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Population genomics of *Escherichia coli* in livestock-keeping households across a rapidly developing urban landscape

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Quantitative evidence for the risk of zoonoses and the spread of antimicrobial resistance remains lacking. Here, as part of the UrbanZoo project, we sampled *Escherichia coli* from humans, livestock and peri-domestic wildlife in 99 households across Nairobi, Kenya, to investigate its distribution among host species in this rapidly developing urban landscape. We performed whole-genome sequencing of 1,338 *E. coli* isolates and found that the diversity and sharing patterns of *E. coli* were heavily structured by household and strongly shaped by host type. We also found evidence for inter-household and inter-host sharing and, importantly, between humans and animals, although this occurs much less frequently. Resistome similarity was differently distributed across host and household, consistent with being driven by shared exposure to antimicrobials. Our results indicate that a large, epidemiologically structured sampling framework combined with WGS is needed to uncover strain-sharing events among different host populations in complex environments and the major contributing pathways that could ultimately drive the emergence of zoonoses and the spread of antimicrobial resistance.

The spread of bacterial pathogens and antimicrobial resistance (AMR) across human and animal populations presents a substantial and growing threat to global health and economic development. Identifying risk factors for emergence and spread is one of epidemiology's most important challenges. Many recent pandemics and newly emergent infectious diseases have animal origins[1,2] and are associated with rapidly urbanizing environments[3,4]. The dynamic interfaces among humans, domestic livestock and wild animals act as conduits by which humans can be exposed to zoonotic pathogens and AMR in an environment with inadequate sanitation infrastructure, limited access to appropriate and effective drugs and unregulated antimicrobial usage[5–8].

The importance of livestock to the transmission of bacteria and AMR remains unclear[9]. The practice of keeping livestock, particularly in urban settings, has been described as a risk factor for the emergence and spread of zoonoses[10,11]. Antimicrobial agents used in human medicine are also used for growth promotion, disease prevention and disease treatment in livestock, enhancing selection pressures on bacterial pathogens for AMR emergence and spread.

Wild birds and mammals have also been documented to carry and exchange drug-resistant bacteria with livestock and humans[6,12,13]. The rapid expansion of urban environments into previously pristine or sparsely populated natural landscapes also increases the potential for greater contact among wildlife, humans and livestock, which can provide conduits for microbiome sharing[14].

Fundamental to whole-genome sequencing (WGS) studies is the availability of systematically sampled bacterial isolates obtained from humans, livestock and wildlife across overlapping geographical regions and time frames, yet data are lacking[15]. In this study, we sampled the bacterium *Escherichia coli* from humans, livestock and peri-domestic wildlife in 99 households and their environs across 33 sublocations in Nairobi, Kenya, in an epidemiologically

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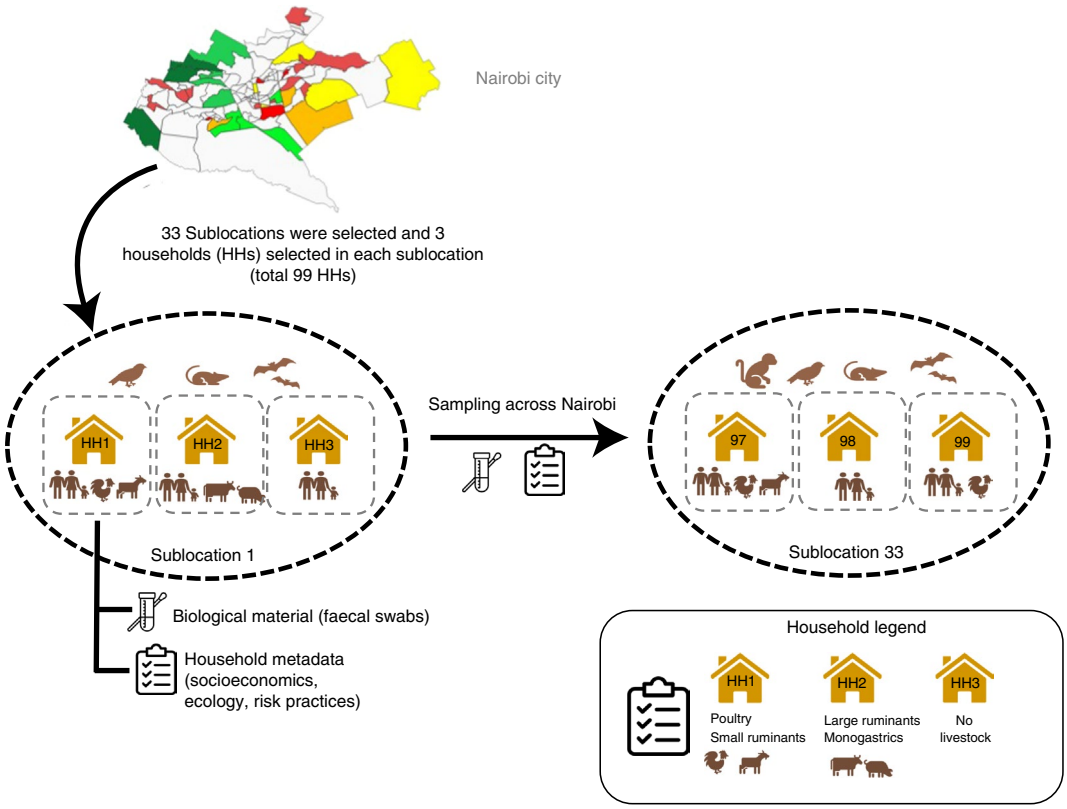

Fig. 1 | Flow diagram of the household selection procedure. Different colours given to the sublocations on the Nairobi city map represent different wealth categories (dark green, wealthy; dark red, poor).

structured study. The rapid development of Nairobi's urban landscape is similar to that of many other cities in the developing world, making it an ideal system in which to explore how people's interactions and co-existence with animals influences pathogen transmission across species[16,17].

This '99 households' study was part of a broader study ('Epidemiology, Ecology and Socio-Economics of Disease Emergence in Nairobi', or 'UrbanZoo' for short) and focused on mechanisms for zoonotic pathogen emergence in urban environments. The broader study included mapping agriculture-sector value chains to understand the flow of animal source food products into the city of Nairobi[18–26] as well as the aetiology of childhood diarrhoea in low-income settlements, studies quantifying antibiotic drug resistance carriage in multiple hosts[6,12] and the roles of different hosts in disseminating clinically important resistance profiles[27,28]. It also included work to explicitly analyse the interplay among urbanization, food supply and pathogen risk[29]. The data presented here explore the phylogeography of bacterial isolates across an urban landscape.

As a common commensal and pathogen in vertebrates, as well as its ease of isolation and culture and its wealth of available genetic information, *E. coli* is an ideal exemplar bacterium to study the more general phenomenon of dispersal of pathogens across host populations. Here we report a genomic investigation of 1,338 *E. coli* isolates sourced from humans, livestock and wildlife across Nairobi to elucidate patterns of bacterial strain sharing as a proxy for transmission potential. We test the hypothesis that the distributions of bacterial strains and their genetic pools are limited to particular defined ecological niches (households and hosts) versus an alternative that they display a cosmopolitan distribution—in essence, recapitulating the famous tenet, "Everything is everywhere, but the environment selects"[30]. By considering both household and host factors, our study captures both neutral (dispersal limitation) and niche (environmental selection) processes in driving bacterial distribution[31]. Our study aims to identify risk factors to help inform surveillance strategies that target potential hotspots for strain sharing and AMR transmission among populations in an urban setting and, more broadly, to understand risks associated with transmission of multi-host pathogens in urban settings.

Results

***E. coli* in Nairobi are from both global and local lineages.** A total of 1,338 *E. coli* isolates were sequenced as part of this study (Supplementary Table 1). In total, 311 genomes were obtained from human isolates; 421 genomes were isolated from 63 wildlife species, primarily composed of wild birds ($n=245$), rodents and bats ($n=130$) isolates; and 606 genomes were obtained from 13 species of livestock that can be grouped into poultry ($n=324$), goat and sheep ($n=109$), cattle ($n=61$), pig ($n=49$) and rabbit ($n=38$) isolates. The isolates were distributed across 99 households from 33 geographic sublocations, spanning the entire urban area of Nairobi, with each sublocation represented by 20–63 isolates (Fig. 1, Extended Data Fig. 1 and Supplementary Methods).

The genomes represent all major lineages of the *E. coli sensu stricto* phylogroup in addition to members of the cryptic clade I. The isolates belong to Clermont phylogroups B1 (45%), A (38%), B2 (6%), D (4%) and E (2%) and, to a lesser extent, clades C, F and G and clade I (<1%). Phylogroup A was strongly associated with humans (41% of human isolates) compared with the other host categories. In the livestock mammal, wild bird and wild mammal categories, genomes from phylogroup B1 were the most frequently isolated.

A total of 537 sequence types (STs), based on the seven-gene Achtman scheme, were represented, with the three most common being ST10 ($n=93$, 7%), ST48 ($n=64$, 5%) and ST155 ($n=54$, 4%)

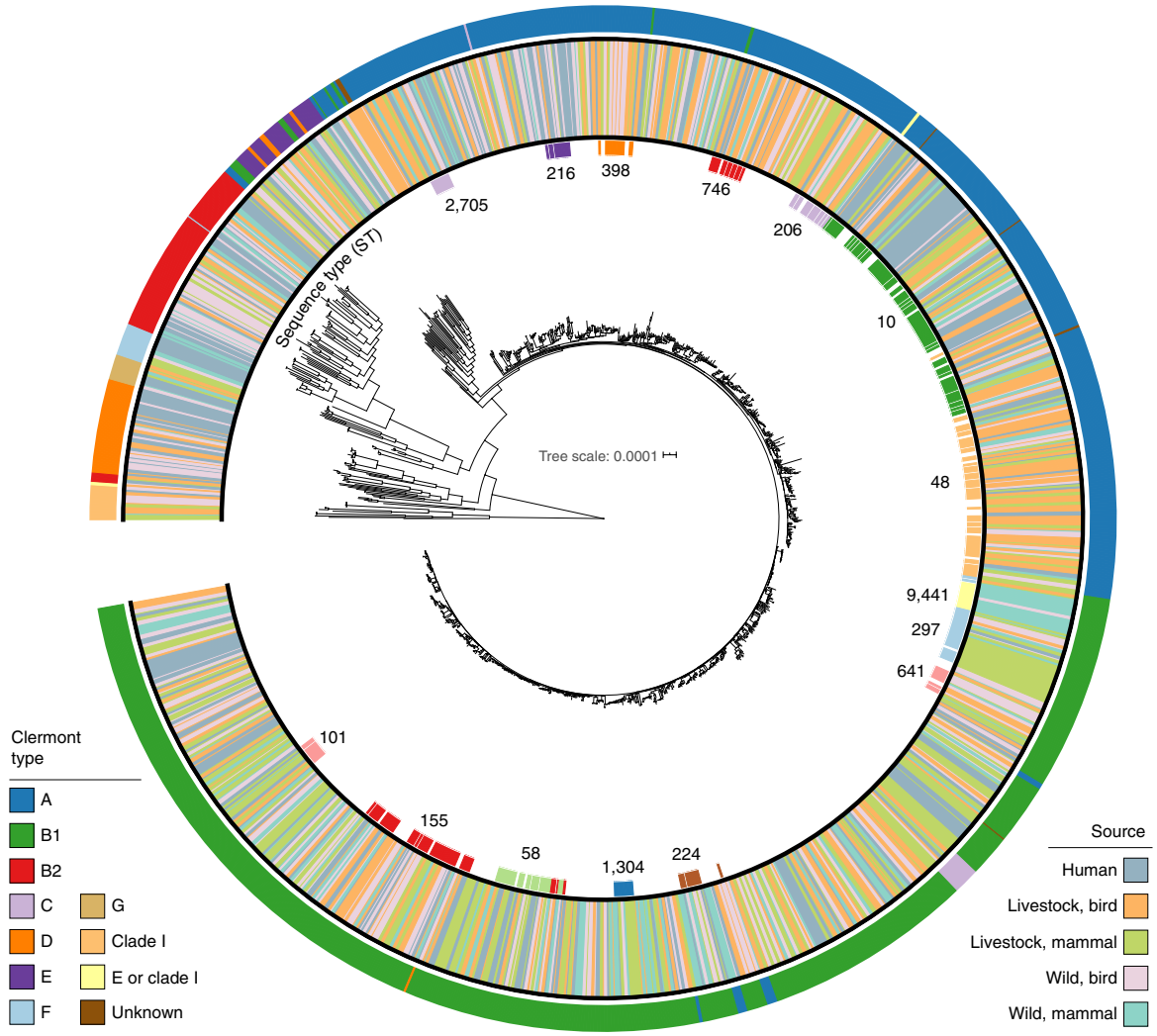

Fig. 2 | Core genome phylogeny of 1,338 *E. coli* isolates. Inner ring: STs (only STs with a minimum of ten isolates are shown); middle ring: source type of isolate; outer ring: Clermont phylotype classifications. The tree is rooted on the clade I group.

(Supplementary Table 2). One hundred and thirty-nine STs, representing 14% (184/1,338) of isolates, have been found only in African countries (Kenya, Madagascar, South Africa and Uganda), based on the genomes that were present in Enterobase at the time this study was carried out. One hundred and thirty-three of the Africa-specific STs in this collection, representing 13% (173/1,338) of the isolates, were unique to Kenya. Most of these novel and unique STs were isolated from livestock (52%, 96/184) and wildlife (34%, 63/184). A core-genome alignment comprising 80,722 nucleotide positions conserved across all 1,338 isolates was used to infer the overall phylogenetic relationship among isolates (Fig. 2). Additionally, we did not find extensive associations of isolates with either host species or sublocation (Fig. 2 and Extended Data Fig. 2).

Clonal strain sharing of *E. coli*. Transmission of bacteria, either directly or indirectly via a common source, can be inferred by the presence of very closely related genomes in two individuals, which we refer to as clonal strain sharing. To identify clonal strain sharing, we used core-genome, multi-locus sequence typing (cgMLST), which is a measure of genetic relatedness that is reproducible and scalable across larger and more diverse datasets[32]. We first plotted the frequency distribution of pairs of isolates differing by fewer than 100 cgMLST loci (Fig. 3). Here, we found a total of 150 pairs of isolates that differed by ten or fewer cgMLST alleles from other isolates in our collection. These pairs comprised 187 (14%) isolates, with some isolates involved in multiple pairs. Data on household and host type for these 150 pairs revealed that most occurred among hosts from the same household ($n=101$, 67%) and 33% ($n=49$) involved hosts from different households. Given the low genetic distances and epidemiological context, we refer to these pairs of ≤10 cgMLST loci as 'sharing pairs' to indicate evidence of recent strain sharing either by direct transmission or acquisition from a common source (Extended Data Fig. 3). We found no significant correlation between host type sharing and inter-household geographical distance ($\chi^2=8.83$, $P=0.64$, Kruskal–Wallis) (Extended Data Fig. 4).

Pairwise core-genome, single-nucleotide polymorphisms (cgSNPs) of these sharing pairs were also investigated to validate the genetic distance as measured by cgMLST. The distribution of closely related pairs (<100 cgSNPs) also showed a similar pattern, with 159 pairs separated by fewer than ten cgSNPs (Extended Data Fig. 5). Both cgMLST and cgSNPs measures captured very closely related

pairs of isolates, with 73% of the sharing pairs ($n=109$) separated by four or fewer cgSNPs and 97% ($n=145$) by a maximum of ten cgSNPs (Extended Data Fig. 6). Only one pair had more than 13 cgSNPs. WGS studies of *E. coli* outbreaks in humans have shown that epidemiologically linked isolates usually differ by up to four cgSNPs when isolated within 30 days of each other and, when separated by 5–10 core cgSNPs, this time frame increases to an average of 8 months[33]. Therefore, the genetic diversity of isolates within the same household agrees with examples of epidemiologically linked *E. coli* in other settings, and we estimate that length of evolutionary time separating two isolates from within the same household is within the range of several months to several years.

Sixty-five percent ($n=97$) of the pairs were between isolates from the same host category (57 (38%) within livestock, 26 (17%) within wildlife and 14 (9%) within humans), and the remaining 36% ($n=53$) were found between host categories (38 (25%) between wildlife and livestock (W–L), ten (6%) between human and livestock (H–L) and five (3%) between human and wildlife (H–W)). Further details on the breakdown of these sharing pairs are provided in Supplementary Table 1. No correlation was evident between sharing pairs and particular *E. coli* lineages, as sharing pairs were distributed across the phylogeny for all six (H–H, L–H, L–L, W–H, W–L and W–W) categories of sharing (Extended Data Fig. 7). However, in seven cases, wildlife isolates that were implicated in sharing pairs were found in the same cluster as isolates involved in sharing pairs with other host categories (Extended Data Fig. 7).

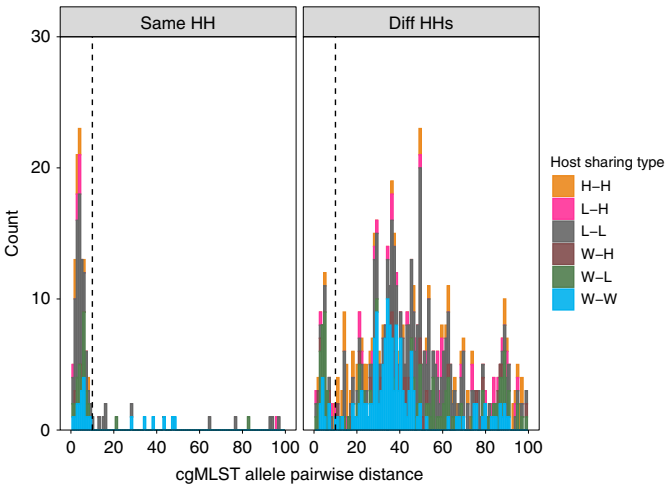

Fig. 3 | Frequency distribution of pairwise distances among isolates from the same household and from different households. cgMLST allele pairwise distances among isolates from the same household (HH; left) and from different (Diff) HHs (right). The sources of isolates in each pair are indicated by the colour. Only pairs that are closer than 100 cgMLST loci apart are shown. The vertical dashed black line indicates the sharing threshold (10 cgMLST alleles). H, human; L, livestock; W, wildlife.

***E. coli* strain sharing between humans and livestock.** We identified ten sharing pairs involving human and livestock isolates belonging to STs that were not host restricted and have been associated with a variety of sources and host species (Table 1).

All sharing pairs involved human males ($P=0.003$, Fisher's exact test). Six of the ten sharing pairs involved humans and livestock in the same household, whereas four humans (not keeping livestock) shared bacteria with livestock from other households. The ten sharing events between humans and livestock did not always occur in a livestock-keeping household. Six of seven persons (we lacked data for three people) had direct contact with livestock through collecting eggs, slaughter, milking or handling, but one person had no history of livestock contact (Table 1).

Sharing is shaped by host and households. Household and host category strongly influenced the distribution of sharing of *E. coli* isolates in both the core genome and the pangenome in Nairobi (Fig. 4a–d). Within households, sharing of *E. coli* isolates was consistently higher than expected within the same host category (Fig. 4a,c). No strong pattern was observed among households where the observed shared *E. coli* isolates fell largely within the expected range (Fig. 4b,d). Resistome similarity was predominantly low among different hosts but high among poultry isolates, irrespective of household structure (Fig. 4e,f). Sharing among poultry (livestock birds (LB)) in the same household was particularly high across all three definitions of sharing and similarity—that is, the core, pangenome and resistome (LB–LB in Fig. 4).

To further investigate resistome similarity between hosts, we performed the same analysis with sharing classed as two isolates sharing resistance genes that confer drug resistance to a given class of antibiotics. We compared eight classes of antibiotic whose resistance genes were found in the population (Extended Data Fig. 8) and found that, between households, poultry–poultry sharing continued to be much greater than the expected range (Extended Data Fig. 8). Resistome similarity among poultry does not, therefore, appear to be driven by resistance to a single or few antibiotic classes. H–H sharing between households was also higher than expected, suggesting similar antibiotic selection pressures on human isolates across the board.

Discussion

Our population genomic analysis, explicitly embedded within an epidemiologically structured sampling framework, provides a comprehensive overview of the genomic landscape of *E. coli* in humans, livestock and peri-domestic wildlife in a rapidly developing city. Our findings have implications for understanding the baseline level of bacterial diversity in settings where there is a potential for interaction between humans and animals. Our results reveal strain sharing occurring within households and a lower but detectable level of connectivity among human and animal populations across the urban environment beyond the household.

Isolates from Africa make up less than 3% ($n=3,626$) of the publicly available *E. coli* genome sequences in the public genome database, Enterobase. Our study provides a substantial contribution to the record of *E. coli* diversity in this part of the world with the identification of 133 unique and novel STs, in addition to a detailed footprint at a city-wide scale. Previous work on the population structure of *E. coli* isolated from human, livestock and wildlife in other both rural and urban settings showed varying degrees of overlap in the genotypes among these populations, driven by frequent contact and close proximity[13,14,34]. The wide range of genotyping methods used in these studies, each with varying levels of resolution, makes it difficult to make direct comparisons between studies. Earlier genotyping methods have lower resolution and are less robust[35]. Other studies measure similarity in microbiome community composition but are less reliable at resolving strain differences between samples[36]. Our approach combines high-resolution WGS with a structured sampling design, which captures more accurately the extent of strain sharing in this location.

In our study, we found that household stratification drives clonal strain sharing. Previous studies have shown an important role of the household as a driver for sharing similar microbiomes or bacteria in humans and companion animals[37–41]. Our findings show that strain sharing can involve humans, livestock and wildlife found in the same household or area.

The use of isolates collected within a time frame of 14 months in this study increased our ability of finding clonal isolates that

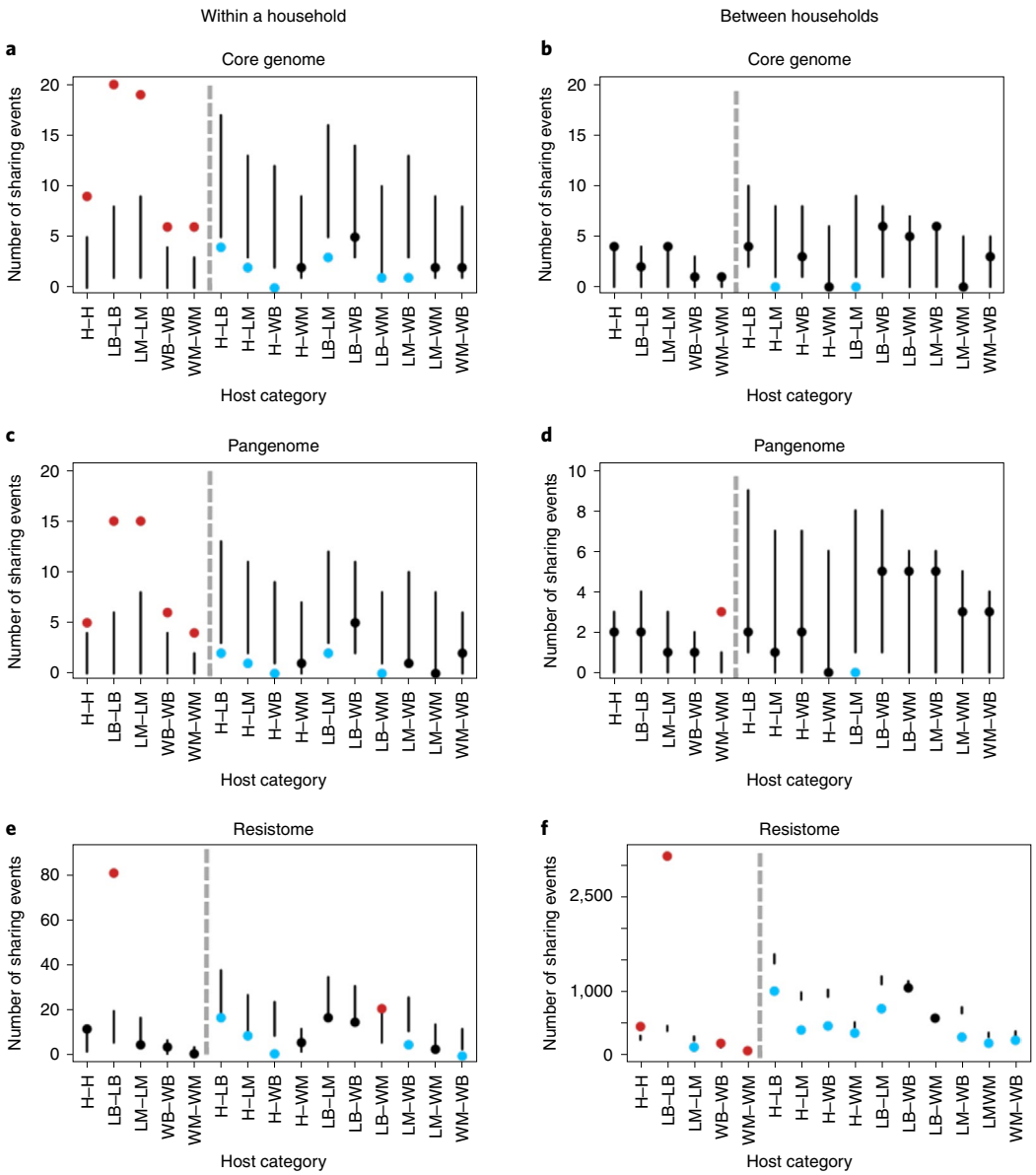

Fig. 4 | Number of sharing pairs for core genomes, pangenomes and resistomes within and between households. a,**c**,**e**, Number of within-household sharing pairs across 15 host category types for core genomes (**a**) ($n = 121$), pangenomes (**c**) ($n = 94$) and resistomes (**e**) ($n = 9{,}502$). **b**,**d**,**f**, Number of between-household sharing pairs across 15 host category types for core genomes (**b**) ($n = 121$), pangenomes (**d**) ($n = 94$) and resistomes (**f**) ($n = 9{,}502$). Panels show the 95% confidence intervals (vertical lines) of the calculated expected distribution using a resampling approach. Points depict the observed number of sharing pairs in each category coloured according to whether they fall above (red), below (blue) or within (black) the expected distribution. Hosts in the same category (for example, H–H) and different categories (for example, H–LB) are separated by grey dashed lines. Source type of isolate pairs is indicated on the *x* axis with human (H), livestock birds (LB), livestock mammals (LM), wildlife birds (WB) and wildlife mammals (WM). In each plot, within-category connections are on the left of the grey dotted line and between-category connections are on the right.

overlap among hosts, households and sublocations. Previous work using whole genomes found either no overlap or isolates that were separated by more than ten cgSNPs, which does not provide strong evidence for a recent sharing event[42,43]. Although challenging in practice, we have demonstrated the importance of large-scale structured sampling to understand strain sharing at the population level.

Genotype similarity of the core and accessory genome within households is posited to be driven by direct and social contact among individual hosts[44,45]. Consistent with expectation, host type was also shown to be a strong driver in *E. coli* isolate sharing within households (Fig. 4). Members of the same host category, particularly in the same household, are more likely to have direct and/or indirect contact within shared environments, creating increased opportunity for bacterial sharing[14,36,37,44–46].

Eight of the ten H–L strain-sharing events that we identified involved various poultry species. Inhalation and ingestion of faecal dust from poultry has previously been identified as a significant risk in the spread of bacteria from one host to another, both within the poultry populations and with humans working in close contact with them[47]. Furthermore, closely related ST131 strains have been previously found in both human and poultry *E. coli* populations, and genetic factors responsible for causing infections in chickens are also found in human pathogenic isolates[48–51]. Humans in direct contact with livestock were more prone to sharing *E. coli* isolates,

Table 1 | Details of humans involved in bacterial sharing with livestock (≤10 cgMLST loci)

Sharing pair	Livestock host	cgMLST distance	ST	Household	Livestock-keeping status	Human–livestock-handling status	Gender
1	Chicken	1	10	Different	Yes	Yes	Male
2	Goose	1	538	Same	Yes	Yes	Male
3	Chicken	3	23	Different	No	–	Male
4	Cattle	3	6,178	Same	Yes	Yes	Male
5	Duck	3	58	Same	Yes	Yes	Male
6	Rabbit	4	9,454	Same	Yes	Yes	Male
7	Turkey	4	9,454	Same	Yes	Yes	Male
8	Chicken	4	206	Same	Yes	–	Male
9	Turkey	8	1,237	Different	Yes	–	Male
10	Chicken	10	48	Different	No	None	Male

–, Information not collected

probably through direct contact with livestock products and/or faecal matter. Although the sample size of such sharing events within our large overall sample is small, this result is consistent with previous work postulating direct contact as a risk for bacterial sharing[39,52]. The results also serve to highlight that detecting connections or common sources among pathogens in spatially distributed hosts in large, complex environments requires carefully structured sampling designs that account for the considerable heterogeneity in natural systems[53]. We note that the strong host-type signal for *E. coli* sharing within a household (Fig. 4a) does not hold true when examining pairs between households (Fig. 4b). This could be due to a higher diversity of *E. coli* in the wider population, leading to a lower probability of detecting closely related strains.

Our resistome similarity analysis also suggests disproportionately higher rates of resistome similarity among poultry, irrespective of the household, compared with the other host groups. As poultry isolates are phylogenetically diverse, the presence of a common selection pressure could explain this observation. Across Nairobi, poultry are routinely exposed to a set regimen of antimicrobial agents (for therapeutic or prophylactic purposes), and such recipes vary minimally geographically from one location to another[54]. Conversely, a wider range of combinations of antimicrobials is available for use in ruminants and monogastrics, including an array of injectable formulations, and these greatly vary from one farm to another. We also find resistome similarity to be higher than expected among human and wildlife isolates, both mammals and birds. The similar availability and usage patterns of antibiotics in the human population across the city could explain the similarity seen in humans, suggesting that resistome similarity occurs from prevailing selective pressures rather than spread from a common source. The presence of manure, rubbish and human waste—all contaminated with potentially similar kinds of AMR pathogens and antimicrobials—across the urban landscape of Nairobi provides a conduit for acquisition and/or selection of similar resistomes in wildlife, which act as a sink population for AMR[12].

We observed a higher-than-expected level of accessory genome sharing among wild mammals (bats and rodents) and among households, apparently involving divergent lineages, as we did not see the same pattern at the core-genome level. Other types of wildlife (for example, wild birds) around the world have been shown to carry and transmit *E. coli* and should be considered a public health risk[55–57]. Our findings suggest that the role of rodents and bats should also be considered.

Our study design focuses on the breadth of sampling over depth, and, as a single isolate is sampled from each host, our approach does not account for intra-host diversity. Previous studies on the intra-host diversity of *E. coli* strains found them to be variable across host populations, and taking single isolates has the potential to underestimate the number of potential strain-sharing events[58]. However, our study using single isolates already reveals sharing events between human and animal hosts, and the scale of sharing can only be higher with incremental samples per host. Future studies should, therefore, consider both inter-host and intra-host diversity to expand on our findings.

Conclusions

Employing an epidemiologically structured sampling framework and using highly discriminatory WGS, our study provides detailed insight into the strain diversity of *E. coli* across a fast-growing African city where livestock-keeping within households is commonplace. To our knowledge, this is one of the largest and most comprehensive surveys of the bacterial genomic landscape in an urban environment so far, and it serves as a model for epidemiologically structured, targeted sampling and WGS of human and animal-borne bacteria. We found evidence of recent clonal sharing between humans and livestock, and we show that the *E. coli* population structure in humans, livestock and wildlife in this environment is shaped by both household and host type. These findings indicate that household bacterial distribution is predominantly, although not exclusively, driven by dispersal limitation, whereas, within the household, the host niche is the strongest driver for bacterial sharing (and their genetic pools) distribution. We also found similarities in the resistome of the isolates that did not match the patterns of shared genomes and presumably reflects common antibiotic usage practices, particularly in poultry. This provides the strongest evidence in our study for direct selection acting on bacteria within a host (shared antibiotic environment). These findings provide empirical support for the hypothesis that 'Everything is everywhere' (frequent sharing of bacteria and AMR genes between households) but 'environment selects' (different households and hosts have different bacterial and resistome persistence). From a disease-control-policy perspective, our study highlights the need to undertake surveillance for emerging pathogens at the appropriate spatial scale (here, households) and to account for patterns of interconnectivity where epidemiological links might be created by livestock, wildlife or humans themselves. Further work, guided by the finding of where clonal sharing is most likely to be found, will be required to quantify spillover risk associated with the main routes of inter-host transmission.

Methods

Study site. A cross-sectional study targeting synanthropic wildlife and sympatric human and livestock populations in Nairobi, Kenya, was carried out from August

2015 to October 2016 as part of the UrbanZoo project. Faecal samples ($n=2,081$) from 75 wildlife species (birds and mammals, $n=794$), 13 livestock species ($n=677$) and humans ($n=333$) were collected from households across Nairobi that were participating in the UrbanZoo 99 households project. Our study design is described in detail in the Supplementary Methods. In brief, Nairobi was split into administrative units, and 33 were chosen based on a socioeconomic stratification, which was weighted by population, such that the larger proportion of low-income households was oversampled while ensuring representation of all other socioeconomic groups. Three households were randomly selected in each sublocation to obtain two livestock-keeping and one non-livestock-keeping household (a total of 99 households), with the aim of maximizing the spatial distribution and diversity of livestock-keeping practices captured within the sampling frame (Fig. 1 and Extended Data Fig. 1). Households in each sublocation had to meet strict inclusion criteria of keeping small mammals (rabbits) or poultry, large mammals (cattle, goats and sheep) or pigs or no livestock within the household perimeter. Wildlife samples were obtained by a range of taxon-specific trapping methods, which are described in the Supplementary Methods.

Sample collection and microbiological testing. Questionnaires detailing household composition and socioeconomic data, as well as livestock ownership and management, were administered at each household using Open Data Kit Collect version 1.4.10 software[59]. Human, animal and wildlife faecal samples were collected and transported on ice to one of two laboratories (University of Nairobi or Kenya Medical Research Institute) within 5 h of collection. Samples were enriched in buffered peptone water for 24 h and thereafter plated onto eosin methylene blue agar (EMBA) and incubated for 24 h at 37 °C. Subsequently, five colonies were selected and subcultured on EMBA before being further subcultured on Müller–Hinton agar. A single colony was picked at random from the plate for each original sample (hereafter referred to as an 'isolate'), and a 10-parameter biochemical test was used (triple sugar iron agar = 4, Simmon's citrate agar = 1, and motility-indole-lysine media = 3, urease production from urea media = 1, oxidase from tetra-methyl-*p*-phenylenediamine dihydrochloride = 1) for identification of *E coli*.

WGS. DNA was extracted from bacterial isolates using commercial kits (Purelink Genomic DNA Mini Kit, Invitrogen, Life Technologies) at the International Livestock Research Institute in Nairobi, Kenya, and transported under licence to the Wellcome Trust Centre for Human Genetics. WGS was carried out at the Wellcome Trust Centre for Human Genetics on the Illumina HiSeq 2500 platform.

Sequence analysis. Sequenced reads were filtered for quality and trimmed for adaptors with BBDuk (version 38.46), using k = 19, mink = 11, hdist = 1, ktrim = r, minoverlap = 12, qtrim = rl and trimq = 15. The following sequencing quality thresholds were used based on Quast: (1) at least 3 Mb aligned to EC958; (2) a maximum assembly length of 6.5 Mb; (3) GC content of between 50% and 51%; and (4) assembly N50 of >30 kb or a maximum of 100 cgMLST missing loci. In total, 1,642 genomes were sequenced that passed this quality threshold.

Genomes were assembled using Spades version 3.13.0 with the '–careful' option. Clermont phylotype of the isolates was determined using the ClermonTyping tool version 1.4.1[60], and the multi-locus sequence type was determined and assigned by Enterobase[61].

The pangenome was estimated using Roary version 3.12.0 with the following options: -s -i 95 -g 100000. Acquired antibiotic resistance genes were identified from the assemblies using starAMR (version 0.4.0) (https://github.com/phac-nml/staramr), with a cutoff of 95% sequence identity and a minimum of 60% alignment to the query sequence, against the ResFinder database downloaded on 25 September 2019[62]. Antibiotic class of each resistant gene was assigned using the ResFinder classification.

Phylogenetic analyses. A core genome alignment was generated using Snippy version 4.6.0 (with default settings) using EC958 as a reference genome (GCA_000285655.3). A phylogenetic analysis of the core genome alignment was performed using IQTREE (version 1.6.12) -m TVM + G4 -bb 1000 -safe. The tree and metadata were visualized in iToL version 4.3 (itol.embl.de). Owing to the large number of isolates and the high level of diversity, we did not mask recombinant regions of the genome.

Ad hoc cgMLST was performed on genome assemblies using chewBBACA (v. 2.0.11) with the 2,513 gene cgMLST profile from Enterobase (downloaded October 2018).

Identification of putative bacterial sharing. A genetic distance matrix was calculated from all pairwise-allelic-profile comparisons using the library 'ape' in R (ref. [63]). The cgMLST cutoff of 11 alleles to define putative *E. coli* (defined here as a sharing pair) transmission clusters was based on the observed bimodal distributions of inter-household and intra-household allele differences (Extended Data Fig. 3). The R package 'cutpointR' was used to validate this cutoff as the optimal value to differentiate pairs that occur within and between households[64]. Pairwise cgSNPs were also calculated using the full consensus genome alignment generated by Snippy version 4.6.0 (snippy-core), followed by custom filtering positions that were fully called and unambiguous with an A, G, C or T that were conserved in at least 99.8% (1,335 of 1,338) of isolates (length = 399,673 nucleotides). Pairwise distances were calculated using Disty McMatrixface version 0.1.0 (https://github.com/c2-d2/disty) with -n 0.002.

Epidemiological analysis of sharing. We established epidemiological links between every possible pair of *E. coli* isolates through a systematic comparison. Household-level sharing was categorized as within-household if a sharing pair involved isolates/hosts from the same household and between-household if a sharing pair involved isolates from a different household. Wildlife isolates that could not be attributed to a specific household were omitted from the sharing analysis (Supplementary Table 2).

We condensed our host types into five broad categories (Supplementary Tables 1 and 2): (1) humans; (2) livestock birds, poultry dominated by chickens; (3) livestock mammals, consisting of ruminants and monogastric livestock, (4) wild birds, predominantly seed-eating birds such as house sparrows; and (5) wild mammals, predominantly rodents, along with bats. Primates were omitted from the sharing analysis as they were associated with only two households, along with some samples derived from populations of bats and wild birds, which could be attributed to sublocation but not household.

Although the sharing threshold for the core genome was ≤10 cgMLST distance, sharing for the pangenome and resistome similarity was based on a Jaccard similarity index (JI) (between 0 and 1, where 1 is identical), where a cutoff threshold was defined, similar to the core genome. For the pangenome/accessory genome, this was determined to be JI ≤ 0.98 (Fig. 3c,d). Resistome sharing was defined as JI = 1 (Fig. 3e,f), with each isolate having a minimum of two AMR genes. In practice, this means that two isolates must share an identical set of AMR genes of length ≥2.

To calculate the number of observed sharing events, we identified clusters of isolates that were within the sharing threshold. So as to count an isolate as 'shared' only once for clusters >2, we applied a Hamiltonian path method[65] such that the number of pairs/connections is counted as $m-1$, where m is the number of isolates that form a cluster (Supplementary Fig. 9).

Having defined the number of observed sharing events among each of our host categories within and between households, we then wanted to know whether these observed events fell above or below what might be expected given the differential sampling effort across host categories. To do this, we first calculated the total number of possible pairs, assuming equal chance of sharing. Within households, this was calculated using the formula $n(n-1)/2$, where n is the number of samples of a given host type within a household. Between-household sharing was calculated as $(n_1) \times (n_2)$, where n_1 is the number of samples of a given host in household 1, and n_2 is the number of samples of a given host in household 2. These values were then calculated as a proportion of the total number of all possible pairwise combinations. We next performed a simulation to see how the observed sharing events were distributed, given the proportion of each pairwise host combination calculated in the previous step.

To do this, we resampled (using the rmultinom function) the total number of observed values for each type of sharing (resampling with replacement 1,000 times) from the calculated proportions. These resampled values were then used to generate the expected range of sharing events (± 95% confidence intervals) for each pairwise combination of host category. From this, we were able to assess whether our observed sharing events fell above, below or within the range that we might expect given the sampling effort. This pattern of sharing events among hosts and households enabled us to highlight cases where we observed sharing among hosts that lay outside from the predicted range. The same approach was applied to all aspects of genome sharing (Fig. 3a–f).

Ethical approval. The collection of data adhered to the legal requirements of the Government of Kenya. The International Livestock Research Institute (ILRI) Institutional Research Ethics Committee is registered and accredited by the National Commission for Science, Technology and Innovation in Kenya and is approved by the Federalwide Assurance for the Protection of Human Subjects in the United States. Ethical approval for human sampling and data collection was obtained from the ILRI Institutional Research Ethics Committee (ILRI-IREC2015/09). Livestock samples were obtained under the approval of the ILRI Institutional Animal Care and Use Committee (reference ILRI-IACUC2015/18), and permits were obtained from the Directorate of Veterinary Services. Wildlife were trapped under approval of an ILRI Institutional Animal Care and Use Protocol (IACUC2015/12), and permits were obtained from the National Museums of Kenya and Kenya Wildlife Service. Written informed consent was obtained from all adult participants and from the parents of underage participants.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Whole-genome sequences used in this study are available under the BioProjects with accession numbers PRJEB32607 and PRJEB41827. The reference genome used for mapping is *E. coli* strain EC958 (GCA_000285655.3). The ResFinder AMR gene database used was downloaded on 25 September 2019 from https://bitbucket.org/genomicepidemiology/resfinder_db. Source data are provided with this paper.

Code availability

The scripts used to perform this analysis can be found at https://git.ecdf.ed.ac.uk/epigroup/urbanzoo.

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Author contributions

E.M.F., T.P.R., M.E.J.W., S.K., E.K., J.M.B. and M.J.W. designed the sample collection design. M.J.W., M.E.J.W. and E.M.F. designed the sequencing design. M.F., J.M.B., J.A., J.M.H., J.M.B., P.M., D.M.M, Y.N., E.F., T.I., L.A., F.A., M.K.M., T.K., E.O. and A.O. collected surveys and samples in the field. V.K., A.K., C.N., N.G., T.O., J.M.H., S.M.N., A.O., J.K. and D.M.M. performed microbiological cultures and DNA extraction. B.A.W., D.M.M. and M.J.W. performed bioinformatic and phylogenetic analysis. L.P., H.P., A.I. and D.J.W. contributed to bioinformatic analysis. D.M.H.M., D.M.M., B.A.W., E.M.F. and M.E.J.W. performed epidemiological and statistical analyses, interpreted results and wrote the paper. J.R., D.J.W., C.K., C.T. and J.D.D. contributed intellectually to the study design and interpretation. All authors provided comments on the manuscript and gave final approval for publication. D.M.M. and B.A.W. contributed equally. M.E.J.W. and E.M.F. contributed equally.

Competing interests

The authors declare no competing interests.

Additional information

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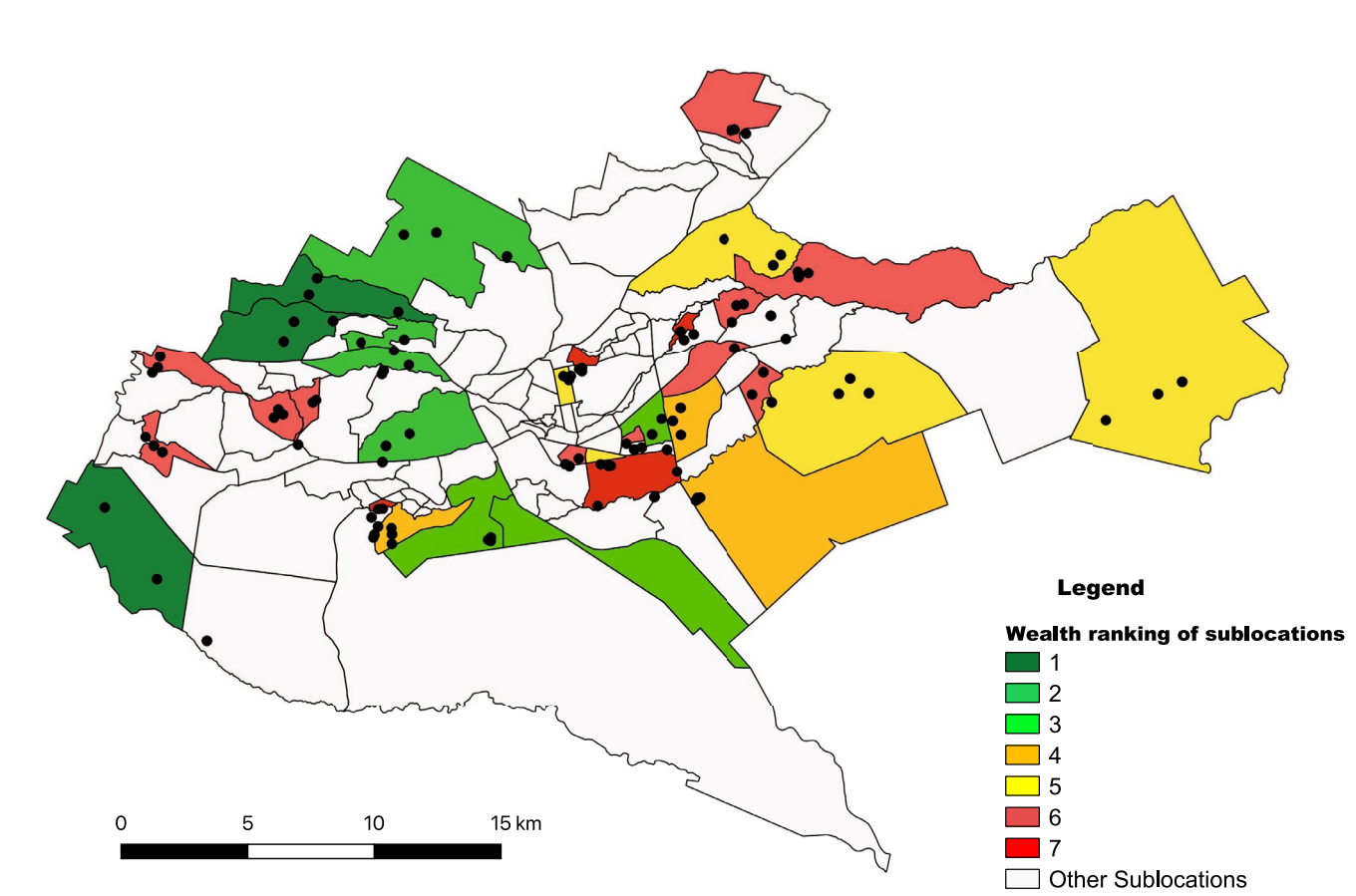
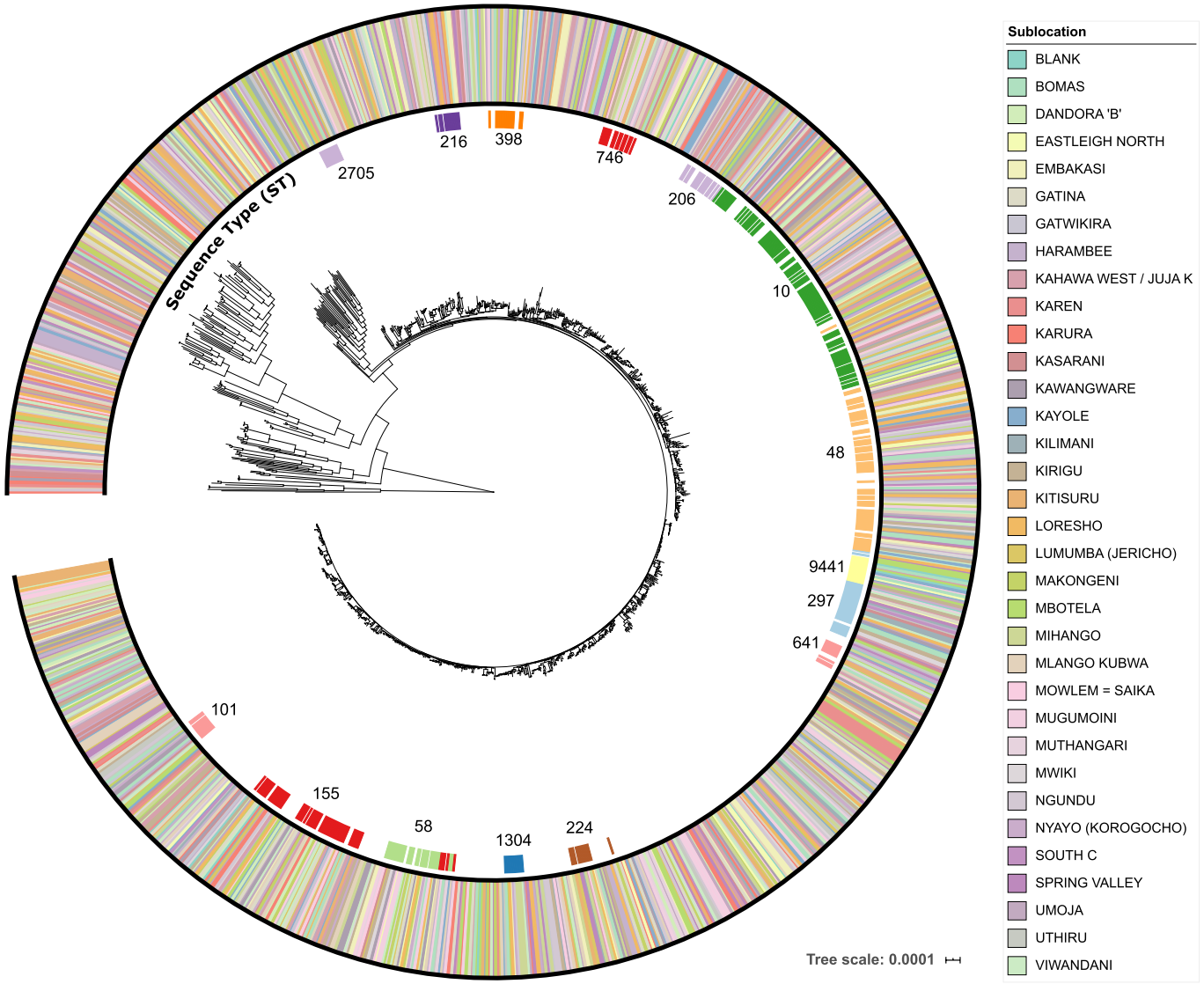
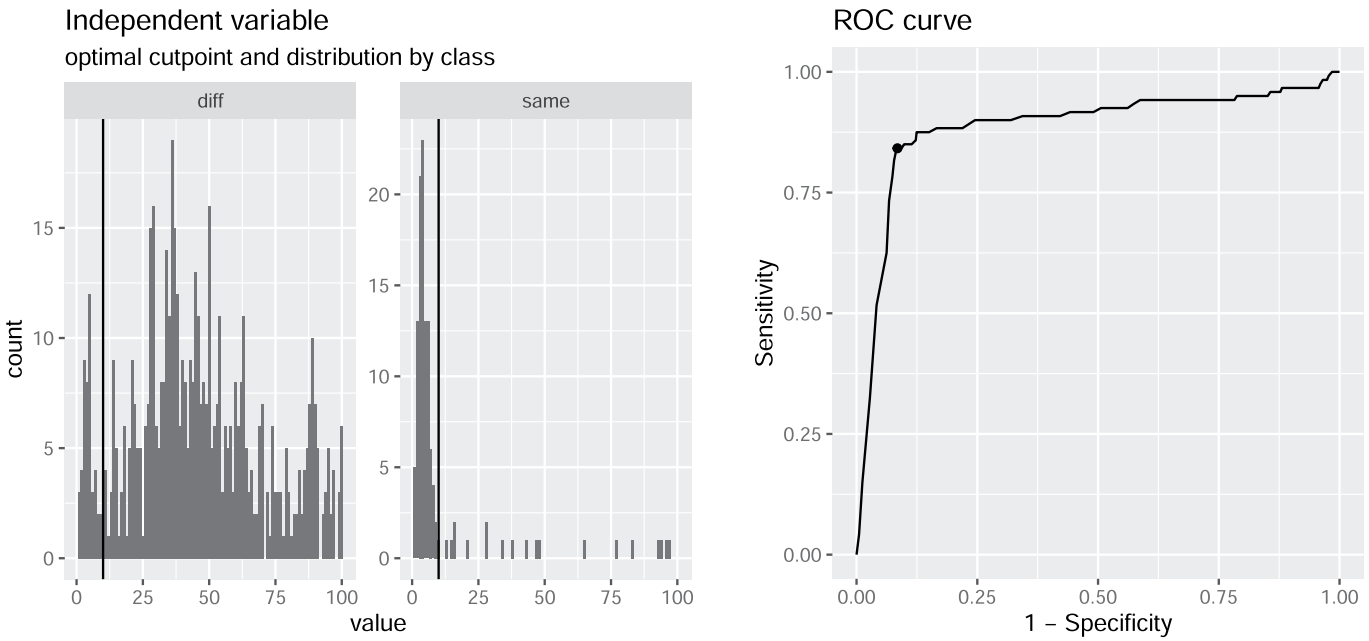


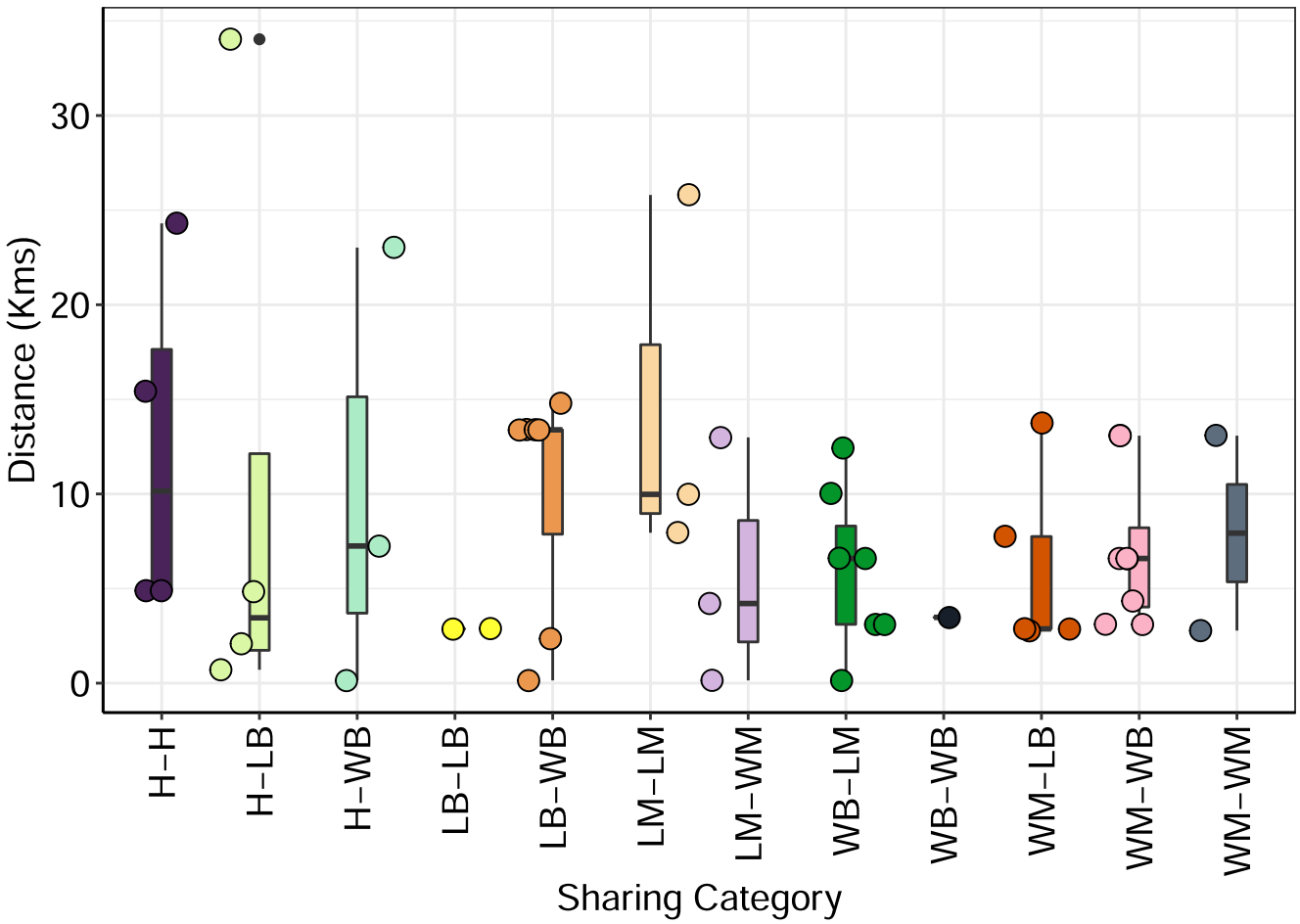
Extended Data Fig. 1 | Geographical distribution of selected sublocations within the city of Nairobi chosen based on a socio-economic stratification, together with locations of each of 99 households selected within each stratum. Different colours given to the sublocations represent different wealth categories (Dark green – wealthy, dark red – poor).



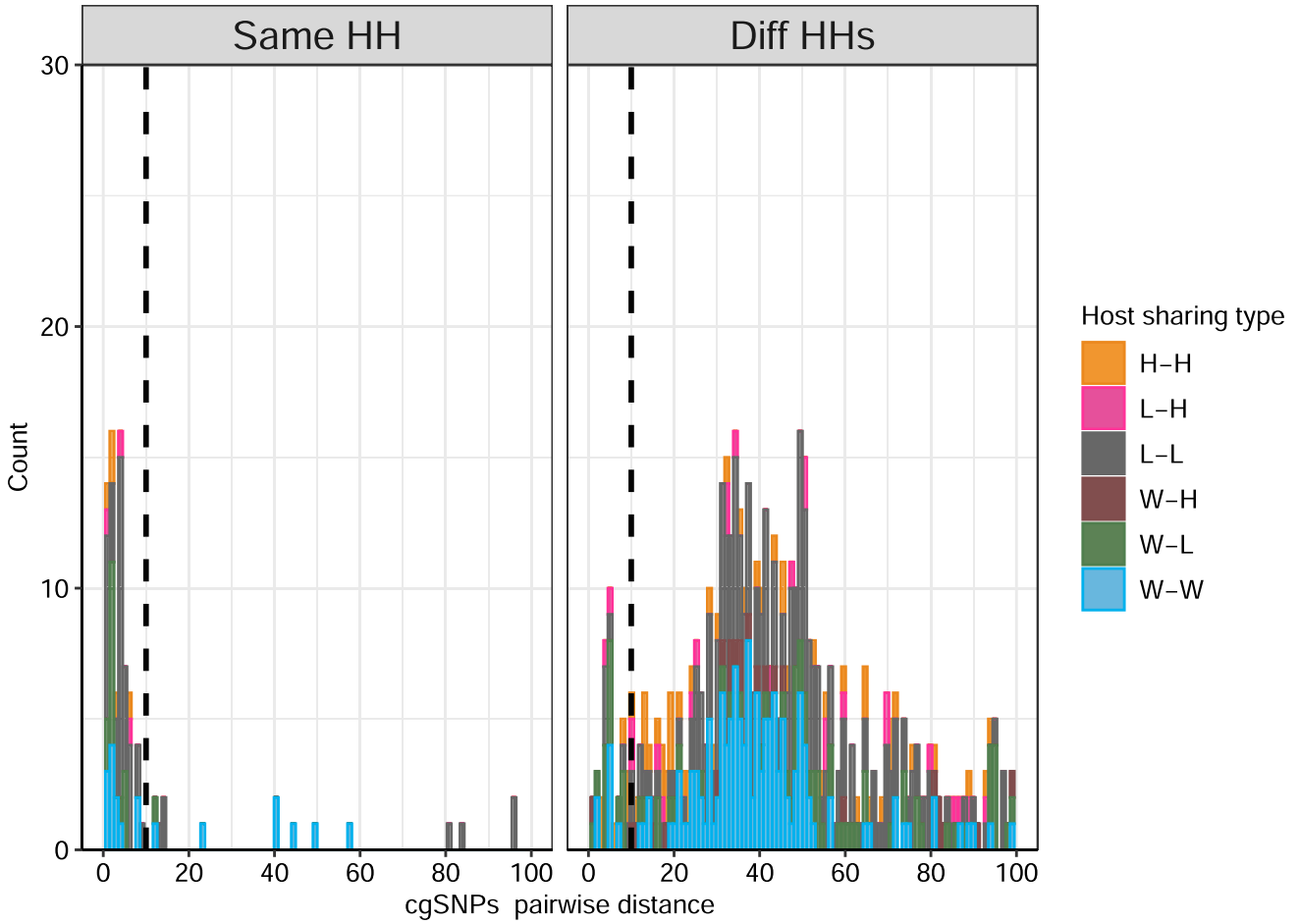
Extended Data Fig. 2 | Maximum-Likelihood tree of a core genome alignment showing the distribution of isolates across 33 sublocations in Nairobi. The outermost ring is colored according to the sublocation of sample origin and innermost ring represents the commonly predicted sequence types (ST) in our isolate collection.



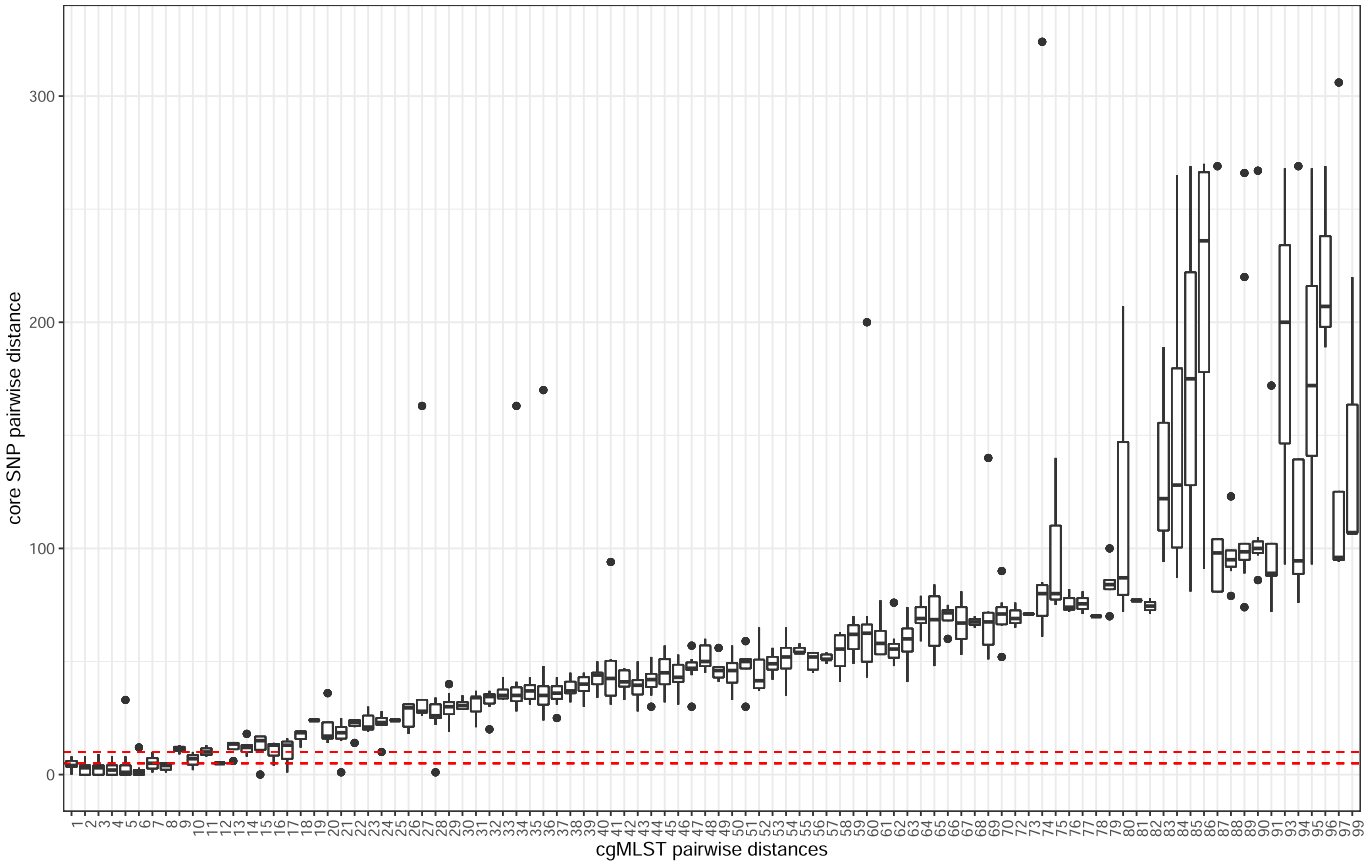
Extended Data Fig. 3 | Output of cutpointR, used to identify the optimal threshold to maximise the differentiation between sharing pairs occurring within households (same) and between households (diff) at 10 cgMLST loci. The black vertical lines on the 2 panels on the left indicate the *optimal_cutpoint* value of 10 as calculated by cutpointR.



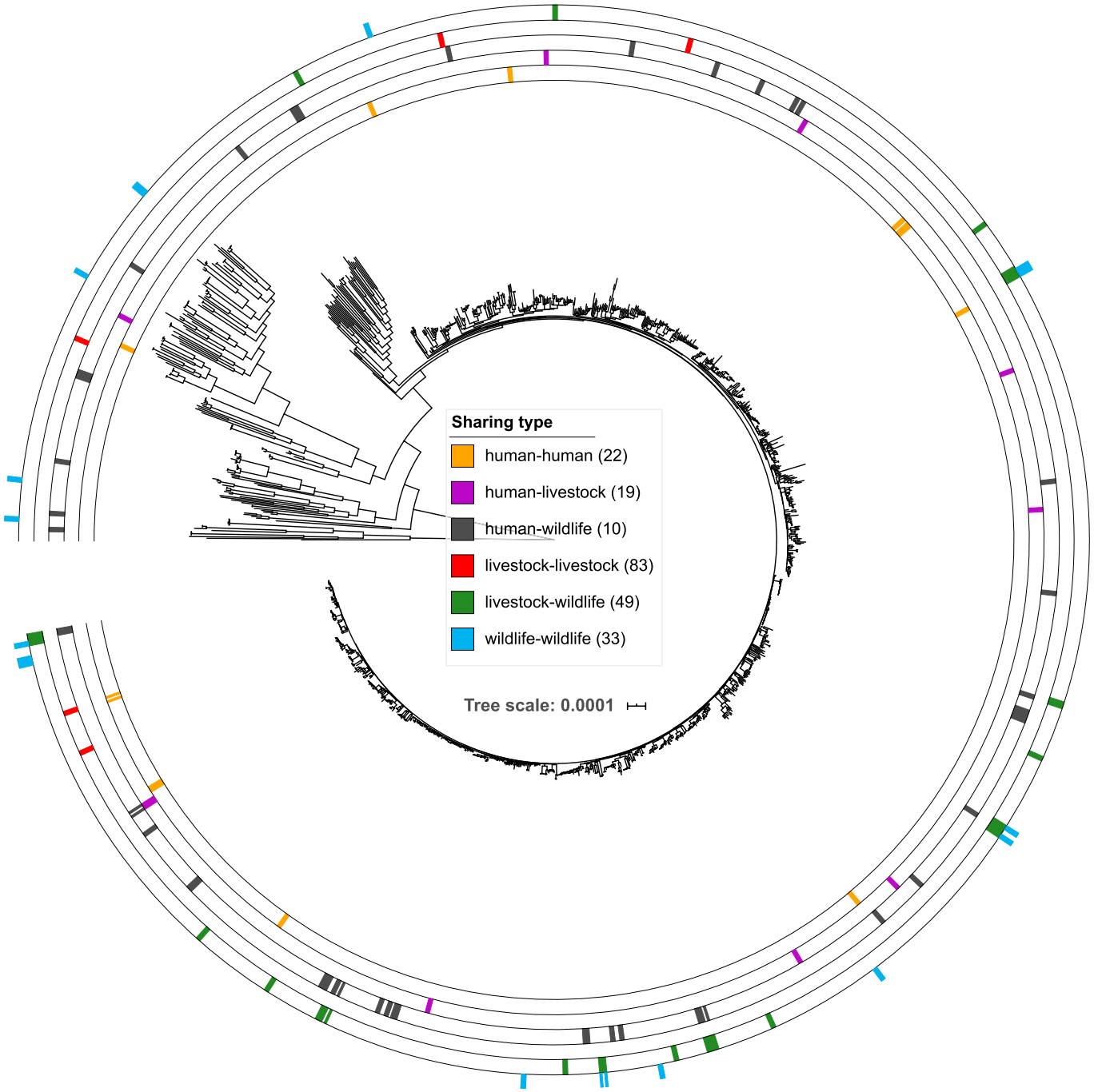
Extended Data Fig. 4 | Plot of geographical distance of bacterial sharing in pairs involving different households (n = 49). Abbreviations: H- Human, LB – Livestock Bird, WB – Wild Birds, LM – Livestock mammals, LB – Livestock Birds. Boxplot centre lines show median value; upper and lower bounds show the 25th and 75th quantile, respectively; upper and lower whiskers show the largest and smallest values within 1.5 times the interquartile range above the 75th percentile and below the 25th percentile, respectively; and points show data points (jittered to improve visualisation).



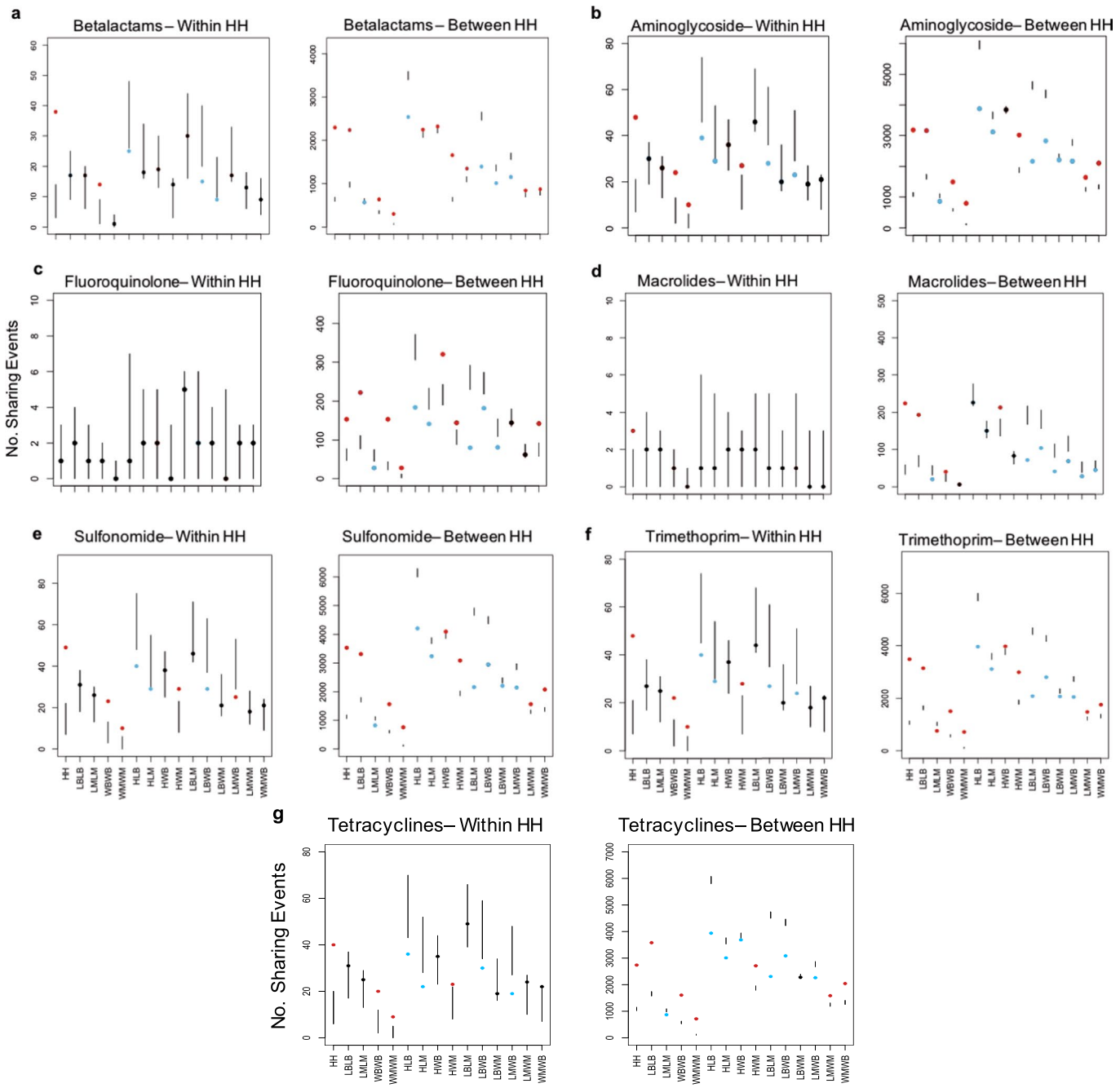
Extended Data Fig. 5 | The distribution of closely related isolates (separated by 100 cgSNPS, n = 660) based on core genome SNPs (cgSNPs). An alignment of 399,673 aligned nucleotide positions were used. Each of these core SNP positions were conserved in at least 99.8% of isolates (1335/1338). The vertical dashed black line indicates the sharing threshold (10 cgSNPs).



Extended Data Fig. 6 | The correlation between pairwise genetic distances measured by core genome single nucleotide polymorphisms (cgSNPs) and core genome multi locus sequence typing (cgMLST) of pairs of isolates with ≤ 100 cgMLST and ≤ 1000 cgSNPs. 73% of pairs with ≤ 10 cgMLST loci apart (n = 109) were separated by ≤ 4 cgSNPs while 97% (n = 145) of these ≤10 cgMLST pairs were separated by ≤10 core SNPs. Only one pair had more than 13 cgSNPs. Two horizontal red dotted lines indicate 4 and 10 cgSNPs. Boxplot centre lines show median value; upper and lower bounds show the 25th and 75th quantile, respectively; upper and lower whiskers show the largest and smallest values within 1.5 times the interquartile range above the 75th percentile and below the 25th percentile, respectively; and points show show samples outside the whisker range.



Extended Data Fig. 7 | Core genome phylogenetic tree showing the distribution of isolates involved in the 6 different categories of sharing events. Ring colours indicate the sharing category (human-human, human-livestock, human-wildlife, livestock-livestock, livestock-wildlife, wildlife-wildlife) from the innermost to the outermost rings, respectively.



Extended Data Fig. 8 | Number of sharing events across 15 host category types, within and between households for each of 7 identified antibiotic classes. (a) Beta-lactam (n = 21686), (b) Aminoglycosides (n = 36934), (c) Fluoroquinolone (n = 2088), (d) Macrolide (n = 1533), (e) Sulfonamide (n = 38125), (f) Trimethoprim (n = 36315), (g) Tetracyclines (n = 36816). Panels show the 95% confidence intervals (vertical lines) of the calculated expected distribution using a resampling approach, points depict the observed number of sharing events in each category coloured according to whether they fall above (red), below (blue) or within (black) the expected distribution. Source type of isolate pairs are indicated on the x-axis with either Human (H), Livestock birds (LB), Livestock mammals (LM), Wildlife birds (WB), Wildlife mammals (WM). In each plot, within-category connections are on the left of the grey dotted line and between-category connections are on the right.

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Field data were collected using the open source software Open Data Kit (ODK) Collect (version 1.4.1).
Data analysis	Genome analysis was performed with the following software, which is also detailed in the Methods and Supplementary Methods. BBDuk (v38.46). Sequenced reads were filtered for quality and trimmed for adaptors with BBDuk (v38.46) k=19 mink=11 hdist=1 ktrim=r minoverlap=12 qtrim=rl trimq=15. Genomes were assembled using Spades v3.13.0 with the `--careful` option. Clermont phylotype of the isolates was determined using the ClermonTyping tool v1.4.1 (downloaded 20 Nov 2019). The pangenome was estimated using Roary v3.12.0 with the following options: -s -i 95 -g 100000. Acquired antibiotic resistance genes were identified from the assemblies using starAMR (v0.4.0) (https://github.com/phac-nml/staramr), with a cutoff of 95% sequence identity and a minimum of 60% alignment to the query sequence, against the ResFinder database downloaded 25 September 2019. A core genome alignment was generated using Snippy v4.6.0 (with default settings) using EC958 as a reference genome (GCA_000285655.3). A phylogenetic analysis of the core genome alignment was performed using IQTREE (v1.6.12) -m TVM+G4 -bb 1000 -safe. The tree and metadata were visualised in iToLv4.3 (itol.embl.de). Pairwise distances were calculated using Disty McMatrixface v0.1.0 (https://github.com/c2-d2/disty) with -n 0.002. Ad hoc core genome multi Locus sequence typing (cgMLST) was performed on genome assemblies using chewBBACA (v. 2.0.11) with the 2513 gene cgMLST profile from Enterobase (Downloaded October 2018). A genetic distance matrix was calculated from all pairwise allelic profile comparisons using the library "ape" in R (Paradis et al., 2004). The R package "cutpointR" was used to validate this cutoff as the optimal value to differentiate pairs that occur within and between households. Custom R scripts to perform sharing distribution analysis is provided at https://git.ecdf.ed.ac.uk/epigroup/urbanzoo

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Whole genome sequences used in this study are available under the BioProjects with accession number PRJEB32607 and PRJEB41827. The reference genome used for mapping is E. coli strain EC958 (GCA_000285655.3). The ResFinder AMR gene database used was downloaded on 25 September 2019 from https://bitbucket.org/genomicepidemiology/resfinder_db.

Field-specific reporting

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

This work presented in this paper formed part of the UrbanZoo project (http://www.zoonotic-diseases.org/project/urban-zoo-project/) a Medical Research Council-funded project that aimed to utilise a landscape genetics approach to understand the movement and sharing of pathogens in a major developing city. A significant component of the UrbanZoo project was the '99 household project' which focused on sampling of households across socio-economic strata of Nairobi to investigate the role of informal livestock keeping practices as a route of zoonotic disease emergence in humans. The project was designed as a cross-sectional study, utilizing multi-stage cluster sampling by stratifying the city into 33 sublocations that were proportionately chosen to represent a gradient of socioeconomic housing types, and thus urbanization across the city. These sublocations represent the first-level of clustering. Within each sublocation, three randomly selected households represented the second level of clustering (which number 99 in total), within which samples of humans, livestock and peri-domestic wildlife represent the cluster sampling. Households were selected with the aim of maximizing the spatial distribution and diversity of livestock keeping practices across Nairobi, and were chosen to capture three main criteria: socio-economic diversity, population distribution and livestock keeping practices. Geospatial mapping data, generated as part of a technical report produced by Institut Français de Recherche en Afrique (IFRA), was used to identify 17 classes of residential neighborhood in Nairobi based on physical landscape attributes, which were subsequently verified by 817 household questionnaires. Each of the 17 classes of neighborhood were then ranked by average income and condensed into seven wealth groups. Administrative sublocations were mapped onto each wealth group, identifying a total of 70 possible sublocations, for which dominant wealth groups were calculated by extracting the proportion of population belonging to each neighborhood class within the sublocation boundaries. A total of 33 sublocations were selected to be included in the study, with the number of sublocations belonging to each wealth group chosen proportionately to the population density and the variety of neighborhood classes in each of the seven wealth groups. Final selection of individual sublocations was aimed at maximizing areas with high livestock densities, whilst ensuring coverage of other neighborhood classes and geographical spread. For each sublocation, three geographical points were selected at random within the dominant housing type, comprising of: two livestock keeping and one non-livestock keeping household. A total of 99 households, 66 of which kept livestock were visited. Livestock keeping households had to meet strict inclusion criteria of: (i) keeping small livestock only (small ruminants - goats/sheep, small monogastrics - poultry/rabbits), and (ii) large livestock (large ruminants (cattle), large monogastrics (pigs), with or without small livestock. To ensure an equal sample of both cattle and pig-keeping households, the combination of livestock keeping households represented in each sublocation was randomised, and had to consist of either large ruminant and small monogastric, or large monogastric and small ruminant species. For sublocations in which households keeping large ruminant or large monogastric species were absent, a replacement household keeping either small monogastic or small ruminant species was recruited. The order in which sublocations were visited was randomized. Within the sublocations, local administrative leaders assisted in recruitment, which was carried out a few days before the sampling date. The three pre-selected geographical points were identified on the ground, and the nearest three households that met the inclusion criteria identified.

Research sample

A total of 1,338 samples were collected as part of this study including: 311 samples from humans, 421 samples from 63 wildlife species [comprising of wild birds (n=245), rodents and bats(n=130)]. 606 samples from 13 species of livestock that can be grouped into poultry (n=324), goat and sheep (n=109),cattle (n=61), 94 pig (n=49) and rabbit (n=38)isolates. The isolates were distributed across 33 geographic sublocations spanning the entire urban area of Nairobi. Humans were sampled irrespective of age and gender. Food producing animals (including cattle,goats, poultry, pigs and rabbits) were sampled as they represent a direct link to humans either through food, direct contact or shared habitats. Avian (wild birds and bats), rodents and non-human primates were the selected wildlife hosts in this urban study system, since they are diversely and widely distributed across urban landscapes, demonstrating epidemiological and ecological responses to land-use change, and interacting closely with livestock and humans

Sampling strategy

Owing to the design of this study – genetic analysis of E .coli population in a unstudied urban population for which it is challenging to predict significance in advance, we were unable to generate robust statistical power calculations or sample sizes posed in the study. As such, the number of samples (human, livestock and wildlife) varied according to the household sizes. Due to large variation in the size of household compounds, trapping effort of wildlife species (i.e. number of rodent traps placed per trapping session) was maintained such that it was proportional to the size of the household compound, and thus standardized across households.

Data collection

Sampling of human: In each household, the household head/owner (or a nominated member) completed a questionnaire, detailing livestock ownership (e.g. abundance of livestock species), management practices (e.g. manure disposal practices), household composition (e.g. number of occupants), and socio-economic variables. Thereafter, following an informed consent, every human member of the household was invited to contribute a faecal sample and answer questionnaires on: their age, gender and occupation, food consumption and medical history. Faecal samples were collected from people not present in the household during the visit, such as school-age children. The number of members per recruited household ranged from one to 19, including staff members and unrelated household residents. However, full participation by every member was only achieved in 20 of the 99 households. Composition of the household varied by wealth group, with households at the lower end of the wealth-scale having more children (median = 2, compared to median 1 child in wealth groups 1 and 4, and median 0 children in wealth groups 2 and 3).

Sampling of livestock: Rectal swabs were obtained from (up to 20) livestock species present in the household (ensuring that all species were represented). Up-to 12 different species of livestock(cattle, pigs, sheep, goats, rabbits, guinea pigs, chickens, ducks, geese, turkeys, guinea fowl and pigeons) were recruited and sampled over the course of the study(Table xx). The distribution of livestock between neighbourhood classes varied according to species.Chickens were the most common species encountered, kept by 83% of the 66 livestock-keeping households; these along with goats, rabbits and other poultry types were distributed relatively evenly across all neighbourhood classes. However, cattle and sheep were found almost exclusively in either the very wealthy areas, the very poor areas, or the areas on the eastern and western periphery of the city. The distribution of pigs was similar, except that they were not found in the higher wealth groups, although one pig-keeper in a dense new-build area (wealth group 5) was recruited.

Sampling of wildlife: Rodents, bats, birds and non-human primates were sampled. Rodents were trapped using medium-sized (23 cm x 7.5 cm x 9 cm) Sherman live traps (H. B. Sherman Traps Inc., Tallahassee, FL) or Victor lethal traps (Woodstream Corp., Lititz,PA) that were baited with dried fish, placed against walls throughout the household and livestock keeping facilities, and left in place for three nights. Traps were set in each household for all trapping nights and checked daily. Mist nets were set at dawn to trap birds, with nets being positioned outside the house and around livestock keeping facilities. For household compounds in which bat activity was deemed likely (as judged based on the presence of fruiting trees and/or 'flyways'), mist nets were set at dusk and monitored for two hours. Where household members reported frequent sightings of non-human primates, wire-mesh live-capture traps were pre-baited with bananas for a minimum of three days. Traps were then set, and monitored regularly for a maximum of three days. Due to large variation in the size of household compounds, trapping effort (i.e. number of traps/mist nets placed per trapping session) was maintained such that it was proportional to the size of the household compound.
Human and animal faecal samples were collected and transported on ice to one of two laboratories (University of Nairobi or Kenya Medical Research Institute) within five hours of collection. Questionnaires and data associated with samples was recorded using Open Data Kit (ODK) Collect software, on electronic tablets, and uploaded to databases held on servers at the International Livestock Research Institute (ILRI). Field teams involved in data collection consisted of two clinical officers, and one to three veterinarians/ animal health workers - all Kenyan nationals, fluent in both Kiswahili and English, and participants could opt to complete the questionnaires in either of these languages.

Timing and spatial scale

Field data was collected between September 2015 and September 2016 across the city of Nairobi. Triplets of households with each sublocation were sampled within the same week. All field data collection for each household was conducted on the same day.

Data exclusions

Isolates deemed not to be E. coli, on the basis of biochemical testing or whole genome sequencing, were removed from the dataset.

Reproducibility

Standard epidemiological, laboratory (microbiology and sequencing) and analytical approaches were used throughout the study and all data used in this study is available in open-source platforms. Sampling effort was maintained such that it was proportional to the household composition and size and sampling was standardised (being conducted by the same team of veterinarians and clinicians). Field samples were sent to one of two laboratories (University of Nairobi and Kenya Medical Research Institute) for microbial culture, and all efforts were undertaken to ensure that this did not introduce bias into the study. Protocols were standardized between laboratories. All analytical processed were conducted in R Statistical environment and code is provided.

Randomization

Participants were not allocated into experimental groups. However, geographical points used to select households within each sublocation were distributed at random. The combination of livestock keeping households represented in each sublocation was randomized, and had to consist of either large ruminant and small monogastric, or large monogastric and small ruminant species. The order in which sublocations were visited for data collection between September 2015 and September 2016 was randomized. One purified E. coli isolate per original sample grown was selected at random. During analysis isolates were grouped according to the host animal species (e.g. human/poultry), household source (e.g. same household).

Blinding

Since the "groups" in question were host species (humans, livestock and wildlife and household it was not feasible to blind samplers to either. No attempts were made at blinding during microbiological processing or DNA extraction. Library preparation and whole genome sequencing was performed in a separate country and personnel, who were blind to the groups. No blinding was attempted during data analysis.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions

Climatic and weather conditions were not investigated in this study however to account their possible impact on study outcomes, field work was conducted over the course of one year, and as such precipitation and temperature varied over the course of the study. Topographical and natural habitat conditions differ markedly across the city – e.g greener and lush in the South and West, and savannah biome in the East and North – and as such between our study households. Fieldwork was conducted between 7 am and 10 am with the exception of bat sampling that happened between 6:00 pm and 7:30 pm.

Location

Sampling was conducted in households across the city of Nairobi, Kenya. Nairobi lies just below the equator with a latitude of -1.286389, and longitude is 36.817223 and lies at 1,795 metres (5,889 ft) above sea level.

Access & import/export	The collection of data adhered to the legal requirements of the International Livestock Research Institute (ILRI), and Government of Kenya regulations. Permission to access study locations was obtained from the administrative authorities and the National Commission for Science, Technology and Innovation in Kenya. Additional permits to sample livestock and wildlife were obtained from the Directorate of Veterinary Services, the National Museums of Kenya and Kenya Wildlife Service respectively. E. coli DNA was exported from Kenya to The Wellcome Trust Centre for Human Genetics, Oxford, UK under a Kenyan Ministry of Agriculture license RES/POL/VOL XXIV/72, in adherence to Nagoya protocol requirements.
Disturbance	Sampling was non destructive or harmful to participants or environments involved.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Study did not involve laboratory animals
Wild animals	Once caught, all birds, and all but two bats caught per trapping session, were live-sampled in the field under manual restraint, before being released unharmed. All live rodents (except for individuals belonging to the genus Cricetomys, which were live-sampled under anaesthesia) and up to two bats caught per trapping session were transferred back to a biosafety level three (BSL3) laboratory at ILRI. Trapped rodents and bats were placed in containers that were resistant to escape, provided adequate ventilation and protection from the elements. Holding containers were transported via project vehicles that were decontaminated after use. at the labortaory, he animals were humanely euthanised by cardiac puncture under isoflurane anaesthesia and a full post-mortem examination then performed, with fresh faeces being collected from the rectum. Rodents caught in lethal traps were also necropsied in the laboratory following the same protocols. Faecal samples were collected non-invasively from small carnivores, by keeping them in the trap for a maximum period of twelve hours. Non human primates were anaesthetised where trapped, using a combination of Medetomidine and Ketamine (under the supervision of a Kenya Wildlife Service veterinary officer), and morphometric data and a suite of biological samples (including faeces if available, or a rectal swab) were collected from each animal. The primate was carefully monitored throughout, and anaesthesia reversed using Atipamezol. Carnivores and NHPs were released unharmed at an appropriate time of day, from the same location at which they were trapped. Rodents were euthanized humanely for two reasons, (i) because they were trapped within people's households and release of species that are deemed as pests (and a potential public health hazard) would not have been a viable option, (ii)In order to collect a fresh fecal samples via post-mortem.
Field-collected samples	Human and animal faecal samples were transported from the field on ice (4 degrees) to the laboratory within 5 h of collection. Extracted DNA was stored in -20 degree freezers and transported on dry ice to Oxford University for whole genome sequencing.
Ethics oversight	The collection of data adhered to the legal requirements of the Government of Kenya. The International Livestock Research Institute Institutional Research Ethics Committee is registered and accredited by the National Commission for Science, Technology and Innovation in Kenya. Livestock samples were obtained under the approval of the ILRI Institutional Animal Care and Use Committee (Reference ILR-IACUC2015/18) and permits obtained from the Directorate of Veterinary Services. Wildlife were trapped under approval of an ILRI Institutional Animal Care and Use Protocol (IACUC2015/12), and permits were obtained from the National Museums of Kenya and Kenya Wildlife Service

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	In each of the 99 households across Nairobi that participated in the study, the human participants that contributed faecal samples had their age, gender, occupation, food consumption and medical history recorded on a questionnaire. The number of members per recruited household ranged from one to 19, including staff members and unrelated household residents. However, full participation by every member was only achieved in 20 of the 99 households. Composition of the household varied by wealth group, with households at the lower end of the wealth-scale having more children (median = 2,

	compared to median 1 child in wealth groups 1 and 4, and median 0 children in wealth groups 2 and 3). Human specific covariates such as age, gender were not included in the analysis.
Recruitment	Within a study sub-location three randomly three points (each representing a household type - two livestock keeping and one non livestock keeping) in GIS were dropped. The nearest household to that point (within the dominant household type) was located - in most cases non-livestock keeping. Local administrative officials assisted to locate the nearest (Euclidian distance) households to that first selected that represent the two other classes, in most cases livestock keeping households. In each household, the household head/owner (or a nominated member) completed a questionnaire, detailing livestock ownership (e.g. abundance of livestock species), management practices (e.g. manure disposal practices), household composition (e.g. number of occupants), and socio-economic variables. Thereafter, following an informed consent, every human member of the household was invited to contribute a faecal sample and answer questionnaires on: their age, gender and occupation, food consumption and medical history. Faecal samples were collected from people not present in the household during the visit, such as school-age children. No bias were identified that could impact on the results.
Ethics oversight	The International Livestock Research Institute Institutional Research Ethics Committee is registered and accredited by the National Commission for Science, Technology and Innovation in Kenya, and approved by the Federal wide Assurance for the Protection of Human Subjects in the USA. Ethical approval for human sampling and data collection was obtained from the ILRI Institutional Research Ethics Committee (ILRI-IACUC2015/09).

Note that full information on the approval of the study protocol must also be provided in the manuscript.