion to 13 in ten million for members of the US population (Table 6-5). If the risk estimation

is based on the hospitalized population, the range among the three models is from 61 in 1 billion to 1.1 in 1 million hospitalized patients in one year (Table 6-6).

Table 6-5. Total Estimated Cases of SREF in One Year, Assuming an Average 10% Attribution to Food Pathways[1]

Model	Mean	Percentiles				
		5%	25%	(Median) 50%	75%	95%
1: ICU BSI	2	0	1	1	3	6
2: Prescriptions	39	5	15	30	52	104
3: Septicemias	9	1	4	7	12	21

[1] ICU = Intensive care unit; BSI = Bloodstream infection

Table 6-6. Unadjusted US Risk Estimates: Number of Chances in 1 Million per Year, Assuming Average 10% Attribution to Food Pathway Sources[1]

Model	Mean	Percentiles				
		5%	25%	(Median) 50%	75%	95%
US Population[2]						
1: ICU BSI	0.007	0.001	0.002	0.005	0.009	0.022
2: Prescriptions	0.14	0.02	0.05	0.11	0.18	0.36
3: Septicemias	0.03	0.00	0.01	0.03	0.04	0.07
Hospitalized Population[3]						
1: ICU BSI	0.06	0.01	0.02	0.04	0.08	0.19
2: Prescriptions	1.19	0.15	0.46	0.92	1.59	3.19
3: Septicemias	0.26	0.04	0.11	0.22	0.36	0.63

[1] ICU = Intensive care unit; BSI = Bloodstream infection.

[2] Midpoint 2001, US Census = 285,317,559

[3] Annual hospital discharges, 2001 = 32,653,000 (Kozak et al., 2004).

Using this assumption, the results show that the mean number of attributable SREF cases might range from 2 to 39 in one year (Table 6-5). The distribution of risk estimates is fairly narrow as exemplified by the 95th percentiles, for which the risk estimates range from 6 to 104 cases in one year. The 95th percentile risk is roughly equivalent to stating that in 95% of the risk estimates, the risk will be less than or equal to the value for the 95th percentile (Table 6-6).

CVM was also interested in risk estimates given an assumption that *all* existing resistance to streptogramins among the human population originated in food animal uses

of virginiamycin. In this scenario, secondary transmission of resistance among humans and primary (index) cases of resistance from consumption of contaminated food are considered to be equivalent. The risk estimates under this scenario increase proportionally to the risk estimates in Table 6-6 and they represent, essentially, the joint probability of VREF and SREF bacteremias. Given this scenario, the average risk estimates range from 6 chances in 10 million to 1.2 chances in 100,000 per person-year among the hospitalized population.

6.7.1 Sensitivity Analysis

One of the benefits of the risk assessment process is that key data gaps are usually identified suggesting areas of needed research and generating testable hypotheses. Toward this end, a simplified sensitivity analysis was performed on the three models using built-in features of Analytica®. A "variable importance" is the absolute rank-order correlation between the sample of output values and the sample for each uncertain input. Importance analysis "is a robust measure of the uncertain contribution because it is insensitive to extreme values and skewed distributions. Unlike commonly used deterministic measures of sensitivity, it averages over the entire joint probability distribution. Therefore, it works well even for models where the sensitivity to one input depends strongly on the value of another." (Analytica® documentation).

The sensitivity (variable importance) analysis results are shown in the following Figures 11, 12 and 13. The analyses show that the community prevalence of SREF is the dominant variable in terms of sensitivity for all three models. The least sensitive variable in Models 1 and 3 is the probability that the infection is *Enterococcus spp*., while the least sensitive in Model 2 is "losses." The sensitivity analysis suggests that it is desirable to reduce uncertainty in the prevalence of SREF, the treatment duration (Model 2) and the probability calculations leading to the days of care estimate in Models 1. As the risk assessment is finalized, a detailed sensitivity analysis might reveal optimal research strategies to reduce uncertainty in the risk estimates.

6.7.2 Apportionment of Risk by Age Groups

All three models in this risk assessment yielded small numbers of estimated SREF cases in one year. The estimates are expected to be highly age-dependent, based on established health medical literature. The rate of hospitalization and the average number of days in the hospital is clearly a function of age. For example, the NHDS groups discharge data by standard epidemiological study age categories (in years) of under 15, 15-44, 45-64, 65-74, 75-84, and 85 or older (Popovic, 2001). The rate of hospital discharge, in number of discharges per 1,000 members of the population is strongly age-dependent (Figure 14). The rate of "days of care" per 1,000 population follows a similar pattern (Figure 15) in which the older age groups bear a significant proportion of the days of hospitalization. For example, in 1999 there were about 4 days of care per person in the 85 years and older group, compared to less than 1 day per person over all ages combined. Finally, a major portion of the population is not hospitalized at all during a given year. Although age adjustment is justifiable, such an adjustment has not been completed at this time because the results are not likely to contribute significantly to the interpretation of the results.

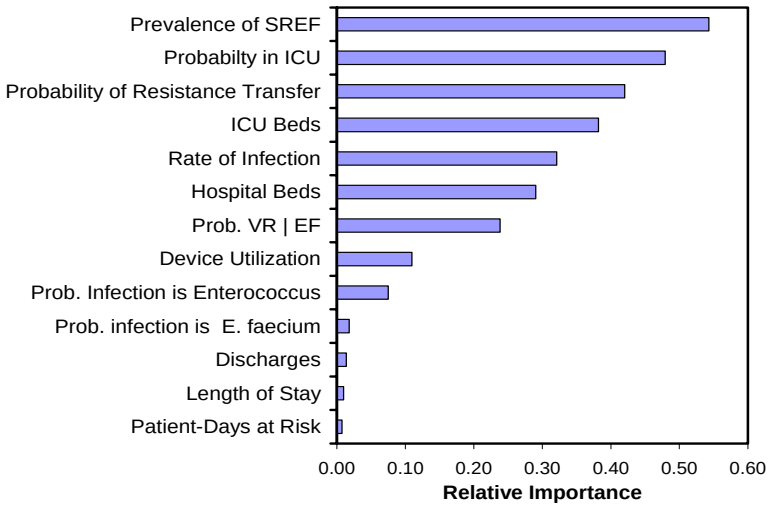

Figure 11. Relative Importance of the Variables in Model 1: ICU-BSI. The figure shows information from a simplified sensitivity analysis. The community prevalence of SREF is the most important variable followed by the probability of being in the ICU. Note that the

probability of being in the ICU is itself derived from the ratio of "ICU Beds" and "Hospital Beds." Thus, there is some redundancy in this simple model.

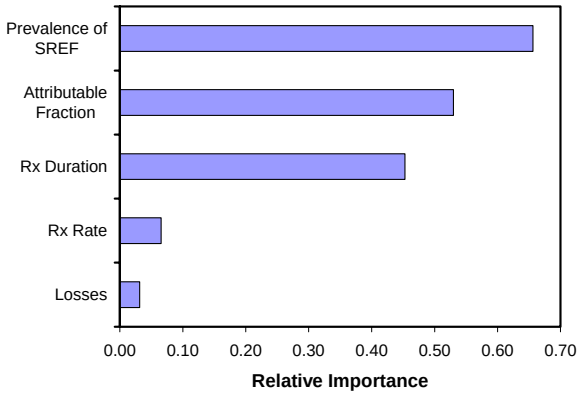

Figure 12. Relative Importance of the Variables in Model 2: Prescriptions. The figure shows information from a simplified sensitivity analysis. The community prevalence of SREF is the most important variable followed by the treatment duration. Not that the subjective variable, "Losses," estimating loss of sold Synercid, is the least important in the estimation of SREF cases.

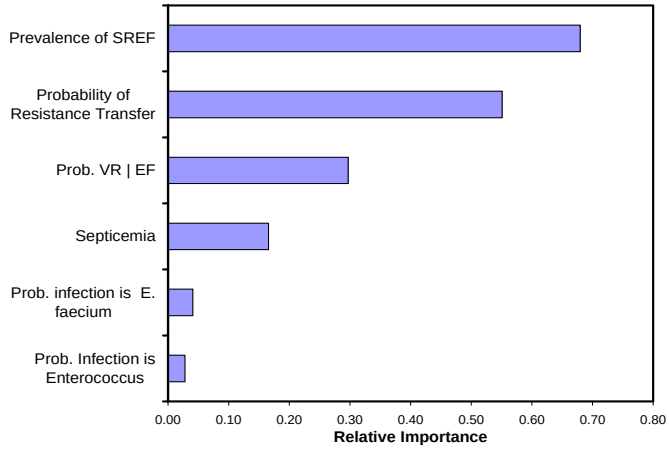

Figure 13. Relative Importance of the Variables in Model 3: Septicemias. The figure shows information from a simplified sensitivity analysis. The community prevalence of SREF is the most important variable followed by the probability that the ICU infection is from a food pathway.

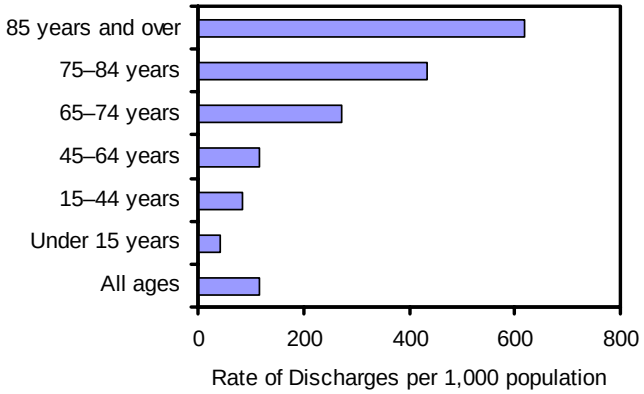

Figure 14. Rate of Hospital Discharges by Age Group, 1999. The NCHS data are plotted showing the strong age-relatedness of the rate of short-term stay, non-federal hospital discharges.

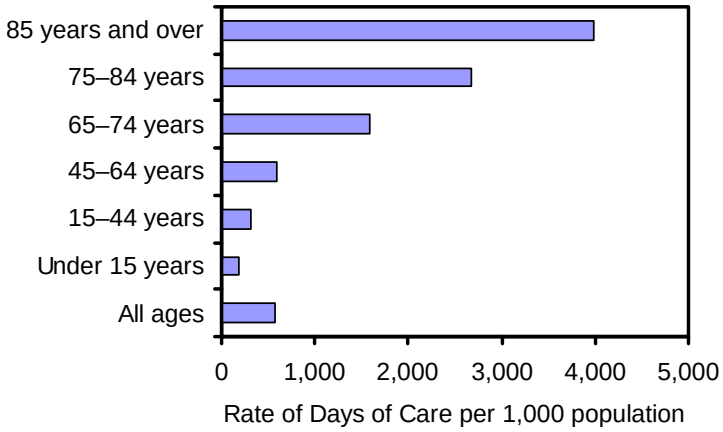

Figure 15. Rate of Hospital Days of Care by Age Group, 1999. The NCHS data are plotted showing the strong age-relatedness of the rate of short-term stay, non-federal hospital discharges

7 CONCLUSIONS

This risk assessment is presented in draft form. It is anticipated that comments from reviewers might lead to changes in the final document. Additionally, CVM has pending research results that potentially add information to the risk assessment; however, the pending studies are not anticipated to affect risk estimates because they focus on exposure pathways and molecular analyses. Given these caveats, the preliminary conclusions from this risk assessment are that

- streptogramin-resistant *E. faecium* are found in isolates obtained from poultry and swine sources in both the US and Europe. The prevalence of resistance appears to be related to the usage pattern of virginiamycin on the farms;
- streptogramin-resistant *E. faecium* can be recovered from food animal products purchased from retail sources;
- low-level streptogramin resistance (primarily, MICs = 4 μ/mL) occurs at low frequencies in the non-hospitalized human population;
- the transfer of streptogramin resistance determinants from animal *E. faecium* to human *E. faecium* through the foodborne pathway is biologically plausible, but the extent of such transfer *in vivo* cannot be estimated at this time;
- molecular genetics studies are providing critical information on the flow of streptogramin resistance determinants, but are also suggesting the existence of undefined resistance determinants;
- SREF isolates from food animals are commonly associated with high level resistance (e.g., MIC ≥ 32 μg/mL) which is generally not observed in the MIC distribution from human SREF isolates;
- the different MIC distribution and the dissimilar pattern of resistance genes between animal and human isolates is inconsistent with the postulated attribution of human streptogramin resistance to animal sources;
- assuming a food pathway attribution of 10%, the mean number of cases of SREF in humans per year attributable to animal uses of virginiamycin ranges from 2 to 39, using three different models for risk estimation;
- assuming a food pathway attribution of 10%, the average risk to a random member of the US population of having SREF attributable to animal uses of virginiamycin and that may

result in impaired Synercid therapy ranges from 7 chances in 1 billion to 14 chances in 100 million in one year;

- the average risk to a random *hospitalized* member of the US population, the most relevant "at-risk" population, of having SREF attributable to animal uses of virginiamycin and that may result in impaired Synercid therapy, ranges from 6 chances in 100 million to 1.2 chances in 1 million in one year;
- however, if the food pathway attribution is assumed to be 100%, then the estimated mean number of cases of SREF in humans per year attributable to animal uses of virginiamycin would increase 10-fold.

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APPENDIX I: GLOSSARY OF TERMS

NOTE: The following definitions are provided for terms as used in this risk assessment. These definitions are a guide for the use of terms in this risk assessment. Definitions in other FDA documents may differ.

Term	Definition
Asymptomatic	Without symptoms, or not exhibiting symptoms.
Attack rate	The numbers of people at risk who develop a disease out of the total number of people at risk. The attack rate is useful in comparing the risk of disease in groups with different exposures.
Colony forming unit, CFU	A cell or cluster of two or more attached sister cells capable of multiplying to form a macroscopic colony of cells.
Consequence Assessment	As used in this risk assessment, a description of the relationship between specified exposures to a biological agent and the consequences of those exposures.
Cumulative Distribution	A representation of a distribution where the values are arranged in ascending or descending order.
Distribution	A series of values or a mathematical equation describing a series of values.
Dose	The amount or number of a pathogen that is ingested or interacts with an organism (host).
Dose-response Assessment	The determination of the relationship between the magnitude of exposure and the magnitude and/or frequency of the adverse effect of interest.
ED_{50}	(Effective dose) The dose of a toxic substance that elicits an effect in 50% of the persons who received that dose.
Empirical Distribution	A series of observed values or data.
Exposure assessment	A component of a risk assessment that characterizes the source and magnitude of human exposure to the hazardous agent.
Food Code	A number representing a food in the food consumption surveys; each food has its own food code.
Foodborne pathogen	A microorganism (bacteria, virus, protozoa) that is capable of causing disease and is transmitted by food.
FoodNet	Foodborne Diseases Active Surveillance Network. A surveillance system led by the Centers for Disease Control and Prevention for compiling epidemiological incidences of foodborne illness
Frequency Distribution	A distribution describing the rate or frequency of occurrence of a value in a series or population.

Hazard characterization	The qualitative or quantitative evaluation of the nature of the adverse effects associated with biological, chemical, and physical agents that may be present in food.
Hazard identification	The identification of known or potential health effects associated with a particular agent.
Immunosuppression	An agent or condition that decreases a person's ability to resist infection.
Incidence	The number of new cases of a disease that occur during a specified period of time, typically taken as one year. The incidence is usually reported as a ratio of new cases per 100,000 members of the population.
Infection	Invasion and multiplication of microorganisms in body tissues, which may be clinically undetected. The infection may remain localized, subclinical and temporary if the body defenses are effective. A local infection may persist and spread to become an acute, subacute or chronic clinical infection or disease state. A localized infection may also become systemic when the microorganisms invade the lymphatic or vascular system.
Intermediate-age subpopulation	Total US population excluding elderly and pregnancy associated groups, and including susceptible populations such as cancer patients, AIDS patients, and transplant patients.
Iteration	A single calculation among a series of calculations. For example, in Monte Carlo methods for estimating uncertainty, a single calculation through the entire model is one iteration.
LD_{50}	(Lethal Dose) The dose resulting lethality to 50% of a population.
Modeling (mathematical)	Attempting to predict aspects of the behavior of some system by creating an approximate mathematical representation of the system. Mathematical models can contribute to understanding portions of a complex system or the entirety of a system.
Monte-Carlo Simulation	A process for making repeated calculations with minor variations of the same mathematical equation, usually with the use of a computer. May be used to integrate variability in the predicted results for a population or the uncertainty of a predicted result. A two dimensional Monte-Carlo in simulation may be used to do both.
Nosocomial Infection	The NNIS system defines a nosocomial infection as a localized or systemic condition 1) that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and 2) that was not present or incubating at the time of admission to the hospital. (Garner et al., 1996).
Outbreak	The occurrence of two or more cases of similar illness resulting from a common source of exposure.

Prevalence	In epidemiology, the number of affected persons present in the population at a specific point in time divided by the number of persons in the population at that time. May be expressed as a ration or as a percentage.
Probability	As used in this risk assessment, probability denotes uncertainty. The term is also sometimes used to denote frequency.
Release Assessment	A description of the biological pathways necessary for the use of an antimicrobial drug in animals to release resistant bacteria or resistance determinants into a particular environment, and estimating the probability of that complete process occurring either qualitatively or quantitatively.
Ribotype	A subtype of a bacterial strain more detailed than the species or serotype level, determination of a ribotype is based on analysis of patterns formed by DNA fragments.
Risk	The likelihood of the occurrence and the magnitude of the consequences of exposure to a hazard on human health.
Risk Analysis	As used in this risk assessment: the process consisting of four components: hazard identification, risk management, risk assessment and risk communication.
Risk Assessment	The scientific evaluation of known or potential adverse health effects resulting from human exposure to hazards. The process consists of the following steps hazard identification, exposure assessment, hazard characterization (dose-response), and risk characterization.
Risk Characterization	Integration of hazard identification, hazard characterization and exposure assessment into an estimation of the adverse effects likely to occur in a given population, including attendant uncertainties.
Risk Estimation	As used in this risk assessment: Integration of the results from the release assessment, exposure assessment, and consequence assessment to produce overall measures of risks associated with the hazards identified at the outset.
SENTRY	The SENTRY program is a longitudinal surveillance program that was established to monitor the predominant pathogens and antimicrobial resistance patterns of nosocomial and community-acquired infections nationally and internationally.
Serotype	A group of related microbes distinguished by its composition of antigens.
Susceptibility	The degree that a host is vulnerable to infection, includes the ability of the host to defend itself.
Susceptible Population	A group of people at increased risk for infection and illness from a pathogen, often caused by a decrease in the effectiveness of the person's immune system.

Uncertainty	An expression of the lack of knowledge, usually given as a range or group of plausible alternatives.
Uncertainty Distribution	A description of the range of plausible values for a prediction.
Variability	A description of differences among the individual members of a series or population.
Virulence	The capacity of a microbial pathogen to invade and/or produce illness in the host. Mediated by the presence of specific genes and their protein products that interact with the host.

APPENDIX II: ACRONYMS AND ABBREVIATIONS

ARS	Agricultural Research Service of the USDA
BSI	Bloodstream infection
CDC	Centers for Disease Control and Prevention
CFSAN	FDA's Center for Food Safety and Applied Nutrition
CFU	Colony forming unit
Codex	Codex Alimentarius Commission
CVM	Center for Veterinary Medicine of the FDA
FDA	US DHHS's Food and Drug Administration
FoodNet	Foodborne Diseases Active Surveillance Network (CDC)
FSIS	USDA's Food Safety and Inspection Service
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
ICARE	Intensive Care Antimicrobial Resistance Epidemiology
ICU	Intensive Care Unit
LTCF	Long-Tern Care Facility
MIC	Minimum Inhibitory Concentration (See Glossary)
NAS	National Academy of Sciences
NaSH	National Surveillance System for Healthcare Workers
NARMS	National Antimicrobial Resistance Monitoring System
NCHS	National Center for Health Statistics
NHANES III	Third National Health and Nutrition Examination Survey
NHDS	National Hospital Discharge Survey
NNHS	National Nursing Home Survey
NNIS	National Nosocomial Infection Survey
NRC	National Research Council
OIE	Office International des Epizooties, Paris
PFGE	Pulsed Field Gel Electrophoresis
PulseNet	Molecular Subtyping Network for Foodborne Bacterial Disease Surveillance
QD	Quinupristin-Dalfopristin
SREF	Streptogramin resistant *Enterococcus faecium*
US DHHS	United States Department of Health and Human Services
USDA	United States Department of Agriculture
UTI	Urinary tract infection
VirREF	Virginiamycin resistant *Enterococcus faecium*
VRE	Vancomycin resistant *Enterococcus spp.*
VREF	Vancomycin resistant *Enterococcus faecium*
WHO	World Health Organization