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Influence of Antibiotic Selection on Genetic Composition of *Escherichia coli* Populations from Conventional and Organic Dairy Farms▽†

Seth T. Walk,[1] Janice M. Mladonicky,[1] Jaclyn A. Middleton,[1] Anthony J. Heidt,[1] Julie R. Cunningham,[1] Paul Bartlett,[2] Kenji Sato,[3] and Thomas S. Whittam[1]*

Microbial Evolution Laboratory, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824[1]; Department of Large Animal Clinical Sciences, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824[2]; and Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82070[3]

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The widespread agricultural use of antimicrobials has long been considered a crucial influence on the prevalence of resistant genes and bacterial strains. It has been suggested that antibiotic applications in agricultural settings are a driving force for the development of antimicrobial resistance, and epidemiologic evidence supports the view that there is a direct link between resistant human pathogens, retail produce, farm animals, and farm environments. Despite such concerns, little is understood about the population processes underlying the emergence and spread of antibiotic resistance and the reversibility of resistance when antibiotic selective pressure is removed. In this study, hierarchical log-linear modeling was used to assess the association between farm type (conventional versus organic), age of cattle (calf versus cow), bacterial phenotype (resistant versus susceptible), and the genetic composition of *Escherichia coli* populations (*E. coli* Reference Collection [ECOR] phylogroup A, B1, B2, or D) among 678 susceptible and resistant strains from a previously published study of 60 matched dairy farms (30 conventional and 30 organic) in Wisconsin. The analysis provides evidence for clonal resistance (ampicillin resistance) and genetic hitchhiking (tetracycline resistance [Tet^r]), estimated the rate of compositional change from conventional farming to organic farming (mean, 8 years; range, 3 to 15 years), and discovered a significant association between low multidrug resistance, organic farms, and strains of the numerically dominant phylogroup B1. These data suggest that organic farming practices not only change the frequency of resistant strains but also impact the overall population genetic composition of the resident *E. coli* flora. In addition, the results support the hypothesis that the current prevalence of Tet^r loci on dairy farms has little to do with the use of this antibiotic.

Escherichia coli is an indicator species for a variety of anthropogenic effects on microbial populations, such as the emergence and spread of antibiotic resistance in agriculture (1, 5–10, 14, 20, 30, 35, 36, 38, 39). Although most strains are commensal bacteria and nonpathogenic to humans and animals, there are well-recognized pathogenic strains that can cause a variety of human and zoonotic diseases, and some commensal populations are known to carry high levels of antibiotic resistance (4, 28, 29). Such resistant populations pose a public and veterinary health risk because of the potential transfer of genetic resistance determinants to pathogens. In addition, certain virulence factors may be mobilized on genetic elements and transferred to normally commensal but antibiotic-resistant strains via horizontal exchange (31, 42, 43).

During antibiotic selection in the laboratory, resistance-conferring mutations often have measurable deleterious effects (i.e., a resistance cost) due to a reduction in the function of genes in which resistance mutations arise. In order to maintain a competitive advantage over other members of the population, it is hypothesized that deleterious effects on fitness are compensated by changes elsewhere in the genome (21, 24, 25, 32, 33, 37). The occurrence of such compensatory fitness mutations makes it difficult to determine whether the abundance and distribution of resistant strains are results of direct selection on the original mutation that caused resistance, selection on compensatory changes, or other ecological factors that limit population diversity (environmental selection, bottlenecks, genetic drift, etc.). There is sound evidence that antibiotic use increases the abundance of resistant phenotypes (34), but it is not clear if the cessation of antibiotic use will decrease abundance after compensatory changes have occurred (2, 22). In addition, resistance loci may be genetically linked to loci under strong selection and be carried at a high frequency in the population (genetic hitchhiking).

Antibiotic use in dairy cattle provides an ideal opportunity to assess the role of natural selection in bacterial populations for several reasons. The source of the antibiotic selective pressure is known, and the dosage is often recorded. The common genetic determinants for certain resistant phenotypes have been characterized, and high-throughput assays are available for their identification. Hypotheses generated under laboratory conditions can be tested in vivo by comparing bacteria from farms that regularly use antibiotics (conventional) and bacteria from farms that rarely use antibiotics (organic) (34). Finally, a number of studies have previously characterized re-

* Corresponding author. Mailing address: Microbial Evolution Laboratory, 165 Food Safety and Toxicology Building, Michigan State University, East Lansing, MI 48824. Phone: (517) 432-3100, ext. 178. Fax: (517) 432-2310. E-mail: whittam@msu.edu.

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sistance dynamics on both farm types and have identified variables that significantly influence the abundance of resistant phenotypes (1, 5, 7, 14, 30, 34–36).

The purpose of the present study was to assess the influence of antibiotic selection on the genetic composition of *E. coli* populations from conventional and organic dairy farms. First, we used a PCR-based assay (12) to quantify the abundance and distribution of four phylogenetic groups in populations cultured during a longitudinal sampling of cattle from matched conventional and organic dairy farms in Wisconsin (34). We then assessed the pattern of statistical dependence for farm type (conventional versus organic), age of cattle (cows versus calves), bacterial phenotype (resistant versus susceptible), and bacterial genetic composition (*E. coli* Reference Collection [ECOR] groups A, B1, B2, and D) using hierarchical log-linear modeling.

MATERIALS AND METHODS

***E. coli* strain collection.** A total of 678 *E. coli* strains (367 random susceptible and 311 resistant strains) were assembled from a collection of 1,121 strains of a longitudinal sampling of 10 randomly selected cows and calves from a matched set of 30 conventional and 30 organic dairy farms in Wisconsin (34). Briefly, a cluster of organic farms was selected, and the geographically closest conventional farm was selected for purposes of comparison so as to minimize the effects of distance (cline effects). All organic farms were certified by a USDA-accredited certification agency as not having treated adult cows with an antibiotic for at least 3 years (mean, 8 years; range, 3 to 15 years) prior to this study. More information about these farms is available (34).

In the original study (34), fecal samples were taken from five lactating cows and five calves (<6 months of age) at each of two visits (once in March and once in September) and conducted with aseptic technique. Laboratory isolation was begun within 72 h, and a single *E. coli* colony was isolated from each fecal sample so as to exclude any single farm or within-animal bias. All isolates were confirmed by standard biochemical assays. MICs of 17 antibiotics were determined for each strain as recommended by the CLSI (formerly NCCLS) (26) using a commercially available semiautomatic broth microdilution test (Sensititre; Trek Diagnostic Systems Inc., Cleveland, OH) and appropriate quality control strains. These antibiotics included ampicillin, amoxicillin-clavulanic acid, cephalothin, cefoxitin, ceftiofur, ceftriaxone, streptomycin, kanamycin, gentamicin, apramycin, amikacin, tetracycline, sulfamethoxazole, trimethoprim-sulfamethoxazole, nalidixic acid, and ciprofloxacin. Ampicillin resistance (Amp^r) and tetracycline resistance (Tet^r) phenotypes were confirmed by the presence of overnight growth on LB broth (Lennox; Becton, Dickinson, and Company, Sparks, MD) agar containing antibiotic at the CLSI cutoff concentrations (32 μg/ml and 16 μg/ml, respectively). More details about the strain collection and isolation procedures can be found in reference 34.

ECOR phylogrouping by multiplex PCR. Strains were grouped into one of four phylogenetic lineages (A, B1, B2, or D) based on methods adapted from those of Clermont et al. (12). Genomic DNA was isolated from 2 ml of overnight culture in LB broth (Lennox; Becton, Dickinson, and Company, Sparks, MD) using the Puregene DNA isolation kit (Gentra Systems Inc., Minneapolis, MN.). DNA preparations were quantified with a NanoDrop ND-1000 UV-visible spectrophotometer (NanoDrop Technologies, Wilmington, DE), diluted to a final concentration of 100 ng/μl, and stored at 4°C. Genomic DNA preparations were tested using primers targeting a 650-bp region of the conserved housekeeping gene *mdh* (see www.shigatox.net/stec/mlst-new/index.html for primer sequences and reaction conditions), and AmpliTaq Gold DNA polymerase (Applied Biosystems). This protocol has produced a positive amplicon in strains representing the genotypic diversity of the species as well as *E. coli*'s most recent common ancestor, *Escherichia albertii*. Genomic DNA was reisolated if the assay was negative. Strains that were negative for duplicate, independent genomic isolations were considered members of species other than *E. coli* and excluded from further analysis. Representative ECOR strains were used as template controls for a duplex PCR targeting the genes *chuA* and *yjaA*. We found that the following duplex conditions yielded higher PCR specificity with AmpliTaq Gold than the published triplex: denaturation at 94°C for 10 min; 35 cycles of 92°C for 1 min, 59°C for 1 min, and 72°C for 30 seconds; and a final elongation at 72°C for 5 min. A separate PCR was run with primers targeting the TspE4.C2 anonymous DNA locus using published conditions (12).

Resistance loci and class 1 integron PCR. Amp^r and Tet^r strains were screened for the presence of six previously identified resistance loci. A multiplex PCR was used to detect the presence of bla_{TEM}, bla_{SHV}, and bla_{OXA-1} in Amp^r strains by the method of Colom et al. (13). Fragments of the *tetA*, *tetB*, and *tetC* genes were targeted in Tet^r strains using the primers and conditions published by Boerlin et al. (9). Three primer sets were used to determine the presence of class 1 integrons in the resistant strains. Primer sets targeting the class 1 integrase locus, *intI1*, the conserved region cassette regions A and B, and the quaternary ammonium compound resistance gene *qacEΔ1* are given along with the reaction conditions in reference 23. Integron presence was defined as amplification of all three loci.

Statistical analyses. Strains were categorized for analysis as follows: *F* for farm type (conventional versus organic), *A* for age of cattle (calf versus cow), *D* for resistant phenotype (resistant versus susceptible) or drug susceptibility level (high or medium versus low), and *E* for ECOR group (A, B1, B2, and D). The numbers of strains in each category were recorded in the cells of contingency tables. Hierarchical log-linear modeling with nested effects was used to assess dependent associations using the CATMOD procedure and SAS statistical software (SAS Institute, Cary, NC). Nonsignificant, higher-order interactions were removed until the most parsimonious model was found based on the likelihood ratio chi-square statistic for testing goodness of fit (G^2). Nonsignificant G^2 values indicated that the fit model was not significantly different from the saturated model. Odds ratios were calculated based on parameter estimates from the most parsimonious models.

We chose to use log-linear modeling because the categorical variables in the analysis were observed simultaneously and also because no assumptions or distinctions needed to be made about whether variables were response or explanatory (40). This methodology is different from other approaches, like the chi-square test, in that it tests the strength of associations between categorical (Poisson distributed) variables and not significance. There are no assumptions that the dependent and independent variables be linearly related or that the relationship between variables be the same along the entire range (homoscedasticity). All variables were assumed to be independent. The expected counts in each cell of the contingency tables were above the rule-of-thumb cutoff of ≥1 and no more than 20% of cells <5. In addition, residuals were small (standardized residuals of <1.96) and were normally distributed.

Higher-order (three-way) interactions for multidrug-resistant phenotypes were visualized in mosaic displays for multiway contingency tables (15), which were obtained online at http://euclid.psych.yorku.ca/cgi/mosaics. The original plots were redrawn and shaded with respect to the significant ($\alpha = 0.05$) associations from the SAS analysis.

RESULTS

Overall abundance of *E. coli* phylogroups. Strains belonging to all four ECOR phylogroups were identified (Fig. 1) among the 678 *E. coli* strains from calves and cows on dairy farms. The relative phylogroup composition of these bacterial populations was used to compare different patterns of antibiotic resistance. The populations analyzed here represent the natural variation in farm type (conventional versus organic), age of cattle (calf versus cow), and resistance phenotype (resistant versus susceptible). It is clear that phylogroup abundance was not evenly distributed among the different types of dairy farms (Fig. 1; see Table S1 in the supplemental material). The most abundant phylogenetic groups were B1 (58.3%) and A (27.4%), whereas groups D (11.5%) and B2 (2.8%) were rare. B2 strains were not sampled at each variable level (no resistant B2 genotypes were found on organic farms), so these data were combined with group D strain data (B2D) for statistical analyses.

Genetic composition of antibiotic-susceptible and -resistant *E. coli* populations. Our initial goal was to test for dependent associations among three nominal variables (*F* for farm type, *A* for age of cattle, and *E* for ECOR phylogroup) by analyzing the number of strains in these categories. The tests for associations in the susceptible population (susceptible to 17 anti-

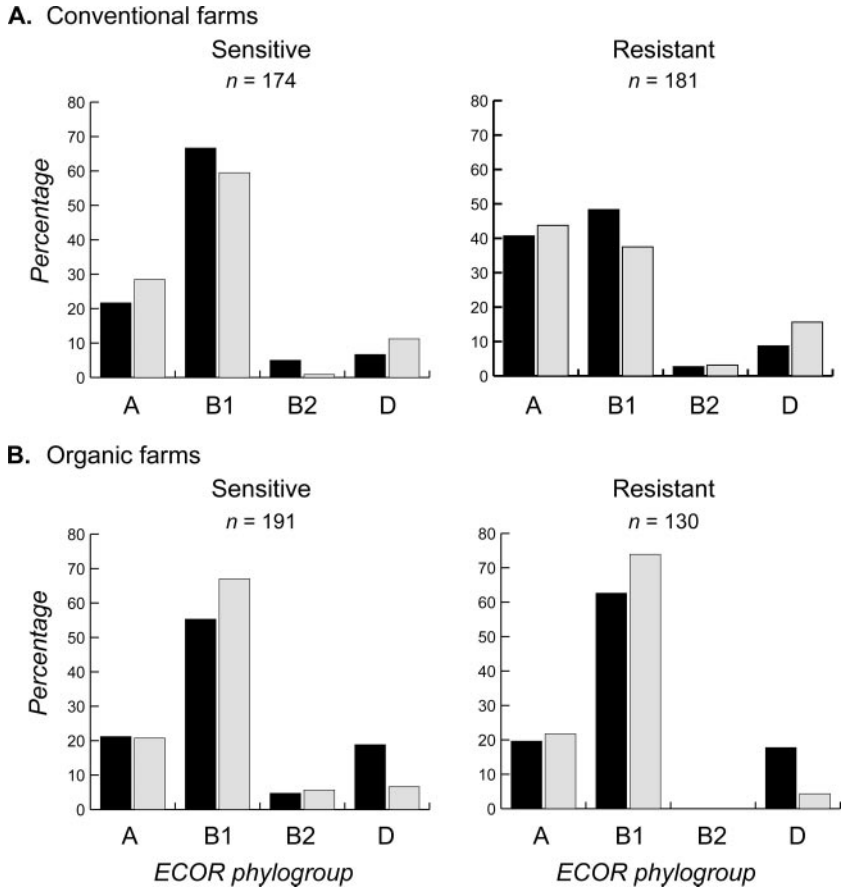

FIG. 1. Histogram plots of ECOR phylogroups for susceptible and resistant *E. coli* populations from conventional (A) and organic (B) farms (strains from calves [black bars] and strains from cows [gray bars]).

microbials) by log-linear modeling of the 376 susceptible strains revealed no significant interactions with ECOR phylogrouping (Table 1). In other words, the distribution of phylogroups in antibiotic-susceptible *E. coli* sampled from calves and cows on conventional and organic dairy farms was similar and not significantly different. A significant negative association was found between conventional farms and the number of susceptible calf strains [i.e., the $F(A = \text{calf})$ interaction in Table 1[). This result was expected because the abundance of resistant strains was higher in calves on conventional farms than in calves on organic farms. Despite this discrepancy in abundance, however, these data indicate that susceptible strains of the four phylogroups were circulating at similar frequencies on both farm types in young and adult animals.

A similar analysis was applied to the 311 resistant strains and revealed a significant association between farm type and ECOR phylogrouping (Table 1). Based on parameter estimates, the odds of recovering resistant *E. coli* of phylogroup A were significantly greater on conventional farms than on organic farms (df = 1, $\chi^2 = 21.1$, probability [Pr] $> \chi^2 < 0.0001$). This overabundance of phylogroup A strains was not seen in the susceptible or resistant population from organic farms. In addition, there were no significant farm-phylogroup (i.e., *F-E*) interactions when the susceptible populations from both farms and the resistant population from organic farms were analyzed together (model not shown). These data suggest that resistance determinants on conventional farms were linked to the genetic backgrounds of phylogroup A and that these strains increased in frequency as a result of antibiotic use. Interestingly, animal age was not associated with the distribution of phylogroups in

TABLE 1. Best-fit models explaining the frequency of antibiotic-susceptible and -resistant *E. coli* from conventional and organic dairy farms[a]

E. coli	Final population model[b]	G^2 (df, Pr $> \chi^2$)[c]	Significant interactions	χ^2 (df, Pr $> \chi^2$)
Susceptible	$\mu + A + E + F(A = \text{calf})$	8.85 (7, 0.26)	$F(A = \text{calf})$	4.27 (1, 0.04)
Resistant	$\mu + A + E + F(E = \text{ECOR group A})$	5.32 (7, 0.62)	$F(E = \text{ECOR group A})$	21.1 (1, <0.0001)

[a] The analysis is based on testing hierarchical log-linear models with nested effects in parentheses. Nominal categorical variables are designated as follows: *A* for animal age (calf or cow), *E* for ECOR phylogroup (A, B1, B2, or D), and *F* for farm type (conventional or organic).
[b] μ designates the overall main effect.
[c] The likelihood ratio chi-square statistic was used to test for goodness of fit of the final population model (compared to the saturated model).

TABLE 2. Log-linear modeling of significant associations between farm type, multidrug resistance, and ECOR phylogrouping

F-D-E model[a]	Final *F-D-E* model[b]	G^2 (df, Pr > χ^2)	Significant interactions with *E*[c]	χ^2(df, Pr > χ^2)
I	$\mu + F + D + E + F\text{-}E + D\text{-}E + F\text{-}D(E$ = ECOR group B1)	5.84 (4, 0.21)	*F-D*(*E* = ECOR group B1)	9.55 (2, 0.008)
II	$\mu + F + D + E + F\text{-}E + D\text{-}E + D\text{-}E(F$ = organic)	1.79 (4, 0.78)	*D-E*(*F* = organic)	18.64 (4, <0.001)
III	$\mu + F + D + E + F\text{-}E + D\text{-}E + F\text{-}E(D$ = high) + *F-E*(*D* = medium) + *F-E*(*D* = low)	Saturated	*F-E*(*D* = high) *F-E*(*D* = medium) *F-E*(*D* = low)	6.25 (2, 0.044) 8.29 (2, 0.016) 10.34 (2, 0.006)

[a] Model of farm type (*F*), multidrug resistance (*D*), and ECOR phylogrouping (*E*).
[b] Three separate parameterizations of the *F-D-E* model are given to show statistical dependence as a function of nested effects. μ designates the overall main effect.
[c] Significant interactions (three-way) with *E*.

the resistant population, suggesting that similar phylogroups circulate at similar frequencies in young and adult dairy cattle.

Influence of multidrug resistance on genetic composition. Strains were categorized according to their level of drug susceptibility (*D*) as defined by the number of antimicrobial-resistant phenotypes (low, one or two antimicrobial-resistant phenotypes; medium, three or four antimicrobial-resistant phenotypes; high, five or more antimicrobial-resistant phenotypes). A log-linear model fit to the data according to farm type (*F*), multidrug resistance level (*D*), and ECOR phylogrouping (*E*) revealed significant heterogeneity in the association between these variables (Table 2), including the presence of a significant three-way (*F-D-E*) interaction. In other words, the best-fit model to these data included all three variables. The model was simplified slightly by reparameterizing and nesting the variables (*F-D-E* models I, II, and III in Table 2), which allowed nonsignificant levels to be removed.

To illustrate the complexity of the interactions affecting bacterial multidrug resistance, we summarized the components of the *E. coli* populations using mosaic plots of three different parameterizations of the *F-D-E* log-linear model (Fig. 2). Odds ratios were estimated for significant interactions with respect to a fixed (nested) factor. For example, when the effect of multidrug resistance was nested, a significant two-way interaction between farm type (*F*) and phylogroup (*E*) was found and can be seen by comparing the size of the shaded box to the size of the nonshaded boxes for a given level of drug susceptibility (*D*). When the low multidrug resistance level is considered (top row of panel B), it is clear that the shaded box representing group B1 strains on organic farms is larger than the one for conventional farms. The opposite is true for phylogroup A or B2D strains (larger boxes for the conventional farm category); hence, a significant interaction is represented by the shaded organic B1 box (df = 2, $\chi^2 = 6.3$, Pr > $\chi^2 = 0.044$). The odds of isolating phylogroup A strains with medium multidrug resistance were significantly higher on conventional farms (df = 2, $\chi^2 = 8.3$, Pr > $\chi^2 = 0.016$), and the odds of isolating highly resistant, phylogroup B2D strains were significantly higher on organic farms (df = 2, $\chi^2 = 10.3$, Pr > $\chi^2 = 0.006$). As mentioned above, there were no resistant phylogroup B2 strains isolated from organic farms, so the shaded B2D box on organic farms represents group D strains only. Phylogroup-specific interactions were also found when model effects were fixed for *E* (df = 2, $\chi^2 = 9.6$, Pr > $\chi^2 = 0.008$) and *F* (df = 4, $\chi^2 = 18.64$, Pr > $\chi^2 < 0.001$). These data suggest that conventional farms are associated with medium and highly resistant phylogroup A and B1 strains, whereas in contrast, organic farms with virtually no antibiotic use are associated with low and highly resistant phylogroup B1 and D strains.

The association between the age of cattle (*A*), multidrug resistance (*D*), and ECOR phylogrouping (*E*) was also found to be heterogeneous. The high abundance of resistant calf strains and limited overall resistance on organic farms resulted in sampling zeros for three of the nine possible categories in cows (no medium resistant ECOR B2D strains, highly resistant phylogroup A strains, or highly resistant phylogroup B1 strains were sampled). After correction for sampling zeros in the resistant cow categories, the three-way interaction term (*A-D-E*) was not significant in the model. These data suggest that the age of the cattle influences the abundance of multidrug-resistant strains but does not influence the genetic composition of this population.

Tetracycline and ampicillin resistance determinants. Of the 311 resistant strains analyzed, 129 (41.5%) were Amp^r, 281 (90.4%) were Tet^r, and 112 (36.0%) were resistant to both drugs. Based on PCR screening for three common *E. coli* β-lactamase genes (bla_{TEM}, bla_{SHV}, and $bla_{OXA\text{-}1}$), 119 (92.2%) Amp^r strains carried the bla_{TEM} locus, while the remaining 10 (7.8%) strains did not produce an amplicon for any of the targeted loci. Similarly, for three genes known to confer *E. coli* tetracycline resistance (*tetA*, *tetB*, and *tetC*), 268 (95.4%) Tet^r strains carried at least one of these loci, while 13 (4.5%) did not. The *tetB* and *tetA* genes were the most abundant (64.8% and 28.1%), while the *tetC* gene was rarely sampled (4.6%).

Genetic composition and resistance determinants. We created four data sets according to the four genetic determinants present in the resistant population. Data from the susceptible population analyzed above were added to each to create a two-level factor (*G*) for log-linear modeling. Factor *G* categorized strains that carried a resistance gene (bla_{TEM}, *tetA*, *tetB*, or *tetC*) or did not (susceptible). Due to the low occurrence in the sample, data for *tetC*$^+$ strains ($n = 7$) were pooled with data for strains that were negative for all three loci ($n = 13$) and called *tetC*/other. Data for strains that were negative for the three β-lactamase loci ($n = 10$) were omitted. Log-linear models were fit to each of the four data sets to test for associations between *F*, *G*, and *E* (Table 3).

Interactions between the resistance loci and ECOR phylogroups were not dependent on farm type (no *F-G-E* interac-

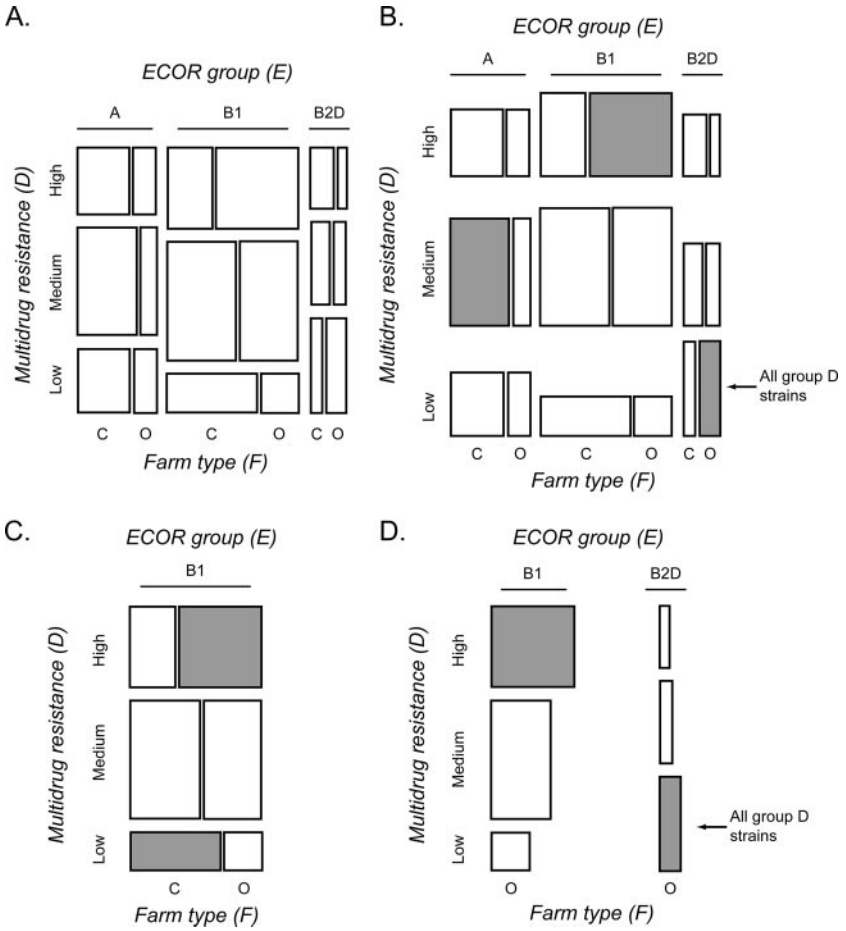

FIG. 2. Mosaic plots of the dependent associations between farm type (organic [o] and conventional [c]) (*F*), multidrug resistance (*D*), and ECOR phylogrouping (*E*). Shaded boxes mark significant odds ratio estimates (positive odds only). (A) Overall mosaic plot for *F-D-E*. (B) *F-E* interactions at fixed levels of *D*. (C) *F-D* interactions at fixed levels of *E*. (D) *D-E* interactions at fixed levels of *F*.

tions). The genetic composition of the susceptible population was not significantly different from those of the *tetA*, *tetB*, or *tetC*/other populations (models not shown). The only significant *G-E* association was found in the $bla_{\mathrm{TEM}}{}^{+}$ population (Table 3), where the odds of sampling the bla_{TEM} locus on conventional farms was significantly associated with ECOR phylogroup A ($df = 1$, $\chi^2 = 5.0$, $Pr > \chi^2 = 0.025$). These data suggest that the genetic composition of resistant *E. coli* populations on dairy farms is dependent on individual resistance determinants.

Genetic composition and class I integrons. All Amp^r and Tet^r strains were screened for the presence of class I integrons based on the presence of three loci (*intI1*, *qacEΔ1*, and the conserved cassette region). Of the total 298 Amp^r and/or Tet^r strains, 59 (19.8%) carried a class I integron. We created a three-level factor called "integron populations" that was comprised of resistant, integron-positive strains (int$^+$); resistant, integron-negative strains (int$^-$); and susceptible, integron negative strains (susceptible). Log-linear models were then used to test for significant associations between farm type (*F*), ECOR phylogrouping (*E*), and integron populations (*I*).

There was no significant phylogroup-integron (*E-I*) interaction with farm type (no *F-E-I* interaction). However, the distribution of ECOR phylogroups was dependent on integron

TABLE 3. Log-linear modeling of farm type, resistance gene, and ECOR phylogrouping

Resistance gene	Final resistance gene model[a]	G^2 (df, $Pr > \chi^2$)	Significant interactions with E[b]	χ^2 (df, $Pr > \chi^2$)
tetA	$\mu + F + G + E + F\text{-}E + F\text{-}G$	7.32 (4, 0.12)		
tetB	$\mu + F + G + E + F\text{-}E + F\text{-}G$	4.10 (4, 0.39)		
tetC/other	$\mu + F + G + E$	11.24 (7, 0.13)		
bla_{TEM}	$\mu + F + E + F\text{-}E + F\text{-}G + G\text{-}E$	3.81 (2, 0.15)	*G-E*	20.84 (2, <0.0001)

[a] Model of farm type (*F*), resistance gene (*G*), and ECOR phylogrouping (*E*). Resistant populations were defined by the determinant they carried. Note that *G-E* was a significant term in the bla_{TEM} population only. μ designates the overall main effect.
[b] Significant *G-E* (two-way) interaction for the bla_{TEM} population.

presence in these populations (df = 4, $\chi^2 = 12.4$, Pr $> \chi^2 = 0.015$). The int$^-$ and susceptible populations were compositionally the same (B1 > A > B2D), but phylogroups in the int$^+$ population were evenly sampled. This analysis suggests that the int$^+$ population had significantly more group A and B2D strains than the other (int$^-$ and susceptible) populations.

DISCUSSION

In this study, we examined the dynamics of antibiotic selection on conventional and organic dairy farms by comparing the relative frequencies of four phylogenetic groups (genetic composition) of antibiotic-resistant and susceptible *E. coli* populations. It is noteworthy that this definition of genetic composition does not closely measure the amount and distribution of genetic diversity at the gene and genotype levels but instead is susceptible to the phylogroup level so that the dynamics are indicative of broad changes in population genetic structure. A number of studies use this compositional definition to look for patterns in complex systems with many interacting variables. For example, phylogroup B1 strains were found to be common in a variety of host species (16), but they were not numerically dominant in healthy swine (11) or some human populations (27). In short, these data allow observational inferences to be made about the ecology of certain phylogenetic groups.

The genetic composition of *E. coli* from dairy farms was not similar (B1 > A > D > B2), suggesting that phylogroup B1 strains colonize at a higher abundance and, therefore, have a higher relative fitness in dairy cattle. This result is different than what is seen in Australian herbivores where group B2 strains dominate, although climate and proximity to human activity can also influence the relative distribution of phylogenetic groups in hosts (16).

Rate of compositional change in antibiotic-resistant populations on conventional farms. A key finding of this study is that there is an overabundance of resistant phylogroup A strains on conventional diary farms compared to phylogroup A strains on organic farms where antibiotic use has been limited. Based on two observations, we are confident that this overabundance has been a consequence of antibiotic use and not some other conventional farm management practice. First, susceptible populations on conventional farms and organic farms are nearly identical in genetic composition and are not statistically different, suggesting that these *E. coli* populations experience similar selective pressures in both agricultural environments. Second, the composition of the resistant populations on organic farms was not significantly different from the composition of the susceptible populations. These observations also suggest the possibility that the resistant population on conventional farms will evolve to that of the susceptible populations on organic farms if antibiotic use was stopped. Given that organic farms in this study were certified as having not used antibiotics for at least 3 years (mean, 8 years; range, 3 to 15 years), we estimate that, when antibiotic selective pressure is removed, it takes at least this long for the compositional transition.

Evidence for clonal resistance dynamics. Although these data do not address questions about the acquisition of resistance determinants by susceptible strains, the observations may adequately describe general dynamics after resistance is conferred. For example, we expected to find a significant difference between the genetic composition of resistant and susceptible populations if a resistant clone swept to high frequency during drug use on conventional farms (clonal expansion). We had the same expectation if clonal interference were operating between multiple resistant clones of the same phylogroup. An additional possibility was that clonal interference was operating between clones of different phylogroups. The expectation here was a more even distribution of phylogroups compared to the susceptible population. The significant association between conventional farms, antibiotic resistance, and phylogroup A strains supports the expectation that strains of this phylogroup were being selected. This observation does not exclude the possibility that resistant strains of other phylogroups were selected but suggests that selection was stronger for group A strains. Further characterization is needed, however, to differentiate between the spread of one clone or multiple closely related clones.

The compositional similarity between susceptible populations and resistant populations on organic farms suggests that there is an optimal genetic composition (OGC) for the farms in this study. In other words, these data suggest that there is a stable relative frequency of ECOR phylogroups in dairy cattle in the absence of antibiotic selection. Interestingly, populations of *E. coli* from freshwater beaches also appear to have an OGC, as defined by a stable composition at six separate beach sites (41). Although defining the precise mechanism of resistance requires further work, the overabundance of phylogroup A strains on conventional dairy farms was significantly associated with the bla_{TEM} locus in the Amp^r population and the presence of class I integrons in the overall resistant population. These data suggest that bla_{TEM} and class I integrons were linked to phylogroup A strains during selection on conventional farms and resulted in a departure from OGC. If this interpretation is correct, we predict that a more discriminant genetic characterization of Amp^r strains from conventional farms will reveal less genetic diversity in group A strains than in resistant strains of phylogroups B1 and B2D.

Ampicillin and other β-lactamase antibiotics are commonly used on dairy farms. In a survey of conventional dairy operations from July 2001 to June 2002 in Pennsylvania, about half of the farms ($n = 17$), for which records were available ($n = 33$), reported using ampicillin to treat pneumonia in calves (36). A wider survey of dairy farms ($n = 131$; 99 conventional and 32 organic) from Michigan, Minnesota, New York, and Wisconsin found no ampicillin use on organic farms compared to 8%, 22%, 26%, 12%, and 4% use on conventional farms for treatment of calf respiratory disease, adult respiratory disease, clinical mastitis, metritis, and foot problems, respectively (44). This difference in the use of ampicillin between conventional and organic dairy practices may be responsible for the higher frequency of resistant phylogroup A strains we observed.

Evidence for hitchhiking of resistance loci. In contrast to the Amp^r population, there was no evidence supporting an underlying clonal model for the dynamics in the Tet^r population. Populations carrying Tet^r determinants (*tetA*, *tetB*, and *tetC*/other) were at OGC on both farm types. This observation is difficult to explain if antibiotic selection and clonal spread were occurring on a single farm type. One explanation is that the organic farms received an occasional flux of Tet^r strains from

conventional farms, and the migration was sufficient to maintain the observed similarity. However, this explanation seems unlikely because the occasional flux would likely bring Amp^r strains from conventional farms as well, which in turn would ameliorate the differences discussed above. If Tet^r loci were linked to other compensatory, beneficial mutations, then the composition of these populations might appear similar regardless of antibiotic use.

Several lines of evidence support the hypothesis for the role of hitchhiking or compensatory mutations in Tet^r antibiotic resistance spread. Bartoloni et al. initially described a resistant *E. coli* population from humans living in a remote Guarani Indian community in Bolivia (3). Individuals of the village had little contact with outsiders and no veterinary or agricultural antibiotic use, relied on rainwater for survival, and had limited available health care (every 3 months). Nevertheless, tetracycline resistance was found in 64% (69 of 108) of the individuals tested. Pallecchi et al. recently characterized the underlying genetic determinants and ECOR phylogroups for 113 resistant strains of the original collection (28). The authors found that of the 103 Tet^r strains analyzed, 52 carried *tetA* and 51 carried *tetB*. These loci were distributed among all four *E. coli* phylogroups (same procedure used in this study) and were found on all five conjugative plasmids identified in this study. The abundance and distribution of Tet^r strains in this remote community support the hypothesis that naturally occurring Tet^r determinants circulate in hosts for reasons other than selection by drug use.

Support of the hitchhiking hypothesis for Tet^r loci is also consistent with the description of a "calf-adapted" *E. coli* population that was multiply resistant to streptomycin, sulfadiazine, and tetracycline (20). Almost all strains (49 of 50) analyzed shared a ~140-kb chromosomal location and the same resistance loci (*strA*, *sul2*, and *tetB*) and were genetically diverse by pulsed-field gel electrophoresis. Khachatryan and colleagues showed that on average the streptomycin-, sulfadiazine-, and tetracycline-resistant population outcompeted susceptible strains in vitro and in neonatal calves (18). They also showed that the resistance loci themselves do not influence this selective advantage (19). Their main conclusion was that the combination of *strA*, *sul2*, and *tetB* in the original resistant population had hitchhiked with some other fitness-conferring locus.

Effect of age on genetic composition. Sato et al. showed that the resistant strains examined here were most prevalent in calves on conventional farms (34). A similar positive association has been reported in other studies of preweaned calves and adult cattle (5, 7, 20). These observations suggest that antibiotic-resistant strains are better at colonizing calves than adult cows. One explanation for these observations is that the cost of resistance (fitness cost) becomes too great as the host gastrointestinal tract matures and competition with other microbes increases. Regardless of its influence on prevalence, the age of cattle had little effect on the distribution of phylogenetic groups in either the susceptible or resistant populations of this study. These data suggest that resistant strains decrease in abundance as the cattle age, while the genetic composition of the population remains stable. Other analyses of human strains showed a significant association between host age and genetic composition, but the time reported for such change may be longer than the average life span of dairy cattle (17).

Effect of multidrug resistance on genetic composition. We found a rather complicated interaction between farm type, multidrug resistance, and ECOR phylogrouping (Fig. 2). Significant associations depended on the way our log-linear model was parameterized. However, all three possible parameterizations resulted in a significant association between low multidrug resistance, group B1 strains, and organic farms. These data suggest an inverse relationship between multidrug resistance and fitness for group B1 strains on organic farms. Since phylogroup B1 strains were the numerically dominant group overall, this result should be encouraging for those seeking to reduce the amount of multidrug-resistant strains in dairy cattle through limited antibiotic usage.

Two of the parameterizations showed an association with high multidrug resistance, group D strains, and organic farms. This result is important because a number of human pathogens, including the strain most associated with human hemorrhagic colitis, O157:H7, belong to this group (according to the PCR method used here). However, we are cautious to base generalizations on this analysis because (i) we did not design our sampling study to directly address this question and (ii) the abundance of strains used for these comparisons were low. For example, the *F-E*(*D* = high) association (Table 2) between highly resistant phylogroup D strains and organic farms becomes nonsignificant if two fewer strains were sampled on organic farms and two additional strains were sampled on conventional farms. Similarly, we are cautious about the association between highly multidrug-resistant phylogroup B1 strains and medium multidrug-resistant phylogroup A strains on conventional farms because the significance of the association depends on the model parameterization.

Conclusions. The genetic composition for the antibiotic-susceptible *E. coli* populations on conventional farms, susceptible *E. coli* populations on organic farms, and resistant *E. coli* populations on organic farms was the same, suggesting a relative steady-state genetic composition for the farms in this study. In contrast, the resistant population on conventional farms had an overabundance of Amp^r, group A strains that could be explained by linked loci (bla_{TEM} and class I integrons) during a selective sweep or clonal interference among closely related strains. Given the amount of time since organic farms had abandoned conventional practices, the rate of compositional change was estimated to be between 3 and 15 years (mean, 8 years). In contrast to the Amp^r population, the Tet^r populations analyzed here showed no clonal dynamics and appeared to achieve a steady-state genetic composition. These data add support to the hypothesis that the abundance and distribution of Tet^r determinants are weakly influenced by antibiotic use. We found that the age of cattle had little influence on the genetic composition of the resistant or susceptible populations. Finally, phylogroup B1 strains with low multidrug resistance were significantly associated with organic farms, suggesting that these dairy farming practices have a proportionately large, negative effect on the prevalence of multidrug-resistant strains.

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