

QUINUPRISTIN-DALFOPRISTIN-RESISTANT *ENTEROCOCCUS FAECIUM* ON CHICKEN AND IN HUMAN STOOL SPECIMENS

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ABSTRACT

Background The combination of the streptogramins quinupristin and dalfopristin was approved in the United States in late 1999 for the treatment of vancomycin-resistant *Enterococcus faecium* infections. Since 1974, another streptogramin, virginiamycin, has been used at subtherapeutic concentrations to promote the growth of farm animals, including chickens.

Methods To determine the frequency of quinupristin-dalfopristin-resistant *E. faecium*, we used selective medium to culture samples from chickens purchased in supermarkets in Georgia, Maryland, Minnesota, and Oregon and stool samples from outpatients.

Results Between July 1998 and June 1999, samples from 407 chickens from 26 stores in four states were cultured, as were 334 stool samples from outpatients. Quinupristin-dalfopristin-resistant *E. faecium* was isolated from 237 chicken carcasses and 3 stool specimens. The resistant isolates from stool had low-level resistance (minimal inhibitory concentration [MIC], 4 μg per milliliter; resistance was defined as a MIC of at least 4 μg per milliliter). The resistant isolates from chickens in general had higher levels of resistance (MICs ranging from 4 to 32 μg per milliliter; MIC required to inhibit 50 percent of isolates, 8 μg per milliliter).

Conclusions Quinupristin-dalfopristin-resistant *E. faecium* contaminates a large proportion of chickens sold in U.S. supermarkets. However, the low prevalence and low level of resistance of these strains in human stool specimens suggest that the use of virginiamycin in animals has not yet had a substantial influence. Foodborne dissemination of resistance may increase, however, as the clinical use of quinupristin-dalfopristin increases. (N Engl J Med 2001; 345:1155-60.)

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VANCOMYCIN-resistant enterococci are an important threat to public health.¹⁻³ According to data from the National Nosocomial Infections Surveillance System, vancomycin-resistant enterococci caused more than 21 percent of nosocomial enterococcal infections in the United States in 1998.⁴ Before the 1990s, it was thought that vancomycin-resistant enterococci were present only in hospitals where vancomycin had been used for many years. However, it has become increasingly evident that vancomycin-resistant enterococci

are easily recovered from farm animals that are fed avoparcin,⁵⁻⁷ an antibiotic growth promoter structurally related to vancomycin that was used in Europe until recently, but not in the United States. Moreover, vancomycin-resistant enterococci have been isolated from commercially available foods and from healthy persons in countries where avoparcin was used in farm animals.⁸⁻¹⁴ In 1997 the European Union banned the use of avoparcin.¹⁵ Since then, the prevalence of vancomycin-resistant enterococci in the food supply and in humans has declined in some areas of Europe.^{16,17} There is concern, however, that cross-resistance and selection pressures from the use of other antimicrobial agents (such as tetracycline and macrolides) on farms are contributing to the persistence of vancomycin-resistant enterococci.¹⁸

Although *Enterococcus faecalis* is a more common cause of disease in humans, resistance to vancomycin is more frequent among *E. faecium* isolates.¹⁻³ In late 1999, the Food and Drug Administration (FDA) approved quinupristin-dalfopristin, a combination of two synergistic streptogramin antibiotics, for intravenous use in people infected with vancomycin-resistant *E. faecium*. Surveys conducted before the approval of quinupristin-dalfopristin suggested that most isolates of *E. faecalis* were resistant to the combination, whereas nearly all clinical isolates of *E. faecium* were susceptible, including isolates that were resistant to vancomycin.^{1,19,20} Quinupristin-dalfopristin represents one of the few options available for treating these pathogens, because vancomycin-resistant *E. faecium* is frequently resistant to multiple drugs.

Virginiamycin, a streptogramin with cross-resistance to quinupristin-dalfopristin,²¹ was approved for use in animal feed at subtherapeutic concentrations to promote the growth of animals used for food, including chickens, in the United States in 1974.²² Turkeys fed virginiamycin in the United States have been shown to be colonized with quinupristin-dalfopristin-resistant *E. faecium*.²³ Similar findings and

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additional studies in Europe²⁴⁻²⁷ led the European Union in 1998 to ban the use of virginiamycin and all other antibiotics used to promote growth in animals (bacitracin, tylosin, and salinomycin) that are related to antimicrobial agents used in humans.²⁸

To assess the potential risk to human health posed by the use of virginiamycin in farm animals in the United States, we determined the prevalence of quinupristin-dalfopristin-resistant strains of *E. faecium* contaminating chicken sold in supermarkets in four regions of the United States and determined whether people in these areas had quinupristin-dalfopristin-resistant *E. faecium* in their intestinal tracts.

METHODS

Survey Design

The survey was conducted between July 1998 and June 1999 by state health departments participating in the Emerging Infections Program sponsored by the Centers for Disease Control and Prevention (CDC). For the first six months of the study, the participating laboratories included two state public health department laboratories (Oregon and Georgia) and a university hospital laboratory (University of Maryland). A third state health public laboratory (Minnesota) then joined the study.

Each month at each site, a sample of 10 whole broiler chickens was purchased from a supermarket located in the same county as the state health department laboratory or hospital laboratory or in an adjacent county. Those buying the chickens were told to choose a different store each month and to choose as many different brands as possible in each store. At three of the four study sites (Oregon, Minnesota, and Georgia), a sample of stool specimens was collected from outpatient specimens submitted to the state health department laboratory for routine culture. All patient identifiers were removed from the stool specimens.

Screening of Chicken Carcasses and Specimens of Human Stool

The chicken carcasses were rinsed in 400 ml of buffered peptone water that was then incubated at 35° to 37°C for 20 to 24 hours; after incubation, 0.5 ml of the fluid was used to inoculate selective enterococcal broth medium. Human stool was cultured by immersing the tip of a cotton swab in the specimen to obtain an estimated 0.5 g of the sample. The swab was then thoroughly inoculated into selective or nonselective enterococcal broth. Enterococcal medium containing quinupristin-dalfopristin was prepared at the University of Maryland laboratory and shipped to the other laboratories.

Selective enterococcal broth consisted of bile esculin azide broth with 4 µg of quinupristin-dalfopristin (Synercid, Rhone-Poulenc Rorer, Collegeville, Pa.) and 2 µg of ampicillin per milliliter. Ampicillin was added to make the broth more selective for *E. faecium* than for *E. faecalis*. Samples were also inoculated into nonselective enterococcal medium consisting of bile esculin azide broth without added antibiotics. After inoculation, both types of broth were incubated for 48 hours at 35° to 38°C, and then 10 µl of medium was subcultured in a different selective or differential agar medium (or both).

Samples obtained from selective enterococcal broth were subcultured in modified Ford agar²⁹ supplemented with 4 µg of quinupristin-dalfopristin and 2 µg of ampicillin per milliliter. Ford agar was modified by replacing raffinose with arabinose. Samples obtained from nonselective enterococcal broth were subcultured in trypticase soy agar with 5 percent sheep's blood, 10 µg of colistin per milliliter, and 10 µg of nalidixic acid per milliliter. After 48 hours of incubation at 35° to 37°C, all colonies from the modified Ford agar that were morphologically typical of *E. faecium* colonies were Gram stained and spot-tested with pyrrolidonyl aryl-

amidase reagent to determine whether they were enterococci. These samples were then sent to the CDC for definitive identification and susceptibility testing. In contrast, each plate of trypticase soy agar containing colistin-nalidixic acid was inspected, and only the most predominant colonies were Gram stained and spot-tested with use of pyrrolidonyl arylamidase reagent, and a single strain of suspected enterococcus, if present, was sent to the CDC for further testing.

Definitive Identification and Susceptibility Testing

Enterococci were identified to the species level according to standard methods developed by the CDC.³⁰ All isolates identified as *E. faecium* were tested for resistance to quinupristin-dalfopristin (minimal inhibitory concentration [MIC], ≥4 µg per milliliter) with use of the broth-microdilution method in accordance with recognized standards.^{31,32} Strains of *E. faecium* recovered from nonselective enterococcal medium were also tested for resistance to penicillin, ampicillin, erythromycin, rifampin, and tetracycline and high-level resistance to gentamicin (MIC, >500 µg per milliliter) and streptomycin (MIC, >1000 µg per milliliter).

RESULTS

Stool Cultures

Enterococci were isolated from 237 of 334 stool specimens (71 percent) cultured in nonselective enterococcal medium, and from 76 of 334 specimens (23 percent) cultured in selective enterococcal medium, which contained quinupristin-dalfopristin and ampicillin (Table 1). Although the selective medium was more specific than the nonselective medium for detecting *E. faecium*, both types had similarly low specificity for detecting quinupristin-dalfopristin-resistant *E. faecium*. Overall, quinupristin-dalfopristin-resistant isolates of *E. faecium* were recovered from three (1 percent) stool specimens cultured in nonselective medium; in the case of all three resistant isolates, the MIC of quinupristin-dalfopristin was 4 µg per milliliter. No quinupristin-dalfopristin-resistant strains of *E. faecium* were recovered from stool samples cultured in selective medium. Two of the quinupristin-dalfopristin-resistant isolates of *E. faecium* were identified in Oregon (2 of 106, or 2 percent), 1 was identified in Minnesota (1 of 60, or 2 percent), and none were identified in Georgia (0 of 168).

Cultures of Specimens from Chickens

Chickens were purchased from 26 supermarket chains; 27 brands were included. Enterococci were isolated from 351 of 407 specimens (86 percent) cultured in nonselective enterococcal medium and from 335 of 407 specimens (82 percent) cultured in selective enterococcal medium (Table 1). Selective medium was more specific than nonselective for detecting *E. faecium* and quinupristin-dalfopristin-resistant *E. faecium*. The overall isolation rate of quinupristin-dalfopristin-resistant *E. faecium* on chickens was 58 percent with the use of selective medium. The rate of isolation of quinupristin-dalfopristin-resistant *E. faecium* with the use of either medium ranged from 17 percent in Minnesota (10 of 58) to 87 percent in Oregon (95 of 109). Quinupristin-dalfopris-

TABLE 1. COMPARATIVE YIELD OF DIFFERENT SCREENING MEASURES FOR ENTEROCOCCI, *ENTEROCOCCUS FAECIUM*, AND QUINUPRISTIN-DALFOPRISTIN-RESISTANT *E. FAECIUM* FROM STOOL SAMPLES AND CHICKEN CARCASSES.*

VARIABLE	TOTAL NO. OF SAMPLES	NONSELECTIVE MEDIUM	SELECTIVE MEDIUM	P VALUE
Stool samples	334			
Positive for enterococci		237/334 (71)	76/334 (23)	<0.001
<i>E. faecium</i>		58/237 (24)	46/76 (61)	<0.001
Quinupristin-dalfopristin-resistant <i>E. faecium</i>		3/58 (5)	0/46	0.25
Positive for quinupristin-dalfopristin-resistant <i>E. faecium</i>		3/334 (1)	0/334	0.25
Chicken samples	407			
Positive for enterococci		351/407 (86)	335/407 (82)	0.15
<i>E. faecium</i>		20/351 (6)	254/335 (76)	<0.001
Quinupristin-dalfopristin-resistant <i>E. faecium</i>		11/20 (55)	237/254 (93)	<0.001
Positive for quinupristin-dalfopristin-resistant <i>E. faecium</i>		11/407 (3)	237/407 (58)	<0.001

*Nonselective enterococcal medium consisted of bile esculin azide broth without antibiotics and trypticase soy agar containing colistin-nalidixic acid. Selective enterococcal medium consisted of bile esculin azide broth and modified Ford agar, each containing quinupristin-dalfopristin and ampicillin.

tin-resistant strains of *E. faecium* were recovered from chickens from 21 of the 26 supermarket chains (81 percent) and 16 of the 27 brands sampled (59 percent); there were no substantial differences in the monthly frequency of isolation.

Susceptibility Tests

The distribution of MICs of quinupristin-dalfopristin varied, depending on whether the isolates from stool samples and chicken were obtained from selective or nonselective enterococcal medium (Fig. 1). The MIC required to inhibit 50 percent (MIC₅₀) of isolates recovered from nonselective medium was 2.0 μg per milliliter with respect to isolates from stool samples and 4.0 μg per milliliter with respect to isolates from chicken. The MIC₅₀ of isolates recovered from selective medium was 2.0 μg per milliliter for stool and 8.0 μg per milliliter for chicken. There was considerable overlap in the MICs, whether the isolates were recovered from nonselective or selective medium.

Many *E. faecium* isolates recovered from stool samples and chicken samples that were cultured in nonselective medium were resistant to antibiotics other than or in addition to quinupristin-dalfopristin (Table 2). Isolates from chicken were generally resistant to more agents than were isolates from stool samples. The only exception was in the case of rifampin: more isolates from stool samples than from chicken samples were resistant to this drug. Among isolates from chicken, quinupristin-dalfopristin-resistant strains were slightly more likely than susceptible strains to be re-

sistant to penicillin and tetracycline but less likely to have high-level resistance to gentamicin (MIC, >500 μg per milliliter) and streptomycin (MIC, >1000 μg per milliliter) or to be resistant to rifampin and erythromycin.

DISCUSSION

We found a high prevalence of quinupristin-dalfopristin-resistant strains of *E. faecium* on chickens purchased from supermarkets in four regions of the United States; the MIC ranged from 4.0 to 32.0 μg per milliliter in isolates from chickens. Although the prevalence varied, at least 17 percent of chickens analyzed at each site yielded quinupristin-dalfopristin-resistant strains of *E. faecium*. In addition, strains of *E. faecium* that were resistant to quinupristin-dalfopristin were isolated from a small number of stool samples from outpatients. Our study was conducted before the FDA approved quinupristin-dalfopristin for use in humans and suggests that the use of virginiamycin in farm animals has created a reservoir of streptogramin-resistant *E. faecium* in our food supply.

Selective medium was more specific for the detection of quinupristin-dalfopristin-resistant strains of *E. faecium* from samples of chicken than from stool samples. The difference in the comparative yield of the selective medium was most likely due to differences in the MIC in the case of isolates from stool samples and chicken. Because both the selective broth and agar used in our study contained 4 μg of quinupristin-dalfopristin per milliliter, the concentration

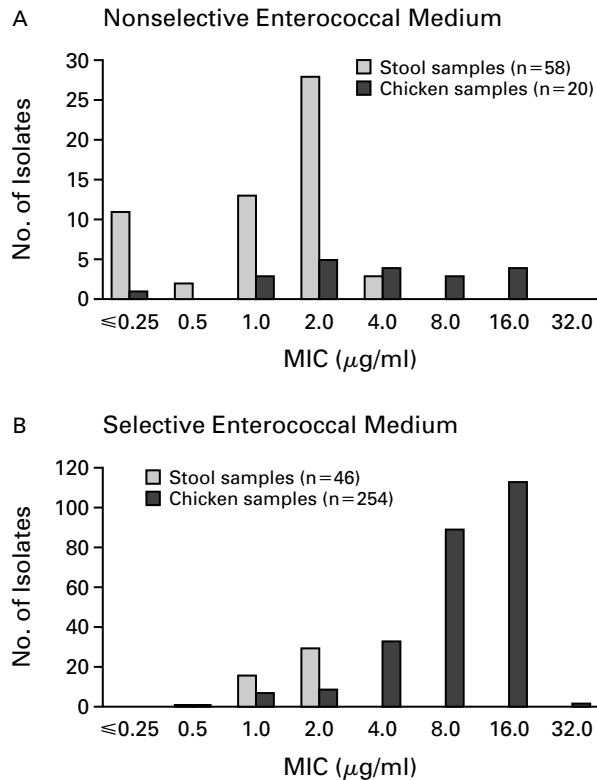


Figure 1. Susceptibility of Isolates of *Enterococcus faecium* from Stool Samples and Chicken Carcasses Cultured in Nonselective Enterococcal Medium (Panel A) and Selective Enterococcal Medium (Panel B).

Results are expressed in terms of the minimal inhibitory concentration (MIC) of quinupristin-dalfopristin. Resistance was defined as a MIC of at least 4 µg per milliliter. Selective medium contained quinupristin-dalfopristin and ampicillin.

of the drug combination may have been too high to detect reliably isolates from stool with low-level resistance (i.e., a MIC of 4 µg per milliliter).

Because the selective medium detected the majority of the quinupristin-dalfopristin-resistant strains isolated from chickens, some of this resistance may have been either induced by or selected for among populations of *E. faecium* with varying levels of resistance (i.e., heteroresistant populations). If resistance was easily induced or selected for in vitro (i.e., after a single passage in antibiotic-containing medium), then it stands to reason that resistance could also be easily induced or selected for in vivo. Nonetheless, induction of or selection for resistance among heteroresistant populations cannot account for all of the difference observed in MICs in the case of isolates from chicken and stool; several quinupristin-dalfopristin-resistant strains that were isolated from chickens with the use of nonselective medium had high

MICs that were similar to those for isolates obtained from selective medium.

Our findings indicate that there was little resistance to quinupristin-dalfopristin among enterococci isolated from people in the United States through mid-1999, despite decades of virginiamycin use in farm animals. Some may find these data reassuring. It is possible that strains of *E. faecium* adapted to chickens and other farm animals colonize humans poorly, or that the determinants of resistance in the animal strains are poorly transferred to *E. faecium* adapted to humans. Alternatively, the rarity of resistance may reflect the absence of selection pressure in humans in the United States.

Although it is approved only for injection, quinupristin-dalfopristin and its active metabolites are eliminated through biliary excretion³³; therefore, even parenteral use may affect bowel flora. As the use of quinupristin-dalfopristin increases in people, the selection pressure on *E. faecium* in the intestines will increase and will probably increase the prevalence of resistance among human isolates. The presence of quinupristin-dalfopristin-resistant strains of *E. faecium* in the food supply increases the likelihood that such an increase could be the result either of direct infection with these strains from food or of the transfer of resistance determinants from these bacteria to *E. faecium* in humans. On the basis of the European experience with vancomycin-resistant enterococci⁵⁻¹⁷ and quinupristin-dalfopristin-resistant *E. faecium*,²⁴⁻²⁷ it appears that both direct infection and transfer of resistance determinants will be increasingly likely to occur in the United States as the use of quinupristin-dalfopristin increases. Because quinupristin-dalfopristin is used principally in hospitalized patients, clinically significant resistance to this drug combination may first appear in hospitals, even if the organism or its resistance determinants originated in the food supply.

The importance of the concomitant use of antimicrobial agents in establishing colonization with resistant *E. faecium* has been demonstrated in several animal models³⁴⁻³⁶ and in human volunteers.⁸ The clinical use of quinupristin-dalfopristin may also select for native strains that acquire resistance traits from animal-derived strains of *E. faecium* that are passing through the intestinal tract. The transferability of streptogramin-resistance determinants in *E. faecium* from isolates from farm animals has been demonstrated both in vitro and in vivo.^{37,38} Broad-scale in vivo transfer of streptogramin-resistance determinants from strains found in animals to strains found in humans has been suggested on the basis of distribution of resistance genes in isolates of *E. faecium* from animals and humans in Europe.²⁶

In the United States, virginiamycin is used to promote the growth of chickens and animals used for food.²² Although data on the total amount of virginiamycin used are not available, the isolation of quin-

TABLE 2. DRUG RESISTANCE AMONG STRAINS OF *ENTEROCOCCUS FAECIUM* ISOLATED FROM CHICKEN AND STOOL SAMPLES CULTURED IN NONSELECTIVE ENTEROCOCCAL MEDIUM.

TYPE OF ANTIBIOTIC	ISOLATES FROM CHICKEN		ISOLATES FROM STOOL	
	RESISTANT TO QUINUPRISTIN-DALFOPRISTIN (N=11)	SUSCEPTIBLE TO QUINUPRISTIN-DALFOPRISTIN (N=9)	RESISTANT TO QUINUPRISTIN-DALFOPRISTIN (N=3)	SUSCEPTIBLE TO QUINUPRISTIN-DALFOPRISTIN (N=55)
	no. resistant (%)			
Beta-lactam				
Penicillin	4 (36)	1 (11)	0	2 (4)
Ampicillin	0	1 (11)	0	1 (2)
Aminoglycoside				
Gentamicin, high-level resistance	2 (18)	5 (56)	0	1 (2)
Streptomycin, high-level resistance	1 (9)	4 (44)	0	3 (5)
Miscellaneous				
Erythromycin	4 (36)	4 (44)	0	4 (7)
Rifampin	0	2 (22)	2 (67)	29 (53)
Tetracycline	11 (100)	7 (78)	1 (33)	13 (24)

upristin-dalfopristin-resistant *E. faecium* from chickens purchased at supermarkets in four states suggests that the use of virginiamycin in chickens is widespread. Virginiamycin is added to chicken feed at a ratio of 5 to 10 g per ton (5.5 to 11 g per 1000 kg) of feed, and 8 billion chickens are raised annually in the United States. In January 2001, it was estimated that more than 192,000 lb (87,000 kg) of virginiamycin is used each year in chicken production in the United States.³⁹

The FDA has recently requested data for an assessment of the effect on human health of the use of streptogramins in food animals and the resulting resistance.⁴⁰ Streptogramin-resistant organisms are now common in the food supply. Studies of the prevalence of quinupristin-dalfopristin-resistant *E. faecium* in the feces of hospitalized patients before and after treatment with quinupristin-dalfopristin would help clarify the risk of colonization and horizontal transfer of resistance determinants. Additional studies are needed to clarify the factors in the practice of animal husbandry, meat processing, cooking, and infection control that affect the frequency of human contact with these resistant organisms and the acquisition of resistance. If such studies demonstrate a role for foodborne transmission in the emergence of quinupristin-dalfopristin-resistant *E. faecium* in humans, restrictions on the continued use of virginiamycin in food animals should be considered.

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