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Introduction

Antimicrobial resistance (AMR) in cattle production systems results in increased morbidity and mortality of livestock, and is consequently important for animal health and welfare. In epidemiological studies of AMR in cattle, it has been demonstrated that calves have a higher prevalence of AMR than older animals, which has important implications regarding the interpretation of surveillance data. Whilst antimicrobial exposure can influence the prevalence of AMR, the impact of this exposure on AMR diversity is not well understood.

The aims of the analyses presented here were to assess the association of host age class (cow or calf), and independently, the association of antimicrobial exposure (treated or untreated), with AMR diversity.

Materials and methods

An extensive dataset on AMR and antimicrobial exposure was utilized, comprising 25,480 isolates of faecal *E. coli* from 115 cows and 103 calves on seven dairy farms in the western United States. Susceptibility testing was performed by disc diffusion; isolates were categorised as susceptible or resistant to each of 12 antimicrobials. A profile was defined as a unique combination of resistances.

Given the heterogeneity of farm type, the analyses were stratified by farm in the first instance. For each farm, all cow isolates were compared to all calf isolates to assess the association of host age class with AMR diversity. Similarly, for each farm, all isolates from treated animals were compared to all isolates from untreated animals to assess the association of antimicrobial exposure with AMR diversity.

The methods used to explore associations between each factor and AMR involved the use of resampling and ecological diversity metrics.

Results

The number of unique profiles in cows only, calves only, and common to both were tabulated for each of the seven farms. Similarly, the number of profiles in treated animals only, untreated animals only, and common to both were also tabulated for each of the seven farms (Fig. 1).

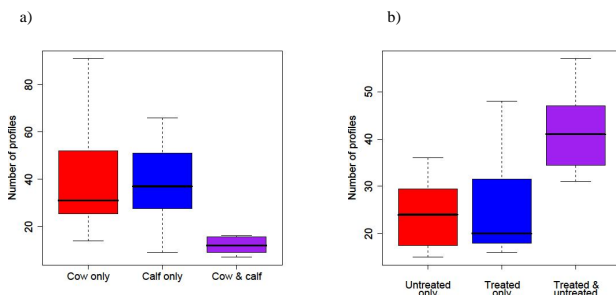


Figure 1. a) Number of AMR profiles found only in cows, only in calves, and in common between cows and calves for the seven farms. b) Number of AMR profiles found only in untreated animals, only in treated animals, and in common between untreated and treated animals for the seven farms. Whiskers extend to the most extreme of the seven data points.

Resampling analysis

A resampling technique was used to determine whether the observed numbers of profiles in treated animals only, untreated animals only, and those common to both treated and untreated animals were comparable to the resampled distributions of expected numbers, given a null hypothesis that isolates were drawn from a common microbial community. At each of 10,000 iterations, the isolates were randomly relabelled as treated or untreated without replacement, and the number of profiles in each of the three categories tabulated. An example of the output for one farm is shown in Fig. 2. The same approach was taken examining the number of profiles in cows only, calves only, and common to cows and calves.

RESULTS OVERVIEW

a) Cow/calf analysis

- # cow only profiles: significantly higher than expected ($p < 0.05$) on 6 farms
- # calf only profiles: significantly higher than expected ($p < 0.05$) on all 7 farms
- # profiles common to cows and calves: significantly lower than expected ($p < 0.05$) on all 7 farms

b) Treated/untreated analysis

- # untreated only profiles: significantly higher than expected ($p < 0.05$) on 3 farms
- # treated only profiles: significantly higher than expected ($p < 0.05$) on 4 farms
- # profiles common to treated and untreated: significantly lower than expected ($p < 0.05$) on all 7 farms

Resampling analysis continued

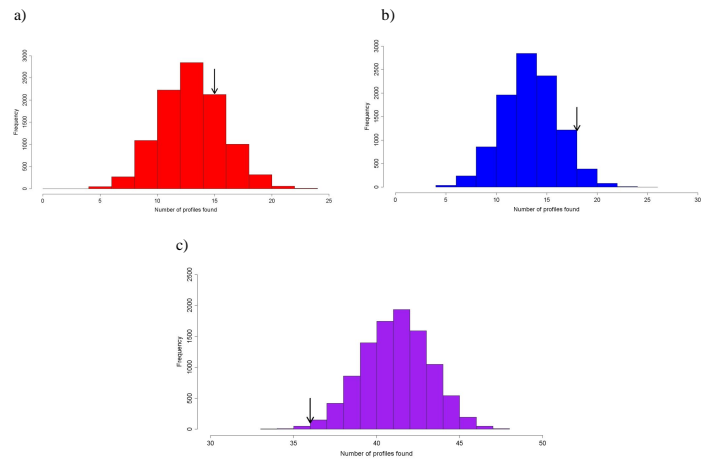


Figure 2. Example output of resampling analysis for Farm 3: Distribution of the expected number from bootstrapping of phenotypic AMR profiles found in *E. coli* in dairy cattle in a) untreated animals only, b) treated animals only, c) common to both treated and untreated animals. Arrows represent the observed number of profiles in each category.

Ecological diversity analysis

A family of related ecological diversity measures, covering all weightings of the contribution of species richness and abundance to diversity, was used to compare the diversity of AMR profiles from treated and untreated animals, and from cows and calves. Four principle measures were examined: Species richness (SR; rare profiles count the same as common profiles), Shannon entropy (SE), Simpson diversity (SD), Berger Parker (BP; rare profiles do not count at all). An example of the output for one farm is shown in Fig. 3.

RESULTS OVERVIEW

a) Cow/calf analysis

- Diversity of calf profiles > diversity of cow profiles on 3 farms
- Diversity of cow profiles > diversity of calf profiles on 1 farm
- Sample with greatest diversity depended on specific measure on 3 farms (for most measures, calf diversity > cow diversity)

b) Treated/untreated analysis

- Diversity of treated profiles > diversity of untreated profiles on 4 farms
- Diversity of untreated profiles > diversity of treated profiles on 1 farm
- Sample with greatest diversity depended on specific measure on 2 farms

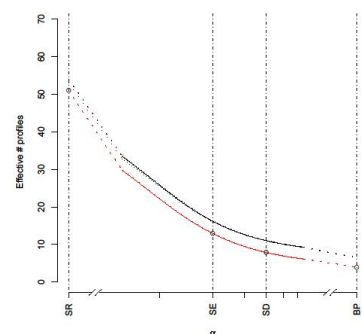


Figure 3. Example output of sample diversity measures (SR, SE, SD, BP) on Farm 3 for phenotypic AMR profiles found in *E. coli* from untreated (red) and treated (black) dairy cattle with confidence intervals (dotted lines) for the treated sample generated by subsampling to the size of the untreated sample.

Discussion

The observation that calves had a greater profile diversity than cows was not unexpected, and is in agreement with previous studies. However, the observation that on many farms, treated animals had greater profile diversity than untreated was unexpected and warrants further investigation.

In general, the results suggest that host age class and antimicrobial exposure have a strong influence on AMR diversity, despite hosts occupying a common environment.

Acknowledgements

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