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Molecular Epidemiology of Antimicrobial-Resistant Commensal *Escherichia coli* Strains in a Cohort of Newborn Calves

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Pulsed-field gel electrophoresis (PFGE) was used to investigate the dissemination and diversity of ampicillin-resistant (Amp^r) and nalidixic acid-resistant (Nal^r) commensal *Escherichia coli* strains in a cohort of 48 newborn calves. Calves were sampled weekly from birth for up to 21 weeks and a single resistant isolate selected from positive samples for genotyping and further phenotypic characterization. The Amp^r population showed the greatest diversity, with a total of 56 different genotype patterns identified, of which 5 predominated, while the Nal^r population appeared to be largely clonal, with over 97% of isolates belonging to just two different PFGE patterns. Distinct temporal trends were identified in the distribution of several Amp^r genotypes across the cohort, with certain patterns predominating at different points in the study. Cumulative recognition of new Amp^r genotypes within the cohort was biphasic, with a turning point coinciding with the housing of the cohort midway through the study, suggesting that colonizing strains were from an environmental source on the farm. Multiply resistant isolates dominated the collection, with >95% of isolates showing resistance to at least two additional antimicrobials. Carriage of resistance to streptomycin, sulfamethoxazole, and tetracycline was the most common combination, found across several different genotypes, suggesting the possible spread of a common resistance element across multiple strains. The proportion of Amp^r isolates carrying sulfamethoxazole resistance increased significantly over the study period ($P < 0.05$), coinciding with a decline in the most common genotype pattern. These data indicate that calves were colonized by a succession of multiply resistant strains, with a probable environmental source, that disseminated through the cohort over time.

Antimicrobial-resistant bacteria are now frequently isolated from the commensal gut flora of food animals (1, 3, 14, 21, 24, 46). While resistance in such commensal bacteria is not a problem in itself, the possible transfer of resistance elements to zoonotic pathogens within the gut has serious implications for public health. Consequently, the need to reduce antimicrobial consumption within a veterinary context has received much press (34, 42, 43). However, while the use of antimicrobials clearly exerts a selective force for resistance development (11, 15), there is also evidence that the prevalence of resistance in a bacterial population may not be simply related to the levels of antimicrobial usage (29, 31, 33) and that resistant organisms sometimes persist long after the selecting agent has been withdrawn (8, 17, 28). Thus, the relationship between the maintenance of resistance within a population and the use of selective agents is complex, and other factors are likely to play a role in the acquisition or persistence of antimicrobial-resistant organisms (11, 39).

Animal age appears to be a decisive factor: several studies have now demonstrated that young animals show a higher prevalence of resistant fecal *Escherichia coli* than older stock held on the same farm (13, 27, 33), while carriage of ampicillin-resistant (Amp^r) *E. coli* by young calves has been shown to decline with calf age (25). Discovering why young animals acquire resistant commensal bacteria and how these are transmitted between animals is now essential for an understanding of the dynamics of antimicrobial resistance spread within commensal bacterial populations to be achieved.

The transmission of resistance between commensal bacteria harbored within the gut can occur both horizontally, through the movement of mobile genetic elements, and vertically, through proliferation and subsequent dissemination of resistant bacterial strains. While a variety of methods, including O serotyping and biochemical and resistance profiling, have previously been used to investigate changes in unselected commensal *E. coli* populations in both cattle and pigs (22, 23, 26), these do not discriminate between bacterial strains on the basis of their genotype and thus cannot adequately distinguish between the vertical and horizontal transmission of resistance traits or the persistences of distinct resistance strains within a population. In this study we examined the possibility that resistance is transmitted through the dissemination of defined bacterial populations, using pulsed-field gel electrophoresis (PFGE), a technique that is commonly used to investigate the epidemiology of bacterial pathogens in human and animal populations and which provides a discriminatory fingerprint of a bacterial strain based upon its genotype. This method can

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therefore provide information about the potential clonal dissemination of resistant strains (2, 38).

In this study we looked at two populations of Amp^r and nalidixic acid-resistant (Nal^r) commensal *E. coli* strains which had been collected over a period of 4 months from an intensively sampled cohort of beef calves born on a single farm. The general epidemiology of both ampicillin- and nalidixic acid-resistant *E. coli* strains in the cohort has been described elsewhere (24) and showed that the prevalence of resistance was high from birth but declined with calf age. Here, we investigated the possibility that resistance was disseminated through this cohort by defined bacterial strains, which would suggest that resistance spread either directly from calf to calf or through contamination via the environment. Common phenotype patterns were also identified by antimicrobial sensitivity testing, in order to highlight the possible spread of transferable resistance elements between strains.

MATERIALS AND METHODS

Sampling and bacterial isolation. Isolates were obtained from a cohort of 48 calves born into a single beef cattle herd in the Highlands, Scotland, and monitored for a 4-month period from September 2001. Calves were run with their dams as a single group, initially on the same field, at pasture. The group was then housed after week 10, in the same barn, for the remainder of the study period. A total of 19 antimicrobial treatments were administered to 12 calves over the study period: six fluoroquinolone, three beta-lactam, one tetracycline, six tylosin, and three florfenicol treatments. In all cases, calves had already generated resistant isolates either prior to or on the same day as drug administration. Calf dams did not receive any antimicrobial treatments over the study period.

Rectal fecal samples were collected on a weekly basis from cohort calves and screened on Chromocult tryptone bile X-glucuronide agar (Merck) for *E. coli* that was resistant to ampicillin (16 mg/liter) and nalidixic acid (8 mg/liter). Antibiotics were selected as examples of beta-lactam and quinolone agents, antibiotic classes that are frequently used in farm animals in the United Kingdom. Samples were diluted 1:10 in maximum recovery diluent (Oxoid), and 10 μl was spread onto nonselective (antibiotic-free) and antibiotic-containing plates and incubated overnight at 44°C. Characteristic *E. coli* colonies of a dark blue color were recorded, indicative of the presence of the enzyme glucuronidase (18). The reference strains *E. coli* NCTC 10418, NCTC 11560, JR 225, and NCTC 12900 were used to confirm the activity of antibiotic-containing plates. Full details of the herd and sampling protocol, together with the temporal and age-related trends in the distribution and prevalence of ampicillin- and nalidixic acid-resistant commensal *E. coli* strains within the cohort, are described fully elsewhere (24).

A single distinct colony was selected from each positive plate, subcultured to ensure purity, and stored on cryogenic beads (Mast Diagnostics) at −70°C until required. In order to confirm that single-pick isolates represented the *E. coli* strain variance found within a single sample, multiple-pick isolates were also taken from samples of a subset of 10 calves over a selected time period (weeks 4 to 13), and the proportions of isolates belonging to the main PFGE groups were then compared with those in the single-pick collection. Multiple picks were taken from the original screening plates after the initial single pick had been taken (number of picks determined by colony availability) (median, 6; maximum, 24) and stored as described above. Totals of 419 Amp^r and 152 Nal^r single-pick isolates and 240 Amp^r and 151 Nal^r multiple-pick isolates were examined.

Characterization of isolates by pulsed-field gel electrophoresis. PFGE was performed using XbaI (Promega) according to standard procedures, with slight modifications (5, 19). DNA fragments were resolved by electrophoresis in 1% agarose gels (pulsed-field certified agarose; Bio-Rad) with a CHEF DRII machine (Bio-Rad), using 0.5× Tris-borate-EDTA as the buffer. Gels were run for 22 h at 14°C, using a linearly ramped switching time from 2.2 s to 55 s and a voltage of 6.0 V/cm. Gels were stained with 0.5 μg/ml of ethidium bromide in distilled water and photographed using a software-based image capturing system (Gel Doc 2000; Bio-Rad). PFGE banding patterns were analyzed using BioNumerics version 3.0 software (Applied-Maths, Ghent, Belgium). A lambda ladder (New England BioLabs Inc.) was included in three lanes of every gel to allow fingerprint comparison between different gels. Fingerprints were clustered into groups by using the Dice coefficient and evaluated by the unweighted-pair group method. A tolerance and an optimization of 1% were allowed to account for differences between gels. Clustered groups where more than two indistinguishable isolates were present were assigned an arbitrary letter classification, to enable temporal and phenotypic trends to be evaluated. Due to the low similarity between clustered groups and the importance of stringency within the context of the current study, a difference of one band from the phylogenetic group core (either absence or presence of a band) was tolerated within a defined group (30, 37).

Antimicrobial susceptibility testing. The MICs for Amp^r and Nal^r single-pick isolates of a panel of eight antimicrobial agents (abbreviations for phenotype patterns are as follows: A, ampicillin; Ap, apramycin; N, nalidixic acid; S, streptomycin; Su, sulfamethoxazole; T, tetracycline; Tr, trimethoprim; and C, cephradine) were determined using the agar dilution method (36). These antimicrobials were selected on the basis that they, or equivalent generation agents from the same class, are commonly used in the therapeutic treatment of cattle in Scotland. Plates were prepared by supplementing Iso-Sensitest agar (Oxoid) with the appropriate dilution of antimicrobial agent and were inoculated with samples via a 36-pin multipoint inoculator (Denley). A drug-free plate was inoculated at the end of each series to confirm isolate viability, while reference strains *E. coli* NCTC 10418, NCTC 11560, and NCTC 12900 were included as controls on each plate. Plates were incubated overnight at 37°C and the MIC for each isolate recorded as the dilution at which growth was inhibited. Resistance breakpoints were based on British Society for Antimicrobial Chemotherapy guidelines (4), except for cephradine and tetracycline, for which breakpoints were taken one dilution up based on the MIC distribution for the isolate collections, and apramycin, for which no breakpoint was quoted. Resistance was defined as an MIC of ≥4 mg/liter (tetracycline and trimethoprim), ≥16 mg/liter (apramycin and streptomycin), ≥32 mg/liter (ampicillin, cephradine, and nalidixic acid), and 64 mg/liter (sulfamethoxazole).

Data analysis. Similarities in the frequencies of the five main Amp^r and two main Nal^r PFGE groupings were compared between the single-pick and multiple-pick studies, for a comparable time frame between weeks 4 and 13, using chi-square analysis. Temporal trends in the overall number of PFGE genotype patterns identified and in the proportion of different PFGE groups present were examined using PROC LOESS (SAS version 8.2), a nonparametric method for estimating local regression surfaces (12). Piecewise linear regression (PROC NLIN, SAS version 8.2) was used to examine the cumulative recognition of all PFGE genotypes identified over the course of the sampling period. The proportions of isolates resistant to streptomycin, sulfamethoxazole, tetracycline, and trimethoprim were examined using simple linear regression to test the hypothesis of an increase in resistance over time (i.e., slope greater than zero). Resistance to cephradine over time was tested using a second-order polynomial regression, which was more appropriate given the structure of the data. A comparison of the slopes for the proportion of isolates resistant to sulfamethoxazole and trimethoprim was performed using a generalized linear model (PROC GLM SAS version 8.2) after the data (p) were transformed ($p' = \arcsin \sqrt{p}$) to correct for nonnormality (49).

RESULTS

PFGE genotype distribution within the cohort. Only two different PFGE groups were identified within the single-pick Nal^r collection, comprising 78.9% (group O) and 18.4% (group G) of isolates. In addition, four isolates with unique fingerprints were found only once and were not grouped. In contrast, the single-pick Amp^r isolate collection was more diverse: 24 different PFGE groups were identified, together with 3 pairs of isolates and 29 unique isolates. Groups O and G identified in the Nal^r collection were also detected in the Amp^r collection, with group O again being more common than group G. Group B was the most frequently identified Amp^r PFGE genotype, comprising 16% of the collection, followed by groups N, O, V, and Y, with most of the remaining groups containing fewer than 4.1% of the total Amp^r single-pick isolates (Fig. 1a). There was considerable variation in banding profile between the majority of the defined PFGE groups, with the exception of groups A and B and groups W and Y, which differed by two bands only (Fig. 1b).

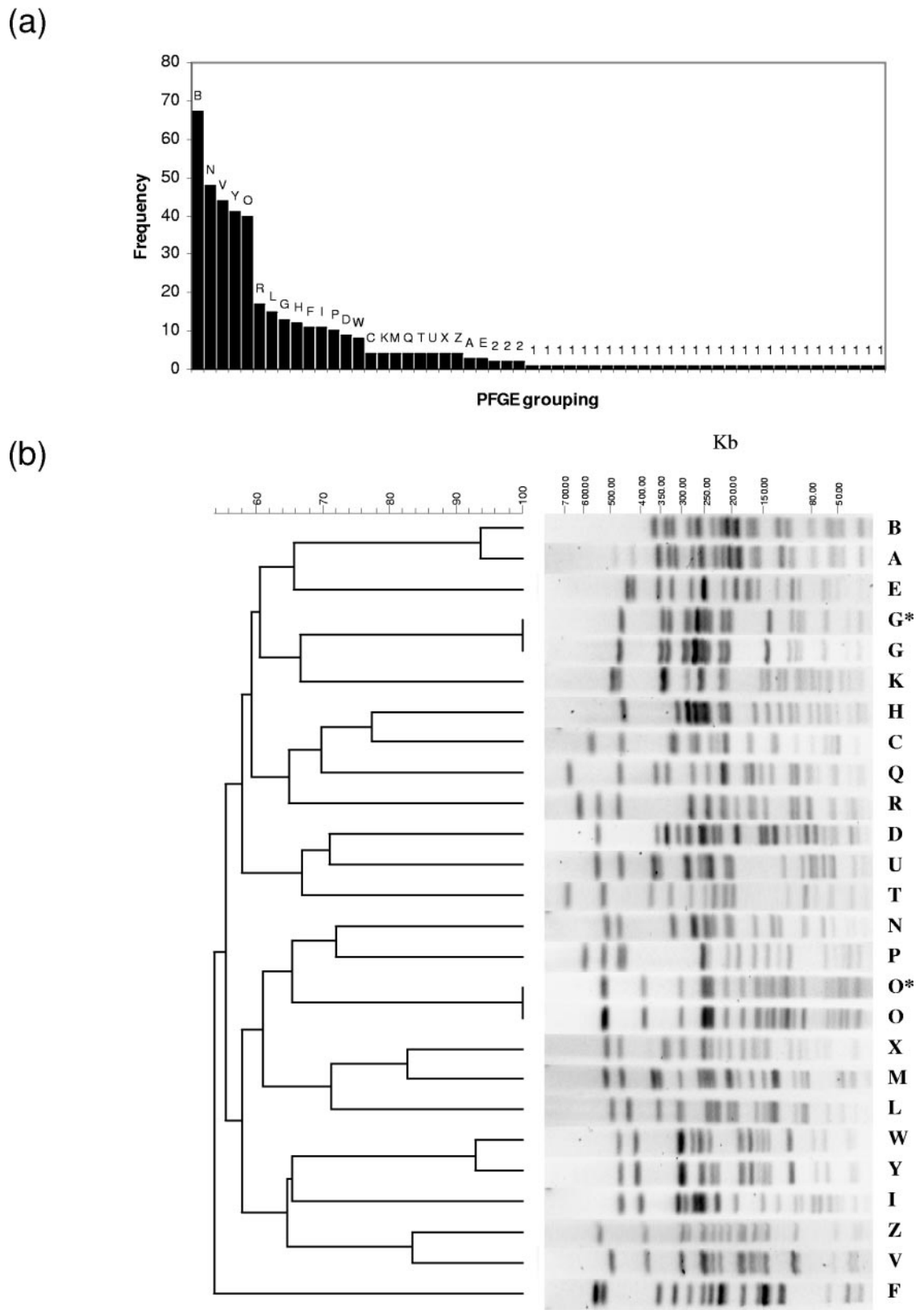

FIG. 1. (a) Rank abundance curve for the PFGE genotypes (n = 56) observed in the Amp^r single-pick collection (n = 419), with nominated groups represented by the relevant letter, isolate pairs by "2," and unique isolates by "1." (b) Comparison of PFGE banding patterns after digestion with XbaI for a typical example from each of the nominated groups in both the Amp^r (n = 24) and Nal^r (*; n = 2) single-pick isolate collections, showing the percent similarity between group types and band sizes in kilobase pairs (kb).

All calves yielded both Amp^r and Nal^r isolates at least once over the study period, with medians of 9 (range, 1 to 16) and 3 (range, 1 to 6) isolates collected per calf, respectively. The majority of calves harbored only one Nal^r genotype over the entire sampling period (58.3%), while a median of 5 (range, 1 to 10) Amp^r genotypes per calf were identified. There was some consistency in the distribution of Amp^r isolates within individual calves: two consecutive indistinguishable isolates were observed at least once in 83.8% of calves and on two occasions or more in 35.4% of calves, while three or four consecutive indistinguishable isolates were observed in 35.4% and 10.4% of calves, respectively. Two calves yielded highly conserved patterns of 6 and 14 consecutive indistinguishable isolates (PFGE genotypes V and Y, respectively).

Diversity within individual calf samples was examined using the multiple-pick data; medians of 2 (range, 1 to 6) Amp^r genotypes and 1 (range, 1 to 2) Nal^r genotypes were identified within individual samples. Eighty-eight percent of nalidixic acid and 40% of ampicillin multiple-pick plates yielded only a single PFGE type. Comparison of the frequencies of the five main Amp^r PFGE groups detected between weeks 4 and 13 in the single-pick collection with the frequencies of those in the multiple-pick collection found that while the proportions of groups B, N, V, and O were similar, group Y was underrepresented in the multiple-pick isolates, resulting in a significant difference between the overall frequencies for the two isolate collections ($P = 0.015$; df = 4). However, group W, a minor group in the single-pick collection which shared only two band differences with group Y (Fig. 1a), was found to be more common in the multiple-pick collection, and when groups W and Y were considered as a single group, no significant difference was observed in the frequencies in the single-pick and multiple-pick data ($P = 0.311$; df = 4). A comparison of the frequencies for the two Nal^r groups O and G showed no significant difference between the multiple- and single-pick data ($P = 0.112$; df = 1).

Temporal trends in genotype distribution. The cumulative recognition of Amp^r single-pick genotypes with time was biphasic, with a linear increase in type detection during the first 6 weeks of the study, which then declined but was followed by a further linear increase between weeks 11 and 14, before a further decline (Fig. 2a). Piecewise linear regression revealed a significant breakpoint in the data at week 11, corresponding to the time point when the calf cohort were housed, between weeks 10 and 11. While, in general, a positive association was identified between the number of isolates collected in a given week and the number of PFGE genotypes identified in that week (Pearson's correlation, 0.689; $P = 0.001$), an increase in sample size did not consistently result in an increase in the number of genotypes identified, with the latter remaining relatively static during the rising phase of isolate collection between weeks 3 and 10 of the study, when a peak mean number of four isolates per genotype was detected (Fig. 2b).

Trends in the proportion of single-pick isolates belonging to a given PFGE group were investigated over the entire study period in order to determine whether particular PFGE groups predominated at any time. For Nal^r isolates, group O predominated during the early and later phases of the study but was displaced by group G during the midpoint of the study, which peaked at week 8 (Fig. 3a). Of the five main Amp^r groups, the strongest trend was observed in group B, which predominated during the first 8 weeks of the study, comprising over 40% of isolates between weeks 4 and 5, but later disappeared at week 14 (Fig. 3b). Distinct trends were also observed in group N, which peaked at 30% of the population during the midphase of the study at week 11, and group V, which was found from week 5 onwards but was most common between weeks 10 and 15 (Fig. 3c and d). Groups O and Y showed no sustained temporal pattern and fluctuated between 0 and 25% of the population throughout the study period (Fig. 3e and f). Amp^r group G isolates were found only between weeks 6 and 11 and peaked at week 10, showing a profile similar to that of Nal^r group G isolates.

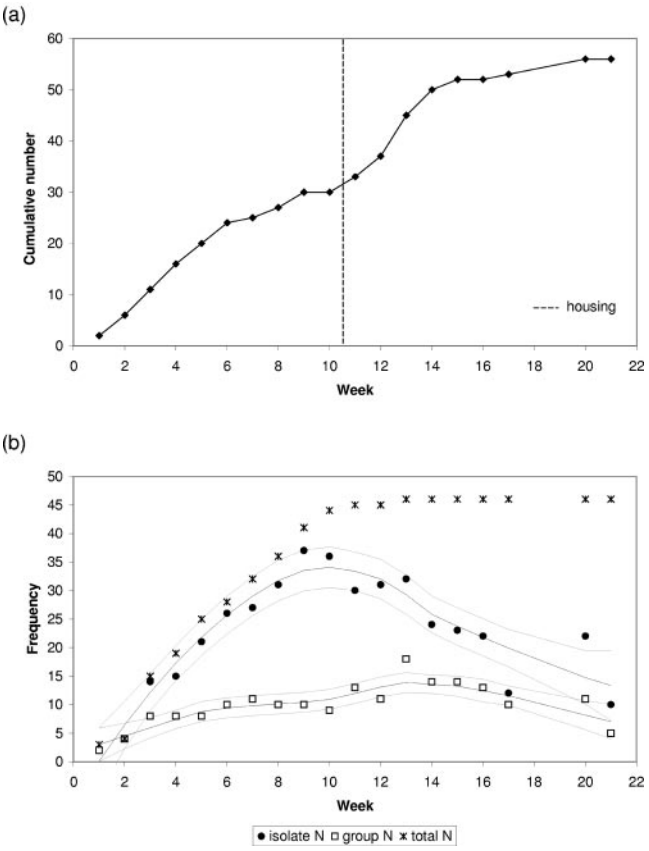

FIG. 2. (a) Observed cumulative recognition of PFGE genotypes in the Amp^r single-pick collection, by sampling week, with the time of cohort housing marked by a dashed line. (b) Number of Amp^r PFGE genotypes identified in each sampling week (group N) and total number of Amp^r single-pick isolates collected per week, equivalent to the number of calves positive for Amp^r in that week (isolate N), showing 95% confidence intervals (gray lines) fitted for both curves by using PROC LOESS (smoothing parameters, 0.5 and 0.8, respectively). The total number of calves sampled per week (total N) is also shown.

Antimicrobial susceptibility patterns. Almost all Amp^r single-pick isolates (99.5%) carried resistance to at least one of the additional antibiotics tested. The majority carried resistance to streptomycin, tetracycline, or sulfamethoxazole (85.7%, 80.0%, and 77.8% of the population, respectively), while resistance to cephradine, trimethoprim, nalidixic acid, and apramycin was detected less frequently (36.5%, 29.1%, 12.9%, and 2.4%, respectively). The carriage of multiple resis-

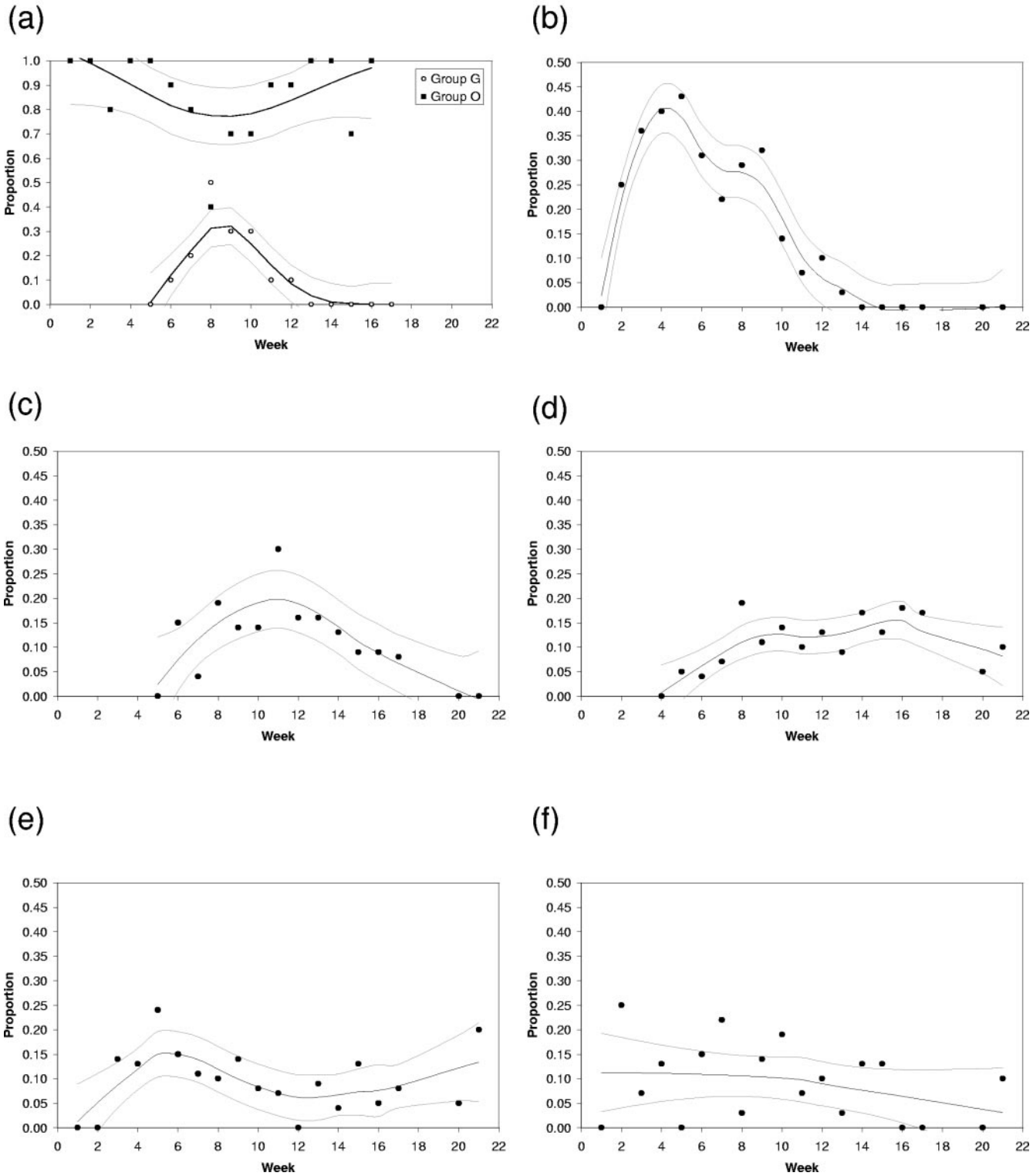

FIG. 3. Proportions of isolates from the single-pick collections present in the two main Nal^r PFGE groups (a) and in the five main Amp^r PFGE groups, i.e., group B (b), group N (c), group V (d), group Y (e), and group O (f), shown by sampling week, with trend lines (black) and 95% confidence intervals (gray) fitted using PROC LOESS (smoothing parameters, 0.5, 0.8, 0.5, 0.8, 1.0, 1.0, and 0.5, respectively).

tance was the most common state, with four additional resistance determinants observed in 36.5% of isolates and two in 32.2% of isolates. Five additional determinants were found in 14.6% of the population. Certain combinations were common, with core phenotypes of ST, SSu, and SuT found in 77.3%, 68.76% and 60.6% of isolates, respectively, while SSuT was present in 60.4% of isolates overall and in all of those with four or five additional resistance determinants.

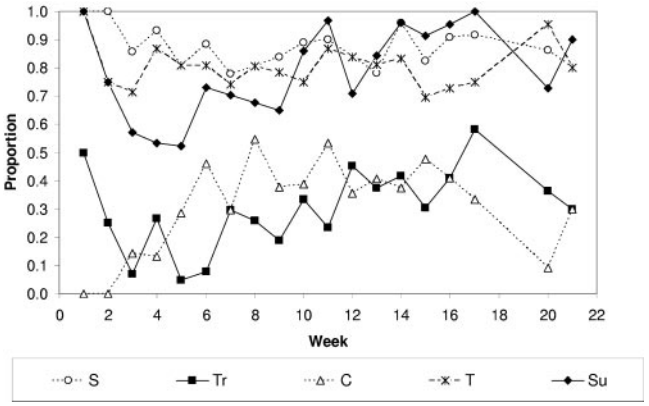

FIG. 4. Proportions of Amp^r single-pick isolates showing resistance to streptomycin (S), trimethoprim (Tr), cephradine (C), tetracycline (T), and sulfamethoxazole (Su), by sampling week.

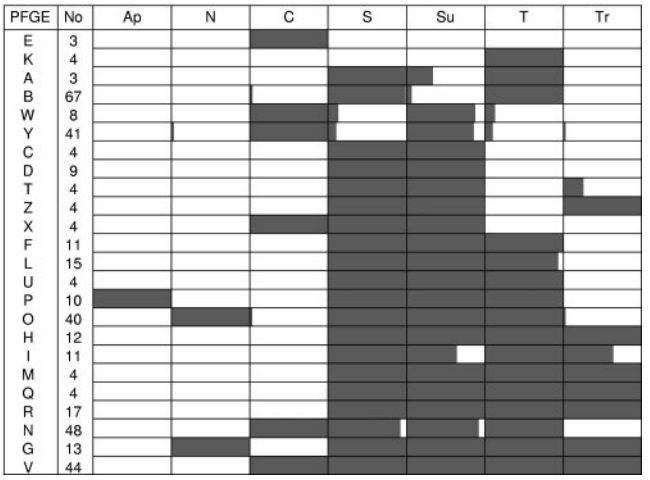

FIG. 5. Comparison of antimicrobial susceptibility phenotype versus genotype for all PFGE-grouped Amp^r single-pick isolates, showing group size (No), with the percentage of isolates within a group resistant to a given antimicrobial indicated by block shading. Ap, apramycin; C, cephradine; N, nalidixic acid; S, streptomycin; Su, sulfamethoxazole; T, tetracycline; Tr, trimethoprim.

The proportion of Amp^r single-pick isolates resistant to the five additional antibiotics was plotted on a weekly basis (Fig. 4) in order to examine whether there was an overall increase or decrease in antimicrobial susceptibility over time. No significant linear trend in the proportion of isolates resistant to streptomycin ($P = 0.198$) or tetracycline ($P = 0.784$) was observed, although there was a significant increase in the proportion of isolates resistant to sulfamethoxazole ($P = 0.037$). The trend in trimethoprim resistance was marginally nonsignificant ($P = 0.051$), although the pattern of resistance to sulfamethoxazole and trimethoprim over the course of the study was the same (for time $\times$ antibiotic, $F = 29.95$ and $P = 0.867$). A nonlinear trend in cephradine resistance was also observed, with an increase in the proportion of isolates resistant during the middle part of the study period ($P < 0.001$). Trends in the proportion of Amp^r isolates resistant to apramycin and nalidixic acid were similar to the weekly prevalence curves observed in the calf cohort (24).

Phenotype profiles of the single-pick Amp^r isolates were compared with the PFGE genotype patterns in order to examine within- and between-group antimicrobial phenotype combinations (Fig. 5). For the majority of PFGE groups (15 patterns), the antimicrobial phenotype was conserved, with no within-group variation in antimicrobial susceptibility profile. Of the five main genotype groups, group V showed no variation in phenotype, while within groups B and O over 94% of isolates and in groups N and Y 91% and 85% of isolates, respectively, had the same profiles. Comparing between groups, the combinations SSu, SSuT, and SSuTTr were found in more than one group and were also found as "core" components in a number of other groups. In contrast, phenotype profiles including Ap, C, N, and Tr determinants tended to be restricted to specific PFGE groups. Two phenotype patterns, ST and CSu, were each found in closely related PFGE groups (groups A and B and groups W and Y, respectively). Unique and paired isolates that were not grouped ($n = 35$) showed resistance to streptomycin, tetracycline, sulfamethoxazole, and trimethoprim, either alone or in combination, with two isolates additionally showing resistance to cephradine.

A comparison of the antimicrobial susceptibility profiles of the two main PFGE genotypes in the Nal^r population, types O and G, found that all isolates in both groups had the resistance phenotype ANSSuT, while only type G isolates also showed resistance to trimethoprim. These profiles matched those observed in the equivalent groups O and G of the Amp^r isolate collection. Additional resistance to cephradine and apramycin was observed in just one out of the 28 type G isolates.

DISCUSSION

In this study we have presented a unique view of the microbial ecology of an antimicrobial-resistant commensal *E. coli* population within a large cohort of calves monitored intensively from birth. The use of a PFGE genotyping technique has enabled us to demonstrate the spread of distinct antimicrobial-resistant strains throughout the cohort over time, with the dominance of certain PFGE-defined genotypes seemingly influenced by both time and the calf environment, rather than by cohort treatment with antimicrobial agents. Carriage of multiple resistance by isolates was the most common state, and a marked conservation of phenotype profiles was observed within genotype groups.

Five major PFGE genotypes were identified within the single-pick Amp^r population, of which groups B, N, and V showed distinct temporal distribution patterns, with group B in particular predominating during the first weeks of the study but then disappearing. Other PFGE groups, such as O and Y, were found throughout the study at a relatively constant prevalence. On average, only five different Amp^r genotypes were isolated from individual calves over the study period, and there was evidence of some consistency in genotype patterns identified within individual animals between consecutive weeks. In contrast, the Nal^r population showed almost no diversity and was entirely dominated by one major PFGE genotype, which was transiently supplanted, for a period of one week only, by a second, less common strain.

These results suggest that antimicrobial-resistant *E. coli*

populations behave in a manner similar to that of unselected commensal *E. coli* populations and show a continual turnover of colonizing strains (22, 23). Two types of commensal gut colonizers have previously been described: resident strains that are found over long time periods at varying levels and transient strains that appear in successive waves (26). Within this cohort both strain types appear to be represented, with the intermittent appearance of possible resident strains probably influenced by the sensitivity of the sampling method employed, together with fluctuations in actual bacterial numbers. The lack of persistence of transient strains, for example, groups B and G within the Amp^r and Nal^r populations, respectively, may be due to a variety of reasons, including an inherently poor colonizing ability or competition with resident or other dominating strains.

Cohort calves were born successively over a 12-week period and were therefore not all exposed to the same initial source of bacteria at the same time. This, together with the distinct temporal trends observed in some of the main genotype groups, suggests that some strains were transmitted through the cohort, probably via environmental contamination. The importance of the environment as a source of new strains is further emphasized by the impact that housing the cohort appeared to have on the cumulative recognition of genotypes. An initial linear increase in new genotype recognition over time was followed by a plateau, suggesting that the majority of genotypes in the available "pool" of strains had been recognized. However, a further increase in new strain recognition was then observed following housing of the cohort, which then leveled off after 3 to 4 weeks. While calves were not naive to the farmstead environment and were run through the main yard for weekly sampling when held at pasture, the change in housing and/or associated changes in their own or their dam's diet resulted in a distinct alteration in the dominant genotype patterns that they harbored. Prevalence data for this cohort showed a clear decline in the carriage of Amp^r and Nal^r *E. coli* strains with calf age (24), while the greater significance of age, rather than time, in the carriage of Amp^r *E. coli* has also been demonstrated in calf cohorts on other farms (25). This suggests that while the susceptibility of calves to colonization by resistant bacteria may be linked with age, the specific bacterial genotypes acquired are more likely to be influenced by environment and time.

Although the main study was based on the analysis of only a single isolate per sample, the multiple-pick data supported the main trends observed in genotype patterns, particularly the predominance of group B during the first few weeks of the study and the transient appearance of group N. The discrepancy in the frequencies of the two groups Y and W between the two data sets may be explained by the high similarity in banding profiles and phenotypes between groups Y and W, suggesting that these two groups may be highly closely related. Therefore, while the selection of just one isolate per sample was not able to provide information about within-calf strain dynamics or diversity, we conclude that the repeated-measure design of this study did allow us to demonstrate general trends present within the cohort as a whole. A similar approach has previously been used to investigate diversity of antimicrobial-resistant commensal *E. coli* strains within pigs and found that sampling time point influenced variability of isolates to a greater extent than within-animal diversity (9).

The difference in the number of genotypes identified within the Amp^r and Nal^r populations is probably related to the mechanisms of bacterial resistance to these two types of antimicrobial agents. Beta-lactam resistance is generally mediated by transferable resistance elements (32), and the presence of many different genotypes within the Amp^r population is therefore not surprising. However, comparison of antimicrobial sensitivity and genotype profiles showed both considerable phenotype consistency within specific genotype groups and high-frequency carriage of the "core" phenotype pattern SSuT among different Amp^r genotypes. This suggests both the possible dissemination of a common resistance element throughout the Amp^r population within the cohort and the direct transmission of resistance traits through defined strains. In contrast, quinolone resistance is primarily due to mutations within the bacterial chromosome, favoring clonal spread (45, 47) of a limited number of genotypes. Of the two Nal^r PFGE groups observed within the calf cohort, both had been previously observed in other cattle groups on the farm before the study start, while group O was also found in a new set of calves after the study end, suggesting transmission of discrete Nal^r strains through different age groups and time periods on the farm (unpublished data). While quinolone resistance cannot be transferred horizontally between cohabiting commensal and pathogenic bacteria within the gut, the dissemination of quinolone-resistant strains within this cohort does suggest that pathogens may also be subject to similar selective pressures on this farm. This highlights the importance of using commensal *E. coli* strains as "indicator" bacteria for monitoring antimicrobial resistance within both human and animal populations (43).

The temporal trends observed within some of the PFGE genotypes may be partly explained by the resistance phenotypes of those groups. For example, the significant increase in the proportion of Amp^r isolates carrying sulfamethoxazole resistance over time coincided with a decrease in the proportion of isolates belonging to the Amp^r genotype group B (sulfamethoxazole susceptible). This genotype may have become less common either because the presence of sulfamethoxazole resistance afforded a fitness advantage such that susceptible strains became uncompetitive within the gut or wider environment or because sulfamethoxazole resistance may be linked to another trait that was beneficial to resistant bacteria. A fitness advantage has recently been reported for a plasmid isolated from human clinical *E. coli* carrying the *sul2*, *strA*, and *strB* resistance genes (16).

Carriage of multiple-resistance phenotypes by both Nal^r and Amp^r isolates was more common than the occurrence of the single-resistance state. Multiple resistance is common in *Enterobacteriaceae* and can be carried on plasmids or transposons (6, 10, 40, 41). A 180-kb plasmid encoding resistance to apramycin, tetracycline, and streptomycin has been identified in Amp^r group P strains (48). The presence of such a transferable element carried by one of the genotypes within this cohort has implications for the possible transmission of resistance to cohabiting pathogens. Verotoxigenic *E. coli* strains were also identified within this cohort over the same period (35), and studies to examine whether these pathogens will accept resis-

tance plasmids isolated from the commensal population are ongoing. Transmission of multiple-resistance phenotypes between epidemiologically unrelated commensal and pathogenic *E. coli* strains has recently been demonstrated in vitro under ileal conditions (7).

The transmission of multiply resistant *E. coli* strains through the calf cohort outlined in this study has implications for the elimination of antimicrobial resistance carriage from the commensal gut flora of food animals. The spread of defined resistant strains, probably via environmental contamination, suggests that on farms reservoirs of fecal flora may remain as important sources of potential resistance for livestock in the absence of specific selection pressures. Furthermore, the dominance of multiply resistant strains carrying the SSuT phenotype, together with the significant increase in the proportion of isolates carrying sulfamethoxazole resistance observed over the study period, suggests a possible link of this phenotype with the high prevalence levels of antimicrobial resistance commonly observed in young livestock. The SSuT phenotype seen here has been identified in fecal *E. coli* strains from cattle in other studies (1, 20, 27, 44, 46), and plasmid-carrying SSuT *E. coli* strains isolated from young calves have been shown to carry a fitness advantage in vivo compared with equivalent SSuT strains isolated from adult cattle (27). We suggest that the predominance of the SSuT phenotype observed in the Amp^r and Nal^r strains isolated in this study implies a distinct role for all or part of this phenotype, possibly through linked genes, with the acquisition of antibiotic-resistant *E. coli* in young calves. Further investigation is now required to examine whether a common gene signal or linkage can be found between resistant strains carrying this phenotype isolated from different farms.

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