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Role of Calf-Adapted *Escherichia coli* in Maintenance of Antimicrobial Drug Resistance in Dairy Calves

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The prevalence of antimicrobial drug-resistant bacteria is typically highest in younger animals, and prevalence is not necessarily related to recent use of antimicrobial drugs. In dairy cattle, we hypothesize that antimicrobial drug-resistant, neonate-adapted bacteria are responsible for the observed high frequencies of resistant *Escherichia coli* in calves. To explore this issue, we examined the age distribution of antimicrobial drug-resistant *E. coli* from Holstein cattle at a local dairy and conducted an experiment to determine if low doses of oxytetracycline affected the prevalence of antimicrobial drug-resistant *E. coli*. Isolates resistant to tetracycline (>4 μg/ml) were more prevalent in <3-month-old calves (79%) compared with lactating cows (14%). In an experimental trial where calves received diets supplemented with or without oxytetracycline, the prevalence of tetracycline-resistant *E. coli* was slightly higher for the latter group ($P = 0.039$), indicating that drug use was not required to maintain a high prevalence of resistant *E. coli*. The most common resistance pattern among calf *E. coli* isolates included resistance to streptomycin (>12 μg/ml), sulfadiazine (>512 μg/ml), and tetracycline (>4 μg/ml) (SSuT), and this resistance pattern was most prevalent during the period when calves were on milk diets. To determine if prevalence was a function of differential fitness, we orally inoculated animals with nalidixic acid-resistant strains of SSuT *E. coli* and susceptible *E. coli*. Shedding of SSuT *E. coli* was significantly greater than that of susceptible strains in neonatal calves ($P < 0.001$), whereas there was no difference in older animals ($P = 0.5$). These data support the hypothesis that active selection for traits linked to the SSuT phenotype are responsible for maintaining drug-resistant *E. coli* in this population of dairy calves.

The increasing prevalence of antimicrobial drug-resistant bacteria is a major concern to human and veterinary medicine (39). Resistant bacteria include both pathogens and commensal organisms, with the latter serving as a potential reservoir for mobile resistance elements (49). Bacteria become resistant through mutations of target genes or horizontal transfer of genes encoding efflux pumps, degradative enzymes, alternative housekeeping enzymes, or ribosomal protection proteins. Horizontal gene transfer has been shown to occur even among different species of bacteria and within multiple environments (9, 38, 40, 42). Maintenance of antimicrobial drug resistance genes is governed by continued exposure to antimicrobial drugs (25, 54), plasmid addiction mechanisms (16), or close genetic linkage to other selectively advantageous genes (26).

In the United States, agriculture could be responsible for as much as 70% of antimicrobial drug consumption (36) and, therefore, agricultural animals are considered an important reservoir and arena for emerging antimicrobial drug resistance (1–3, 6, 51, 53, 55, 56). Reducing consumption of antimicrobial drugs in some cases has been associated with a decreasing prevalence of resistant organisms (27, 28, 46), but in other cases the resistance level remained constant or it even increased independent of antimicrobial drugs (8, 17, 45, 50). Persistence of antimicrobial resistance after removal of the selective agent has been correlated with a prolonged duration and the amount of antimicrobial drugs used (15). Extended application of antimicrobial drugs selects for resistant clones, and after removal of the selective agent(s) these clones may be sufficiently adapted to the genetic load of resistance genes that they cannot be easily displaced by susceptible flora (31). We are unaware of documented cases of complete loss of resistance following withdrawal of antimicrobial drugs.

The prevalence of resistant organisms is not equally distributed across all age groups, with younger animals usually having higher levels (4, 19–22, 24, 29, 35, 44, 57). We can speculate that one reason younger animals shed more resistant organisms is that this cohort may be exposed to greater amounts of antimicrobial drugs for medication or growth promotion. In addition, the intestinal physiology of younger animals is different from that in older animals and, thus, there may be niche-specific clones that are better suited to the calf intestinal environment. If antimicrobial drug resistance in young animals results from linkage between resistance genes and genes conferring selective advantage in neonatal intestines, then removal of antimicrobial drug selection pressure is unlikely to reduce this potentially important resistance reservoir in the short term.

In this paper we examined the relationship between age and antimicrobial drug resistance at a research dairy in Washington state. The prevalence of resistant bacteria was significantly greater in younger animals than in older animals. Experimental manipulations demonstrated that resistant *E. coli* had higher fitness in the calf enteric environment independent of exposure to antimicrobial drugs.

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MATERIALS AND METHODS

Herd survey. The study was performed at a Holstein dairy farm (Washington State University [WSU], Pullman, Wash.). This operation has been using an oxytetracycline (Terramycin; TM-50)-containing milk supplement for at least 10 years. The supplement, for calves less than 6 weeks old, includes 4.54% TM-50 (final concentration in the milk, ≈26 μg/ml), 2.65% vitamin A, 1.72% vitamin D, and 91.1% dried skim milk and is added directly to bulk or waste milk at feeding time. Bulk milk conformed to Food and Drug Administration standards for residual antimicrobials for human consumption. Calves were fed milk twice a day, but supplement was added only once (daily). We initially examined the prevalence of antimicrobial drug-resistant *E. coli* at the dairy. Sixty fecal samples (5 g) were collected weekly from fresh fecal pats from pens containing calves <3 months old (10 samples), heifers 3 to 6 months old (10 samples), heifers 6 to 14 months old (10 samples), lactating cows (20 samples), and nonlactating cows (10 samples). Samples were processed as described below.

TM-50 supplement study. To assess the impact of the TM-50-supplemented diet on the prevalence of resistant bacteria, we performed the following experiment. Newborn calves were consecutively assigned to either the control or experimental group. Calves were physically separated from each other in individual pens, and control and treatment group calves were held in different locations within the calf facility to avoid any chance of physical contact. Calves in the control group ($n = 9$) were fed bulk milk twice daily, which was supplemented once daily but contained no TM-50 (−TM-50). The diet of the experimental group ($n = 9$) was identical except it included TM-50 in the milk supplement (+TM-50). The calves left the experiment at 12 weeks of age. The calf diet included, in addition to the milk, a grain concentrate free of antimicrobials. Calves were weaned at 4 to 6 weeks of age. There were no documented instances of use of other antimicrobial drugs (therapeutic) during this study.

Sampling, bacterial isolation, and characterization for herd survey and supplement studies. Fresh fecal samples (20 g) were taken two to three times per week from each calf. Feces were collected with sterile tongue depressors and placed into sterile bags. The collected samples were streaked for isolation on violet red bile agar with 4-methylumbelliferyl-β-D-glucuronide (VRB-MUG; VRB from Remel and MUG from Biosyth Ag Switzerland) plates within 4 h after collection and were incubated overnight at 37°C. Eight presumptive *E. coli* colonies (pink coloration and luminescence under UV light) per animal per sample were used to inoculate EC medium (Remel) with MUG (EC-MUG) broth (200 μl) in a 96-well plate format, leaving 8 to 24 noninoculated, negative control wells. The 96-well plates were then incubated at 44.5°C overnight, and the MUG reaction was reconfirmed under UV light.

Presumptive *E. coli* isolates were tested for antimicrobial drug susceptibility using agar dilution at breakpoint concentrations. Antimicrobial drug (Sigma) susceptibilities were tested using IsoSensitest agar medium (Oxoid) supplemented with ampicillin (16 μg/ml), tetracycline (4 μg/ml), chloramphenicol (16 μg/ml), streptomycin (12 μg/ml), or sulfadiazine (512 μg/ml). Replicated test plate series included a final plate of antimicrobial drug-free medium to confirm inoculum delivery. The results of replicator assays were recorded after overnight incubation at 37°C. Results for antimicrobial drug plates were coded as a dichotomous variable: 0 for no growth and 1 for growth. These results were used to calculate the frequencies for different resistance patterns. The accuracy of the replicator assay was validated by testing two to four samples from each 96-well plate by a disk diffusion assay conforming to NCCLS guidelines ($n = 173$) (32). Quality control organisms used for the disk diffusion assay included *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 10536.

Data were entered into Microsoft Access (Microsoft Corp., Redmond, Wash.) and were analyzed using Microsoft Excel and NCSS 2001 (NCSS Statistical Software, Kaysville, Utah). An exact binomial test was used to test the distribution of resistance phenotypes for calves versus cows and for treatment versus control groups. Proportionality was adjusted for sample size differences, and α was adjusted for multiple comparisons using a Bonferroni correction.

Molecular characterization of SSuT isolates. Fifty SSuT isolates (resistant to streptomycin [>12 μg/ml], sulfadiazine [>512 μg/ml], and tetracycline [>4 μg/ml]) from calves were examined for the prevalence of specific resistance genes, shared plasmids, and clonal diversity. Antimicrobial drug resistance genes were screened using PCR (*tet*A and *tet*B [5], *str*A and *str*B [17], and *sul*2 and *sul*2 [17]). Plasmids were isolated by alkaline lysis method (43), and the profiles were visualized on 1% agarose gels stained with ethidium bromide. Pulsed-field gel electrophoresis (PFGE) was used to examine clonal diversity using the *Xba*I enzyme (12), and band profiles were analyzed using Bionumerics (Applied Maths, Austin, Tex.).

TABLE 1. Percentages of antimicrobial drug-resistant *E. coli* from the WSU dairy distributed by age of cattle, 2001

Age	n[a]	% Resistant[b] to:				
		AMP	TET	STR	SUL	CHL
Preweaned	3,221	14.9	79.2	58.0	58.1	9.8
3–6-mo heifers	3,224	14.5	35.7	26.4	26.2	1.2
>6-mo heifers	3,245	5.9	17	10.9	10.9	0.1
Lactating cows	6,321	4.6	14.2	6.2	10.7	0.1
Dry cows	2,877	2.4	13.3	7.3	12.3	0.5

[a] Number of isolates tested.
[b] AMP = ampicillin; TET = tetracycline; STR = streptomycin; SUL = sulfamethoxazole; CHL = chloramphenicol.

In vitro and in vivo competition experiments. *E. coli* isolates with the SSuT pattern (resistant to streptomycin, sulfadiazine, and tetracycline), the dominant resistance pattern isolated from calves, were tested in vitro and in vivo to determine their differential fitness compared to susceptible *E. coli* (susceptible to the antimicrobials listed above). For in vivo studies, the *E. coli* strains of interest were selected for nalidixic acid resistance (Nal^r; 20 μg/ml) following the methods of Marshall et al. (33). The design for the in vitro competitions included mixing 10^5 CFU (Luria-Bertani medium) of five SSuT and five susceptible *E. coli* isolates. All 10 *E. coli* isolates were from individual calves, on different dates, and represented three distinct SSuT and three distinct susceptible fingerprints (defined by PFGE). We used mixtures of strains in the event that a particular lineage might be responsible for the pattern of high SSuT prevalence. Similar in vitro experiments were accomplished using Nal^r strains of SSuT and susceptible *E. coli.* The mixtures were incubated overnight at 37.0°C on a shaker (200 rpm). Overnight culture (10 μl) was transferred into fresh Luria-Bertani broth (3 ml) for eight consecutive days. On days 0, 4, and 8, a competition index (CI) was calculated by estimating the CFU per milliliter for the SSuT and susceptible strains. The number of SSuT colonies was counted on fresh MacConkey agar supplemented with sulfadiazine (512 μg/ml), whereas the number of susceptible colonies was determined by subtracting the number of SSuT colonies from the same dilution grown on MacConkey agar medium without antimicrobial drugs. Three replicate counts were made at each time point and averaged. The CI was calculated as follows: $(X - Y)/(X + Y)$, where X was the number of SSuT colonies and Y was the number of susceptible colonies. CI values near +1 indicated dominance by SSuT strains, whereas CI values near −1 indicated dominance by susceptible strains.

In vivo experiments involved neonatal calves (2 to 3 days old; $n = 7$) and heifers (12 to 14 months; $n = 12$). For in vivo studies, five SSuT Nal^r and five susceptible Nal^r *E. coli* isolates were mixed (10^8 CFU of each for calves and 10^{10} CFU of each for heifers) and administered per os (oral administration was used for neonatal calves and gastric tubes were used for heifers). Neonatal calves were sampled every other day until shedding of inoculum bacteria was not detectable (up to 21 days postinoculation). Heifers were sampled every three days for 27 days postinoculation. In vivo experiments with neonatal calves were performed in a vivarium, whereas experiments with the heifers were conducted in the farm setting. The 10 strains of *E. coli* were identical for both in vitro and in vivo studies. To determine the CFU per milliliter for SSuT and susceptible *E. coli* in vivo experiments, 1 g of freshly collected fecal samples was serially diluted in peptone-buffered saline and 10-fold dilutions were plated on specific selective medium. CI values were calculated as described above, where colony counts were averaged within test tube (in vitro) or within animal (in vivo) across time points and the null hypothesis (CI = 0) was tested using Student's t test. This analysis eliminated pseudoreplication and temporal variance by averaging within animals across time points. To determine whether any particular SSuT strain completely dominated in calves, PFGE was used to screen Nal^r *E. coli* isolates ($n = 29$) from all the calves on the final days of the experiment. All in vivo inoculation studies were approved by the WSU Institutional Animal Care and Use Committee.

RESULTS

Herd survey. Survey results from a dairy herd at WSU reflected a pattern of high prevalence of antimicrobial drug resistance in younger animals for *E. coli*, which in most instances decreased with age (Table 1). The predominant pattern for

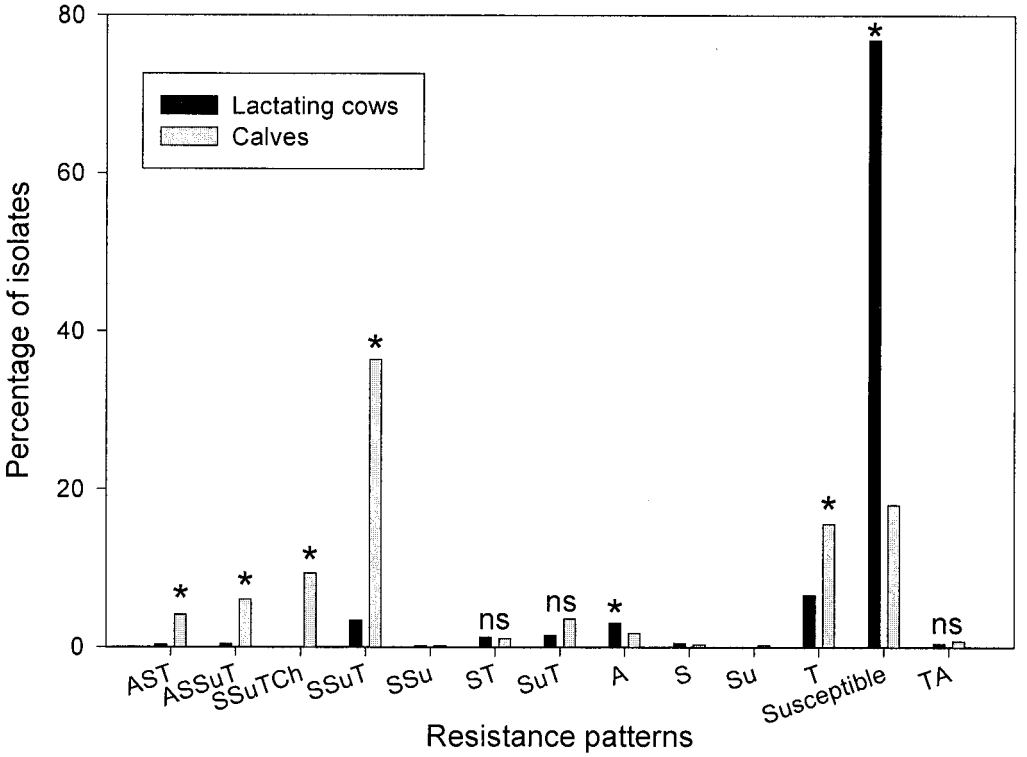

FIG. 1. Frequency of resistance patterns for all fecal *E. coli* shed from preweaned calves (n = 3,221) or lactating cows (n = 6,321). Resistance patterns are denoted by letters, where A = ampicillin, Ch = chloramphenicol, S = streptomycin, Su = sulfamethoxazole, and T = tetracycline. Susceptible isolates were susceptible to all antimicrobial drugs tested (see Materials and Methods). *, statistically significant binomial test ($P <$ 0.0006 with Bonferroni correction for 10 tests); ns, nonsignificant. Only phenotypes having >50 isolates were compared.

neonatal calves was resistance to streptomycin, sulfadiazine, and tetracycline (SSuT) (Fig. 1). In contrast, most *E. coli* isolates from cows were susceptible to all tested antimicrobial drugs.

Experimental results. The addition of oxytetracycline was associated with a higher prevalence of several antimicrobial resistance phenotypes relative to *E. coli* isolated from calves that received no antimicrobial supplement (Fig. 2). Nevertheless, *E. coli* samples collected from calves receiving oxytetracycline had a statistically larger proportion of susceptible *E. coli* and significantly smaller proportion of *E. coli* having an SSuT phenotype (Fig. 2). In addition, when data from all tetracycline-resistant phenotypes was pooled (3,750 isolates), removing oxytetracycline from the diet was associated with a slightly higher-than-expected proportion of tetracycline-resistant *E. coli* (observed = 0.55, expected = 0.533; P = 0.039). We interpreted this result as indicating that removing oxytetracycline did not result in a reduction in the proportion of tetracycline-resistant *E. coli*. The prevalence of the SSuT resistance pattern peaked during the period when calves were fed milk, after which the prevalence began to decline (Fig. 3).

The agar dilution antimicrobial drug susceptibility results were validated with the disk diffusion method (97.1% congruence; n = 173). Based on bacterial growth in negative control wells, we estimated that the contamination rate associated with the agar dilution assay was 4%. The approximate rate of failure to inoculate the plates with replicator was measured as the growth on the antimicrobial drug plate and no growth on the carbohydrate plate for the same isolate and was equal to 3.15%; these samples were excluded from the analysis. Finally, our assay showed a 100% correspondence between resistance for tetracycline and oxytetracycline, at different MICs starting from 4 to 40 μg/ml (data not shown).

In vitro competition results. SSuT *E. coli* isolates consistently out-competed susceptible strains in vitro in the absence of antibacterial selective pressure (Student's t test; $P < 0.001$). There was no significant difference between competition results for Nal^r and Nal^s strains (Student's t test; P = 0.41). Finally, all Nal^r strains had approximately equal MICs for nalidixic acid (200 μg/ml). These latter results were consistent with similar mutations conferring the Nal^r phenotype and with a negligible impact on differential fitness due to the Nal^r phenotype.

In vivo competition results. SSuT *E. coli* isolates consistently out-competed susceptible strains in neonatal calves in the absence of antibacterial selective pressure (Student's t test; $P <$ 0.001) (Fig. 4). In older cows (heifers), SSuT *E. coli* isolates did not show any competitive advantage over susceptible *E. coli* isolates (Student's t test; P = 0.54) (Fig. 5). Throughout the experiment, all heifers presented both positive and negative CI values. None of the animals in the in vivo studies shed detectable fecal Nal^r *E. coli* prior to inoculation (0 to 3 days). All strains of Nal^r *E. coli* present in the SSuT inoculum were also identified in feces, as demonstrated by PFGE (data not shown).

Molecular characteristics of SSuT isolates. Of 50 SSuT isolates obtained from calves, 49 harbored a large plasmid (~140 kb). All the SSuT isolates encoded *str*A, *sul*2, and *tet*B antimicrobial resistance genes. PFGE profiles revealed a very diverse

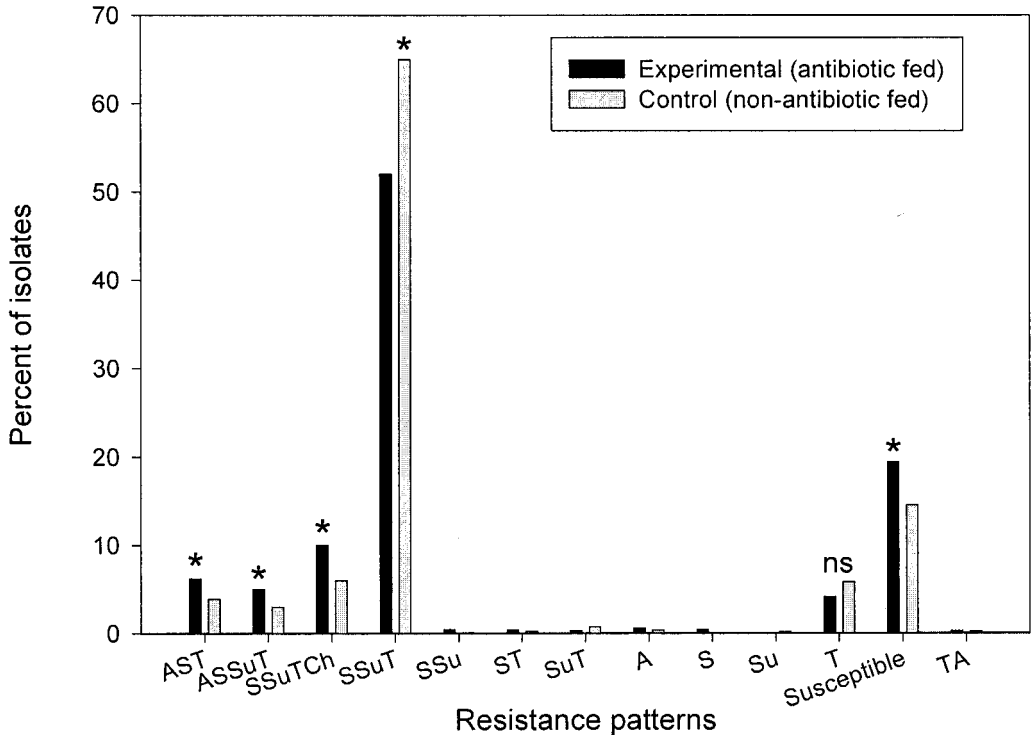

FIG. 2. Frequency of resistance patterns for all fecal *E. coli* shed from calves; experimental group (antibiotic fed; $n = 2,129$) and control group (non-antibiotic fed; $n = 2,432$). See Fig. 1 legend for an explanation of the codes used. *, statistically significant binomial test ($P < 0.0017$ with Bonferroni correction for six tests); ns = nonsignificant. Only phenotypes having >50 isolates were compared.

population of SSuT *E. coli* in calves. Given a similarity coefficient of 90% (Dice index), there were 18 distinct band patterns for these 50 isolates (data not shown).

DISCUSSION

We studied antimicrobial drug resistance in commensal *E. coli* isolates, which are considered a potential reservoir for resistance genes in farm animals (41). On-farm reservoirs of resistant bacteria provide a potential source for resistance gene transfer between bacteria as well as an environment for dissemination to new animals, environments, and food products. Therefore, identifying these reservoirs and mechanisms of persistence will be a key to reducing the load of resistant bacteria in commercial facilities.

In this study, preweaned calves had the greatest prevalence

FIG. 3. Resistance pattern of fecal *E. coli* over weeks of life for all calves ($n = 18$). See Fig. 1 legend for an explanation of the codes used.

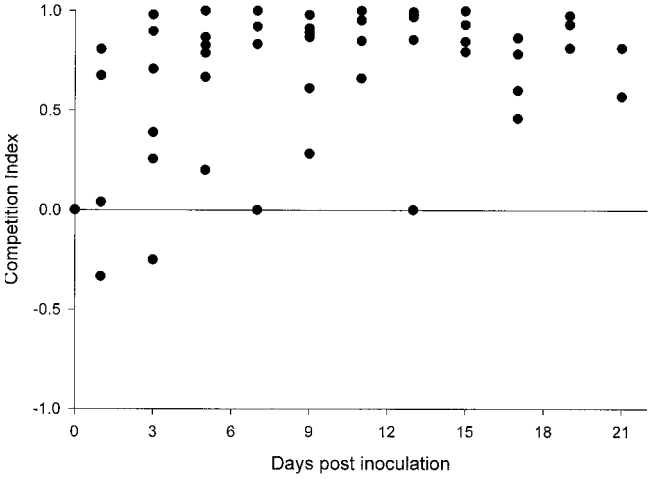

FIG. 4. In vivo competition experiments in neonatal calves ($n = 6$) between Nal^r SSuT and Nal^r susceptible strains. Each circle represents a sample from a single calf. $CI = (X - Y)/(X + Y)$ where X is the number of SSuT colonies and Y is the number of susceptible colonies. When $CI = 0$, there is an equal proportion of Nal^r SSuT and Nal^r susceptible strains in the sample.

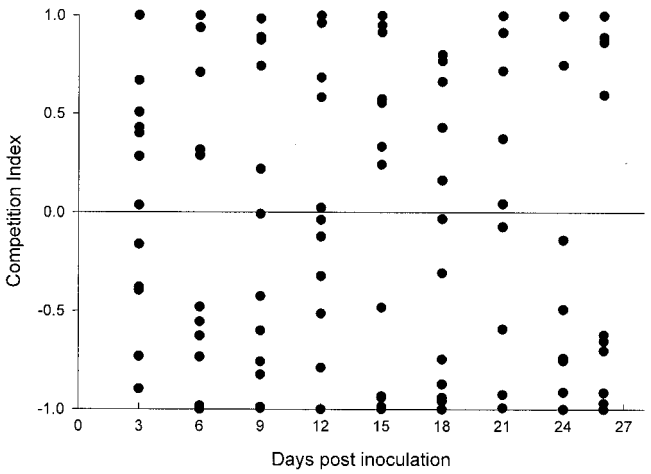

FIG. 5. In vivo competition experiments in heifers ($n = 12$) between Nal^r SSuT and Nal^r susceptible strains. Each circle represents a sample from a single heifer. CI = $(X - Y)/(X + Y)$ where X is the number of SSuT colonies and Y is the number of susceptible colonies. When CI = 0 there is an equal proportion of Nal^r SSuT and Nal^r susceptible strains in the sample.

of resistant *E. coli*, and we found an inverse relationship between prevalence of resistant *E. coli* and animal age. We also demonstrated experimentally that this high degree of resistance could be maintained in the absence of antimicrobial drug selection. In the absence of antimicrobial drugs, a high prevalence of antimicrobial drug-resistant *E. coli* could occur if the resistant strains had a fitness advantage in calves, but this could also occur if the animals received high doses of these strains from the environment. To test the former hypothesis, we compared the fitness of SSuT strains with susceptible strains in vivo. It was clear from these experiments that the SSuT strains had a fitness advantage in calves (Fig. 4), but not in older animals (Fig. 5). In the latter case the data were consistent with random colonization by either SSuT or susceptible strains. Given these results, environmental sources probably play a role in initial inoculation, but active competition in the calf gut leads to expansion of SSuT populations in calves. Finally, we found a very interesting correlation between the highest prevalence of the SSuT resistance pattern and the period when calves received milk, suggesting that the selective advantage in calves might be related to diet. In vitro experiments showed that SSuT *E. coli* strains had a higher fitness than susceptible strains under no specific selective pressure. We have no a priori reason to expect that similar mechanisms were responsible for in vitro and in vivo results, but they may be related.

The prevalence of resistant bacteria is usually correlated with previous use of antimicrobial drugs, but withdrawal of antimicrobial drugs does not always result in an immediate decrease in the prevalence of resistant bacteria (8, 17, 45, 50). Our experimental results support the observation that antimicrobial drug selection is not required for short-term maintenance of resistant organisms. In the absence of antimicrobial pressure, resistance genes probably represent a metabolic burden. Nevertheless, it is possible that this burden is so low that the half-life for persistence can be measured in years or decades (e.g., reference 47). It is also possible that occult selection pressure exists in a direct ecological context (e.g., antibiotic- or colicin-producing bacteria) or via some other effective advantage derived from resistance genes other than antimicrobial drug resistance. Finally, it is plausible that resistance genes could persist if they were closely linked to other selectively advantageous genes. For example, resistance genes might be linked to adhesin or siderophore genes. Others have shown that some adhesin genes have higher prevalence in the *E. coli* flora of younger calves (10, 23, 34), and adhesin genes have also been found on plasmids that also encode antimicrobial drug resistance genes (14, 30, 47, 48). Lactoferrin is an abundant iron chelator in milk, and iron is required for bacterial growth (37, 52). Iron acquisition genes (aerobactin and enterobactin) have been associated with plasmids that also encode antimicrobial drug resistance genes (7, 11, 13, 18).

In our system, the correspondence between milk diet and the highest prevalence of SSuT *E. coli* in the feces suggests either a direct benefit of the resistance genes themselves or linkage to other genes that are adaptive in this environment. A subset of SSuT isolates were characterized, and we found a diverse group of clones (based on PFGE) that harbored a common set of resistance genes (*tet*B, *sul*2, and *str*A) and a common plasmid (~140 kb). It is possible that this plasmid harbors the gene(s) responsible for selective advantage in calves. Studies are under way to identify the mechanisms responsible for the increased fitness of SSuT *E. coli* in calves, and with this information we expect to devise measures to promote displacement of antimicrobial drug-resistant bacteria in this age group.

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REFERENCES

1. **Aarestrup, F. M.** 1999. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. Int. J. Antimicrob. Agents **12:**279–285.
2. **Aarestrup, F. M., Y. Agerso, P. Gerner-Smidt, M. Madsen, and L. B. Jensen.** 2000. Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. Diagn. Microbiol. Infect. Dis. **37:**127–137.
3. **Aarestrup, F. M., H. Kruse, E. Tast, A. M. Hammerum, and L. B. Jensen.** 2000. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. Microb. Drug Resist. **6:**63–70.
4. **Brophy, P. O., P. J. Caffrey, and J. D. Collins.** 1977. Sensitivity patterns of *Escherichia coli* isolated from calves during and following prophylactic chlortetracycline therapy. Br. Vet. J. **133:**340–345.
5. **Call, D. R., M. Bakko, M. Krug, and M. Roberts.** 2003. Identifying antimicrobial resistance genes using DNA microarrays. Antimicrob. Agents Chemother. **47:**3290–3295.
6. **Caprioli, A., L. Busani, J. L. Martel, and R. Helmuth.** 2000. Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies. Int. J. Antimicrob. Agents **14:**295–301.
7. **Cercenado, E., F. Baquero, A. Delgado-Iribarren, and J. L. Martinez.** 1986. Epidemiology of aerobactin production in Enterobacteriaceae. Ann. Inst. Pasteur Microbiol. B **137:**297–303.
8. **Chaslus-Dancla, E., G. Gerbaud, M. Lagorce, J. P. Lafont, and P. Courvalin.** 1987. Persistence of an antibiotic resistance plasmid in intestinal *Escherichia coli* of chickens in the absence of selective pressure. Antimicrob. Agents Chemother. **31:**784–788.

9. **Chee-Sanford, J. C., R. I. Aminov, I. J. Krapac, N. Garrigues-Jeanjean, and R. Mackie.** 2001. Occurrence and diversity of tetracycline resistance in lagoons and groundwater underlying two swine production facilities. Appl. Environ. Microbiol. **67:**1494–1502.
10. **China, B., V. Pirson, and J. Mainil.** 1998. Prevalence and molecular typing of attaching and effacing *Escherichia coli* among calf populations in Belgium. Vet. Microbiol. **63:**249–259.
11. **Colonna, B., L. Ranucci, P. A. Fradiani, M. Casalino, A. Calconi, and M. Nicoletti.** 1992. Organization of aerobactin, hemolysin, and antibacterial resistance genes in lactose-negative *Escherichia coli* strains of serotype O4 isolated from children with diarrhea. Infect. Immun. **60:**5224–5231.
12. **Davis, M. A., D. D. Hancock, T. E. Besser, and D. R. Call.** 2003. Evaluation of pulsed-field gel electrophoresis as a tool for determining the degree of genetic relatedness between strains of *Escherichia coli* O157:H7. J. Clin. Microbiol. **41:**1843–1849.
13. **Der Vartanian, M.** 1988. Differences in excretion and efficiency of the aerobactin and enterochelin siderophores in a bovine pathogenic strain of *Escherichia coli.* Infect. Immun. **56:**413–418.
14. **Di Martino, P., D. Sirot, B. Joly, C. Rich, and A. Darfeuille-Michaud.** 1997. Relationship between adhesion to intestinal Caco-2 cells and multidrug resistance in *Klebsiella pneumoniae* clinical isolates. J. Clin. Microbiol. **35:** 1499–1503.
15. **Dunlop, R. H., S. A. McEwen, A. H. Meek, R. C. Clarke, W. D. Black, and R. M. Friendship.** 1998. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev. Vet. Med. **34:**283–305.
16. **Engelberg-Kulka, H., and G. Glaser.** 1999. Addiction modules and programmed cell death and antideath in bacterial cultures. Annu. Rev. Microbiol. **53:**43–70.
17. **Enne, V. I., D. M. Livermore, P. Stephens, and L. C. Hall.** 2001. Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. Lancet **357:**1325–1328.
18. **Harel, J., J. Fairbrother, C. Forget, C. Desautels, and J. Moore.** 1993. Virulence factors associated with F165-positive *Escherichia coli* strains isolated from piglets and calves. Vet. Microbiol. **38:**139–155.
19. **Hinton, M.** 1985. The sub-specific differentiation of *Escherichia coli* with particular reference to ecological studies in young animals including man. J. Hyg. (London) **95:**595–609.
20. **Hinton, M., A. J. Hedges, and A. H. Linton.** 1985. The ecology of *Escherichia coli* in market calves fed a milk-substitute diet. J. Appl. Bacteriol. **58:**27–35.
21. **Hinton, M., A. H. Linton, and A. J. Hedges.** 1985. The ecology of *Escherichia coli* in calves reared as dairy-cow replacements. J. Appl. Bacteriol. **58:**131–138.
22. **Hinton, M., P. D. Rixson, V. Allen, and A. H. Linton.** 1984. The persistence of drug resistant *Escherichia coli* strains in the majority faecal flora of calves. J. Hyg. (London) **93:**547–557.
23. **Holland, R. E., R. A. Wilson, M. S. Holland, V. Yuzbasiyan-Gurkan, T. P. Mullaney, and D. G. White.** 1999. Characterization of eae$^+$ *Escherichia coli* isolated from healthy and diarrheic calves. Vet. Microbiol. **66:**251–263.
24. **Howe, K., and A. H. Linton.** 1976. A longitudinal study of *Escherichia coli* in cows and calves with special reference to the distribution of O-antigen types and antibiotic resistance. J. Appl. Bacteriol. **40:**331–340.
25. **Jorgensen, S. E., and B. Halling-Sorensen.** 2000. Drugs in the environment. Chemosphere **40:**691–699.
26. **Kehrenberg, C., and S. Schwarz.** 2001. Occurrence and linkage of genes coding for resistance to sulfonamides, streptomycin and chloramphenicol in bacteria of the genera Pasteurella and Mannheimia. FEMS Microbiol. Lett. **205:**283–290.
27. **Klare, I., D. Badstubner, C. Konstabel, G. Bohme, H. Claus, and W. Witte.** 1999. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb. Drug Resist. **5:**45–52.
28. **Langlois, B. E., K. A. Dawson, T. S. Stahly, and G. L. Cromwell.** 1984. Antibiotic resistance of fecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. J. Anim. Sci. **58:**666–674.
29. **Larsen, H. E., and J. L. Larsen.** 1975. Persistence of resistant *Escherichia coli* in a calf herd. Nord. Vet. Med. **27:**65–76.
30. **Le Bouguenec, C., and Y. Bertin.** 1999. AFA and F17 adhesins produced by pathogenic *Escherichia coli* strains in domestic animals. Vet. Res. **30:**317–342.
31. **Lipsitch, M., and M. H. Samore.** 2002. Antimicrobial use and antimicrobial resistance: a population perspective. Emerg. Infect. Dis. **8:**347–354.
32. **Lorian, V.** 1996. Antibiotics in laboratory medicine, 4th ed. Williams and Wilkins, Baltimore, Md.
33. **Marshall, B., D. Petrowski, and S. B. Levy.** 1990. Inter- and intraspecies spread of *Escherichia coli* in a farm environment in the absence of antibiotic usage. Proc. Natl. Acad. Sci. USA **87:**6609–6613.
34. **Martin, C., E. Rousset, and H. De Greve.** 1997. Human uropathogenic and bovine septicaemic *Escherichia coli* strains carry an identical F17-related adhesin. Res. Microbiol. **148:**55–64.
35. **Mathew, A. G., A. M. Saxton, W. G. Upchurch, and S. E. Chattin.** 1999. Multiple antibiotic resistance patterns of *Escherichia coli* isolates from swine farms. Appl. Environ. Microbiol. **65:**2770–2772.
36. **Mellon, M., C. Benbrook, and K. L. Benbrook.** 2001. Hogging it: estimates of antimicrobial abuse in livestock. UCS Publications, Cambridge, Mass.
37. **Mevissen-Verhage, E. A., J. H. Marcelis, W. C. Harmsen-Van Amerongen, N. M. de Vos, and J. Verhoef.** 1985. Effect of iron on neonatal gut flora during the first three months of life. Eur. J. Clin. Microbiol. **4:**273–278.
38. **Miller, R. V.** 1998. Bacterial gene swapping in nature. Sci. Am. **278:**66–71.
39. **National Academy of Science.** 1999. The use of drugs in food animals: benefits and risks. National Academy Press, Washington, D.C.
40. **Nikolich, M., H. G., N. B. Shoemaker, and A. A. Salyers.** 1994. Evidence for natural horizontal transfer of *tetQ* between bacteria that normally colonize humans and bacteria that normally colonize livestock. Appl. Environ. Microbiol. **60:**3255–3260.
41. **O'Brien, T. F.** 2002. Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. Clin. Infect. Dis. **34**(Suppl. 3)**:**S78–S84.
42. **Ochman, H., J. G. Lawrence, and E. A. Groisman.** 2000. Lateral gene transfer and the nature of bacterial innovation. Nature **405:**299–304.
43. **Portnoy, D. A., S. L. Moseley, and S. Falkow.** 1981. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect. Immun. **31:**775–782.
44. **Raška, K., H. Rašková, Z. Urbanová, D. Matejovská, V. Matejovská, V. Palounek, and L. Polák.** 1979. Resistance of gram-negative bacteria to antibiotics in large calf agglomerations. Acta Trop. **36:**163–170.
45. **Rollins, L. D., S. A. Gaines, D. W. Pocurull, H. D. Mercer, and L. T. Frobish.** 1976. Persistence of transferable drug resistance in the lactose-fermenting enteric flora of swine following antimicrobial feeding. Can. J. Comp. Med. **40:**175–183.
46. **Seppala, H., T. Klaukka, J. Vuopio-Varkila, A. Muotiala, H. Helenius, K. Lager, P. Huovinen, et al.** 1997. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. N. Engl. J. Med. **337:**441–446.
47. **Shimizu, M., T. Sakano, and S. Satoh.** 1988. Linkage of K99 production and STa activity in a plasmid of an *Escherichia coli* porcine isolate. Microbiol. Immunol. **32:**635–639.
48. **Shimizu, M., and T. Terashima.** 1982. Appearance of enterotoxigenic *Escherichia coli* in piglets with diarrhea in connection with feed changes. Microbiol. Immunol. **26:**467–477.
49. **Shoemaker, N. B., H. Vlamakis, K. Hayes, and A. A. Salyers.** 2001. Evidence for extensive resistance gene transfer among *Bacteroides* spp. and among *Bacteroides* and other genera in the human colon. Appl. Environ. Microbiol. **67:**561–568.
50. **Smith, H. W.** 1975. Persistence of tetracycline resistance in pig *E. coli.* Nature **258:**628–630.
51. **van den Bogaard, A. E., and E. E. Stobberingh.** 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. Int. J. Antimicrob. Agents **14:**327–335.
52. **Wharton, B. A., S. E. Balmer, and P. H. Scott.** 1994. Faecal flora in the newborn. Effect of lactoferrin and related nutrients. Adv. Exp. Med. Biol. **357:**91–98.
53. **White, D. G., L. J. V. Piddock, J. J. Maurer, S. Zhao, V. Ricci, and S. G. Thayer.** 2000. Characterization of fluoroquinolone resistance among veterinary isolates of avian *Escherichia coli.* Antimicrob. Agents Chemother. **44:** 2897–2899.
54. **Winckler, C., and A. Grafe.** 2001. Use of veterinary drugs in intensive animal production: evidence for persistence of tetracycline in pig slurry. J. Soils Sedim. **1:**66–70.
55. **Witte, W.** 2000. Ecological impact of antibiotic use in animals on different complex microflora: environment. Int. J. Antimicrob. Agents **14:**321–325.
56. **Witte, W.** 2000. Selective pressure by antibiotic use in livestock. Int. J. Antimicrob. Agents **16:**S19–S24.
57. **Zhang, X. L., F. Wang, D. M. Zhu, S. Wu, P. C. Wu, Y. D. Chen, Y. Q. Wang, and L. Zhou.** 1998. The carriage of *Escherichia coli* resistant to antibiotics in healthy populations in Shanghai. Biomed. Environ. Sci. **11:**314–320.