An ecological approach to assessing the epidemiology of antimicrobial resistance in animal and human populations

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We examined long-term surveillance data on antimicrobial resistance (AMR) in Salmonella Typhimurium DT104 (DT104) isolates from concurrently sampled and sympatric human and animal populations in Scotland. Using novel ecological and epidemiological approaches to examine diversity, and phenotypic and temporal relatedness of the resistance profiles, we assessed the more probable source of resistance of these two populations. The ecological diversity of AMR phenotypes was significantly greater in human isolates than in animal isolates, at the resolution of both sample and population. Of 5200 isolates, there were 65 resistance phenotypes, 13 unique to animals, 30 unique to humans and 22 were common to both. Of these 22, 11 were identified first in the human isolates, whereas only five were identified first in the animal isolates. We conclude that, while ecologically connected, animals and humans have distinguishable DT104 communities, differing in prevalence, linkage and diversity. Furthermore, we infer that the sympatric animal population is unlikely to be the major source of resistance diversity for humans. This suggests that current policy emphasis on restricting antimicrobial use in domestic animals may be overly simplistic. While these conclusions pertain to DT104 in Scotland, this approach could be applied to AMR in other bacteria–host ecosystems.

Keywords: antimicrobial resistance; Salmonella Typhimurium DT104; ecological diversity

1. INTRODUCTION

Antimicrobial resistance (AMR) presents a major and growing threat to effective treatment of bacterial infections. For almost a century, antimicrobials have been used to control bacterial infections and disease in humans and animals. However, with increasing microbial resistance to these drugs, despite current interventions, we face a return to nineteenth century levels of morbidity [1–3]. Hitherto, investigation and policy development for the control of AMR using surveillance data have focused largely on patterns of resistance to individual antimicrobials [4,5]. Quantitative studies have concentrated on theoretical frameworks using simulated and in vitro experimental data [6–8], while much of our current understanding of the impacts of individual antimicrobials has been derived from small scale clinical epidemiological studies [9,10].

Only recently has the broader ecological landscape occupied by the bacteria and their hosts been considered [11–13]. Novel approaches may bring new perspectives on the origins and spread of AMR, or assist in the development of new or revised targeted interventions.

The use of antimicrobials in agriculture as a major driver of AMR in pathogenic bacteria of significance to humans is an issue over which opinions are divided [14–18]. The prophylactic and metaphylactic use in animal populations has been a particular concern [19,20], especially when the drug classes are the same as, or related to, the pharmaceuticals used in the control of human infections. Exposure of microbial populations to antimicrobials evidently selects for resistance; however, the critical and unresolved issue is the relative contribution to resistance in these populations from the different host communities. In addressing the basic tenet that the use of antimicrobials in animal populations is the major influence on the emergence and maintenance of resistance in human pathogens [14,15], three critical questions arise. First, are the resistance phenotypes in human and animal microbial populations the same, and do they arise from common or distinguishable microbial communities? Second, in which microbial
population are the resistances and resistance profiles common to both animals and humans first identified? Third, can we identify the most probable origins of all resistance phenotypes recovered from human and animal populations, and thereby infer the directionality of resistance transmission in these microbial communities?

To address these questions appropriately requires field data collected from naturally infected hosts occupying their natural ecological niches, and characterized using the same microbiological techniques where we consider the full spectrum of multiple resistant phenotypes. Many approaches to date, including studies on Salmonella Typhimurium DT104 (hereafter, DT104) [21–25], have been limited by the lack of concurrency, continuity, and sympathy of data, short time-scales, or inconsistent standardization of microbiological methods. Using data for which these issues do not apply and which cover the entire epidemic period of DT104 in Scotland, we take a novel population biology approach to the ecology of resistance, recognizing that the epidemiology of resistance determinants and the epidemiology of the host bacterium are not inextricably linked.

Multi-drug resistant DT104 was identified in England and Wales in 1984 [26], and over the next two decades became one of the most commonly isolated serovars of Salmonella from animals and humans [23,27,28]. The use of DT104 data from sympatric animal and human populations is particularly relevant even when the route of transmission cannot be established definitively; its zoonotic potential from animal contact is well recognized, with cattle density or cattle contact in particular identified as risk factors for human DT104 infections [21,24,29–32]. Our analyses encompass all potential routes of transmission, including additional unidentified sources, which lead to the observed distribution of resistance patterns in the host populations, as captured by a national surveillance programme.

In this study, we combine aspects of ecology and epidemiology, and show that animals and humans have distinguishable populations of resistance phenotypes, differing in prevalence, linkage and diversity of resistance. By examining the temporal ordering of first appearance, and phenotypic similarities of resistance profiles, we can infer the directionality of resistance transmission in these microbial communities.

2. METHODS

(a) Data

The data comprised 2439 animal and 2761 human DT104 isolates submitted from 1990 to 2004 to the Scottish Salmonella, Shigella and Clostridium difficile Reference Laboratory (SSSCDRL), and are part of an ongoing passive surveillance effort for Salmonella (see electronic supplementary material, figure S1). Salmonella is a reportable animal and human pathogen in the UK; all veterinary diagnostic laboratories isolating Salmonella spp. from livestock species in Scotland are required to forward suspect isolates for confirmation and typing to the SSSCDRL, as all medical diagnostic laboratories are required to forward suspect isolates from humans. For both human and animal DT104 isolates, the isolates were derived from samples submitted for diagnostic investigation of clinical disease; no information on prior antimicrobial treatment was available. Human isolates were derived from domestically acquired infections of DT104, from cases with no history of recent foreign travel.

The species origins of the animal isolates are presented in the electronic supplementary material, table S1. Sero-typing of the isolates and phage typing was accomplished according to internationally standardized methods [33–35]. Antimicrobial susceptibility was assessed using a modified breakpoint method, involving solid agar plates containing a pre-determined concentration of antimicrobial (see electronic supplementary material, table S2), and isolates were classified as non-resistant or resistant. Resistance to each individual antimicrobial, and each resistance profile were classified by their population of first observation, viz. humans or animals. The resistance profiles were then classified by whether they were unique to a given population, whether they occurred in both, and if common to both, the relative time of first identification. Due to the preponderance of cattle isolates in the animal dataset and the documented epidemiological link between human DT104 infection and cattle [21,29–31], these analyses were also performed for the animal isolates exclusively of bovine origin.

(b) Assessment of directionality of AMR movement; seeding and transmission models

In order to assess from which population each given resistance profile was more likely to have originated, the most probable potential precursor for the first observation of each unique profile in each population (35 profiles in animals, 52 profiles in humans), starting in 1991, was identified. This technique assessed only the data from these animal and human populations, and does not preclude another source of resistance, such as imported food or the environment, from contributing to the overall resistance diversity in either population. For each profile, the most probable precursor was defined as the profile with the fewest number of resistance changes and which occurred earliest in the sampling time frame. Determining the fewest number of resistance changes involved assessing both changes in resistance to individual antimicrobials (lowest Hamming distance), and simultaneous changes in groups of resistances which are known to be genetically linked. In DT104, resistance to ampicillin, chloramphenicol, spectinomycin, streptomycin, sulphamethoxazole and tetracycline are commonly chromosomally encoded, the genes located in a 43 kb region termed Salmonella genomic island 1 (SGI1) [36]. SGI1 variants, with different corresponding resistance profiles, have also been reported in DT104 [36–39]. Three additional putative genetically linked resistances in these isolates, determined by the pairwise association of resistances to individual antimicrobials as described in the electronic supplementary material (table S3, figure S2), were also identified. Thus, the most probable precursor could be a profile with resistance to one antimicrobial different (one change, one event), or a profile with resistances to one of the groups of linked resistances either gained or lost (e.g. six changes, one event). The population, animal or human, from which the closest precursor isolate was identified was recorded; where equally probable potential precursors were found in both populations, the source population was recorded as a tie.

In order to eliminate bias due to the greater number of isolates from the human population, the algorithm was applied 1000 times, each using a different sub-dataset sampled, without replacement, from the human dataset to the size of the animal dataset, thus creating equal sample sizes at each of the 1000 iterations. For each population, a Mann–Whitney U test was used to determine whether the
number of putative precursors originating in animals and humans was significantly different.

A similar model was used to determine whether resistance profiles circulated mainly within a population or were commonly transmitted between the two populations. Putative precursors were identified for each isolate found in each population starting from 1991. For any isolate of interest, the most probable precursor was defined as that isolate with a profile that had the fewest number of resistance changes and occurred closest in the sampling time frame to the isolate of interest. The putative precursor isolate was either: (i) an isolate with the same profile, i.e. no resistance change; (ii) an isolate with the fewest resistance changes, again looking at both changes to resistance to individual antimicrobials (lowest Hamming distance) and movement of groups of linked resistances. The population, animal or human, from which the closest precursor isolate was identified was recorded, with ties allowed as before. Again, the Mann–Whitney U test was used to test statistical significance. As for the seeding model above, this analysis does not preclude transmission occurring from another source of resistance diversity not monitored by this surveillance programme.

(c) Assessing whether or not the human and animal profiles are part of a single population of resistance phenotypes

In order to determine whether the numbers of different profiles found in humans and animals could have resulted from sampling a single combined population of phenotypes, the number of resistance profiles found in animals only, humans only and in both animals and humans were tabulated. The two datasets were combined, with consistent identifiers for the unique profiles and for population of origin. Datasets of the same size were then bootstrapped 10 000 times, without replacement, from this combined dataset, using a non-parametric approach. The number of profiles found in each of the three categories (animal only, human only, both animals and humans) were tabulated at each iteration for each of the bootstrapped datasets and compared with those observed. This analysis was also performed restricting the animal isolates to those of exclusively bovine origin.

(d) Assessing the ecological diversity of resistance profiles

Multiple diversity measures, related to Řényi’s measures of general diversity [40, 41], were calculated to compare the overall diversity of the animal and human data. The exponential of Řényi’s entropy measure gives an estimate of the effective number of species $D_n$, with its single parameter $\alpha$ determining the extent to which rare species contribute towards overall diversity (equation (2.1)). In our study, species refers to resistance profiles. Diversity measures representing all species, $D_\alpha$, of species richness, $\alpha = 0$, $1, 2, \infty$ determine the commonly used measures of diversity—species richness (SR), Shannon entropy (SE), Simpson diversity (SD) and Berger–Parker (BP).

To measure the diversity of each sample (animal and human), true abundances of the sample were derived directly from the count data ($P_1 = n_1/\sum n_i$), and the different diversities calculated from the abundance proportions. However, because sample diversity measurements depend heavily on sample size [43], direct comparison of the animal and human samples was conducted by repeatedly subsampling the larger (human) sample to the size of the smaller (animal) without replacement, and confidence intervals were generated by resampling. For the extension of this approach to the level of the population, see the electronic supplementary material. These analyses were also performed to compare the animal isolates exclusively of bovine origin and human isolates.

(e) Connectivity analysis of the human and animal resistance profiles

We used the eBURST algorithm [44] to visualize the connectivity of the animal and human resistance profiles as a means of assessing the sampling coverage of the surveillance programme. Although originally designed to be used with MLST data, the algorithm can also be used with any type of data that are represented by a string of integers. The most stringent definition of group, i.e. putative clonal complex, was used, with all members in a group having identical resistance outcomes for more than 12 of the 13 antimicrobials to at least one other member in the group. The analysis was repeated using less stringent criteria, reflecting the potential for multiple resistances to move in a single genetic event. No evolutionary processes were inferred from the results.

3. RESULTS AND DISCUSSION

Using long-term surveillance data, we demonstrate important differences in the ecology and epidemiological distribution of resistance phenotypes of DT104 in contemporaneous and sympatric animal and human populations. Over the period 1990–2004, there were 2761 human DT104 isolates from patients with no history of recent foreign travel and 2439 animal DT104 isolates, of which 1932 were derived from cattle.

First, we assess the similarity of microbial populations isolated from humans and animals based on their resistance phenotypes. Here, the overall resistance phenotype is characterized in terms of the combination of resistance phenotype to each antimicrobial for each isolate. Given the binary coding—non-resistant or resistant—for each of the 13 antimicrobials, there is a theoretical maximum of $2^{13}$ phenotypic combinations, or profiles, possible in these data. However, observed numbers are constrained by linkage and prevalence of the individual resistances: we encounter just 65 distinct resistance profiles overall, 52 profiles in the human dataset and 35 in the animal dataset (see electronic supplementary material, table S4). Twenty-two profiles are found in both animals and humans, suggesting some overlap between the microbial communities of animals and humans, but the 30 profiles unique to humans, and the 13 unique to animals, point to a degree of insularity. Restricting attention to isolates from only cattle and humans identifies 59 profiles overall,
28 profiles in cattle isolates and 52 in human isolates. Seven profiles were unique to cattle isolates, 31 unique to human isolates, with 21 profiles common to both cattle and humans. Further examination of the data reveals additional important differences between the two microbial communities with differing prevalences of resistance to the individual antimicrobials as well as estimated differences in the genetic linkage of the putative resistance determinants, as evidenced by the phenotype (see electronic supplementary material, figures S2 and S3).

Next we examine when, and in which population, each resistance phenotype is first identified and, therefore, for those held in common, the predominant probable direction of transmission of putative resistance determinants and profiles between humans and animals. Only these two populations are examined as potential sources; other possible sources, such as imported food or the environment, cannot be evaluated as no pertinent data are routinely collected in Scotland. It is also important to recognize that while it was resistance in DT104 isolates that was assessed in this study, it is known that resistance elements can be shared between bacteria; it is therefore possible that the ‘source’ of the resistance determinants of DT104 within an individual may be other co-infecting bacteria, although this cannot be assessed with these data.

When considering individual antimicrobials and ignoring linkage, there is no consistent pattern of prior identification in either population. Resistances to three antimicrobials are identified first in animals, eight are identified simultaneously in humans and animals and two are identified first in humans (see electronic supplementary material, figure S4); the same pattern was observed when comparing human and cattle isolates. However, viewed by profile, the picture is rather different: of the 22 profiles that are common to the two populations, five are identified first in animals, six are identified simultaneously in humans and animals and 11 are identified first in humans (figure 1a). Of the 21 profiles common to both cattle and humans, four are identified first in the cattle isolates, five are identified simultaneously in human and cattle isolates and 12 are identified first in the human isolates.

While this analysis only relates to profiles found in both populations, we increase our confidence in this finding by identifying the first occurrence of each of the profiles in each host population and determining in which population was the most probable phenotypic precursor to be found. Although we cannot dependably measure the evolutionary proximity of these isolates to each other due to the phenotypic nature of the data, we have incorporated the genetic linkages of resistances that are known to occur in DT104, and the putative genetic linkages we have estimated in these isolates, to allow variable numbers of resistance elements to be simultaneously gained or lost. Using this method, we establish that significantly more ($p < 0.001$) putative precursors occur first in the human than in the animal population (figure 1b,c). The results are more emphatic when the putative origin of each individual isolate, based on phenotype, is examined; again, when a precursor can be identified in one population before the other, the number of human isolates putatively originating in the human population is an order of magnitude greater than the number of human isolates originating in the animal population ($p < 0.001$) and significantly more ($p < 0.001$) of the animal isolates appear to have originated in the human population (figure 1d,e). Similar results are obtained when examining isolates from humans and cattle. While this does not rule out the possibilities of other linked resistances not included here or other sources of resistance for the human community, such as non-sympatric animals via imported food, the results suggest that the majority of resistance in these human cases of DT104 is unlikely to originate in sympatric animal populations, in contrast to prevailing orthodoxy [5,22,25,45].

We also assess whether or not these profiles are derived from common or distinguishable microbial communities. If these isolates arise from a common well-mixed microbial population, blinding ourselves to the animal or human origins of the isolates and resampling from the combined dataset would generate distributions of numbers of profiles consistent with those observed. Although we find the number of animal-only profiles is as predicted (with the observed number falling in the 12th percentile of the expected distribution), there are many more human-only profiles observed than expected (falling in the 99th percentile of the expected distribution; see electronic supplementary material, figure S5). Conversely, the 22 profiles observed in both animals and humans are much fewer than expected, falling in the 1st percentile of the bootstrapped values (figure 2a). Similar patterns are observed when comparing human and cattle isolates (see electronic supplementary material, figure S6). We infer from this finding that there is less mixing between the populations than would be anticipated were the isolates and their resistance patterns to have originated from a common community, supporting the observation of the different prevalences and timing of first identification of the different resistance phenotypes.

Having assessed the transmission question using temporal data, we strengthen our findings by comparing the differential diversities of the resistance phenotypes in the two populations, on the premise that the population with the lesser profile diversity is unlikely to be the source of resistance. Using measures of ecological diversity related to Rényi’s entropy measures [40,41,46] that differentially weight species richness and relative abundance, we demonstrate differences between the expected and observed diversities by every measure examined (SR, SE, SD and BP), indicating that samples from human and animal microbial communities differ (figure 2h), and that the human sample is significantly more diverse than both the entire animal sample (figure 2c) and the cattle-only sample (see electronic supplementary material, figure S7).

Extending this method to the resolution of the population and exploiting the finite upper bound on the number of distinct profiles ($2^{15}$), we calculate the scenarios that both maximize and minimize diversity and the associated confidence intervals for the diversity distributions (see the electronic supplementary material).

In general, the human microbial population is significantly more diverse than the general animal microbial population and the cattle microbial population for SE, SD and BP (figure 2d; see the electronic supplementary material, figure S8). These results are consistent across a range of measures that differentially weight the contributions of relative abundance and species richness to diversity, and suggest that our results are robust to aspects of data structure that might be sensitive to different
surveillance methods. Given that one would expect a population acting as the source of resistance to have greater profile diversity than any to which it transfers resistance, these findings, again, are consistent with the hypothesis that the sympatric animal population is unlikely to be the major source of the diversity of resistance for the human population.

These analyses examine the sympatric animal and human populations in Scotland. Cattle density and cattle or animal contact have been shown to be associated with an increased likelihood of human DT104 infection [21,29–31] and analysis of exclusively cattle-derived isolates corroborates our inference. However, given that Salmonella infections in humans are associated with the food chain [47] and imported food is not derived from the sympatric animal population, imported food as a source of infection and/or resistance must also be addressed. There are two relevant issues: first, does the fact that imported food may be acting as a source of resistance in DT104 in humans in Scotland invalidate the conclusion we draw from demonstrating that the sympatric animal population is unlikely to be the major source for humans? The answer is clearly, no; we are restricting our conclusions to the sympatric animal population, in which we observe less diversity and a smaller number of profiles first identified in this population. Second, there is a danger of conflating the epidemiology of DT104 with that of the resistance determinants. Again, we make no

Figure 1. Putative origins of the phenotypic antimicrobial resistance profiles for animals and humans in S. Typhimurium DT104. (a) Relative time of first identification in years for the 22 profiles found in both animals and humans (i.e. the first profile in black was identified in the human population 5 years before being identified in the animal population (grey)). (b) Boxplot of the number of closest precursors, where a putative source population could be identified, to each unique observed profile in animals found in the animal population and the human population from 1991 to 2004 (whiskers extend to most extreme data point which is not more than 1.5 × (interquartile range) from box; open circles represent outliers). (c) Boxplot of the number of closest precursors, where a putative source population could be identified, to each unique observed profile in humans found in the animal population and the human population from 1991 to 2004. (d) Boxplot of the number of closest precursors, where a putative source population could be identified, to each observed isolate in animals from 1991 to 2004 found in the animal population and the human population. (e) Boxplot of the number of closest precursors, where a putative source population could be identified, to each observed isolate in humans from 1991 to 2004 found in the animal population and the human population.
Figure 2. Differences in patterns and diversities of phenotypic antimicrobial resistance profiles in *S. Typhimurium* DT104 from animals and humans. (a) Bootstrap distribution of the expected number of profiles shared between animals and humans, compared to the observed number of profiles (vertical line: 22). (b) Observed sample diversities for species richness (SR), Shannon entropy (SE), Simpson diversity (SD), Berger–Parker (BP) shown as red circles, compared to the expected values (black clouds) generated using bootstrap relabelling of the human and animal profiles. (c) Sample diversity measures (SR, SE, SD, BP) for animals (red) and humans (black) with confidence intervals (dotted lines) for the human sample generated by subsampling to the size of the animal sample. (d) Population diversity measures (SR, SE, SD, BP) for animals (red) and humans (black). Dotted lines are the extrema of the 95% confidence intervals generated using the constraints on the number of theoretically possible profiles (from the observed number up to $2^{13}$). Shaded areas show the range of expected population diversities under these constraints.

claim as to the probable source of DT104 as our focus is on the resistance—it may well be that the organism is sourced from the sympatric animal population but our evidence suggests strongly that there are other sources for the resistance that is then expressed phenotypically in the organism in humans. Indeed, an extension of our inference is that if the observed resistance is not arising *de novo* in the human population then there must be other sources of resistance. While this type of data is not routinely collected in Scotland, previous studies implicate imported food as one possible source [4].

Restating the particular relevance of these data to this issue, in terms of the sympathy, contemporaneity and consistency of microbiological techniques employed, we recognize that passive surveillance data potentially may be biased. It is possible that the subtypes of bacteria obtained from clinical cases may not represent those found in the healthy population [48], and similarly, that the subtypes infecting animals and humans are different. However, a recent genotypic study on *Salmonella* Typhimurium has suggested that this is not the case [49]. By examining the distribution and contiguity of this surveillance sample over the resistance-phenotype space we can obtain a useful indication of the effective sampling coverage. In the animal dataset, 83 per cent of the profiles belong to one contiguous complex, while in the human dataset only four profiles are unconnected to any other, and the remaining 92 per cent of the profiles belong to one putative complex (see electronic supplementary material, figures S9 and S10). The more intriguing observation is that, when considered together, 95 per cent of all profiles can be connected (figure 3). This is remarkable considering that the samples were collected over a period of 15 years, through passive surveillance, and provides some reassurance that the sampling coverage was sufficient to have encountered the vast majority of phenotypic combinations present. Analysing the data under less stringent conditions, reflecting the movement of multiple resistances in one event, results in the same connectivities (see electronic supplementary material, figures S11 and S12). The observation that the majority of profiles can be grouped together may suggest that most profiles belong to a single population, but this is not what we find using a more complete analysis, which clearly demonstrates that the isolate populations are different. Furthermore, it is not differental sampling of the populations, i.e. more intensive sampling of humans, which has led to the observation of greater diversity and more profiles first observed in the human isolates. Given the number of DT104 isolates submitted over the study period, and the size of the human and cattle populations, the odds of a DT104 isolate being submitted for a bovid was 1.80-fold greater (95% CI: 1.50–2.17, $p < 0.001$) than that for a human, thus providing even greater confidence in our results (see the electronic supplementary material). Moreover, of the 35 profiles observed in the animal DT104 isolates, 13, or 37 per cent, were unique to animals and were not detected at all in the human DT104. If animals were the source of resistance for human DT104, and the surveillance of humans was more rigorous than that for animals, we would not expect to detect such a large proportion of resistance profiles in animals that were not also detected in humans—but this is what we observe. We also address a variety of
other potential biases (see the electronic supplementary material) and determine that they are not present in these data.

In common with many AMR surveillance systems, there are no reliable antimicrobial usage data available for the populations from which these isolates were obtained. This type of information would be necessary to evaluate more directly the impact of differing antimicrobial usage patterns on AMR diversity in the two populations, although this would still not evaluate the impact of historical antimicrobial use. It is possible that individuals of one population were more likely to have been treated with an antimicrobial prior to sample submission than individuals from the other population. This may bias the diversity of resistance in the DT104 sampled in that population, but without the relevant data this cannot be evaluated. However, neither population had significantly more multi-drug resistant profiles than the other (see electronic supplementary material, figure S13), which is at least consistent with the notion that differential use of antimicrobials is not responsible for the observed results. In addition, the median number of resistances per isolate was six for both animal and human isolates. We do not dispute the potential for antimicrobial use to give rise to new resistance, such as following the introduction of fluoroquinolone use in animals [50], although this was not observed in all geographical regions and not in Scotland [30]. Indeed, antimicrobial use in any population may select for new resistance but this does not negate our observations and conclusions. We also note that some argue that clonal expansion rather than antimicrobial use contributes to the patterns observed in different populations [51]. This may be the case but would only influence the prevalence of resistance(s) and dissemination, and not the diversity or time of first emergence on which we base our arguments.

Inevitably, the issue of whether phenotypic data are appropriate for these studies will be raised. The data used for these investigations were generated using a breakpoint method and it is possible that had different breakpoints been used, different results may have been obtained. However, clinical surveillance systems that generate the data on AMR used in monitoring and policy formulation are based on phenotypic susceptibility testing methods. Genotypic methods are expensive and time-consuming (although clearly becoming more affordable and faster) to perform on large numbers of samples and have yet to be implemented on the scale necessary for population level studies of this kind. Molecular data on the scale of the data we present here would provide additional evidence and it is worth considering what inference might be drawn from such information and how it might validate, or refute, our phenotypic approach. The molecular data will provide information on the genetics of the bacteria and on the resistance determinants; for our purposes, it is the data on resistance determinants that are relevant. There are three possible outcomes: first, if the molecular data map directly to the phenotypes described, then our conclusions are supported. Second, if genotype does not map directly to phenotype, and there are distinguishable resistance determinants at the molecular level, then this is additional evidence in support of our conclusions that the populations are distinguishable with respect to AMR. It is only in the third scenario where genotype does not map to phenotype and where either the resistance determinants are represented equally in both populations or where the greater diversity is in animals that there would be a contradiction of our conclusions. However, given the diversity of phenotypes observed and the known genetics of DT104 [52]—resistance genes if present are generally expressed—we regard this as an unlikely outcome. We conclude that while genetic data would be a valuable addition, it does not invalidate our inference from the phenotypic data; importantly we make no claims about genotype in this study—we demonstrate that in ecological terms the populations of DT104 arising from passive surveillance of animal and human enteric bacterial isolates are phenotypically distinguishable.

4. CONCLUSIONS

Our approach offers insights and perspectives into AMR that would not be apparent using traditional methods of analysis, and has yielded answers to the three critical questions regarding the contribution of resistance in the domestic animal population to that in humans. First, are the resistance phenotypes in animal and human DT104 the same, and do they arise from common or distinguishable microbial communities? While a number of the phenotypes are common to both populations, there are a significant number of phenotypes that are unique to each population. The resampling analysis has indicated that there are distinguishable microbial communities, with significantly fewer shared profiles and significantly more human-only profiles than would be expected if the animal and human microbial communities were well mixed. Second, in which microbial population are the resistances first identified? For resistance to individual antimicrobials, there is no clear pattern, but the majority of resistance profiles that are common to both populations are identified first in humans. Third, can the origins of resistance profiles be estimated? The answer is yes; when a putative source population can be identified for each profile, and for each isolate, significantly more of these putative precursors were identified in the human population.

Figure 3. Connectivity diagram of animal and human phenotypic antimicrobial resistance profiles of S. Typhimurium DT104. Profiles found only in animals are green squares, profiles found only in humans are black triangles, profiles found in both animals and humans are pink circles. Profiles are connected to each other by lines representing either the loss or acquisition of resistance to a single antimicrobial, using eBURST.
For this exemplar of *Salmonella* Typhimurium DT104 in animals and humans in Scotland, we demonstrate that, while ecologically connected, even at the level of resistance phenotype, animals and humans have distinguishable microbial communities, differing in prevalence, linkage and diversity. While transmission is likely to occur in both directions, animal-to-human and human-to-animal, the critical issue is the proportion of transmission that occurs in each direction. Examination of temporal and phenotypic similarity suggests that a plausible and justifiable conclusion based on these data is that the sympatric animal population is unlikely to be the major source of resistance diversity for human DT104. Further research on other potential sources of resistance diversity in human pathogens in Scotland, such as imported food, would provide important supplementary information. Although we detected no evidence of the impact of differential sampling coverage on our results, such differences are hard to exclude definitively, given the nature of passive surveillance. However, these concerns have not prevented similar phenotypic surveillance data being used to support alternative views [5,31] and to inform media comment and policy formulation and debate. Furthermore, our conclusions are based on examination of the entire resistance profile of isolates, rather than inference based on resistance to a single antimicrobial, often from restricted or unspecified data sources.

With the significant milestone of the Swann Report [53] over 40 years ago, the debate surrounding the issue of antimicrobial use in veterinary medicine remains current, demonstrated by the Chief Medical Officer in the UK recently calling for the withdrawal of specified antimicrobials from usage in animals [54]. Responsible use of antimicrobials in all species is, of course, imperative in order to minimize the emergence, maintenance and transmission of resistant organisms. While human health must remain a priority, bacterial infections nevertheless threaten health and welfare in both animals and humans, and appropriate and measured policy must be developed through a complete understanding of the ecology of resistance in both populations, as well as in the broader ecological web. Having provided evidence that for *Salmonella* Typhimurium DT104 the majority of new resistance profiles do not appear first in the sympatric animal population, we recognize that the broader issue requires consideration of other bacterial species and other host species. Formulation of policy to address this critical issue and to prioritize interventions must make the fullest use of the evidence from surveillance programmes. Our approach contributes to this prioritization by providing a framework for similar evaluations in other host-pathogen combinations in other settings and developing a richer understanding of resistance on which actions to reduce AMR can be based.

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