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Host-specific antimicrobial selection shapes antimicrobial resistance in commensal *Escherichia coli*: A genome-based one health study in Kolkata, India

Shunsuke Iriguchi [a,1], Goutam Chowdhury [b,c,1], Alok Kumar Deb [d], Yuki Hoshiko [a,e], Kei Kitahara [c,f,g], Debjani Ghosh [b], Miki Okuno [a], Ayumu Ohno [c,f,g], Takeshi Yamamoto [a], Shin-Ichi Miyoshi [f,g], Santasabuj Das [h], Asish K. Mukhopadhyay [b], Yoshitoshi Ogura [a,*]

[a] Division of Microbiology, Department of Infectious Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan
[b] Division of Bacteriology, ICMR - National Institute for Research in Bacterial Infections, Kolkata, India
[c] Collaborative Research Centre of Okayama University for Infectious Diseases, ICMR - National Institute for Research in Bacterial Infections, Kolkata, India
[d] Division of Epidemiology, ICMR - National Institute for Research in Bacterial Infections, Kolkata, India
[e] Laboratory of Environmental Hygiene, Department of Health Science, School of Allied Health Sciences, Kitasato University, Kanagawa, Japan
[f] Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
[g] Research Centre for Intestinal Health Science, Okayama University, Okayama, Japan
[h] Division of Clinical Medicine, ICMR - National Institute for Research in Bacterial Infections, Kolkata, India

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ABSTRACT

We conducted a genome-based One Health investigation of 255 non-redundant commensal *Escherichia coli* isolates obtained from humans, cattle, poultry, and pigeons in Kolkata, India. Phylogenomic analysis revealed substantially greater genetic diversity among human-derived isolates than among those from animal hosts. Human and poultry isolates harboured significantly higher burdens of antimicrobial resistance genes (ARGs) and exhibited a greater prevalence of genotypic multidrug resistance compared with cattle and pigeon isolates. Although resistance burdens were comparable between human and poultry populations, their ARG repertoires differed markedly, likely reflecting host-specific antimicrobial selection pressures and usage practices. Near-identical clones were infrequently shared across host species, indicating limited cross-sectoral transmission. Comparative analysis with publicly available *E. coli* genomes from India demonstrated that commensal isolates possessed ARG profiles broadly similar to those of diarrheagenic *E. coli*, whereas extraintestinal pathogenic *E. coli* (ExPEC) carried substantially higher ARG burdens. Notably, four commensal isolates clustered with ExPEC strains in principal coordinate analysis and displayed extensive multidrug resistance. Collectively, these findings suggest that antimicrobial resistance in commensal *E. coli* within this setting is driven primarily by host-specific antimicrobial selection rather than frequent transmission between human and animal reservoirs.

1. Introduction

Antimicrobial resistance (AMR) is a major global public health challenge that limits the effective treatment of bacterial infections. In 2021, bacterial AMR was associated with an estimated 4.71 million deaths, including 1.14 million directly attributable deaths, and this burden is projected to increase substantially by 2050 [1]. Addressing AMR requires a comprehensive understanding of its ecological and evolutionary dynamics, including the host populations in which resistant bacteria persist, the environments that facilitate their maintenance, and the genetic mechanisms underlying their dissemination [2,3].

In high-income countries, antimicrobial stewardship and improved infection prevention have contributed to reductions in the prevalence of a wide range of resistant pathogens in clinical settings [4,5]. However, antimicrobial use in nonclinical sectors, such as livestock production and aquaculture, remains substantial and often insufficiently regulated, facilitating the spread of resistance determinants across sectors [6].

* Corresponding author.
E-mail address: y_ogura@kurume-u.ac.jp (Y. Ogura).
[1] These authors contributed equally.

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Under the One Health framework, efforts to optimize antimicrobial use and strengthen surveillance are also being implemented in livestock sectors, although challenges remain [7].

In many low- and middle-income countries (LMICs), appropriate antimicrobial use in healthcare is not fully implemented, and integrated One Health surveillance remains limited [8,9]. In these settings, AMR surveillance continues to rely largely on phenotypic antimicrobial susceptibility testing, and genome-based investigations are still underutilized. Whole-genome sequencing (WGS) enables high-resolution characterization of resistance genes, mobile genetic elements, and population structure and facilitates the reconstruction of transmission across humans, animals, and the environment [10–12].

Escherichia coli is a key organism for AMR surveillance and is a major cause of both intestinal and extraintestinal infections [13]. The global spread of multidrug-resistant *E. coli*, particularly extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing strains, represents a major clinical concern [14,15]. As a common commensal of the gastrointestinal tract in humans and animals, *E. coli* serves as an important reservoir of antimicrobial resistance genes (ARGs), which can disseminate via horizontal gene transfer [16]. In addition, commensal strains can cause extraintestinal infections following translocation from the gut, and some *E. coli* strains can be transmitted between animals and humans, contributing to zoonotic infections [17,18]. Characterizing commensal *E. coli* populations is therefore essential for understanding the baseline resistome and its role in AMR dissemination.

In India and other LMICs, genomic studies of *E. coli* have largely focused on clinical isolates or on animal-derived strains selectively isolated for antimicrobial resistance [19–23]. Previous studies in West Bengal have investigated ESBL- and AmpC-producing *E. coli* in pigs, poultry, and backyard livestock, as well as β-lactamase-producing Enterobacterales in pig-farm environments and multidrug-resistant *E. coli* from pet animals and backyard livestock [24–27]. While effective for identifying high-risk AMR clones, these approaches introduce selection bias, leaving the genomic landscape of commensal *E. coli* isolated from humans and animals without antimicrobial selection poorly understood.

To address this gap, we focused on Kolkata, eastern India, where high antimicrobial use and high rates of AMR in *E. coli* have been reported [28–31]. As one of the most densely populated cities in India (~24,000 persons/km^2), Kolkata provides an important setting for investigating interactions among human and animal AMR reservoirs. Although antibiotic use in livestock in rural West Bengal is primarily for therapeutic purposes and disease prevention rather than growth promotion, the use of antibiotics important to human medicine raises concerns about AMR transmission between animals and humans [32]. Using a One Health approach, we performed whole-genome sequencing of *E. coli* isolates obtained without antimicrobial selection from humans, livestock (cattle and poultry), and wild birds (pigeons), together with publicly available genome data from human clinical *E. coli* isolates in India. By integrating these genomic data, we characterized antimicrobial resistance gene distribution, population structure, and potential transmission pathways, providing an unbiased genomic perspective on AMR ecology in an LMIC setting.

2. Methods

2.1. Isolation of commensal E. coli strains from humans and animals

Cross-sectional sampling was conducted in Kolkata, India, between 2023 and 2025. Human commensal *E. coli* strains were isolated from faecal or rectal swab samples obtained from patients with diarrhoea at the Infectious Diseases and Beleghata General Hospital and the Dr. B. C. Roy Postgraduate Institute of Paediatric Sciences. A loopful of each stool sample was streaked onto MacConkey agar (Difco, BD, USA) plates and incubated at 37 °C for 16–18 h. Up to 12 typical pink lactose-fermenting colonies from each plate were selected and subcultured on Luria–Bertani (LB) agar (Difco, BD, USA). All selected isolates were confirmed to be oxidase negative, acid and gas producing on triple-sugar iron (TSI) agar, and indole positive. To selectively obtain commensal *E. coli* strains rather than diarrhoeagenic pathogens, isolates negative for diarrhoeagenic virulence genes by routine PCR-based diagnostic screening [22] were selected, and one isolate per individual was included in this study.

Animal-derived isolates were obtained noninvasively from faecal samples collected from cattle (197samples), poultry (64 samples), and pigeons (36 samples). For livestock, the study objectives and sampling procedures were explained to the respective farmers, and verbal informed consent was obtained before faecal sample collection. Only apparently healthy cattle and poultry were selected for faecal sample collection. Their health status was assessed based on general appearance and the absence of apparent signs of illness or diarrhoea, with assistance from veterinary personnel. *E. coli* strains were isolated using the same procedures as those used for human samples, and one to five isolates were selected from each sample. As described below, to avoid redundancy, isolates differing by fewer than five SNPs were excluded from subsequent genomic analysis. For animal-derived isolates, the presence or absence of virulence genes was not considered during isolate selection.

2.2. Genome sequencing, quality assessment, and filtering

Genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Genomic libraries were prepared using the xGen DNA Library Prep EZ Kit (Integrated DNA Technologies) and NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs) (New England Biolabs). Sequencing was performed on the Illumina NovaSeq X Plus platform to generate 151 bp paired-end reads.

Genome assembly was performed using Platanus_B v1.3.2 [33] with default parameters, and genome annotation was conducted using Prokka v1.14.6 [34] with default settings. Assembly quality was assessed using CheckM v1.2.0 28 [35], and genomes with ≥95% completeness and < 3% contamination were retained for further analysis. Species identification was confirmed using fastANI [36], with a threshold of >95% average nucleotide identity against the *E. coli* reference strain ATCC 11775. Redundant clones isolated from the same host were excluded if they differed by fewer than five SNPs identified within core genes detected by Roary [37]. The isolates retained after filtering are summarized in Supplementary Table 1.

The genome sequences generated and included in this study were deposited in the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJDB42326. In addition, genomic data from three previous studies of human-derived *E. coli* isolates in India were included for comparative analysis, comprising those of commensal *E. coli* (PRJNA611810) [38], diarrhoeagenic *E. coli* (PRJDB18117) [22], and extraintestinal pathogenic *E. coli* (INRP000050) [39]. Publicly available genome data from nonselectively collected commensal *E. coli* isolates from cattle (PRJDB5579) [40] and chickens (PRJDB9460) in Japan were also included for comparative analysis. Details of all publicly available genomes included in the comparative analyses are provided in Supplementary Table 2.

2.3. Phylogroup and sequence type (ST) determinations and ARG identification

Phylogroups were determined using ClermonTyping v20.03 [41]. STs were assigned on the basis of the Achtman seven-gene multilocus sequence typing (MLST) scheme [42,43] using mlst v2.32.2 (https://github.com/tseemann/mlst). ARGs and mutations within the QRDRs of *gyrA, gyrB, parC,* and *parE* were identified using NCBI AMRFinderPlus v4.0.22 with default parameters [44].

2.4. Core gene-based phylogenetic analysis

Core genes were identified by pangenome analysis using Roary v3.13.0 [37] with the options "-i 90" and "-cd 99". SNP sites were extracted from the core gene alignment using SNP sites [45]. Maximum-likelihood phylogenetic trees were constructed using RAxML-NG v1.1.0 [46] with the GTR + G4 substitution model and 100 bootstrap replicates. The phylogenetic trees were visualized and annotated using iTOL v7 [47].

2.5. Statistical analysis

All the statistical analyses were performed using R v4.5.1 (R Project for Statistical Computing). To identify ARGs associated with specific

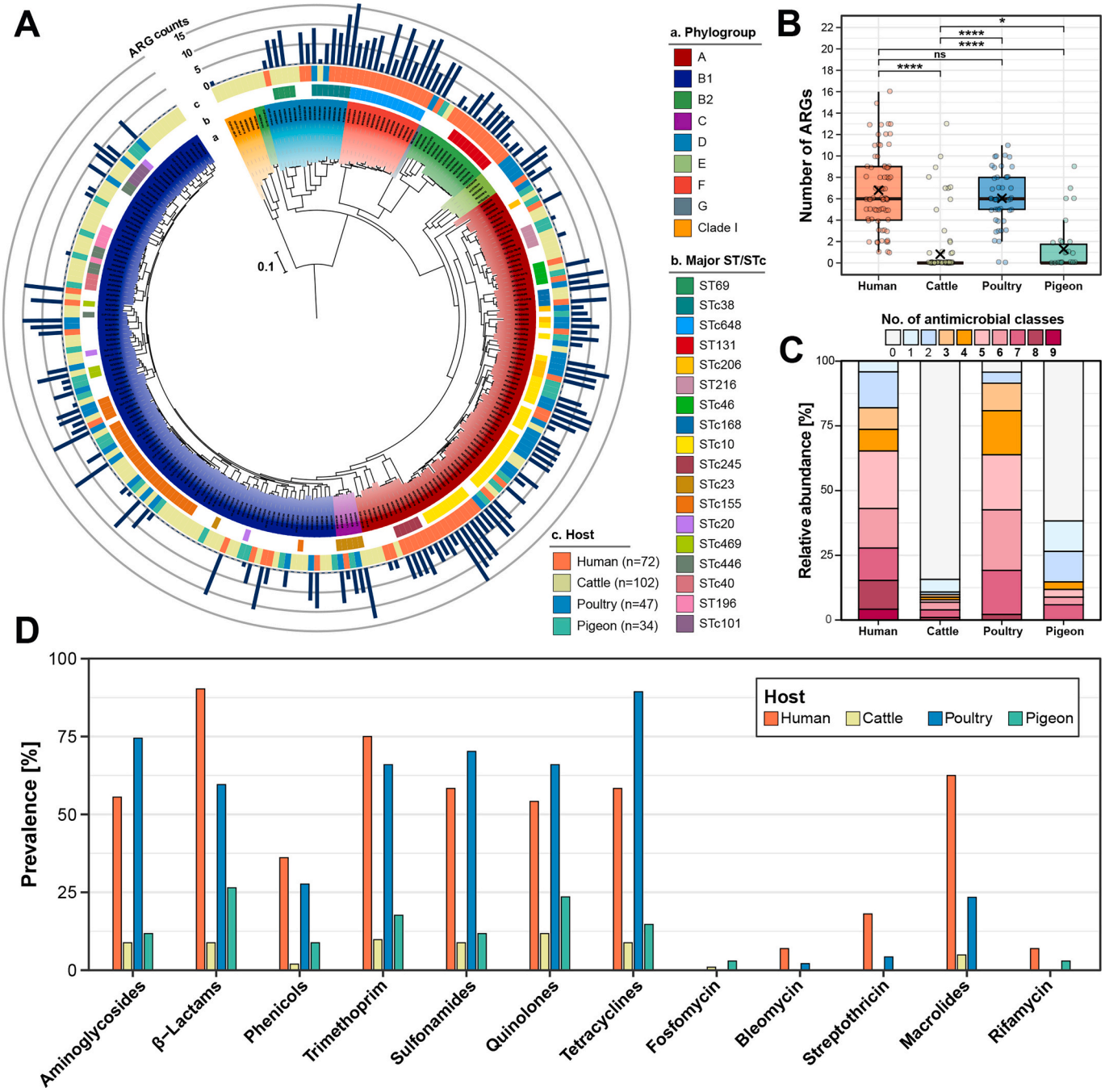

Fig. 1. Phylogenetic diversity and host-associated antimicrobial resistance profiles of commensal *E. coli* in Kolkata, India. (A) Core-genome maximum-likelihood phylogeny of 255 commensal *E. coli* isolates from Kolkata, India. The tree was constructed from an alignment of 228,918 informative SNP sites identified within 2349 core genes defined at ≥90% sequence identity. Associated metadata, including phylogroup, major ST/STc (represented by at least three isolates), host, and the number of ARGs per isolate, are shown. (B) Number of ARGs detected per isolate. Statistical comparisons were performed using the Wilcoxon rank-sum test (****$P <$ 0.0001, *$P < 0.05$; ns, not significant). The bold horizontal line and cross indicate the median and mean values, respectively. (C) Distribution of genotypic multidrug resistance (gMDR), defined as the presence of ARGs conferring resistance to three or more antimicrobial classes. The bars are stratified by the number of antimicrobial classes represented in each isolate. ARGs were assigned to 12 antimicrobial classes according to the NCBI Reference Gene Catalogue. (D) Prevalence of ARGs by host and antimicrobial class. Each bar represents the proportion of isolates from each host carrying at least one ARG belonging to the indicated antimicrobial class. The bars are colour-coded by host.

hosts, indicator species analysis was conducted using the indicspecies R package. The indicator value calculated by this package represents the strength of the association between a given group and an individual feature and is defined as the product of specificity and sensitivity. In this study, these metrics were applied to the bidirectional associations between each host and individual ARGs to calculate the attribution score using the following formula:

Attribution score $_{(geneA,\ hostA)}$ (%) = Sensitivity $_{(geneA,\ hostA)}$ × Specificity $_{geneA}$×100 (1).

The statistical significance of the attribution scores was evaluated using permutation tests with 999 permutations, and P values <0.05 were considered to indicate statistical significance. The detailed results are summarized in Supplementary Table 3.

PCoA and permutational multivariate analysis of variance (PERMANOVA) were performed using the vegan package in R. When significant differences were detected by PERMANOVA, pairwise PERMANOVA was subsequently conducted as a post hoc analysis using the pairwiseAdonis package. The resulting P values were adjusted using the Benjamini–Hochberg method. Statistical summaries of each analysis are provided in Supplementary Tables 4 to 5.

3. Results

3.1. Phylogenetic diversity and clonal relationships of commensal E. coli isolates from diverse hosts

Between 2023 and 2025, commensal *E. coli* strains were isolated from humans, cattle, poultry, and pigeons in Kolkata, India. Human isolates were obtained from the stool samples of patients with diarrhoea undergoing routine bacteriological testing, and isolates lacking diarrhoeagenic virulence genes and distinct from the identified diarrhoeal pathogens were selected as commensal strains. Animal isolates were recovered from freshly collected faecal samples of apparently healthy animals. All the isolates were subjected to WGS, filtered on the basis of sequence and assembly quality, and confirmed as *E. coli*, and the elimination of redundant clones within the same host (<5 core-genome SNPs) was performed. After filtering, 255 nonredundant genomes were retained for analysis, comprising 72 human, 102 cattle, 47 poultry, and 34 pigeon isolates (Supplementary Table 1).

Core gene-based phylogenetic analysis revealed substantial phylogenetic diversity, with the isolates classified into nine phylogroups/clades (Fig. 1A). Human-derived isolates were phylogenetically diverse and belonged mainly to phylogroups A (38.9%), F (19.4%), B2 (16.7%), B1 (12.5%), and D (9.7%) (Supplementary Fig. 1). In contrast, most cattle-derived (83.3%), poultry-derived (80.9%), and pigeon-derived (94.1%) isolates belonged to phylogroup A or B1. Six cattle-derived isolates (5.9%) were classified into clade I.

Sequence typing revealed 85 sequence types or sequence type complexes (STs/STcs) (Supplementary Table 1). Among these, 147 isolates (57.6%) belonged to the major STs/STcs represented by three or more isolates. The most common were STc10 (n = 33), STc155 (n = 27), and STc648 (n = 14). Among the major STs/STcs, ST648 (n = 14), ST131 (n = 9), and STc245 (n = 5) were composed exclusively of human-derived isolates, whereas ST69 (n = 4) was composed exclusively of cattle-derived isolates (Supplementary Fig. 2). All the other major STs/STcs included isolates from multiple host species.

Pairwise core-genome SNP distance analysis between isolates from different hosts revealed only one human–poultry isolate pair differing by five or fewer SNPs, a threshold suggestive of recent epidemiological linkage (Supplementary Table 6). Within 10 to 99 SNPs, only two additional closely related pairs were identified: one human–poultry pair and one poultry–pigeon pair. These results indicate that strain-level transmission of *E. coli* between hosts was infrequent in this study population.

3.2. Prevalence of ARGs in human and animal commensal E. coli isolates

In total, 70 ARGs belonging to 12 antimicrobial classes were identified and broadly distributed across the phylogeny without marked lineage specificity (Fig. 1A and Supplementary Fig. 3). At least one ARG was detected in 146 isolates (57.3%), with up to 16 ARGs identified in a single isolate (Supplementary Table 1). The mean number of ARGs per isolate was significantly greater in human- and poultry-derived isolates (6.8 and 6.0, respectively) than in cattle- and pigeon-derived isolates (0.8 and 1.3, respectively) (Fig. 1B). Genotypic multidrug resistance (gMDR), defined as the presence of ARGs conferring resistance to three or more antimicrobial classes, was highly prevalent in human- and poultry-derived isolates (81.9% and 91.5%, respectively) but was much less common in cattle- and pigeon-derived isolates (9.8% and 14.7%, respectively) (Fig. 1C). Across multiple antimicrobial classes, human- and poultry-derived isolates presented high ARG prevalence (Fig. 1D). The clearest differences between human- and poultry-derived isolates were observed for β-lactam and macrolide resistance genes, which were more prevalent in human-derived isolates, and tetracycline resistance genes, which were more prevalent in poultry-derived isolates.

For comparison with a high-income country setting, we analysed publicly available genome data from commensal *E. coli* isolates collected from humans and livestock in Miyazaki Prefecture, Japan. The mean numbers of ARGs per isolate were 0.5 for human-derived isolates (n = 200), 2.1 for cattle-derived isolates (n = 200), and 4.8 for poultry-derived isolates (n = 300) (Supplementary Fig. 4). As observed in India, poultry-derived isolates harboured more ARGs than cattle-derived isolates. In contrast, human-derived isolates carried the fewest ARGs, in marked contrast to the pattern observed in Kolkata.

3.3. Host-associated distribution of ARGs

Interestingly, the ARGs frequently detected in human-derived isolates differed from those frequently detected in poultry-derived isolates (Fig. 2A). To identify host-associated ARGs, we performed indicator species analysis and calculated attribution scores, where a score of 100% indicates that an ARG is present in all the isolates from one host and absent from all the isolates from the other hosts. Numerous ARGs were significantly associated with either human- or poultry-derived isolates (Fig. 2B). In human-derived isolates, $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, *dfrA17*, *sul1*, *tet(B)*, and *mph(A)* had high positive attribution scores (>50%). In poultry-derived isolates, *tet(A)*, *qnrS13*, *sul3, sul2, dfrA14, blaTEM-1*, *aph (3′)*-Ia, and *aadA1* also presented high positive attribution scores (>50%). No ARGs showed significant positive associations with cattle- or pigeon-derived isolates.

Principal coordinate analysis (PCoA) based on the ARG profiles of isolates carrying at least one ARG clearly separated human- and poultry-derived isolates (R^2 = 0.120, p = 0.001), further demonstrating that these two host groups harboured distinct ARG repertoires (Fig. 2C).

Mutations in the quinolone resistance–determining regions (QRDRs) were detected in 98.6% of the human-derived isolates and 53.2% of the poultry-derived isolates, substantially higher than in the cattle- and pigeon-derived isolates (15.7% and 14.7%, respectively) (Supplementary Fig. 5). The predominant QRDR genotype also differed between hosts. In human-derived isolates, the most common combination was *gyrA* (D87N and S83L), *parC* (S80I), and *parE* (S458A), which were detected in 51.4% of the isolates. In contrast, the most frequent QRDR mutation in poultry-derived isolates was the single *gyrA* S83L substitution, which was detected in 14.7% of the isolates.

3.4. Comparison of ARG distribution between human commensal and clinical isolates

To compare ARG profiles among human-derived *E. coli* populations in India, we analysed 72 commensal isolates from the present study together with publicly available genomes of 45 commensal isolates from

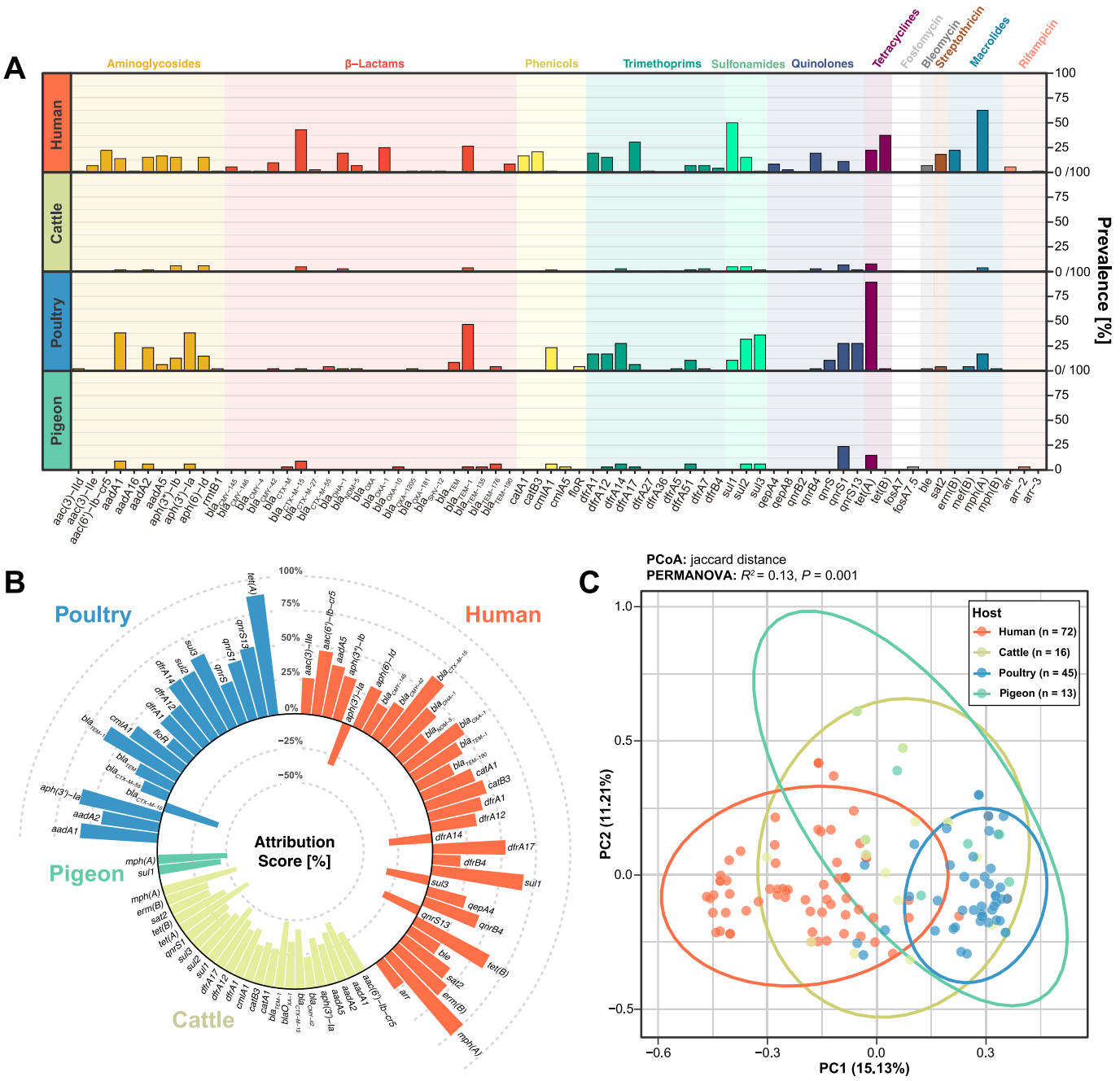

Fig. 2. Host-specific ARG signatures in commensal *E. coli.* (A) Prevalence of ARGs among commensal *E. coli* isolates by host. The bars are colour-coded according to antimicrobial class. (B) Bidirectional host attribution analysis of ARGs. Attribution scores were calculated as the product of sensitivity (within-host prevalence) and host specificity. Positive values indicate ARG enrichment and identify significant host-associated signature ARGs, whereas negative values indicate ARG depletion and highlight genes that are significantly underrepresented in a given host. Statistical significance was assessed using permutation tests ($P < 0.05$). (C) Principal coordinate analysis (PCoA) of ARG profiles among 146 commensal *E. coli* isolates carrying at least one ARG. Each point represents an individual isolate and is colour coded by host; ellipses indicate 95% confidence intervals. Overall differences in ARG composition among hosts were significant according to PERMANOVA ($P = 0.001$), and all pairwise comparisons were significant after multiple testing correction ($P < 0.05$).

the Global Enteric Multicenter Study (GEMS) [38], all of which were collected without antimicrobial selection, 47 diarrhoeagenic *E. coli* (DEC) isolates [22] and 35 extraintestinal pathogenic *E. coli* (ExPEC) isolates [39], all of which were collected in India (Supplementary Table 2). Overall, the phylogenetic distributions were broadly similar across these datasets, except for a higher proportion of phylogroup F among the commensal isolates and a higher proportion of phylogroup C among the ExPEC isolates (Supplementary Fig. 6).

Among the commensal isolates, only three ARGs differed significantly in prevalence between the present study and the GEMS study (Fig. 3A). Similarly, comparisons between commensal isolates from this study and DEC revealed significant differences in only four ARGs. In contrast, comparison with ExPEC revealed significant differences in 23 ARGs, 19 of which were more prevalent in ExPEC. The number of ARGs per isolate was also substantially greater in ExPEC than in commensal *E. coli* and DEC (Fig. 3B). Consistent with these findings, PCoA revealed that the ExPEC isolates formed a distinct cluster separate from the commensal *E. coli* and DEC, indicating a markedly different ARG repertoire ($R^2 \geq 0.130$, $p = 0.001$) (Fig. 3C). Notably, four human commensal isolates from the present study (NICED00041, NICED00286,

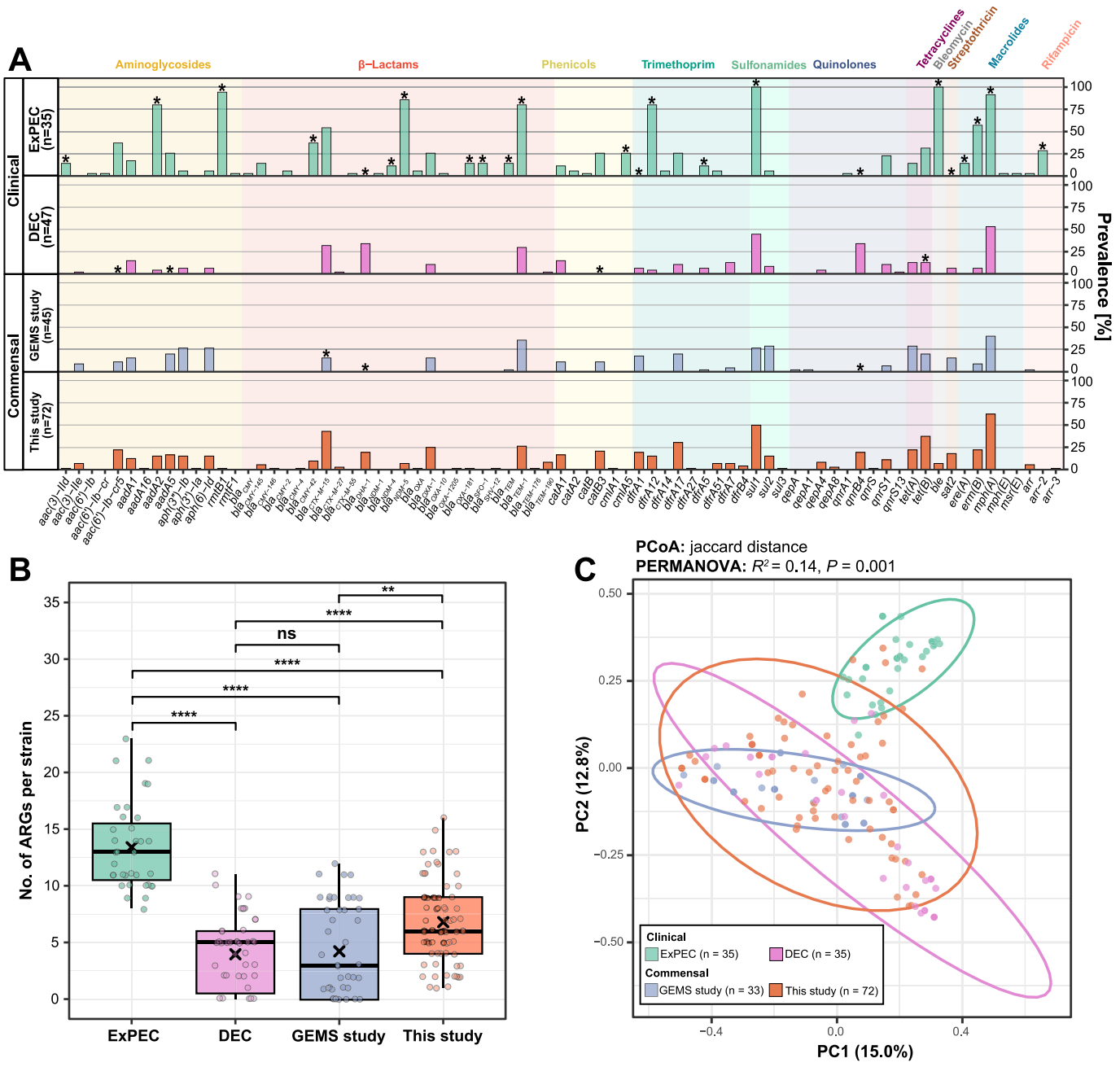

Fig. 3. Comparison of ARG profiles among human-derived *E. coli* populations in India. (A) Prevalence of ARGs among four nonselective collections: extraintestinal pathogenic *E. coli* (ExPEC; $n = 35$), diarrhoeagenic *E. coli* (DEC; $n = 47$), commensal isolates from the Global Enteric Multicenter Study (GEMS; $n = 45$), and human commensal isolates from the present study ($n = 72$). The bars are colour-coded by study group. Asterisks indicate significant differences in ARG prevalence relative to the human commensal isolates from the present study (Fisher's exact test with false discovery rate correction; $^*P < 0.05$). (B) Number of ARGs detected per isolate. Statistical comparisons were performed using the Wilcoxon rank-sum test ($^{****}P < 0.0001$, $^*P < 0.05$; ns, not significant). The bold horizontal line and cross indicate the median and mean values, respectively. (C) Principal coordinate analysis (PCoA) of ARG profiles among 175 *E. coli* isolates carrying at least one ARG. Each point represents an individual isolate and is colour coded by study group; ellipses indicate 95% confidence intervals. Overall differences in ARG composition among the study groups were significant according to PERMANOVA ($P = 0.001$), and all pairwise comparisons were significant after multiple testing correction ($P < 0.05$).

NICED00292, and NICED00320) were positioned within the ExPEC cluster in the PCoA plot. These isolates were highly multidrug resistant, carrying 9–13 antimicrobial resistance genes belonging to seven or eight antimicrobial classes, including aminoglycosides, β-lactams, trimethoprim, sulfonamides, and macrolides (one isolate lacked a macrolide resistance gene) (Supplementary Table 1). All four isolates also harboured QRDR mutations associated with fluoroquinolone resistance.

4. Discussion

In this study, we used a genome-based One Health approach to compare the population structure and ARG profiles of commensal *E. coli* from humans, livestock, and wild birds in Kolkata, India. Compared with animal-derived isolates, human-derived isolates were phylogenetically more diverse and were dominated by phylogroups A and B1 (Fig. 1 and Supplementary Fig. 1). This pattern is consistent with the findings of previous studies [38,40,48–51] and suggests that host species are among the major determinants of the population structure of commensal *E. coli*.

Previous One Health studies have shown that specific resistant clones, particularly ESBL-producing *E. coli*, can circulate among humans, animals, and the environment [52,53]. In LMICs, close contact between humans and livestock and inadequate sanitation are thought to increase opportunities for cross-sectoral transmission of resistant bacteria and ARGs [54]. Nevertheless, nearly identical commensal *E. coli* clones were rarely shared between host species in our study (Supplementary Table 6). Similar findings have been reported in Kenya, where direct sharing of commensal *E. coli* clones between humans and animals was detected but remained uncommon [12]. These observations suggest that although interhost transmission occurs, its contribution to the overall population structure of commensal *E. coli* is limited.

The most prominent finding of this study was the markedly elevated ARG burden in human- and poultry-derived isolates (Fig. 1). In contrast, cattle- and pigeon-derived isolates harboured relatively few ARGs. Comparisons with publicly available genomes from Japan revealed a similar pattern in livestock, with poultry-derived isolates carrying more ARGs than cattle-derived isolates, but a markedly different pattern in humans, where commensal isolates carried very few ARGs (Supplementary Fig. 4). Similar host-associated differences have also been reported in the Netherlands and other European countries, where antimicrobial resistance in commensal *E. coli* is consistently higher in poultry than in cattle, while resistance levels in human commensal isolates are generally lower than those observed in poultry [50,55].

These findings highlight host-associated differences in the ARG burden of commensal *E. coli*, which may reflect differences in antimicrobial use across human and animal sectors. In India, antibiotic consumption in human medicine is dominated by broad-spectrum agents such as azithromycin, cefixime, amoxicillin–clavulanate, cefpodoxime, and ofloxacin [31]. In poultry production, commonly used antimicrobials, such as ciprofloxacin, nalidixic acid, tetracycline, ampicillin, gentamicin, cefuroxime, and cotrimoxazole [56]. In contrast, antimicrobial use in cattle is generally lower than that in intensive poultry production in many settings [6], which likely explains the very low ARG burden observed in cattle-derived isolates, comparable to or lower than that observed in pigeon-derived isolates.

Despite similarly high rates of genotypic MDR, human- and poultry-derived isolates clearly harbour distinct ARG repertoires. Human-derived isolates were enriched in the azithromycin resistance gene *mph(A)* and several clinically important β-lactam resistance genes, including the clavulanate-insensitive β-lactamase gene $bla_{\text{OXA-1}}$, the ESBL gene $bla_{\text{CTX-M-15}}$, the AmpC β-lactamase gene $bla_{\text{DHA-1}}$, and the metallo-β-lactamase gene $bla_{\text{NDM-5}}$ (Fig. 2). In contrast, poultry-derived isolates were characterized by a high prevalence of the ampicillin resistance gene $bla_{\text{TEM-1}}$ and the tetracycline resistance gene *tet(A)*. Similar host-specific differences were observed for phenicol, sulfonamide, and trimethoprim resistance genes. These findings indicate that distinct antimicrobial selection pressures, rather than frequent horizontal transfer of dominant resistance determinants, are the primary drivers of resistome composition in each sector.

Comparison with publicly available clinical isolates from India provided further insight into the relationship between colonizing and pathogenic *E. coli*. ARG profiles were highly similar between commensal isolates from this study, commensal isolates from the GEMS [38], and DEC [22], indicating a shared baseline resistome (Fig. 3). In contrast, ExPEC [39] carried substantially more ARGs and formed a distinct cluster according to principal coordinate analysis. In India, where self-medication with antimicrobials is common, the elevated resistance burden in ExPEC may partly reflect selective enrichment of highly resistant strains among patients requiring hospital-based diagnosis and treatment. Notably, four commensal isolates from our study clustered with ExPEC in the PCoA plot (Fig. 3C) and exhibited extensive multidrug resistance, suggesting that the human intestinal microbiota can serve as a reservoir of strains capable of causing difficult-to-treat infections.

This study has several limitations. First, although we identified occasional sharing of closely related strains between hosts, the sample size was insufficient to quantify rare transmission events with precision. Detecting strain-level transmission in unbiased collections of commensal *E. coli* will require substantially larger studies. Similarly, larger sample sizes are needed to validate the observed differences in ARG repertoires between human and poultry isolates. Second, short-read assemblies did not allow detailed reconstruction of plasmids and other mobile genetic elements, limiting our ability to trace the movement of specific ARGs across hosts. Large-scale complete genome analyses using long-read sequencing will be essential for clarifying how plasmids mediate ARG transmission in One Health settings.

In conclusion, compared with isolates from cattle and wild birds, commensal *E. coli* from humans and poultry in Kolkata carried a markedly greater burden of ARGs, reflecting intense antimicrobial exposure in these sectors. Despite the shared high resistance burden, human and poultry isolates possessed distinct ARG repertoires, indicating that host-specific antimicrobial selection pressure is more important than frequent cross-sectoral transmission in shaping resistome composition. Although the occasional sharing of clones and resistance genes likely occurs, its contribution to the antimicrobial resistance burden in human clinical settings appears limited. Moreover, some human commensal isolates already exhibited resistance profiles comparable to those of highly resistant ExPEC, underscoring the urgent need to strengthen antimicrobial stewardship in human healthcare in India.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT (OpenAI) to improve the readability, grammar, and overall language of the manuscript. The AI-generated output was reviewed, revised, and verified by the authors, who take full responsibility for the content of the published article.

CRediT authorship contribution statement

Shunsuke Iriguchi: Writing – original draft, Investigation, Formal analysis, Data curation. **Goutam Chowdhury:** Resources, Investigation, Conceptualization. **Alok Kumar Deb:** Resources, Investigation. **Yuki Hoshiko:** Visualization, Investigation, Data curation. **Kei Kitahara:** Resources, Conceptualization. **Debjani Ghosh:** Resources, Investigation. **Miki Okuno:** Visualization, Supervision, Data curation. **Ayumu Ohno:** Investigation. **Takeshi Yamamoto:** Investigation, Data curation. **Shin-Ichi Miyoshi:** Supervision, Conceptualization. **Santasabuj Das:** Supervision. **Asish K. Mukhopadhyay:** Writing – review & editing, Software, Conceptualization. **Yoshitoshi Ogura:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

Ethical approval

This study was approved by the Institutional Ethics Committee (IEC) of the National Institute for Research in Bacterial Infections, Kolkata, India (registration number: A-1/2015-IEC). Written informed consent was obtained from all the adult participants and from the parents or legal guardians of the paediatric participants prior to sample collection. Animal faecal samples were collected noninvasively. Oral informed consent was obtained individually from the owners of the livestock animals included in this study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2026.101646.

Data availability

All sequence data generated in this study have been deposited in the NCBI BioProject database under accession number PRJDB42326.

References

[1] GBD 2021 Antimicrobial rResistance cCollaborators, Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050, Lancet 404 (2024) 1199–1226, https://doi.org/10.1016/S0140-6736(24)01867-1.
[2] A.H. Holmes, L.S. Moore, A. Sundsfjord, M. Steinbakk, S. Regmi, A. Karkey, P. J. Guerin, L.J. Piddock, Understanding the mechanisms and drivers of antimicrobial resistance, Lancet 387 (2016) 176–187, https://doi.org/10.1016/S0140-6736(15)00473-0.
[3] M. Woolhouse, M. Ward, B. van Bunnik, J. Farrar, Antimicrobial resistance in humans, livestock and the wider environment, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 370 (2015) 20140083, https://doi.org/10.1098/rstb.2014.0083.
[4] D. Baur, B.P. Gladstone, F. Burkert, E. Carrara, F. Foschi, S. Dobele, E. Tacconelli, Effect of antibiotic stewardship on the incidence of infection and colonisation with antibiotic-resistant bacteria and Clostridium difficile infection: a systematic review and meta-analysis, Lancet Infect. Dis. 17 (2017) 990–1001, https://doi.org/10.1016/S1473-3099(17)30325-0.
[5] World Health Organization, Global antimicrobial resistance and use surveillance system (GLASS) report 2022 (WHO,, https://iris.who.int/server/api/core/bitstreams/e5cc3da6-f46e-4355-a352-8d16820e0dd1/content, 2022.
[6] T.P. Van Boeckel, C. Brower, M. Gilbert, B.T. Grenfell, S.A. Levin, T.P. Robinson, A. Teillant, R. Laxminarayan, Global trends in antimicrobial use in food animals, Proc. Natl. Acad. Sci. USA 112 (2015) 5649–5654, https://doi.org/10.1073/pnas.1503141112.
[7] K.L. Tang, N.P. Caffrey, D.B. Nobrega, S.C. Cork, P.E. Ronksley, H.W. Barkema, A. J. Polachek, H. Ganshorn, N. Sharma, J.D. Kellner, W.A. Ghali, Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis, Lancet Planet Health. 1 (2017) e316–e327, https://doi.org/10.1016/S2542-5196(17)30141-9.
[8] J.A. Ayukekbong, M. Ntemgwa, A.N. Atabe, The threat of antimicrobial resistance in developing countries: causes and control strategies, Antimicrob. Resist. Infect. Control 6 (2017) 47, https://doi.org/10.1186/s13756-017-0208-x.
[9] D. Limmathurotsakul, R. Ahmad, E.A. Ashley, R. Atun, R.H. van Doorn, J. Joshi, S. S. Kanj, J. Midega, M. Mpundu, P. Turner, K. Walia, S.J. Peacock, N.A. Feasey, Surveillance and epidemiology of drug resistant infections consortium (SEDRIC), transitioning to people-centred antimicrobial resistance surveillance, Lancet Infect. Dis. 26 (2025) e308–e313, https://doi.org/10.1016/S1473-3099(25)00662-0.
[10] X. Didelot, R. Bowden, D.J. Wilson, T.E.A. Peto, D.W. Crook, Transforming clinical microbiology with bacterial genome sequencing, Nat. Rev. Genet. 13 (2012) 601–612, https://doi.org/10.1038/nrg3226.
[11] S.R. Partridge, S.M. Kwong, N. Firth, S.O. Jensen, Mobile genetic elements associated with antimicrobial resistance, Clin. Microbiol. Rev. 31 (2018) e00088–17, https://doi.org/10.1128/CMR.00088-17.
[12] D.M. Muloi, B.A. Wee, D.M.H. McClean, M.J. Ward, L. Pankhurst, H. Phan, A. C. Ivens, V. Kivali, A. Kiyong'a, C. Ndinda, N. Gitahi, T. Ouko, J.M. Hassell, T. Imboma, J. Akoko, M.K. Murungi, S.M. Njoroge, P. Muinde, Y. Nakamura, L. Alumasa, E. Furmaga, T. Kaitho, E.M. Ohgren, F. Amanya, A. Ogendo, D. J. Wilson, J.M. Bettridge, J. Kiiru, C. Kyobutungi, C. Tacoli, E.K. Kang'ethe, J. D. Davila, S. Kariuki, T.P. Robinson, J. Rushton, M.E.J. Woolhouse, E.M. Fevre, Population genomics of *Escherichia coli* in livestock-keeping households across a rapidly developing urban landscape, Nat. Microbiol. 7 (2022) 581–589, https://doi.org/10.1038/s41564-022-01079-y.
[13] J.B. Kaper, J.P. Nataro, H.L. Mobley, Pathogenic *Escherichia coli*, Nat. Rev. Microbiol. 2 (2004) 123–140, https://doi.org/10.1038/nrmicro818.
[14] M.S. Aljohni, M. Harun-Ur-Rashid, S. Selim, Emerging threats: antimicrobial resistance in extended-spectrum beta-lactamase and carbapenem-resistant *Escherichia coli*, Microb. Pathog. 200 (2025) 107275, https://doi.org/10.1016/j.micpath.2024.107275.
[15] C. Rana, V. Vikas, N. Narwal, K. Korpela, S. De, Dynamics of extended spectrum cephalosporin and carbapenem resistance genes in *Escherichia coli*: an emerging environmental hazard, J. Hazard. Mater. 509 (2026) 141955, https://doi.org/10.1016/j.jhazmat.2026.141955.
[16] M.M. Tawfick, A.A. Elshamy, K.T. Mohamed, N.G. El Menofy, Gut commensal *Escherichia coli*, a high-risk reservoir of transferable plasmid-mediated antimicrobial resistance traits, Infect. Drug Resist. 15 (2022) 1077–1091, https://doi.org/10.2147/IDR.S354884.
[17] X. Li, H. Hu, Y. Zhu, T. Wang, Y. Lu, X. Wang, Z. Peng, M. Sun, H. Chen, J. Zheng, C. Tan, Population structure and antibiotic resistance of swine extraintestinal pathogenic *Escherichia coli* from China, Nat. Commun. 15 (2024) 5811, https://doi.org/10.1038/s41467-024-50268-2.
[18] A.R. Manges, H.M. Geum, A. Guo, T.J. Edens, C.D. Fibke, J.D.D. Pitout, Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages, Clin. Microbiol. Rev. 32 (2019) e00135–18, https://doi.org/10.1128/CMR.00135-18.
[19] N.K. Devanga Ragupathi, B. Veeraraghavan, D.P. Muthuirulandi Sethuvel, S. Anandan, K. Vasudevan, A.R. Neeravi, J.L.K. Daniel, S. Sathyendra, R. Iyadurai, A. Mutreja, First Indian report on genome-wide comparison of multidrug-resistant *Escherichia coli* from blood stream infections, PLoS One 15 (2020) e0220428, https://doi.org/10.1371/journal.pone.0220428.
[20] R.P.L. Srijith, S. Sridharan, A. Paul, K. Perumal, Genomic analysis of ST117, 155, 1011, 167, 744, and 17391 in poultry-associated multidrug resistant *Escherichia coli* isolates from India, Sci. Rep. 16 (2026), https://doi.org/10.1038/s41598-026-38232-0.
[21] N. Gheewalla, V. Karthikeyan, Y. Jadhav, K. Kulkarni, A. Tyagi, J. Jagannadham, S. Budhiraja, B. Tarai, M. Kavathekar, S. Karve, Genomic landscape of antimicrobial resistance in India: findings from a multi-species surveillance study, NPJ Antimicrob Resist. 4 (2026) 13, https://doi.org/10.1038/s44259-026-00185-9.
[22] Y. Hoshiko, G. Chowdhury, K. Kitahara, D. Ghosh, D.S. Nagano, A. Ohno, S. I. Miyoshi, M. Okuno, T. Yamamoto, S. Dutta, A.K. Mukhopadhyay, Y. Ogura, Genomic features of three major diarrhoeagenic *Escherichia coli* pathotypes in India, Microb. Genom. 11 (2025) 001430, https://doi.org/10.1099/mgen.0.001430.
[23] D.J. Ingle, M.M. Levine, K.L. Kotloff, K.E. Holt, R.M. Robins-Browne, Dynamics of antimicrobial resistance in intestinal *Escherichia coli* from children in community settings in South Asia and sub-Saharan Africa, Nat. Microbiol. 3 (2018) 1063–1073, https://doi.org/10.1038/s41564-018-0217-4.
[24] A. Banerjee, S. Pal, P. Goswami, K. Batabyal, S.N. Joardar, S. Dey, D.P. Isore, T. K. Dutta, S. Bandyopadhyay, I. Samanta, Docking analysis of circulating CTX-M variants in multi-drug resistant, beta-lactamase and biofilm-producing *E. coli* isolated from pet animals and backyard livestock, Microb. Pathog. 170 (2022) 105700, https://doi.org/10.1016/j.micpath.2022.105700.
[25] A. Samanta, A. Mahanti, S. Chatterjee, S.N. Joardar, S. Bandyopadhyay, T.K. Sar, et al., Pig farm environment as a source of beta-lactamase or AmpC-producing Klebsiella pneumoniae and *Escherichia coli*, Ann. Microbiol. 68 (2018) 781–791, https://doi.org/10.1007/s13213-018-1387-2.
[26] I. Samanta, S.N. Joardar, P.K. Das, T.K. Sar, Comparative possession of Shiga toxin, intimin, enterohaemolysin and major extended spectrum beta lactamase (ESBL) genes in *Escherichia coli* isolated from backyard and farmed poultry, Iran. J. Vet. Res. 16 (2015) 90–93.
[27] I. Samanta, S.N. Joardar, A. Mahanti, S. Bandyopadhyay, T.K. Sar, T.K. Dutta, Approaches to characterize extended spectrum beta-lactamase/beta-lactamase producing *Escherichia coli* in healthy organized Vis-a-Vis backyard farmed pigs in India, Infect. Genet. Evol. 36 (2015) 224–230, https://doi.org/10.1016/j.meegid.2015.09.021.
[28] T. Goudar, S. Desai, M. Basappa, Strategies and recent advances in tackling antibacterial resistance in India: a comprehensive narrative review, Cureus 17 (2025) e95613, https://doi.org/10.7759/cureus.95613.
[29] B. Sihombing, R. Bhatia, R. Srivastava, T.Y. Aditama, R. Laxminarayan, S. Rijal, Response to antimicrobial resistance in South-East Asia region, Lancet Reg Health Southeast Asia. 18 (2023) 100306, https://doi.org/10.1016/j.lansea.2023.100306.
[30] T.P. Van Boeckel, J. Pires, R. Silvester, C. Zhao, J. Song, N.G. Criscuolo, M. Gilbert, S. Bonhoeffer, R. Laxminarayan, Global trends in antimicrobial resistance in animals in low- and middle-income countries, Science 365 (2019) eaaw1944, https://doi.org/10.1126/science.aaw1944.
[31] S.F. Koya, S. Ganesh, S. Selvaraj, V.J. Wirtz, S. Galea, P.C. Rockers, Consumption of systemic antibiotics in India in 2019, Lancet Reg. Health Southeast Asia 4 (2022) 100025, https://doi.org/10.1016/j.lansea.2022.100025.
[32] J.C. Arnold, D. Day, M. Hennessey, P. Alarcon, M. Gautham, I. Samanta, A. Mateus, If it works in people, why not animals?: a qualitative investigation of antibiotic use in smallholder livestock settings in rural West Bengal, India, Antibiotics (Basel). 10 (2021) 1433, https://doi.org/10.3390/antibiotics10121433.
[33] R. Kajitani, D. Yoshimura, Y. Ogura, Y. Gotoh, T. Hayashi, T. Itoh, Platanus_B: an accurate de novo assembler for bacterial genomes using an iterative error-removal process, DNA Res. 27 (2020) dsaa014, https://doi.org/10.1093/dnares/dsaa014.
[34] T. Seemann, Prokka: rapid prokaryotic genome annotation, Bioinformatics 30 (2014) 2068–2069, https://doi.org/10.1093/bioinformatics/btu153.
[35] D.H. Parks, M. Imelfort, C.T. Skennerton, P. Hugenholtz, G.W. Tyson, CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes, Genome Res. 25 (2015) 1043–1055, https://doi.org/10.1101/gr.186072.114.

[36] C. Jain, R.L. Rodriguez, A.M. Phillippy, K.T. Konstantinidis, S. Aluru, High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries, Nat. Commun. 9 (2018) 5114, https://doi.org/10.1038/s41467-018-07641-9.

[37] A.J. Page, C.A. Cummins, M. Hunt, V.K. Wong, S. Reuter, M.T. Holden, M. Fookes, D. Falush, J.A. Keane, J. Parkhill, Roary: rapid large-scale prokaryote pan genome analysis, Bioinformatics 31 (2015) 3691–3693, https://doi.org/10.1093/bioinformatics/btv421.

[38] T.H. Hazen, J.M. Michalski, S.M. Tennant, D.A. Rasko, Genomic diversity of non-diarrheagenic fecal *Escherichia coli* from children in sub-Saharan Africa and South Asia and their relatedness to diarrheagenic *E. Coli*, Nat. Commun. 14 (2023) 1400, https://doi.org/10.1038/s41467-023-36337-y.

[39] T. Mehrotra, D. Konar, A.K. Pragasam, S. Kumar, P. Jana, P. Babele, D. Paul, A. Purohit, S. Tanwar, S. Bakshi, S. Das, J. Verma, D. Talukdar, L. Narendrakumar, A. Kothidar, S.P. Karmakar, S. Chaudhuri, S. Pal, K. Jain, C.V. Srikanth, M. J. Sankar, K. Atmakuri, R. Agarwal, R. Gaind, M. Ballal, N. Kammili, R.K. Bhadra, T. Ramamurthy, G.B. Nair, B. Das, Antimicrobial resistance heterogeneity among multidrug-resistant gram-negative pathogens: phenotypic, genotypic, and proteomic analysis, Proc. Natl. Acad. Sci. USA 120 (2023) e2305465120, https://doi.org/10.1073/pnas.2305465120.

[40] Y. Arimizu, Y. Kirino, M.P. Sato, K. Uno, T. Sato, Y. Gotoh, F. Auvray, H. Brugere, E. Oswald, J.G. Mainil, K.S. Anklam, D. Dopfer, S. Yoshino, T. Ooka, Y. Tanizawa, Y. Nakamura, A. Iguchi, T. Morita-Ishihara, M. Ohnishi, K. Akashi, T. Hayashi, Y. Ogura, Large-scale genome analysis of bovine commensal *Escherichia coli* reveals that bovine-adapted *E. Coli* lineages are serving as evolutionary sources of the emergence of human intestinal pathogenic strains, Genome Res. 29 (2019) 1495–1505, https://doi.org/10.1101/gr.249268.119.

[41] J. Beghain, A. Bridier-Nahmias, H. Le Nagard, E. Denamur, O. Clermont, ClermonTyping: an easy-to-use and accurate in silico method for *Escherichia* genus strain phylotyping, Microb Genom. 4 (2018), https://doi.org/10.1099/mgen.0.000192.

[42] T. Wirth, D. Falush, R. Lan, F. Colles, P. Mensa, L.H. Wieler, H. Karch, P.R. Reeves, M.C. Maiden, H. Ochman, M. Achtman, Sex and virulence in *Escherichia coli*: an evolutionary perspective, Mol. Microbiol. 60 (2006) 1136–1151, https://doi.org/10.1111/j.1365-2958.2006.05172.x.

[43] Z. Zhou, N.F. Alikhan, K. Mohamed, Y. Fan, G. Agama Study, M. Achtman, The EnteroBase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core genomic diversity, Genome Res. 30 (2020) 138–152, https://doi.org/10.1101/gr.251678.119.

[44] M. Feldgarden, V. Brover, N. Gonzalez-Escalona, J.G. Frye, J. Haendiges, D.H. Haft, M. Hoffmann, J.B. Pettengill, A.B. Prasad, G.E. Tillman, G.H. Tyson, W. Klimke, AMRFinderPlus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence, Sci. Rep. 11 (2021) 12728, https://doi.org/10.1038/s41598-021-91456-0.

[45] A.J. Page, B. Taylor, A.J. Delaney, J. Soares, T. Seemann, J.A. Keane, S.R. Harris, SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments, Microb. Genom. 2 (2016) e000056, https://doi.org/10.1099/mgen.0.000056.

[46] A.M. Kozlov, D. Darriba, T. Flouri, B. Morel, A. Stamatakis, RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference, Bioinformatics 35 (2019) 4453–4455, https://doi.org/10.1093/bioinformatics/btz305.

[47] I. Letunic, P. Bork, Interactive tree of life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool, Nucleic Acids Res. 52 (2024) W78–W82, https://doi.org/10.1093/nar/gkae268.

[48] J. Marin, O. Clermont, G. Royer, M. Mercier-Darty, J.W. Decousser, O. Tenaillon, E. Denamur, F. Blanquart, The population genomics of increased virulence and antibiotic resistance in human commensal *Escherichia coli* over 30 years in France, Appl. Environ. Microbiol. 88 (2022) e0066422, https://doi.org/10.1128/aem.00664-22.

[49] E. Karakaya, F. Aydin, T. Kayman, S. Abay, *Escherichia coli in different animal feces: phylotypes and virulence genes*, World J. Microbiol. Biotechnol. 39 (2022) 14, https://doi.org/10.1007/s11274-022-03451-w.

[50] H.P. Kaspersen, M.S. Brouwer, J. Nunez-Garcia, I. Cardenas-Rey, M. AbuOun, N. Duggett, N. Ellaby, J. Delgado-Blas, J.A. Hammerl, M. Getino, C. Serna, T. Naas, K.T. Veldman, A. Bossers, M. Sunde, S.S. Mo, S.B. Jorgensen, M. Ellington, B. Gonzalez-Zorn, R. La Ragione, P. Glaser, M.F. Anjum, *Escherichia coli from six European countries reveals differences in profile and distribution of critical antimicrobial resistance determinants within one health compartments, 2013 to 2020*, Euro Surveill. 29 (2024) 2400295, https://doi.org/10.2807/1560-7917.ES.2024.29.47.2400295.

[51] N.M. Thomson, R. Gilroy, M. Getino, E. Foster-Nyarko, A.H.M. van Vliet, R.M. La Ragione, M.J. Pallen, Remarkable genomic diversity among *Escherichia* isolates recovered from healthy chickens, PeerJ 10 (2022) e12935, https://doi.org/10.7717/peerj.12935.

[52] M. Milenkov, C. Proux, T.L. Rasolofoarison, F.A. Rakotomalala, S. Rasoanandrasana, V.L. Rahajamanana, C. Rafalimanana, Z. Ravaoarisaina, I. T. Ramahatafandry, E. Westeel, M. Petitjean, V. Berti, J. Marin, J. Mullaert, L. Han, O. Clermont, L. Raskine, H. Endtz, A. Andremont, E. Denamur, F. Komurian-Pradel, L.H. Samison, L. Armand-Lefevre, Implementation of the WHO tricycle protocol for surveillance of extended-spectrum beta-lactamase producing *Escherichia coli* in humans, chickens, and the environment in Madagascar: a prospective genomic epidemiology study, Lancet Microbe 5 (2024) 100850, https://doi.org/10.1016/S2666-5247(24)00065-X.

[53] P. Musicha, M.A. Beale, D. Cocker, F.A. Oruru, A. Zuza, C. Salifu, G. Katende, S. Nanono, F. Isaasi, K. Chidziwisano, L. Mugisha, H. Kajumbula, D. Musoke, T. Morse, S.T. Jacob, N.A. Feasey, N.R. Thomson, Transmission of extended spectrum beta-lactamase-producing *Escherichia coli* and antimicrobial resistance gene flow across one health compartments in eastern Africa: a whole-genome sequence analysis from a prospective cohort study, Lancet Microbe. 7 (2026) 101224, https://doi.org/10.1016/j.lanmic.2025.101224.

[54] E.K. Rousham, L. Unicomb, M.A. Islam, Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and One Health approaches, Proc. Biol. Sci. 285 (2018), https://doi.org/10.1098/rspb.2018.0332.

[55] A. Hesp, K. Veldman, J. van der Goot, D. Mevius, G. van Schaik, Monitoring antimicrobial resistance trends in commensal *Escherichia coli* from livestock, the Netherlands, 1998 to 2016, Euro Surveill. 24 (2019) 1800438, https://doi.org/10.2807/1560-7917.ES.2019.24.25.1800438.

[56] H. Malik, R. Singh, S. Kaur, P. Dhaka, J.S. Bedi, J.P.S. Gill, G. Gongal, Review of antibiotic use and resistance in food animal production in WHO South-East Asia region, J. Infect. Public Health 16 (Suppl. 1) (2023) 172–182, https://doi.org/10.1016/j.jiph.2023.11.002.