Use of Streptogramin Growth Promoters in Poultry and Isolation of Streptogramin-Resistant Enterococcus faecium from Humans

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(See the editorial commentary by Frimodt-Møller and Hammerum, on pages 1191–3, and the article by Jalava et al., on pages 1209–16.)

Background. Virginiamycin use in poultry selects for Enterococcus faecium with cross-resistance to quinupristin-dalfopristin, a drug for vancomycin-resistant E. faecium in humans. We conducted an epidemiologic study of poultry exposures as risk factors for human carriage of quinupristin-dalfopristin–resistant E. faecium.

Methods. Rectal or fecal samples for E. faecium testing were obtained from 567 newly admitted hospital patients and 100 healthy vegetarians. Participants were interviewed regarding poultry exposure. Retail poultry washes (160 conventional and 26 antibiotic free) were also tested for the presence of E. faecium. Constitutive and inducible quinupristin-dalfopristin resistance were assessed in E. faecium isolates, and resistance genes were identified by polymerase chain reaction.

Results. E. faecium was isolated from 105 patients, 65 vegetarians, and 77 conventional and 23 antibiotic-free poultry washes. Constitutive quinupristin-dalfopristin resistance was absent in human E. faecium, but 56% of conventional poultry isolates were quinupristin-dalfopristin resistant. Inducible quinupristin-dalfopristin resistance was more common in samples from patients than in those from vegetarians and in washes of conventional than antibiotic-free poultry. Higher poultry consumption was associated with inducible quinupristin-dalfopristin resistance. vatE was present in 38% of E. faecium isolates from patients and none from vegetarians. Touching raw poultry was associated with the presence of vatE.

Conclusions. Poultry exposure is associated with a quinupristin-dalfopristin resistance gene and inducible quinupristin-dalfopristin resistance in human fecal E. faecium. The continued use of virginiamycin may increase the potential for streptogramin-resistant E. faecium infection in humans.

Nosocomial enterococcal infections have become a major cause of sepsis and wound infections in recent years, and the prevalence of vancomycin and ampicillin resistance in Enterococcus faecium has increased dramatically in hospital populations [1, 2]. The semisynthetic antimicrobial quinupristin-dalfopristin is 1 of 3 drugs licensed by the US Food and Drug Administration (FDA) for the treatment of serious vancomycin-resistant E. faecium infections in the United States. A related streptogramin antibiotic, virginiamycin, has been used as a growth promoter in US livestock since 1975. Virginiamycin is administered to poultry in feed to increase weight gain [3]. The drug is also used as a growth promoter in swine. Several studies have demonstrated a relationship between the prevalence of quinupristin-dalfopristin–resistant E. faecium in food animals and the magnitude of virginiamycin use as a growth promoter [4–6]. Denmark banned virginiamycin use as a growth promoter in 1997, and the European Union subsequently banned virginiamycin and 3 other antimicrobial growth promoters [7].

Quinupristin-dalfopristin is a mixture of 2 streptogramin compounds (A and B) that inhibit bacterial
protein synthesis through different mechanisms. Clinical quinupristin-dalfopristin resistance was reported in a small number of patients during prelicensure studies [8]. In *E. faecium*, streptogramin A resistance is encoded by the acetyltransferase genes vatD and vatE, which are located on plasmids [9–11]. Streptogramin B resistance is mediated by erythromycin resistance methylase (*ermB*) genes [12, 13]. These genes do not account for all quinupristin-dalfopristin–resistant *E. faecium*, and other mechanisms of resistance remain undefined [14–16].

Microbiological studies have demonstrated that quinupristin-dalfopristin–resistant *E. faecium* are prevalent on poultry farms and in retail poultry in the United States [16–19]. Other studies have shown that feeding virginiamycin to chickens and turkeys leads to the emergence of quinupristin-dalfopristin–resistant *E. faecium* [20–22]. However, the testing of selected human fecal samples has suggested that human colonization with quinupristin-dalfopristin–resistant *E. faecium* is rare in the United States [19]. The latter observation was a key factor in a proposed FDA risk-assessment model regarding streptogramin use in animals and human infections with quinupristin-dalfopristin–resistant *E. faecium*. We conducted an epidemiologic study of quinupristin-dalfopristin susceptibility in *E. faecium* colonizing humans and retail poultry and assessed the association between poultry exposure and the isolation of quinupristin-dalfopristin–resistant *E. faecium* from the gastrointestinal tract of humans.

**PATIENTS, MATERIALS, AND METHODS**

**Study design and population.** This was a cross-sectional survey of quinupristin-dalfopristin–resistant *E. faecium* in recently admitted hospital patients, healthy vegetarians, and retail poultry in 4 communities. The enrollment community hospitals included St. Joseph’s Hospital, Marshfield, Wisconsin; Gunderson Lutheran Hospital, La Crosse, Wisconsin; Sacred Heart Hospital, Eau Claire, Wisconsin; and Rice Memorial Hospital, Willmar, Minnesota.

**Participant recruitment.** Newly admitted hospital patients were enrolled from June 2002 through May 2003. Subjects were enrolled to yield ~150 participants per hospital, distributed evenly throughout the 12-month period. Patients ≥14 years old admitted to a medical, surgical, or intensive care unit (ICU) bed were eligible if they were alert, stable, and capable of giving informed consent. Patients with acute rectal bleeding, recent use of cathartics, or neutropenia were excluded. Rectal-swab samples were obtained within 36 h of hospital admission for all patients.

Vegetarian participants were recruited from January through July 2004 at health-food cooperatives located in or near the 4 study communities. Adult vegetarians were eligible to participate if they reported no consumption of meat products for at least 1 year. Stool samples were obtained from vegetarian participants for the isolation and characterization of *E. faecium*.

Hospital patients and vegetarians were interviewed by telephone regarding potential dietary and environmental sources of *E. faecium*. Interviews were conducted within 62 days of enrollment for hospital patients and at the time of enrollment for vegetarians. The main exposures of interest included touching raw meat, consuming meat products, and contact with live food animals. For hospital patients, information on recent antibiotic use and chronic diseases was obtained through abstraction of medical records. The study protocol was approved by the Marshfield Clinic’s institutional review board, and all participants provided written, informed consent.

**Retail poultry samples.** Retail poultry samples were collected 4 times during the 12-month hospital recruitment period from grocery stores in the 4 study communities. During each visit to a community, we purchased a convenience sample of ~8 chicken and 2 turkey products from various retail locations. These retail samples, designated “conventional retail poultry,” were selected without regard to label information concerning antibiotic use on the farm. During an additional collection period, retail poultry products were purchased from food cooperatives and natural-food stores in or near the study communities. These poultry products, designated “antibiotic-free retail poultry,” were confirmed to be raised without antibiotics on the basis of the product label or by contacting the manufacturer or distributor.

**Laboratory methods.** Stool samples and rectal swabs were suspended in brain-heart infusion (BHI) broth overnight at 37°C. Retail poultry samples were massaged in buffered peptone water, and the rinsate was centrifuged at 3070 g to yield 1 mL of concentrate for inoculation into BHI. *E. faecium* was isolated on colistin–naladixic acid agar, identified using standard biochemical procedures, and confirmed by polymerase chain reaction (PCR) for a species-specific *ddl* amplicon [23]. A total of 5–6 randomly selected isolates from each sample were evaluated for resistance to quinupristin-dalfopristin. Quinupristin-dalfopristin susceptibility was measured by E-test using a recognized reference standard for the interpretation of MICs [24]. Isolates with MICs ≥4 μg/mL were classified as resistant, and those with MICs between 1 and 4 μg/mL were classified as having intermediate resistance.

An *E. faecium* isolate representing each level of quinupristin-dalfopristin resistance (susceptible, intermediate, or resistant) found in each sample (5–6 isolates) was selected for further evaluation. Ten genetic markers were evaluated initially in the hospital patients: *vatA*, *vatB*, *vatC*, *vgA*, *vgA*, *vgaB*, *vgbA*, *vgbB*, *vatE*, *vatD*, and *ermB* [13–16, 25, 26]. Only the latter 3 were identified, and subsequent PCR testing for genetic markers was limited to this group. Positive results were confirmed by DNA sequencing. Representative isolates from humans and retail poultry were char-
characterized using pulsed-field gel electrophoresis (PFGE) at the Minnesota Department of Health (St. Paul) [27].

*E. faecium* isolates that were sensitive or had intermediate resistance to quinupristin-dalfopristin (MICs ≤4 µg/mL) were tested for the detection of inducible resistance [28]. Initially, 5 × 10^8 *E. faecium* (measured by optical density) in the log phase of growth were transferred to 5 mL of BHI broth that contained 0.25 µg/mL virginiamycin and cultured at 37°C for 24 h. The bacterial density was readjusted to 1 × 10^7/mL in fresh BHI broth, challenged with 8 µg/mL quinupristin-dalfopristin, and incubated for another 24 h at 37°C. The level of inducible quinupristin-dalfopristin resistance was expressed as relative growth, defined as the 24-h optical density (at 600 nm) of the isolate preexposed to virginiamycin and challenged with quinupristin-dalfopristin divided by the optical density of the same isolate cultured in BHI broth for 24 h without virginiamycin before exposure or quinupristin-dalfopristin challenge. This ratio was multiplied by 100 to yield the relative percentage of growth, with possible values ranging from 0% (no inducible resistance) to 100% (high level of inducible resistance). The inducibility assay was also performed for isolates from hospital patients after preexposure to 0.25 µg/mL of quinupristin-dalfopristin instead of virginiamycin.

**Statistical analysis.** Statistical analyses were based on the *E. faecium* isolate with the highest level of constitutive quinupristin-dalfopristin resistance in each sample. The primary outcomes included the presence of the vatE gene and the level of inducible resistance, measured as the relative percentage of growth. Contact with raw poultry and frequency of poultry consumption were the primary exposures of interest. The latter was dichotomized at the median consumption level for hospital patients. Vegetarians made up a separate category of poultry consumption.

Unadjusted associations between the exposure and outcome measures were assessed using Fisher’s exact test, the Wilcoxon rank-sum test, or the Kruskal-Wallis test. Multivariable regression models were used to estimate adjusted measures of association. Separate models were constructed for each combination of population (hospital patients alone or the combined group of hospital patients and vegetarians), outcome, and exposure. Variables were screened for inclusion in an initial multivariable model. Candidate variables with were retained. Collinearity was assessed in the resulting model [29, 30]. Final models included variables that achieved statistical significance (P ≤ 0.05) or whose exclusion altered the outcome-exposure measure of association by >10%.

For the vatE models, adjusted prevalence ratios were computed using methods described by Zou [31]. Linear regression was used for the inducible resistance models. Because the distribution of inducible resistance was skewed, the natural log transformation was applied before models were fitted. Applying the inverse transformation to the fitted linear model resulted in a model of the median response on the original scale [32]. The measure of association for the inducible resistance models was the median relative percentage of growth in the exposed group divided by that in the unexposed group. For both measures of association (prevalence ratios for vatE and ratios of medians for inducible resistance), a value of 1.0 indicated no

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hospital patients</th>
<th>Vegetarians</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, mean ± SD, years</strong></td>
<td>63 ± 17</td>
<td>39 ± 15</td>
</tr>
<tr>
<td><strong>Male sex</strong></td>
<td>62 (59)</td>
<td>23 (35)</td>
</tr>
<tr>
<td><strong>White race</strong></td>
<td>104 (99)</td>
<td>63 (97)</td>
</tr>
<tr>
<td><strong>College graduate</strong></td>
<td>21 (20)</td>
<td>42 (65)</td>
</tr>
<tr>
<td><strong>Employed</strong></td>
<td>46 (44)</td>
<td>47 (72)</td>
</tr>
<tr>
<td><strong>&gt;5 physician visits during the preceding year</strong></td>
<td>56 (53)</td>
<td>7 (11)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%), unless otherwise indicated. Missing values for hospital patients included: 1 for college graduate, 2 for exposure to live poultry, 3 for touching raw poultry, 3 for poultry consumption, and 6 for frequency of poultry consumption. One vegetarian had missing information for touching raw poultry.

a Antibiotic therapy was based on self-report for vegetarians and on medical record review for hospital patients.
RESULTS

There were 1614 eligible patients contacted within 36 h after hospital admission, and 622 (39%) agreed to participate. Of these, 55 were excluded from the analysis because of specimen shipping delays (n = 12) or because the risk-factor interview was not completed (n = 43). For the remaining 567 patients, the median interval from hospital admission to fecal specimen collection was 25 h (range, 11–36 h). The median time between enrollment and completion of the risk-factor interview was 22 days (range, 5–62 days). One hundred vegetarians provided stool samples and completed the risk-factor interview. E. faecium was isolated from 105 hospital patients and 65 vegetarians; further analyses were restricted to this group. Compared with vegetarians, the hospital patients were more likely to be older and male, with less formal education (table 1).

None of the human E. faecium isolates had constitutive resistance to quinupristin-dalfopristin (MIC $\geq$4 $\mu$g/mL) by E-test, but a majority of isolates from both hospital patients and vegetarians had intermediate quinupristin-dalfopristin resistance (table 2). Neither vatE nor ermB was found in vegetarian E. faecium isolates; vatE was commonly found in isolates from the hospital patients. ErmB was present in <10% of hospital patient isolates, and vatD was found in 1 E. faecium isolate from a hospital patient. The rarity of ermB and vatD genes in the study population prevented the effective evaluation of their role in the association between poultry exposure and quinupristin-dalfopristin resistance.

E. faecium was isolated from 77 (48%) of 160 conventional retail poultry samples and 23 (88%) of 26 antibiotic-free retail poultry samples. Quinupristin-dalfopristin resistance was more common in isolates from conventional than antibiotic-free retail poultry (table 2). Conventional retail poultry isolates were 4 times more likely to contain vatE ($P = .004$) and ermB ($P = .005$) than were isolates from antibiotic-free retail poultry. All PFGE patterns from humans and retail poultry were distinct, and no common clones were identified in both sources (data not shown).

The level of inducible resistance to quinupristin-dalfopristin (expressed as relative percentage of growth) varied greatly in E. faecium isolates from different sources (figure 1). Higher inducible resistance levels were found in E. faecium isolates from hospital patients than in those from vegetarians. The median relative percentage of growth was 6.5% (range, 1.3%–56.4%) for isolates from hospital patients and 2.4% (range, 1.2%–4.6%) for isolates from vegetarians ($P < .001$). For isolates from hospital patients that were preexposed to quinupristin-dalfopristin, the median relative percentage of growth was 6.1% (range, 0.8%–45.8%). Without preexposure to virginiamycin or quinupristin-dalfopristin, isolates from hospital patients did not grow when challenged with 8 $\mu$g/mL quinupristin-dalfopristin (median relative percentage of growth, 2.0%; range, 0.9%–8.2%). When measured by E-test, 25 (24%) of 105 isolates from hospital patients became resistant to quinupristin-dalfopristin (MIC $\geq$4 $\mu$g/mL after preexposure to virginiamycin; the median MIC of these resistant isolates after induction was 8 $\mu$g/mL (range, 4–32 $\mu$g/mL). The relative percentage of growth after preexposure to virginiamycin and quinupristin-dalfopristin challenge was correlated with the MIC measurement of resistance after induction (Spearman’s correlation, 0.55 [95% confidence interval [CI], 0.40–0.67]).

Inducible quinupristin-dalfopristin resistance was associated with the presence of vatE. The median relative percentage of

Table 2. Quinupristin-dalfopristin susceptibility (E-test) and genetic determinants of resistance in Enterococcus faecium from different ecologic sources.

<table>
<thead>
<tr>
<th>E. faecium source</th>
<th>Susceptibility to quinupristin-dalfopristin</th>
<th>Resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Hospital patients (n = 105)</td>
<td>25 (24)</td>
<td>80 (76)</td>
</tr>
<tr>
<td>Vegetarians (n = 65)</td>
<td>8 (12)</td>
<td>57 (88)</td>
</tr>
<tr>
<td>Conventional retail poultry (n = 77)</td>
<td>11 (14)</td>
<td>23 (30)</td>
</tr>
<tr>
<td>Antibiotic-free retail poultry (n = 23)</td>
<td>11 (48)</td>
<td>9 (39)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of isolates.

* Antibiotic-free retail poultry samples were obtained from food cooperatives or natural food stores and were confirmed to be raised without antibiotics by product label or information provided by the producer or distributor.
growth after preexposure to virginiamycin was 15.8% (interquartile range [IQR], 9.0%–26.2%) for isolates that contained vatE and 2.6% (IQR, 2.0%–3.7%) for those without vatE ($P < .001$). The PFGE patterns of the inducible isolates were distinct from each other. For each isolate, the pattern after induction and quinupristin-dalfopristin challenge was indistinguishable from the original pattern before induction, which is consistent with inducible resistance rather than selection of a contaminating isolate with constitutive resistance.

*E. faecium* isolates from conventional retail poultry had higher levels of inducible resistance than isolates from antibiotic-free retail poultry. The median relative percentage of growth was 30.6% (range, 1.4%–47.8%) for conventional retail poultry isolates and 2.2% (range, 1.5%–6.6%) for antibiotic-free retail poultry isolates ($P < .001$).

**Risk-factor analysis.** The association between poultry exposures and carriage of *E. faecium* with vatE differed among hospital patients with and without prior antibiotic use. Forty-five (43%) of 105 hospital patients with *E. faecium* had used antibiotics during the month before enrollment. The most common antibiotics used were β-lactams (70%), fluoroquinolones (29%), and macrolides (9%). Among these patients, touching raw poultry did not increase the risk of carrying *E. faecium* with vatE (prevalence ratio, 0.55 [95% CI, 0.31–0.96]). By contrast, the hospital patients without recent antibiotic use had a increased risk of carrying *E. faecium* isolates with vatE if they had touched raw poultry (prevalence ratio, 1.94 [95% CI, 0.73–5.15]; $P = .05$ for both exposures, Breslow-Day test) [34]. None of the vegetarians with *E. faecium* had used antibiotics during the month before enrollment. On the basis of these findings, results are reported for participants without recent antibiotic use.

Carriage of *E. faecium* with vatE was significantly associated with both touching raw poultry and higher poultry consumption in the combined hospital patient and vegetarian group (table 3). Inducible resistance was significantly associated with higher poultry consumption in this group. In the multivariable models, we found higher point estimates for both raw poultry contact and poultry consumption in persons without recent antibiotic use, but not all of these were statistically significant (table 4). Carriage of *E. faecium* with vatE was significantly associated with touching raw poultry in the combined hospital patient and vegetarian group ($P = .032$). Because none of the vegetarian isolates had the vatE gene, we were unable to assess the association between vatE and poultry consumption in the

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**Figure 1.** Plots of inducible resistance for *Enterococcus faecium* isolated from humans and retail poultry with initial *E*-test MICs $< 4 \mu g/mL$ for quinupristin-dalfopristin. Each symbol represents 1 isolate. Inducible resistance (relative percentage of growth) was determined by dividing the 24-h growth density of isolates after preexposure to virginiamycin and quinupristin-dalfopristin challenge by the 24-h growth density of untreated isolates and multiplying by 100.
Table 3. Unadjusted association between \( vatE \) or inducible resistance (relative percentage of growth) and poultry exposure in \( Enterococcus faecium \) from hospital patients and vegetarians.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. with ( E. faecium )^b</th>
<th>( vatE ) present</th>
<th>Inducible resistance^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>( P )^c</td>
<td>Median ( P )^c</td>
</tr>
<tr>
<td>Hospital patients only (( n = 45 ))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Touched raw poultry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>7 (39)</td>
<td>4.6</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>5 (20)</td>
<td>3.6</td>
</tr>
<tr>
<td>Poultry consumption^d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>20</td>
<td>8 (40)</td>
<td>7.8</td>
</tr>
<tr>
<td>Low</td>
<td>24</td>
<td>4 (17)</td>
<td>3.2</td>
</tr>
<tr>
<td>Hospital patients and vegetarians (( n = 110 ))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Touched raw poultry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24</td>
<td>7 (29)</td>
<td>3.0</td>
</tr>
<tr>
<td>No</td>
<td>83</td>
<td>5 (6)</td>
<td>2.5</td>
</tr>
<tr>
<td>Poultry consumption^d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>20</td>
<td>8 (40)</td>
<td>7.8</td>
</tr>
<tr>
<td>Low</td>
<td>24</td>
<td>4 (17)</td>
<td>3.2</td>
</tr>
<tr>
<td>Veg</td>
<td>65</td>
<td>0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

NOTE. Results are shown for participants without antibiotic use during the preceding month.

^a Inducible resistance (relative percentage of growth) was determined by dividing the 24-h growth density of isolates after preexposure to virginiamycin and quinupristin-dalfopristin challenge by the 24-h growth density of untreated isolates and multiplying by 100.

^b Two and 1 hospital patients who did not use antibiotics in the past month were missing data on touching raw poultry and frequency of poultry consumption, respectively. One vegetarian was missing data on touching raw poultry.

^c Fisher’s exact \( P \) for the dichotomous \( vatE \) measure. For the continuous inducible resistance (relative percentage of growth) measure, either the Wilcoxon rank-sum test (dichotomous exposure) or the Kruskal-Wallis test (3-category exposure) was used.

^d Poultry consumption was defined as “high” for those who consumed poultry above the median for the hospital patients (9 times/month), “low” for hospital patients who consumed less, and “veg” for vegetarians.

combined group using statistical methods. However, this association was marginally statistically significant (\( P = .058 \)) in the hospital patients. Carriage of \( E. faecium \) with inducible resistance (higher relative percentage of growth) was significantly associated with touching raw poultry in the hospital patients (\( P = .027 \)). Inducible resistance was also significantly associated with higher poultry consumption in the combined group of hospital patients and vegetarians (\( P < .001 \) for both consumption levels in hospital patients vs. vegetarians).

DISCUSSION

The results of the present investigation suggest that virginiamycin use in poultry contributes to human carriage of \( E. faecium \) that contains streptogramin resistance genes with readily inducible resistance. A previous study in the United States found that fecal \( E. faecium \) with constitutive quinupristin-dalfopristin resistance are rare in humans, but that study did not evaluate the prevalence of streptogramin resistance genes or inducible resistance [19]. In the present study, susceptible and intermediate isolates were screened for both resistance genes and inducible resistance. The acetyltransferase gene \( vatE \) was commonly found in both human fecal \( E. faecium \) and in isolates colonizing retail poultry. In the risk-factor analysis, touching raw poultry and more frequent poultry consumption were independently associated with the presence of \( vatE \) and a phenotype of inducible quinupristin-dalfopristin resistance. In particular, we found striking differences in the characteristics of \( E. faecium \) from recently hospitalized patients who ate meat, compared with healthy vegetarians. The \( vatE \) gene was not found in any vegetarian \( E. faecium \) isolates, but it was found in approximately one-third of isolates from hospital patients. The median level of inducible resistance was also 3-fold higher in persons with a high level of poultry consumption, compared with vegetarians. These positive associations with poultry contact and consumption were found only in persons without recent antibiotic use.

The identification of poultry-related risk factors in the present study is supported by previous studies that have reported a high prevalence of quinupristin-dalfopristin-resistant \( E. faecium \) in retail poultry in the United States [16–18]. Other investigators have also shown that streptogramin-resistant \( E. faecium \) occur less frequently in animals raised without antibiotics.
and we found that inducible resistance was substantially lower in antibiotic-free retail poultry isolates, relative to those in conventional poultry. The biologic plausibility for the foodborne acquisition of streptogramin resistance genes is further supported by the observation that vatE is located on plasmids and can be transferred by conjugation [16, 35, 36]. In an experimental study, ingestion of streptogramin-resistant E. faecium led to transient colonization for up to 14 days, which provides sufficient time for conjugative transfer of resistance determinants to endogenous flora [37]. In the present study, PFGE failed to identify similar strains in humans and retail poultry, which is consistent with the hypothesis that the horizontal transfer of resistance genes is more important than clonal spread of E. faecium from livestock to humans.

The continued nontherapeutic use of virginiamycin and other growth promoters in livestock has been controversial. Virginiamycin use has been banned in Denmark and the European Union, but no regulatory action has been taken by the FDA to prohibit its use in the United States. The FDA has classified streptogramin antibiotics as "highly important" for use in human medicine (available at: http://www.fda.gov/cvm/Documents/fguide152.pdf), and a risk-assessment model for streptogramin use in food-producing animals has been under development (available at: http://www.fda.gov/cvm/Documents/SREF_RA_FinalDraft.pdf). However, the lack of critical information about the probability of acquiring streptogramin resistance through the food supply and the potential for human health consequences has made it difficult to develop meaningful risk estimates. The FDA risk assessment assumed that only 10% of quinupristin-dalfopristin-resistant E. faecium infections originated from food pathways. The results of the present study suggest that the FDA model may underestimate the true risk of foodborne acquisition, because streptogramin resistance genes are commonly found in human fecal E. faecium, despite the absence of constitutive streptogramin resistance.

The presence of vatE and inducible streptogramin resistance in the endogenous fecal flora of newly hospitalized patients creates a genetic reservoir for the emergence of streptogramin-resistant, vancomycin-resistant E. faecium in the hospital environment. E. faecium genogroups have been shown to be associated with different ecologic sources, and isolates carried by healthy people are distinct from those causing nosocomial infection [38, 39]. However, other investigators have suggested that the previous spread of vancomycin-resistant E. faecium infections was due to nosocomial selection of a specific ampicillin-resistant E. faecium genotype, with subsequent horizontal transfer of the vanA transposon [39]. We hypothesize that a similar mechanism of spread could occur with streptogramin-resistant E. faecium, although the genetic basis for streptogramin resistance has not been fully elucidated. The selection and horizontal transmission of streptogramin-resistant E. faecium may be clinically unimportant in the present era because of the infrequent use of quinupristin-dalfopristin in hospitals. However, if the use of quinupristin-dalfopristin increases in future years, the presence of vatE and other genes might facilitate the rapid emergence of streptogramin resistance.

There are several caveats with regard to the design and interpretation of the present study. Healthy vegetarians differ from hospitalized patients in many characteristics besides poultry consumption. Although the multivariable analysis adjusted for many of these factors, confounding may have occurred, and other factors associated with vegetarian status may have contributed to the observed associations. In addition, this was a cross-sectional study, and the temporal relationship between poultry exposures and E. faecium characteristics could not be determined. Finally, the food-consumption patterns and other

<table>
<thead>
<tr>
<th>Group</th>
<th>vatE present</th>
<th>Inducible resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted prevalence ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Hospital patients only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Touched raw poultry</td>
<td>2.3</td>
<td>0.7–6.9</td>
</tr>
<tr>
<td>High poultry consumption</td>
<td>3.0</td>
<td>1.0–9.2</td>
</tr>
<tr>
<td>Hospital patients and vegetarians</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Touched raw poultry</td>
<td>3.4</td>
<td>1.1–10.4</td>
</tr>
<tr>
<td>High poultry consumption vs. vegetarianb</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Low poultry consumption vs. vegetarianb</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** Results are shown for participants without antibiotic use during the preceding month. CI, confidence interval.

a Estimated median relative percentage of growth in the exposed group divided by that in the unexposed group. An adjusted ratio >1 indicates an association between the exposure and relative percentage of growth.

b Prevalence ratios for vatE were undefined because none of the vegetarians carried E. faecium with vatE.
exposures were self-reported, and we could not independently validate the responses. Misclassification of poultry exposures may have occurred, although we expect that any such misclassification would be nondifferential. Additional prospective studies in different populations would be helpful in confirming the major findings. In particular, the relationship between the presence of streptogramin resistance genes and poultry exposure should be assessed in the general population, where recent antibiotic exposure is uncommon. It would also be helpful to conduct a similar study in a country where virginiamycin is not used as a growth promoter.

These findings raise additional concerns regarding the continued use of virginiamycin in food animals. Therapeutic options for serious human vancomycin-resistant enterococcal infections are limited, and quinupristin-dalfopristin is one of few drugs that remain effective. With few new antimicrobials under development, a high priority should be placed on assessing the human health impact of continued streptogramin use in food animals and on developing evidence-based policies to prevent the emergence of streptogramin-resistant infections in hospitals.

**MARSHFIELD ENTEROCOCCAL STUDY GROUP MEMBERS**

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**References**