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Leading article

Occurrence and epidemiology of resistance to virginiamycin and streptogramins

Lee Ann Thal[a] and Marcus J. Zervos[a,b]*

[a]*Departments of Medicine and Clinical Pathology, Section of Infectious Diseases, William Beaumont Hospital, Royal Oak, MI;* [b]*Wayne State University School of Medicine, Detroit, MI, USA*

Introduction

Non-human sources have been increasingly suspected as reservoirs for some antibiotic-resistant bacteria. Antibiotics are used in animals both to treat infections and as growth promoters. The potential role that antibiotic use in veterinary medicine and animal husbandry plays in the transfer of antibiotic-resistant bacteria to humans is a controversial issue. There is clear evidence that the increase in consumption of antibiotics by animals has been accompanied by a similar rise in the number of antibiotic-resistant strains isolated.[1–6] It has been suspected that antibiotic use in food animals has resulted in the novel resistance genes and multiresistant pathogens that have emerged in these animals.[7–20] Dupont & Steele[20] reported that 45% of antimicrobial use in the USA was for animal feed supplementation. Studies of salmonella,[14] *Escherichia coli*,[12] enterococci[6–11,15–17] and campylobacter[13] support the claim that novel resistance genes may be selected in the bacterial flora of animals as a direct consequence of antibiotic use.

The streptogramin antibiotics are naturally occurring compounds isolated from *Streptomyces pristinaspiralis*.[22–24] The streptogramin family comprises several classes of antibiotics, including the mikamycins, pristinamycins, oestreomycins and virginiamycins.[23] Oral and topical pristinamycins have been used in humans in France for many years, primarily in the management of staphylococcal infections.[22–24]

The streptogramins are divided into two groups.[24] Members of group A are polyunsaturated cyclic peptidolide compounds that include virginiamycin M and pristinamycin IIA, while group B compounds are cyclic hexadepsipeptides such as virginiamycin S and pristinamycin IA. Quinupristin/dalfopristin (Synercid) is a new antimicrobial agent that has completed phase III clinical studies in Europe and the USA. It has recently been approved by the FDA in the USA for skin and soft tissue infection and nosocomial pneumonia (as part of combination therapy) for vancomycin-resistant enterococci (VRE). It consists of a combination of quinupristin and dalfopristin in a w/w ratio of 30:70.[25–31] Quinupristin and dalfopristin are derivatives of pristinamycin IA and IIA, respectively. Molecular modifications of the natural compounds were required to increase their aqueous solubility, thus enabling the drug to be formulated as an injectable agent for use in the management of serious infections.

Individual pristinamycin compounds exhibit bacteriostatic activity against Gram-positive bacteria. However, combinations containing one streptogramin from group A and one from group B are usually synergic and have bactericidal activity. This has been demonstrated for the quinupristin/dalfopristin combination.[25–31] In-vitro studies have documented lower MICs against isolates of staphylococci and streptococci for quinupristin/dalfopristin than with either component alone.[26,30] Streptogramins act by binding the 50S subunit of the bacterial ribosome. Dalfopristin and quinupristin are thought to bind sequentially to different sites on the 50S subunit and it has been proposed that the binding of dalfopristin alters the conformation of the ribosome such that its affinity for quinupristin is increased. This results in a stable ternary drug–ribosome complex, and the newly synthesized peptide chains cannot be extruded from the ribosome of that complex. Consequently, protein synthesis is interrupted, thus leading to cell death.

Numerous in-vitro susceptibility studies have been performed with quinupristin/dalfopristin and other streptogramin antibiotics.[25–31] In general, quinupristin/dalfopristin has excellent in-vitro activity against *Staphylococcus*

*Corresponding author. William Beaumont Hospital, 3601 West 13 Mile Road, Royal Oak, MI 48073, USA.
Tel: +1-248-551-0419; Fax: +1-248-551-8880; E-mail: mzervos@beaumont.edu

aureus (including methicillin-resistant strains), coagulase-negative staphylococci, streptococci (including penicillin-resistant *Streptococcus pneumoniae*), *E. faecium*, *Neisseria* spp., *Haemophilus influenzae*, *Moraxella catarrhalis*, *Legionella* spp. and *Listeria monocytogenes*. *Enterococcus faecalis* is resistant *in vitro* to quinupristin/dalfopristin by an undetermined mechanism. Quinupristin/dalfopristin is also active against Gram-positive and Gram-negative anaerobic organisms from several genera, including *Bacteroides*, *Prevotella*, *Fusobacterium*, *Clostridia*, *Actinomyces*, *Peptostreptococcus* and *Lactobacillus*.

Resistance to streptogramins is mediated by: enzymatic modification of the antibiotic; active transport or efflux mediated by an ATP-binding protein; and alteration of the target site. The most commonly known resistance to streptogramins is the MLS_B resistance conferred by the *erm* genes.[32,33] These genes encode an enzyme that dimethylates an adenine residue in the 23S ribosomal RNA, which results in decreased binding of macrolides, lincosamides and streptogramins of group B.[34] In staphylococci, other genes responsible for resistance to streptogramin B antibiotics include *vgb*,[35,36] which is rare, plasmid mediated, and found only in staphylococci; it encodes a streptogramin-inactivating enzyme (hydrolase).[37] Also rare is the *msr*A gene, which confers resistance to streptogramin B antibiotics by active transport after induction with erythromycin and is also found only in staphylococci.[38] Because quinupristin/dalfopristin is a combination of a streptogramin A (quinupristin) and a streptogramin B (dalfopristin), one would expect that it would remain active against MLS_B-positive organisms. This has been demonstrated with both constitutive and inducible strains of MLS_B positive *S. aureus*.[30,33,39] Mechanisms of resistance to streptogramin A antibiotics include the *vat*[40] and *vatB*[41] genes which encode acetyltransferases that inactivate the antibiotic. The *vga* gene[42] confers resistance to streptogramin A antibiotics by active transport and the *lsa* gene[43,44] to lincosamide/streptogramin antibiotics in staphylococci. These genes have been shown to be more important as a cause of resistance to quinupristin/dalfopristin in staphylococci when a combination of *vat*, *erm* and *vgb* was present in the same strain of bacteria.[30] The prevalence of such quinupristin/dalfopristin-resistant strains of staphylococci has been estimated to be <3% in France. These isolates were from skin after the topical use of streptogramins and were not clinically significant. Cross-resistance to macrolides may be seen although this is probably a relatively rare and clinically insignificant occurrence.

Resistance of *E. faecium* to quinupristin/dalfopristin has been reported in a urine isolate from France; the resistance gene, *satA*, specifies an acetyltransferase that is 58% homologous with the one encoded by *vat*.[45] Thus far, cross-resistance between the streptogramin antibiotics and other classes of drugs such as the glycopeptides, β-lactams and aminoglycosides has not been reported. A concern is that resistance will develop when patients are treated with quinupristin/dalfopristin. In clinical trials of quinupristin/dalfopristin, resistance of staphylococci and *E. faecium* appearing during therapy has been rare. In one study we evaluated whether combining quinupristin/dalfopristin with ciprofloxacin or tetracycline would reduce development of resistance.[46] A strain of *E. faecium* (EF3) with MICs of quinupristin/dalfopristin of 0.25 mg/L, ciprofloxacin 0.5 mg/L and tetracycline 0.5 mg/L was used. This strain was resistant to vancomycin (MIC > 125 mg/L), ampicillin (MIC > 32 mg/L) and gentamicin (MIC > 2000 mg/L). Strain EF3 was from a patient with a blood isolate of *E. faecium* that had become less susceptible to quinupristin/dalfopristin (MICs increasing from 0.25 to 2.0 mg/L) after therapy.[47] The *satA* gene was not detected following DNA amplification by PCR. Strain EF3 was grown in overnight culture and ten-fold serial dilutions were plated on to Brain Heart Infusion Agar (Difco, Detroit, MI, USA) plates containing quinupristin/dalfopristin 1 mg/L, ciprofloxacin 0.125 mg/L or tetracycline 0.125 mg/L and plates containing combinations of quinupristin/dalfopristin 1.0 mg/L with ciprofloxacin 0.125 mg/L or tetracycline 0.125 mg/L. With quinupristin/dalfopristin alone, resistant colonies were detected at 48 h at a frequency of 1×10^{-3}. The combination of quinupristin/dalfopristin and ciprofloxacin resulted in resistant colonies at a frequency of 1×10^{-4} at 48 h. With the combination of quinupristin/dalfopristin and tetracycline, no resistant colonies were detected at 48 h. The high rate of resistance with quinupristin/dalfopristin alone was probably a result of strain selection since another study indicated rates of mutational resistance for *E. faecium* of $<1 \times 10^{-9}$.[48] The findings that, *in vitro*, less resistance develops with the combination of quinupristin/dalfopristin and tetracycline than with quinupristin/dalfopristin alone need confirmation and further clinical studies to determine the importance of these observations *in vivo*.

There is little information on the amount, regional differences in use, and the impact of streptogramins on resistance in animal husbandry and veterinary use. Virginiamycin is a mixture of virginiamycin M (a group A streptogramin) and virginiamycin S (a group B streptogramin). Virginiamycin is used worldwide and has been approved by the FDA in the USA for use in chickens, turkeys, swine and cattle (fed in confinement for slaughter). It is used in all these species to promote weight gain. It is also used to prevent necrotic enteritis caused by *Clostridium perfringens* (chickens) and to prevent coccidiosis (chickens and turkeys), to treat and control swine dysentery and, in cattle, to reduce the incidence of liver abscesses.

Non-human sources of resistant enterococci include the inanimate environment and farm animal and other veterinary isolates. Studies in 1985 indicated the presence of Tn*917* sequences in enterococci cultured from humans and farm animals[49] and the MLS_B phenotype (encoded by Tn*917*) is now probably widely distributed in human and

animal isolates of enterococci.[49,50] In Europe, where the glycopeptide avoparcin has been used in animal feed, there has been increasing evidence that the use of this agent is associated with VRE in animal isolates and humans.[51–53] Of great concern is that enterococci causing human disease can be acquired through food animal sources. In the USA, glycopeptides are not approved for use in animals and VRE have been found predominantly in hospitals or extended-care facilities.[54–57]

Streptogramin-resistant *E. faecium* in Europe

In the UK, isolates of quinupristin/dalfopristin-resistant and vancomycin-resistant *E. faecium* (*van*A phenotype) have been found in two centres.[58] Three isolates were cultured from raw chickens and one from a hospital patient (MICs 24–32 mg/L). In these isolates, resistance was shown to be transferable.[58]

In Germany, 150 isolates of *E. faecium* from different clinical origins collected in 1995–1996 were evaluated for sensitivity to vancomycin and quinupristin/dalfopristin before quinupristin/dalfopristin was approved for use in Germany (G. Werner and W. Witte, personal communication). Quinupristin/dalfopristin resistance occurred in all of four *E. faecium* isolates cultured from pig manure collected at a farm using virginiamycin and other microbials and in none of four *E. faecium* isolates from a farm primarily using avoparcin. Quinupristin/dalfopristin resistance (MIC = 8.0 mg/L) was detected in three vancomycin-resistant isolates. The *satA* gene was detected by PCR for all but one quinupristin/dalfopristin resistant strain in this study. Streptogramin-resistant enterococci have also been isolated from pigs in the Netherlands,[59] and from pigs and broilers fed virginiamycin in Denmark.[60] The *satA* gene was detected in *E. faecium* isolates from broilers and pigs in Denmark (A. Hammerum, personal communication). The acquisition of quinupristin/dalfopristin resistance by already multiply-resistant enterococci has serious treatment implications.

Streptogramin-resistant enterococci in the USA

In studies undertaken in Michigan, USA, we evaluated the in-vitro antibiotic susceptibility and molecular relatedness of enterococcal isolates from turkeys fed virginiamycin in animal feed.[61] We found that, in *E. faecium*, ampicillin resistance occurred in up to 78% of isolates (from 45% of animals from which cultures were obtained), and high-level gentamicin resistance occurred in up to 18% of isolates (15% of animals from which cultures were obtained). For *E. faecalis*, high-level gentamicin resistance occurred in up to 47% of the isolates studied (88% of animals from which cultures were obtained). No VRE were detected. Importantly, in isolates of *E. faecium*, resistance to quinupristin/dalfopristin occurred in up to 100% of isolates (25% of animals from which cultures were obtained). Results of molecular typing by pulsed field gel electrophoresis (PFGE) indicated identical clones of ampicillin-, quinupristin/dalfopristin- and gentamicin-resistant strains from turkeys in different culture groups, suggesting dissemination of strains between animals.

In older turkeys, we found a significantly greater incidence of resistance to ampicillin and quinupristin/dalfopristin, which was probably related to their longer exposure to antibiotics and to animals with resistant strains. In this study, we did not evaluate any possible link between resistant strains in animals and those in humans. Since there was no control group of animals that were not given virginiamycin, conclusions about virginiamycin causing resistance are limited. In a separate study of farm animal isolates from south-eastern Michigan,[62] however, we found that gentamicin- or ampicillin-resistant enterococci were rare in animals that had not received antimicrobial agents and, hence, the amount of streptogramin resistance we found was higher than might be expected in virginiamycin-naïve animals. It is possible that animals given antimicrobials other than virginiamycin in feed might also be colonized by streptogramin-resistant enterococci. The significant finding of this study is that we found quinupristin/dalfopristin-resistant strains in animals before the drug combination had been used in humans.

Studies done in Maryland, USA, reported recovery of multiply-resistant enterococci from processed chicken parts.[63] Faecal droppings from 25 chickens, 20 pigs and 25 cows at state and county fairs were evaluated. The majority of the samples were from animals kept at different farms. The associated feed of the cows and pigs was also cultured. Enterococci were recovered from 24 of 25 faecal and eight of 18 feed samples. Three strains of quinupristin/dalfopristin-resistant *E. faecium* were recovered: no *vanA* or *vanB* isolates were present. Enterococci expressing high-level gentamicin resistance (HLGR) were isolated from two of nine chickens, and four of seven pigs. Four of the seven samples of pig feed also contained HLGR enterococci. Conversely, HLGR enterococci were absent from all nine sampled cows and associated feed, although antibiotic-susceptible enterococci were present in all cow faeces. These results suggested that multiply-resistant enterococci colonize chicken, swine and other animal feed, and that this may be a reservoir for such resistant enterococci.

Earlier studies have suggested a link between the use of antibiotics in animal feed and resistant bacteria in humans. Recently streptogramin-resistant enterococci have been found in animals fed virginiamycin. Further work needs to be done to determine what implications this has for humans. It has been recommended that streptogramin use in animals should be limited.[64] In Denmark, virginiamycin has recently been banned as a growth-promoting agent (H. Wegener, personal communication) and, since antibiotic-resistant bacteria that can cause human infection may be transferred via food from animals to humans, until further

information is available, great caution should be exercised in the use of streptogramins in animals.

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