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Authors

Nadimpalli, Maya

Stegger, Marc

Viau, Roberto

et al.

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Plugging the leaks: antibiotic resistance at human–animal interfaces in low-resource settings

Maya L Nadimpalli[1,2,*], **Marc Stegger**[3,4,5], **Roberto Viau**[2,6], **Vuthy Yith**[7], **Agathe de Lauzanne**[8], **Nita Sem**[7], **Laurence Borand**[8], **Bich-tram Huynh**[9,10], **Sylvain Brisse**[11], **Virginie Passet**[11], **Søren Overballe-Petersen**[3], **Maliha Aziz**[4], **Malika Gouali**[7,12], **Jan Jacobs**[13,14], **Thong Phe**[15], **Bruce A Hungate**[16,17], **Victor O Leshyk**[16,17], **Amy J Pickering**[2,18], **François Gravey**[19,20], **Cindy M Liu**[4], **Timothy J Johnson**[21], **Simon Le Hello**[12,19,20], **Lance B Price**[4]

[1]Gangarosa Department of Environmental Health, Emory Rollins School of Public Health, Atlanta, GA ;

[2]Stuart B Levy Center for Integrated Management of Antimicrobial Resistance, Tufts University, Boston, MA;

[3]Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark;

[4]Department of Environmental and Occupational Health, Milken Institute School of Public Health, The George Washington University, Washington, DC;

[5]Antimicrobial Resistance and Infectious Diseases Laboratory, Harry Butler Institute, Murdoch University, Perth, Australia;

[6]Department of Geographic Medicine and Infectious Diseases, Tufts Medical Center, Boston, MA;

[7]Laboratory of Environment and Food Safety, Institut Pasteur in Cambodia, Phnom Penh, Cambodia;

[8]Epidemiology and Public Health Unit, Institut Pasteur in Cambodia, Phnom Penh, Cambodia;

[9]Université Paris-Saclay, UVSQ, Inserm, Anti-Infective Evasion and Pharmacoepidemiology Team, CESP, Montigny le Bretonneux, France;

[10]UMR 1181, Inserm, University of Versailles Saint-Quentin-en-Yvelines, Saint-Quentin-en-Yvelines, France;

[11]Institut Pasteur, Université Paris Cité, Biodiversity and Epidemiology of Bacterial Pathogens, Paris, France;

[12]Enteric Bacterial Pathogens Unit, Institut Pasteur, Paris, France;

[13]Institute of Tropical Medicine, Antwerp, Belgium;

[14]Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium;

[15]Sihanouk Hospital Center for Hope, Phnom Penh, Cambodia;

[*](maya.l.nadimpalli@emory.edu);.

[16]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ;

[17]Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ;

[18]Department of Civil and Environmental Engineering, University of California–Berkeley, Berkeley, CA;

[19]Université de Caen Normandie, Université de Rouen Normandie, Inserm, DYNAMICURE UMR 1311, CHU Caen, Caen, France;

[20]Department of Bacteriology, CHU Caen, Caen, France;

[21]Department of Veterinary and Biomedical Sciences, University of Minnesota, St Paul, MN

Abstract

Antibiotic resistance is one of the greatest public health challenges of our time. International efforts to curb resistance have largely focused on drug development and limiting unnecessary antibiotic use. However, in areas where water, sanitation, and hygiene infrastructure is lacking, we propose that bacterial flow between humans and animals can exacerbate the emergence and spread of resistant pathogens. Here, we describe the consequences of poor environmental controls by comparing mobile resistance elements among *Escherichia coli* recovered from humans and meat in Cambodia, a middle-income country with substantial human–animal connectivity and unregulated antibiotic use. We identified identical mobile resistance elements and a conserved transposon region that were widely dispersed in both humans and animals, a phenomenon rarely observed in high-income settings. Our findings indicate that plugging leaks at human–animal interfaces should be a critical part of addressing antibiotic resistance in low- and especially middle-income countries.

Antibiotic resistance is one of the top threats facing humanity (WHO 2021). Antibiotic-resistant bacterial infections are already responsible for nearly 1.3 million deaths globally each year (ARC 2022), and some estimates suggest annual deaths from antimicrobial-resistant infections could reach 10 million by 2050 if this crisis remains unaddressed (O'Neill 2016). Due to a higher infectious disease burden, widespread informal antibiotic use, and limited resources for controlling antibiotic-resistant infections (Bebell and Muiru 2014; Klein *et al*. 2018), low- and middle-income countries (LMICs) are projected to experience the greatest mortality and economic fallout from this looming crisis (O'Neill 2016).

To address this challenge, global public health organizations are collaborating with countries to develop "One Health"-oriented national action plans aimed at improving antibiotic stewardship in both humans and animal production (WHO 2016; World Bank Group 2019). To date, the importance of environmental controls at the human–animal interface has not received the same level of attention as antibiotic use. This lack of prioritization may be due to high-income country bias, as studies from the UK (Day *et al*. 2019) and the EU (Mughini-Gras *et al*. 2019; Martak *et al*. 2021) have identified only minimal sharing of antibiotic-resistant bacteria between humans and food animals. However, the situation could be vastly different in low- and especially middle-income countries, where environmental controls at the human–animal interface are often deficient or lacking altogether (Figure 1).

Water, sanitation, and hygiene (WASH) in human communities and in food animal production – including on farms, in markets, and along the value chain – aim to control the flow of pathogens in and out of these systems. In settings where humans and food animals live in close proximity, the absence or breakdown of these controls – hereafter referred to as "leakiness" – has profound implications: the continuous, circular exchange of resistant bacteria selected through unregulated antibiotic use may facilitate the exchange of resistance genes among bacteria in both sectors. To our knowledge, leakiness as a potentiator of global antibiotic resistance has not been widely considered by the scientists and public health agencies who are driving policy and funding priorities in this field. Here, we provide evidence for the consequences of leakiness by describing the spread of mobile resistance elements between humans and animals in Phnom Penh, Cambodia, a highly leaky urban center in Southeast Asia.

Insufficient environmental controls and unregulated antibiotic use in Cambodia

Southeast Asia is a hotspot for emerging zoonotic infectious diseases such as highly pathogenic avian influenza H5N1, severe acute respiratory syndrome (SARS-CoV), and Nipah virus, all of which have emerged in the past few decades (Coker *et al.* 2011). Cambodia is among the poorest countries in Southeast Asia, and 76% of the estimated 16 million residents live in rural areas (NIS 2018). As in other Southeast Asian countries, Cambodian livestock production is rapidly increasing to support growing demand. Between 2012 and 2016, domestic poultry production increased by 53%, to 35.7 million chickens per year, and pig production by 34%, to 2.9 million pigs per year (MAFF 2017), with meat demand projected to increase by another 20% by 2024 (Sen 2018). While commercial livestock production has nearly doubled during the past decade (MAFF 2017), at least 80% of livestock continues to be raised by resource-constrained households in rural areas (MAFF 2017; NIS 2018). This distribution of livestock production between commercial farms and small-scale, household farms is similar in many LMICs in Asia and sub-Saharan Africa (Ikhimiukor *et al.* 2022).

WASH conditions and animal husbandry practices in Cambodia suggest ample opportunity for bidirectional exchange of fecal–oral pathogens between humans and livestock (Figures 1 and 2). First, Cambodia has the highest rate of open human defecation in the region (UNICEF Cambodia 2019), with 26.2% of rural residents and 7.7% of urban residents outside the capital continuing to defecate in fields or other open spaces (NIS 2018). Because most livestock raised in rural and urban households are allowed to roam freely during the day, these animals (especially pigs) may consume human feces (Sikasunge *et al.* 2007; Thomas *et al.* 2013). Second, nearly half of livestock-owning households report dumping untreated animal manure directly into the environment (Ström *et al.* 2018a), suggesting that untreated drinking water may be a source of animal fecal bacteria. Cambodia has the lowest access to piped drinking water in Southeast Asia, and nearly 1 in 5 urban residents outside the capital and 1 in 2 rural residents cannot reliably access clean water (NIS 2018). Third, poor WASH practices along the food supply chain in Cambodia, especially in antiquated slaughterhouses (Tum 2015) and crowded live-animal markets, facilitate the contamination

of meat and produce by animal and human fecal bacteria. Finally, such behaviors as using untreated animal manure to fertilize crops for human consumption (Ström *et al.* 2018a), allowing animals to enter food preparation areas (Osbjer *et al.* 2015), not removing manure from the household environment (Atterby *et al.* 2019), handling slaughtering byproducts (Atterby *et al.* 2019), and eating undercooked chicken and fish (Osbjer *et al.* 2015; Nadimpalli *et al.* 2019) likely exacerbate the spread of zoonotic pathogens. With so many leaks between human communities and animal production systems, antibiotic use in both sectors could lead to the emergence and spread of antibiotic-resistant bacteria.

We compared published lists of antibiotics commonly used in human medicine and food animal production in Southeast Asia and found substantial overlap. Antibiotics administered to chickens and pigs included all antibiotics that are commonly used in human medicine, including third-generation cephalosporins, penicillins, fluoroquinolones, gentamicin, and co-trimoxazole (Carrique-Mas *et al.* 2015; Om *et al.* 2017; Ström *et al.* 2018b). Farmers in Cambodia reported purchasing human antibiotics for their animals when veterinary drugs were ineffective (Ström *et al.* 2018b), and antibiotics that are critically important for human medicine (eg colistin) were used extensively in poultry production (Carrique-Mas *et al.* 2015; Coyne *et al.* 2019). Such practices mean that the same antibiotic resistance genes could confer a selective advantage in multiple hosts.

Pathogen and mobile resistance element overlap across humans and food animals in Cambodia

We examined overlap among human- and animal-origin extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, which are bacteria resistant to third-generation cephalosporins and other commonly used antibiotics (Panel 1). In Cambodia, gut colonization with ESBL-producing Enterobacterales has steadily increased over the past 20 years, and more than 90% of healthy community members may now be carrying these organisms in their gastrointestinal tract (Singh *et al.* 2020). This prevalence rate is markedly higher than what is observed in high-income countries (Bezabih *et al.* 2021) like the US (<5%; Islam *et al.* 2018), the UK (11%; Day *et al.* 2019), and Denmark (12%; Dall *et al.* 2019). Previously, we found that ESBL-producing *E coli* colonizing healthy community members in Phnom Penh were highly similar to strains recovered from meat and fish sold at markets (Nadimpalli *et al.* 2019). For example, one-third of human-origin ESBL-producing *E coli* strains encoded the same ESBL gene type ($bla_{CTX-M-55}$ gene) that predominated among *E coli* recovered from meat and fish. This sharply contrasts with findings from multiple high-income countries with robust WASH infrastructure in human communities and along the food value chain (Day *et al.* 2019; Mughini-Gras *et al.* 2019); for example, a recent study from the UK found only 5% of ESBL-producing *E coli* strains from healthy humans harbored ESBL genes that predominated among meat (Figure 3a; Day *et al.* 2019). Substantial leakiness between humans and animals in Cambodia, combined with similar antibiotic pressures in both reservoirs, could result in the synergistic amplification and mutual exchange of mobile resistance elements.

For this case study, we performed long-read DNA sequencing of ESBL-producing *E coli* of human and animal origin and leveraged existing *E coli* datasets to determine if strains from these two sources shared a common pool of mobile resistance elements. Mobile resistance elements contain repeat regions that are challenging to assemble using standard, short-read DNA sequencing technologies; using long-read sequencing allows for substantially more accurate characterization of these elements. First, we used long-read sequencing to assemble high-quality draft genomes of five ESBL-producing *E coli* isolates from the feces of healthy humans ($n = 2$) and from pork meat ($n = 2$) and chicken ($n = 1$) sold at Phnom Penh markets (WebPanel 1; WebTable 1; WebFigure 1). We identified the ESBL-encoding plasmids they harbored and annotated them. Next, we screened for these plasmids among previously published Cambodian collections of ESBL-producing *E coli* from healthy, gut-colonized humans (88 positive of 130 humans screened), clinical specimens ($n = 15$), and meat and fish from markets (93 positive of 150 samples screened) (WebTable 2).

We identified four distinct ESBL-encoding plasmids that were shared with slight variations among *E coli* isolates of both human and animal origin (WebPanel 2; WebFigure 2). One plasmid (pC27A-CTX-M-55) co-encoding resistance to third-generation cephalosporins, fluoroquinolones, phenicols, tetracyclines, sulfonamides, trimethoprim, and aminoglycosides was present in *E coli* from chicken meat ($n = 3$), fish ($n = 12$), pork meat ($n = 2$), and healthy humans ($n = 2$). A second plasmid (pP59A-CTX-M-55) co-encoding resistance to β-lactams, fluoroquinolones, macrolides, phenicols, sulfonamides, and aminoglycosides was prevalent among pork meat isolates ($n = 6$) and identified in a healthy human ($n = 1$). A third plasmid (pP225M-CTX-M-55) co-encoding resistance to third-generation cephalosporins, tetracyclines and aminoglycosides was primarily found among isolates from healthy humans ($n = 6$) but was also carried by *E coli* from a human urinary tract infection ($n = 1$) and from fish ($n = 1$). Finally, a fourth, novel plasmid type (pP276M-CTX-M-55) co-encoding resistance to third-generation cephalosporins, fluoroquinolones, phenicols, and tetracyclines was identified from a healthy human isolate ($n = 1$) and from chicken meat ($n = 1$). Although we only sequenced five ESBL-producing *E coli* isolates for this case study, which represent only a small fraction of the bacteria colonizing the guts of humans and food animals sampled in this setting, the multiple instances of overlap that we observed suggest that plasmid sharing among vertebrate hosts is not uncommon.

We then compared the four ESBL-encoding plasmids identified in this case study to determine whether the ESBL genes themselves shared a common origin. We identified a putative, bla_{CTX-M}-encoding transposon region that was integrated across three of the four plasmids (WebFigure 3). This ~6-kilobase transposon region, hereafter referred to as Tn*CTX-M/qnrS*, also encoded *qnrS1*, which confers low-level fluoroquinolone resistance. Among the three plasmids we sequenced, Tn*CTX-M/qnrS* was flanked by different combinations of insertion sequences (IS26/ISEcp1/ISKpn19). Recent studies have described this same region, flanked by other combinations of insertion sequences, among *E coli* and *Salmonella enterica* recovered from retail meats, food animals, healthy humans, and clinical specimens across China and Southeast Asia (Zhang *et al.* 2019; Li *et al.* 2021; Zeng *et al.* 2022). This suggests that Tn*CTX-M/qnrS* has been mobilized by diverse mobile resistance elements and is likely under high selection pressure.

Finally, we examined existing collections of ESBL-producing Enterobacterales from Cambodia as well as a global *Escherichia/Shigella* database (https://enterobase.warwick.ac.uk/species/index/ecoli) for the Tn*CTX-M/qnrS* region. The $bla_{CTX-M-55}$ and $bla_{CTX-M-15}$ variants of this transposon region, which differed by only a single nucleotide polymorphism, were highly prevalent among Enterobacterales, including *E coli*, *Klebsiella pneumoniae*, and *S enterica* from human and animal products in Cambodia (Figure 3b; WebFigure 4; WebTable 2). Several strains in our existing collections were recovered from the same meat samples (Nadimpalli *et al.* 2019); for seven of 20 samples that were co-contaminated with different ESBL-producing species, Tn*CTX-M/qnrS* was detected in all recovered species (WebTable 2). Examination of the global database revealed that the $bla_{CTX-M-15}$ variant was broadly dispersed, while the $bla_{CTX-M-55}$ variant was mainly identified in isolates from Asia, including Vietnam, Laos, Thailand, Taiwan, and China (WebPanel 2; WebTable 3). The mobilization of the $bla_{CTX-M-55}$ variant across different plasmids, bacterial lineages, and hosts confirms that bacterial exchanges among humans and animals are exceptionally frequent in this region. Our findings also underscore that in settings with high leakiness, the full scope of antibiotic resistance exchange between humans and animals cannot be quantified by measuring bacterial strain overlap alone; rather, the exchange of resistance-encoding elements across bacterial lineages must also be considered.

Rebalancing priorities to combat antibiotic resistance

There is no doubt that antibiotics are the greatest selective force for the emergence of new antibiotic-resistant bacteria (Lipsitch and Samore 2002); however, poor environmental controls may be critically overlooked potentiators for such strains. In this case study, detection of the same mobile resistance elements across multiple vertebrate hosts indicates that antibiotic stewardship alone may be insufficient to stem the growing problem of antibiotic resistance in resource-poor settings. Although our analysis was limited to Cambodia, recent surveillance studies in several LMICs in Asia (Tansawai *et al.* 2019; Huang *et al.* 2020), South America (Murray *et al.* 2021), and Africa (Falgenhauer *et al.* 2019; Muloi *et al.* 2022) have also reported overlapping ESBL gene types among Enterobacterales from humans and food animals. Although most of these studies did not perform resource-intensive long-read sequencing to characterize the mobile resistance elements harboring these ESBL genes, as we did here, such consistent findings – again, in stark contrast to high-income settings – suggest that dissemination of mobile resistance elements across hosts is not unique to Cambodia or Southeast Asia. Overall, these data indicate that antibiotic stewardship efforts in LMICs must be complemented by implementing environmental controls at the human–animal interface (that is, "plugging the leaks"). In addition to stemming the circular flow of antibiotic-resistant bacteria, infrastructural WASH improvements in human communities and food animal production have the potential to also reduce disease and consequently antibiotic demand in both humans and animals (Pickering *et al.* 2019).

Higher demand for animal-based protein in low- and especially middle-income countries suggests that the need to plug leaks is becoming increasingly urgent. Specifically, as these countries develop, these dietary changes are driving up food animal production and concomitant antibiotic use (Van Boeckel *et al.* 2015). As a result, several countries that

lack universal access to clean water and sanitation are expected to double their antibiotic use in animal production over the next 10 years (Van Boeckel *et al.* 2015). Currently, less than 4% of funding through the Joint Programming Initiative for Antimicrobial Resistance (https://www.jpiamr.eu) is allocated toward identifying cost-effective environmental controls that curb microbial exchange (World Bank Group 2019). Furthermore, of the 70-plus countries that have developed national antimicrobial resistance action plans in collaboration with global public health organizations, nearly all have omitted commitments to community-based WASH infrastructure (Essack 2021). This lack of prioritization may reflect a high-income country bias among scientists and policy makers who accept the generalizability of research conducted in settings with strong environmental controls, where the exchange of mobile resistance elements between food animals and the broader community is limited (Day *et al.* 2019; Mughini-Gras *et al.* 2019; Martak *et al.* 2021).

Conclusion

Overall, in settings with pervasive leakiness between humans and animals, a rebalancing of policy and funding priorities is needed if the efficacy of new and existing antibiotics is to be preserved. The case study presented here strongly suggests that the lack of environmental controls along with widespread antibiotic use is leading to the exchange of resistance elements and the evolution of antibiotic-resistant human pathogens in Southeast Asia. Additional studies are necessary to determine the impacts of leakiness on the dissemination of antibiotic resistance in other settings and to ascertain which leaks are the most critical and most cost-effective to plug. We propose that evaluating and implementing WASH initiatives in both the community setting and food animal production should be prioritized along with antibiotic stewardship to address the antibiotic-resistance crisis in resource-poor settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Data Availability Statement

The accession numbers for the five *Escherichia coli* draft genomes presented in this study are available in the National Center for Biotechnology Information's GenBank (https://www.ncbi.nlm.nih.gov/genbank) under Bioproject number PRJNA566431. The accession numbers for the Illumina sequences generated from the ESBL-producing *E coli* ($n = $ 196), *Salmonella enterica* ($n = 26$), and *Klebsiella pneumoniae* ($n = 8$) isolates described in this study are available in the European Nucleotide Archive (https://www.ebi.ac.uk/ena) under accession numbers PRJEB25898, PRJEB27759, and PRJEB34504, respectively.

Newick tree files used to create Figure 3b and WebFigure 4 are available at https://doi.org/ 10.15139/S3/3OMUI2.

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Panel 1.

The importance of ESBL-producing Enterobacterales

- Third-generation cephalosporins are broad-spectrum antibiotics that are generally well-tolerated, affordable, and accessible.

- Bacteria that produce ESBL enzymes can degrade third-generation cephalosporins, leading to treatment failure.

- Genes encoding ESBL enzymes spread among bacteria via mobile resistance elements.

- Enterobacterales like *Escherichia coli* and *Klebsiella pneumoniae* have increasingly acquired these genes and now cause >50,000 deaths per year globally (ARC 2022).

- ESBL-producing *E coli* are used as "indicator organisms" by the World Health Organization to gauge the magnitude and interconnectedness of antibiotic resistance between humans, animals, and the environment, as well as the effectiveness of potential interventions.

In a nutshell:

- Antibiotic resistance is an ecological challenge, with low- and middle-income countries bearing the greatest burden

- These countries have many unique interfaces at which bacteria and their resistance-encoding elements can be exchanged between humans and food animals – including on farms, at markets, and along food value chains – that are minimized or non-existent in high-income settings

- We describe evidence for the consequences of poor environmental controls at these interfaces on the bidirectional spread of antibiotic resistance between humans and animals in Cambodia

- Implementing water, sanitation, and hygiene infrastructure in human communities and food animal production must be prioritized to address global antibiotic resistance
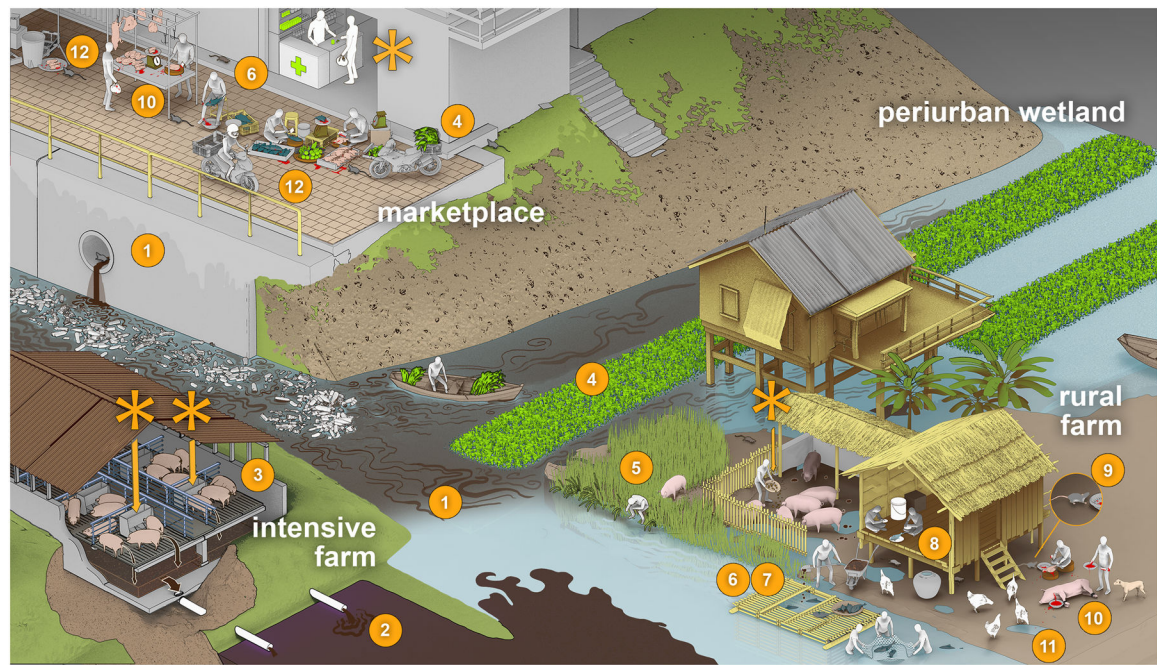
Figure 1.

Unchecked environmental leaks and antibiotic use create myriad opportunities for the exchange of bacteria and mobile resistance elements among humans and animals. Potential transmission pathways include (1) inadequate sewage treatment infrastructure leads to contamination of human and animal drinking water; (2) animal waste contaminates drinking water; (3) raising many animals in confinement propagates disease; (4) raw sewage fertilizing crops for human consumption; (5) open defecation leads to fecal–oral transmission among humans and animals; (6) contact with animal waste; (7) farmed fish are fed pig and poultry feces; (8) consumption of undercooked chicken and fish; (9) pests contaminate food preparation areas; (10) meat contaminated by feces during processing; (11) multiple species in contact in unhygienic conditions; and (12) poor hygiene in markets. Layout stylized to indicate connectivity. Asterisks indicate antibiotic inputs.
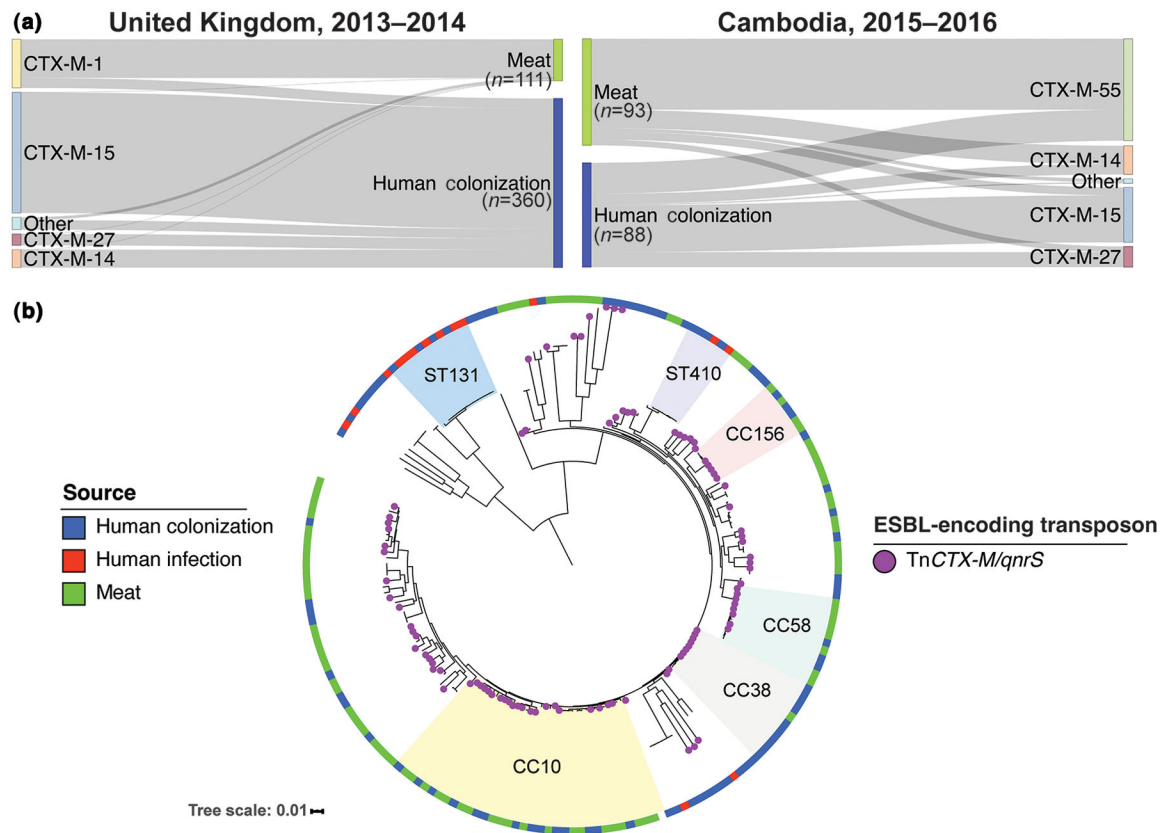
Figure 2.
Frequent mixing of human- and animal-adapted microbes creates opportunities for the exchange of antibiotic resistance at multiple scales (ie of bacteria, plasmids, and transposable elements).

Figure 3.
High antibiotic use and environmental leakiness is associated with the mobilization of an extended-spectrum β-lactamase (ESBL)-encoding transposon region across different bacterial lineages and hosts in Cambodia. (a) Sankey diagram depicting the flow of ESBL genes among *Escherichia coli* from human and animal origin. ESBL genes flow freely among human and animal-origin *E coli* in the leaky Cambodian ecosystem but are largely isolated to specific hosts in the less leaky UK ecosystem. (b) Core-genome phylogenetic tree depicting diverse ESBL-encoding *E coli* lineages circulating in Cambodia. A bla_{CTX-M}-encoding transposon region has been acquired by multiple *E coli* sub-lineages from humans and meat, underscoring rampant genetic exchange in a highly leaky system with substantial antibiotic selective pressure. Tree is mid-point rooted and scale indicates substitutions per site.