nature reviews genetics

https://doi.org/10.1038/s41576-023-00649-y

Review article

Check for updates

Genomic surveillance for antimicrobial resistance — a One Health perspective

Steven P. Djordjevic[1,2] ✉, Veronica M. Jarocki[1,2], Torsten Seemann[3,4], Max L. Cummins[1,2], Anne E. Watt[4], Barbara Drigo[5,6], Ethan R. Wyrsch[1,2], Cameron J. Reid[1,2], Erica Donner[6,7] & Benjamin P. Howden[3,4]

Abstract

Antimicrobial resistance (AMR) — the ability of microorganisms to adapt and survive under diverse chemical selection pressures — is influenced by complex interactions between humans, companion and food-producing animals, wildlife, insects and the environment. To understand and manage the threat posed to health (human, animal, plant and environmental) and security (food and water security and biosecurity), a multifaceted 'One Health' approach to AMR surveillance is required. Genomic technologies have enabled monitoring of the mobilization, persistence and abundance of AMR genes and mutations within and between microbial populations. Their adoption has also allowed source-tracing of AMR pathogens and modelling of AMR evolution and transmission. Here, we highlight recent advances in genomic AMR surveillance and the relative strengths of different technologies for AMR surveillance and research. We showcase recent insights derived from One Health genomic surveillance and consider the challenges to broader adoption both in developed and in lower- and middle-income countries.

Sections

[1]Australian Institute for Microbiology and Infection, University of Technology Sydney, Sydney, New South Wales, Australia. [2]Australian Centre for Genomic Epidemiological Microbiology, University of Technology Sydney, Sydney, New South Wales, Australia. [3]Centre for Pathogen Genomics, University of Melbourne, Melbourne, Victoria, Australia. [4]Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology and Immunology, University of Melbourne at the Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia. [5]UniSA STEM, University of South Australia, Adelaide, South Australia, Australia. [6]Future Industries Institute, University of South Australia, Adelaide, South Australia, Australia. [7]Cooperative Research Centre for Solving Antimicrobial Resistance in Agribusiness, Food, and Environments (CRC SAAFE), Adelaide, South Australia, Australia. ✉e-mail: steven.djordjevic@uts.edu.au

Introduction

Antimicrobial resistance (AMR) can occur when microorganisms acquire genetic information, either by mutation, recombination or horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs) from the bacterial gene pool (Fig. 1). These genetic acquisitions enable bacteria to survive antimicrobial selection pressures, including antimicrobial residues, metals, biocides, and industrial and pharmaceutical chemicals, and renders pharmacological agents used to constrain or kill pathogens partially or completely ineffective. In some cases, antimicrobial selection pressures can select and promote multiple drug resistance (MDR), defined as resistance to three or more classes of antibiotics, in a single genetic exchange[1–4]. Sometimes, resistance genes can reside in bacteria that do not cause disease (such as commensal bacteria) and be passed to pathogenic bacterial hosts; thus, healthy humans and animals and environmental populations of bacteria can be carriers of ARGs. Although AMR develops naturally in bacteria, MDR has increased markedly since the advent and widespread use of antibiotics for the treatment of human, animal and plant diseases. Resistance, often MDR, is increasing in hospital and community-acquired bacterial infections and in intensive animal production systems globally[5–8] (Box 1).

AMR is a critical public and global health challenge that is recognized by the scientific community, water and agricultural industries, and regulators as a health, food security and environmental pollution issue (Fig. 2). To address this challenge, population-level surveillance programmes have been established worldwide that aim to collect data on the prevalence of resistant organisms and antimicrobial prescribing practices. These surveillance programmes typically collect phenotypic, genotypic and, increasingly, genomic data from healthcare activities in both the community and hospitals across wide geographical areas and time periods[9]. Isolated studies have drawn inferences regarding AMR transmission dynamics from bacterial genomic data collected from humans and wildlife[10,11] and from humans and food production animals[12]. Although effective phenotypic AMR surveillance remains important and phenotypic resistance testing can guide antibiotic treatment choice in clinical and veterinary settings, genetic and genomic approaches have become increasingly important in AMR surveillance and are essential to understand how infectious agents, and the mobile genetic elements (MGEs) that carry ARGs, move in clinical, veterinary, agricultural and environmental spheres. Although far from complete, knowledge of microbial resistomes is rapidly expanding, as bioinformatic methods are used to identify latent ARGs in human, animal and environmental microbiomes and curate them in searchable reference databases[13]. Bolstered by the successful application of genomics during the COVID-19 pandemic, there has been accelerated development and adoption of genomic technologies for AMR surveillance, including rapid advancements in the fields of long-read metagenomics, phylodynamics and artificial intelligence-based surveillance systems. These recent developments can enhance the integrated surveillance of AMR across multiple sectors (that is, human, animal and environmental sectors) for One Health AMR surveillance (Fig. 2).

Here, we review the role of genomics in AMR surveillance and how to better use these technologies to mitigate the spread of AMR and improve planetary health outcomes. We shine a spotlight on *Escherichia coli* and its significance to and utility for implementing One Health AMR surveillance.

Genomics toolbox for studying AMR

Increasingly, systems for monitoring AMR seek to incorporate genomic surveillance. Compared with phenotypic surveillance of AMR, which involves culture-based isolation of the microorganism and testing for antibiotic activity in vitro, whole-genome sequencing (WGS) of microorganisms offers higher resolution, more precise characterization of MDR and greater scope for analysing mechanisms, tracing and forecasting trends in AMR[14]. Phenotypic susceptibility to an antibiotic does not reveal the many different types of genetic elements (for example, mutations, plasmids, insertion sequences, integrons, transposons, virulence and metabolic determinants) that can contribute to AMR dissemination and evolution, and neither does it reveal co-resistances such as those to heavy metals or disinfectants. Because genomics can service targeted and untargeted detection and monitoring requirements, it can be applied to both isolated microorganisms and samples of whole microbial communities taken from a range of environments. Sequencing of genomes from individual microbial strains that have been isolated from samples and then cultured is known as microbial genomics. Sequencing entire microbial communities involves the sequencing of all genetic material recovered directly from a sample without culturing and is known as metagenomics. Initially, the sequencing technology typically used for AMR genomic surveillance produced short-read sequences ~150 bp in length, which have the disadvantage of not being able to resolve repeated regions within an isolate genome or differentiate very similar parts of individual genomes within a metagenome, including ARGs. As the price of sequencing continues to drop and new sequencing technologies become established, long-read sequencing is becoming more feasible, which can overcome many, but not all, of the limitations of short-read sequencing. Examples are emerging of systematic genomic surveillance for monitoring AMR, such as routine genomics-enabled surveillance for carbapenemase-producing organisms in Australia[15].

Genomic detection of AMR through ARGs

Monitoring the mobilization, persistence and abundance of ARGs and mutations within and between microbial populations is a central activity for AMR research and enhanced surveillance. Strong correlation has been shown between the presence of an ARG within a microbial genome and phenotypic susceptibility to antimicrobials[16,17]. Although concordance between AMR phenotype and genotype does vary between species and antimicrobial agent combinations[18], identifying an ARG within a genomic sequence remains a useful predictor of resistance and has now reached the level of accuracy where genomic data are used routinely for AMR surveillance of pathogens such as *Salmonella* spp. in large public health agencies[19]. Critically, accurate determination of ARG carriage by in silico genotyping, and therefore phenotypic inferences derived from these data, depends on the quality of the ARG database being used. Many ARG databases and software tools for cataloguing and identifying ARGs in bacterial genomes have been developed[20,21]. However, available ARG databases and software vary in their content, quality and level of expertise required for interpretation and degree of validation. This has created discrepancies and may, potentially, in cases of naïve use, lead to misinformation in the literature[20,21]. This section will describe the landscape of ARG databases and software tools, current best practices in AMR genotyping and phenotypic inference, and key considerations in navigating the wealth of choice faced by AMR researchers. As genomic and phenotypic databases grow, machine learning and other statistical genomics techniques will have a greater role in identifying novel mechanisms of resistance[22], feeding back into enhanced surveillance activities.

The first choice to be made by AMR researchers is whether to use assembled or raw genome sequence data for matching with an

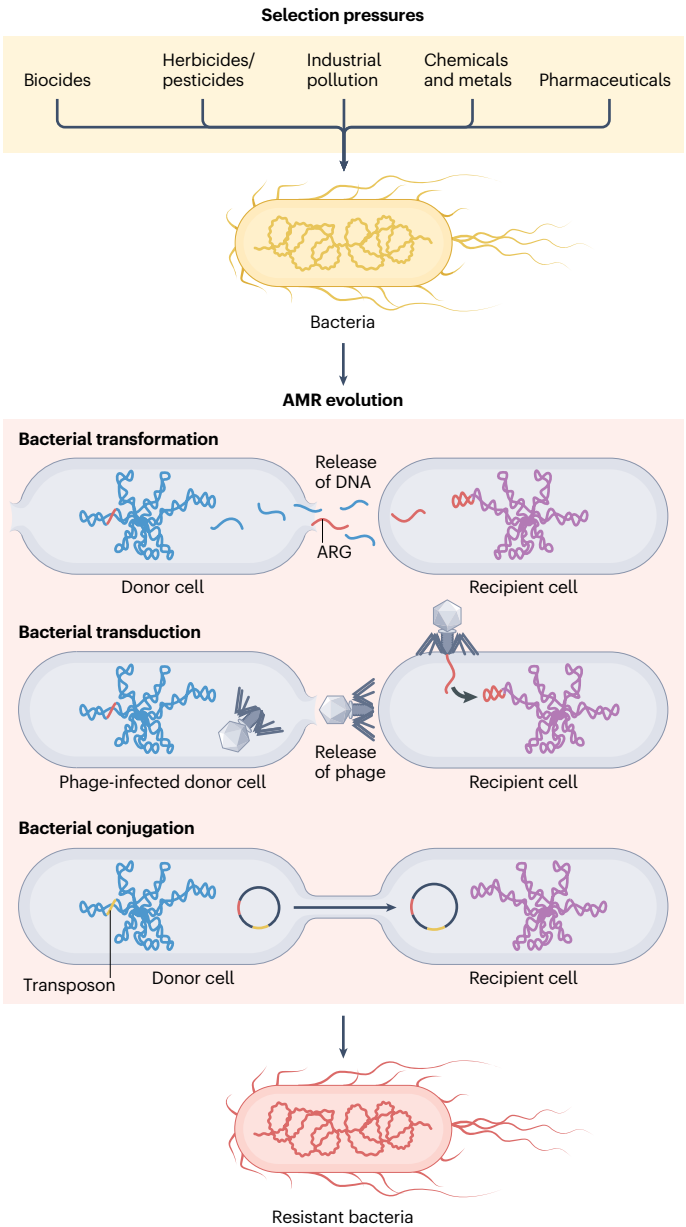

Fig. 1 | Development of antimicrobial resistance. In bacteria, antimicrobial selection pressures such as biocides, pesticides, air pollution, chemicals, metals and antibiotics influence three key mechanisms — transformation, transduction and conjugation — by which bacteria acquire and transfer genetic material, including antimicrobial resistance genes (ARGs). These processes contribute to the evolution and dissemination of antimicrobial resistance (AMR) in bacterial populations. Additionally, pre-existing resistance genes from diverse bacterial flora, aided by the action of insertion sequences, gene cassettes and transposons, can be transferred onto larger mobile genetic elements (MGEs), such as plasmids, enhancing their mobilization between bacterial cells.

ARG database (Fig. 3). For bacterial isolates, using de novo assembled genomes is preferred over raw sequence reads because doing so ensures the full genomic context is interrogated, including MGEs and chromosomes. For metagenomes, the choice to use assembled or raw sequence data depends on the expected quality of the metagenome-assembled genomes (MAGs) obtained. For extremely complex data sets (for example, high diversity associated with soil samples), it may not be possible to perform assembly due to computational constraints or to achieve a satisfactory assembly due to short-read limitations. This means that mapping-based approaches using raw sequencing reads are required instead. This approach can be fast but comes at the potential cost of confounding similar ARGs and not being able to link them to specific replicons. Continuous improvements in the quantity, quality and length of long-read sequencing methods will provide greater confidence in MAGs[23]. Techniques such as Hi-C may also improve the contiguity of MAGs[24] and provide direct molecular evidence of plasmid–chromosome linkage even with short-read data (Fig. 3), but at an increased financial and time cost, which currently would prohibit its use in routine surveillance.

Having chosen whether to use assembled or raw sequence genome data, the next choice is which tool and ARG database to use. There are three main databases, all of which now support identification of both ARGs and AMR mutations: ResFinder/PointFinder[25,26], CARD[27] and NCBI (National Center for Biotechnology Information)[28]. Both ResFinder and NCBI offer additional hidden Markov models such as ResFams. Although there have been efforts to combine these primary sources[29], this may not be necessary because in recent years there has been increasing coordination and sharing amongst the three main players, with initiatives such as hAMRonization from the PHA4GE Consortium providing a path to consistency and completeness. Where the databases differ is in the level and type of metadata. NCBI has strict audit trails back to publications with phenotypic and genotypic evidence and provides scoring cut-off thresholds to minimize false positives. CARD incorporates the Antimicrobial Resistance Ontology (ARO), a structured vocabulary for annotating its entries, which may aid machine learning approaches for interrogating the relationships within AMR surveillance projects.

ARG software provides the method and parameters to control how the genome data are matched to the database. It is common for the ARG tool and ARG database to be a bundle, but this does preclude the use of custom or combined databases. ResFinder uses a tool with the same name, CARD uses RGI (Resistance Gene Finder) and NCBI uses AMRFinderPlus. These three command line tools require assembled contigs as input and are well suited to bacterial isolates and good-quality metagenome assemblies. NCBI's AMRFinderPlus has emerged as the most thoroughly maintained tool in this space and has the added advantage of falling back to hidden Markov models for the ARG protein family to identify more remote ARG orthologues. This could also be done separately using ResFAMS[30], which consists of hidden Markov models derived from clustering and aligning CARD entries, but this is no longer maintained. For detecting AMR directly from raw sequence reads, the hybrid approach of ARIBA[31] can be used with short-read data where assembly is poor or difficult, or KMA[32] can be used to align reads efficiently to the highly redundant ARG database itself.

Although potentially more relevant in the clinical and public health laboratory setting, recent progress has been made in the validation of genome-based AMR phenotype inferences to meet International Organization for Standardization (ISO) certification standards[19]. These types of approaches allow highly reproducible prediction of clinically relevant AMR phenotypes from genomic data, and in this case demonstrate utility for *Salmonella* spp. using the AMRFinderPlus tool and the NCBI database. At this point, these approaches can only identify those resistance determinants that are in the databases, meaning there may

be limitations with unusual species or resistance mechanisms, and biases towards clinically relevant species.

Phylodynamics for AMR surveillance

AMR presents a constantly evolving threat. To understand and mitigate the impact of AMR it is thus insufficient to target only ARGs for genomic surveillance. The complexity of the AMR threat results, in part, from the inextricable linkage between the evolution, proliferation and transmission of ARGs, MGEs and their microbial hosts. A promising approach for gaining insights into this linkage is phylodynamics, which combines microbial epidemiological data with phylogenomic data to model and understand evolutionary histories and microbial ecology. It can be performed at different spatiotemporal scales and has the potential to enable greater insight than microbial epidemiology or phylogenomics alone. Phylodynamics can reveal, for instance, how highly resolved bacterial strains are spatially disseminated, how the prevalence of a genomic feature has changed over time and how the evolution of pathogenicity links to the evolution of AMR loci[33,34]. Although phylodynamic methods are mature for viruses, bacterial phylodynamics is an emerging field[35,36], because larger genomes lead to exponential increases in computation. Ultimately, the expectation is that phylodynamics can be incorporated into the genomic surveillance toolkit for analysis and data visualization (Fig. 3). The potential applications of this approach are reviewed by Ingle et al.[37], with an early example of its application applied to understanding outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA) in Papua New Guinea[34].

Microbial genomics and metagenomics for AMR surveillance

WGS and metagenomics have emerged as valuable tools for monitoring AMR in various environmental contexts. When applied to hospital wastewater and sewage, these techniques enable the identification of AMR genes, tracking their prevalence and monitoring the dissemination of resistance[38]. By analysing urban wastewater and sewage treatment plants, important information regarding AMR profiles, the abundance of resistant bacteria and genes in urban environments, and the effectiveness of sewage treatment in curbing AMR transmission can be obtained[39]. In agricultural settings, WGS and metagenomics enable the tracing of AMR transmission among animals, the environment and humans, thereby supporting efforts to promote responsible antibiotic use and prevent the spread of resistance[20]. Furthermore, the analysis of environmental samples from aquatic and terrestrial ecosystems using WGS and metagenomics provides valuable insights into the presence, diversity and sources of AMR dissemination, enhancing our understanding of resistance dynamics in the environment[40].

Both microbial genomics and metagenomics are powerful tools for AMR surveillance, but each has its own advantages and limitations.

Box 1

The growing global threat of antimicrobial resistance

The evolution of antimicrobial resistance (AMR) is a complex process sustained by many interconnecting factors, including the overuse and misuse of antibiotics, metals and disinfectants in medicine and agriculture, and widely varying standards of water, sanitation and hygiene[160]. These stressors can generate reactive oxygen species, interfere with DNA replication or inhibit cell wall synthesis, processes that are known to trigger the SOS system[161–163], a potent inducer of horizontal gene transfer (HGT)[164–166]. Various mechanisms can contribute to the dissemination of resistant bacteria and antibiotic resistance genes (ARGs), including waste streams[38–41,167,168], migratory, wild and urban-adapted animal species[10,119–122,169], international travel[170,171], food mobility and trade[64], prevailing winds[172,173], ship ballast water discharge[174,175], companion animals[176,177], insects[178] and manuring practices[179,180].

Faecal contamination plays a part in AMR dissemination through a One Health lens. Antibiotic consumption by humans continues to increase[181] but is outweighed, on a tonnage basis, by antibiotic usage in agriculture[182,183], where it is expected to rise based on current trajectories[184,185]. For many antibiotics, a considerable fraction of the administered dose is poorly absorbed and is, instead, excreted into wastewater and downstream environments as pharmaceutical waste[186]. If organic fertilizers such as manure are not appropriately managed, antibiotic residues and heavy metals (also selective agents for AMR) can be released into agricultural and aquatic environments, as can already antibiotic-resistant bacteria, where they form reservoirs of agents capable of exerting selective pressure for AMR[168,187]. In 2014, the total faecal mass, mostly generated by animal production, was estimated at 3.9×10^{12} kg per year[188]. Animal industries and regulators are increasingly aware of the issue, but recognition (and subsequent action) varies greatly across jurisdictions, and the ability and willingness to intervene and change practice also differs geographically.

Climate change is also predicted to have an impact on AMR, as it is expected to broaden the geographic locations to which diseases are currently restricted[189,190]. Moreover, higher temperatures have been linked to an increased incidence of pathogenic bacteria in humans and AMR infections[191] as well as a higher frequency of HGT[192]. Climate change is also linked to increasing incidence of extreme weather and associated events, such as floods, droughts and cyclones, all of which affect disease prevalence, outbreaks and other interlinked stresses, such as food and water insecurity, crop failure, malnourishment and migration[193]. Failures in water, sanitation and hygiene caused by such events will significantly impact on disease risk and, hence, antimicrobial use and resistance. This is true not only for humans but also for animals, and will thus increase the already significant challenge of improving antimicrobial stewardship in agribusiness and food production. The impact of climate change has been modelled for vector-borne, foodborne and zoonotic infections[190,194] and is also relevant to soil-borne and water-borne pathogens[195,196]. A systematic analysis using empirical examples of human pathogenic disease affected by climatic hazards sensitive to greenhouse gas emissions found that 58% (218 out of 375) of known human pathogenic diseases can be affected by climate change[197].

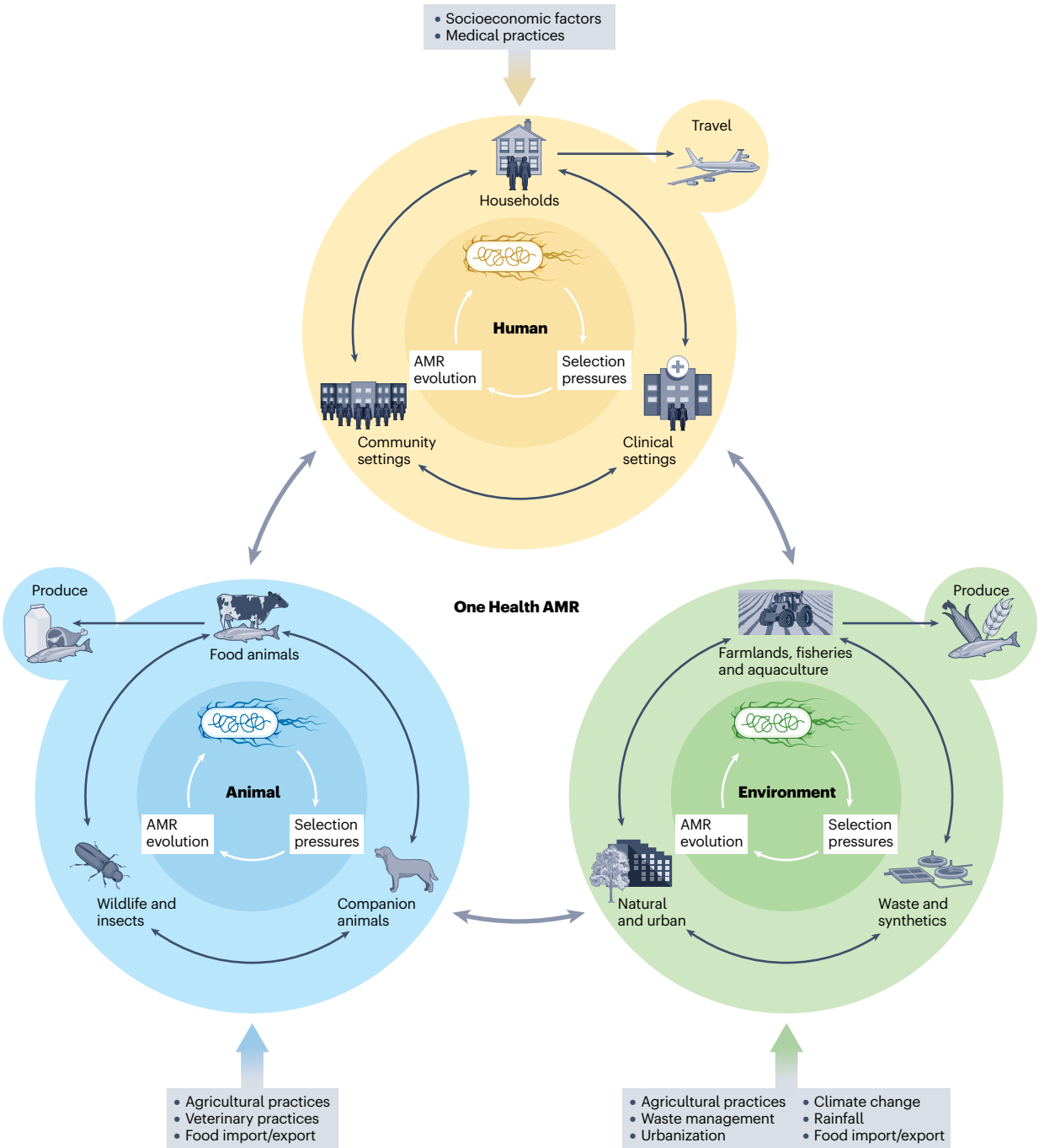

Fig. 2 | One Health antimicrobial resistance. A One Health system depicting the dissemination of antimicrobial resistance (AMR). Boxes show influences that spread AMR in the animal (blue), human (yellow) and environmental (green) sectors.

The main advantage of sequencing single isolates is that it provides a higher level of resolution, enabling the identification of not only ARGs and AMR mutations but also their genetic context. Microbial genomics advances our knowledge of complex resistance regions (CRRs) and pinpoints the precise mechanisms behind the transfer of AMR between bacterial species and their hosts. However, microbial genomics may not capture the diversity of a studied population. Conversely, metagenomics offers a major benefit to AMR genomic surveillance over sequencing of an isolated individual microorganism: the ability to detect and quantify large numbers of ARGs in a single sample[41]. Metagenomic studies have drawn attention to reservoirs of ARGs within humans, animals and environments, such as wastewater, and suggested routes of transmission of ARGs[42,43]. Many microbes cannot be cultured, and a significant benefit of metagenomics is that it allows

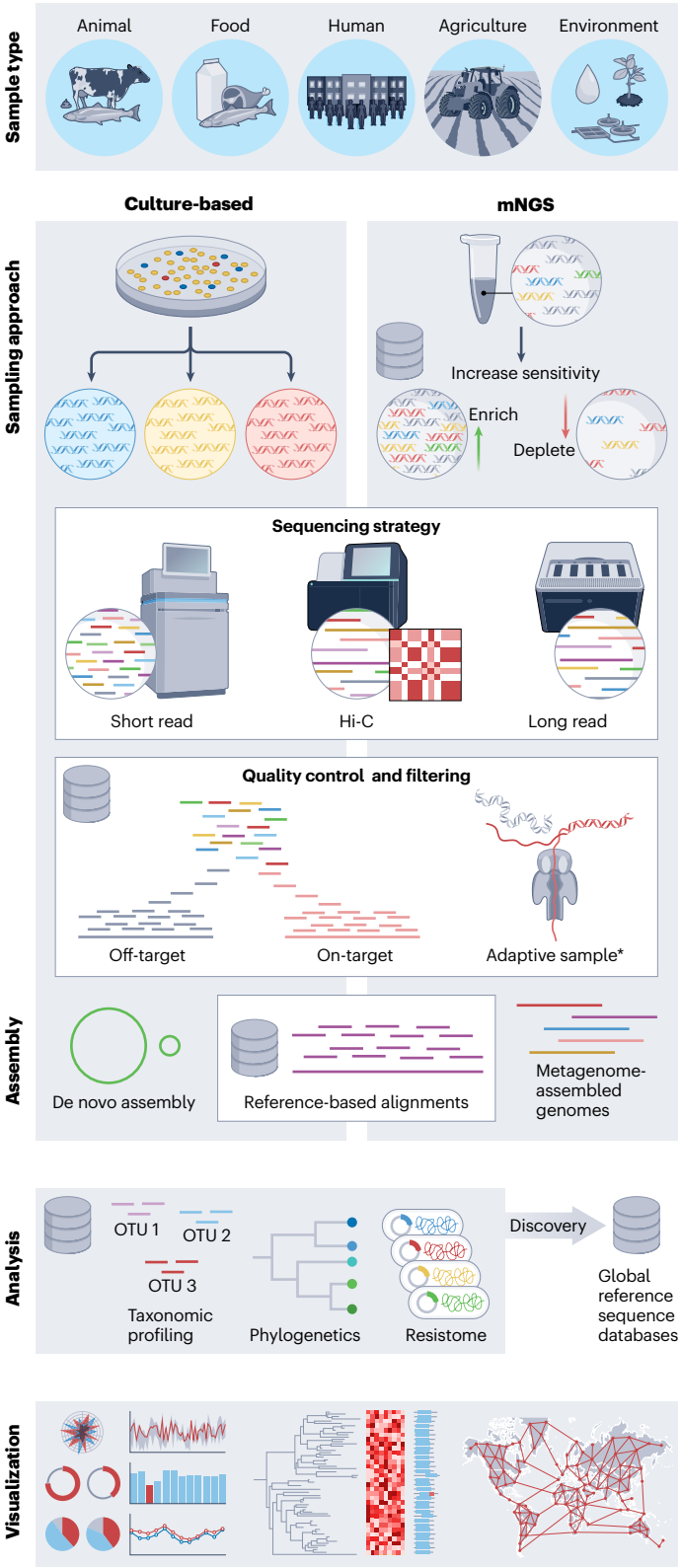

Fig. 3 | One Health genomics toolbox. Steps from sampling through to analysis and visualization based on a range of available genomics approaches. Sample type: clinical, animal and environmental sampling. Sampling approach: nucleic acid may be captured via targeted culture, large-scale culturomics or directly from the sample. Direct samples can be enriched for nucleic acids of interest via various capture techniques (for example, hybridization arrays, CRISPR or poly(A) tail selection) or, conversely, depleted (for example, using riboZero or CRISPR). Sequencing strategy: short-read sequencing provides short-range genomic context but is cheap and deep. Long-read sequencing can be done without amplification and gives long-range context. Molecular techniques such as Hi-C allow the chromosomal range and plasmid source to also be captured. Quality control and filtering: sequencing reads can be filtered in multiple ways. Trimming or removal based on quality scores or length is common. In silico enrichment or depletion can be performed by comparison with known target sequence databases. Removal of human or host reads is a common step in bioinformatics pipelines. Assembly: cultured isolates can be reconstructed using de novo assembly. Short sequence reads will result in a draft genome made on many contigs; depending on downstream applications, genomes may be required to meet more or less stringent quality control criteria pertaining to genome contiguity. Long-read sequencing and Hi-C generally result in a finished, closed genome sequence. Metagenome assembly is more challenging, and results vary depending on the diversity of the microbial community being processed. After metagenome assembly, binning of contigs into metagenomics-assembled genomes (MAGs) representing candidate isolate genomes is often performed. Analysis: analyses can be performed directly on the filtered reads (or k-mers within), or on the genome assemblies and MAGs. Appropriate analyses depend on the questions being asked, but common tasks are looking at taxonomic content, examining relationships of genomes to previously collected genomes for surveillance, discovery of new lineages and genotypes, and monitoring of antimicrobial resistance genes and mechanisms. Visualization: the presentation and reporting of data from a One Health surveillance programme will vary with the audience and potential actions. It is at this point that metadata outside the genomic domain will be integrated and presented in various forms, such as overview dashboards, annotated phylogeographics, statistical summaries and alerts. Discovery: there is an important feedback cycle in a genomic surveillance programme. As genomic data are collected over time, they are added back into global sequence databases, allowing improvements in enrichment panel designs, better in silico databases for filtering and enhanced understanding of diversity within our sampling domain. *Sequencing instrument can selectively sequence or reject on the basis of database similarity. mNGS, metagenomic next-generation sequencing; OTU, operational taxonomic unit.

for surveillance of a much broader range of microorganisms than possible by traditional culture-based microbial genomics. Observing this broader range is important because of the high levels of genetic exchange that occur between different bacterial species and strains. The propensity for exchange creates a need to observe ARGs present in non-pathogenic strains as well as pathogens[44–47].

However, the direct detection of ARGs, virulence determinants or MGEs from metagenome sequence reads is less straightforward than using sequence data from isolates, due to noise in the more complex metagenome data set. Key among these challenges is low abundance amidst large quantities of unhelpful sequence data. Consider, for example, the context of short-read metagenomics and screening wastewater samples for ARGs. Standard approaches such as untargeted shotgun metagenomics, which sequences to a screening depth constrained by staying within reasonable costs, are likely to miss or produce low coverage and confidence around the presence, absence or abundance of rare genes (such as those in emerging resistant pathogenic pandemic strains). The low abundance also has implications for application of machine learning methods to the data. Machine learning methods typically require many training samples to develop

accurate predictions. To address the issue of unwanted nucleic acid amplification, several depletion (reducing the unwanted nucleic acid) or enrichment (increasing the target nucleic acid) steps are possible, including hybridization arrays, CRISPR, poly(A) tail selection for enrichment or riboZero (Fig. 3).

The lack of AMR genetic contextual data faced by short-read metagenomics is being addressed by long-read metagenomics. Coupled with improvements in high molecular weight DNA extraction techniques, MAG binning algorithms and long read-specific metagenomic assemblers[48–51], the recent advent of long-read metagenomics has enabled the retrieval of completed plasmid sequences directly from complex metagenomic samples[48,49,52]. This was previously a severe limitation of short-read MAG binning approaches because of problems in assembling plasmid sequences from short-read data and the inability to provide the genetic context for ARGs. Although significant advances have been made in the approach to metagenomic sampling for pathogen and AMR surveillance, there remains much scope for optimization, which will further enable metagenomics to become a major component of AMR surveillance globally.

Combining genomic technologies to generate actionable insights

The digital nature of genomic data makes them highly interoperable, reproducible and amenable to machine learning and geographic information systems, and they can be combined with other data streams to enhance tracking, tracing and tackling of AMR and emerging pathogens. Integration with artificial intelligence and machine learning systems can enable powerful decision support. Artificial intelligence and machine learning models have recently been developed for a breadth of applications within this space, including identification of AMR outbreaks[53], prediction of resistance and virulence phenotypes[22,54], the host range[55] and the source attribution of strains and their genetic material[56–58]. Source attribution models and geographic information systems, through effective combination and use of WGS data and high-quality metadata, are likely to play important parts in the deconvolution of complex bacterial transmission networks and assist in the targeting of intervention strategies to mitigate the impact of high-risk bacterial populations. As these and other machine learning technologies start to roll out across health jurisdictions at an ever-increasing pace, they are likely to become critical infrastructure in the fight against infectious disease and AMR.

Notwithstanding the power of genomic technologies, it is evident that multi-method approaches are required to provide actionable insights to prioritize and manage AMR mitigation strategies and evaluate the effectiveness of interventions. For example, multi-method surveillance (that is, metagenomics combined with WGS and quantitative PCR) is essential to quantify AMR removal efficiencies by domestic wastewater infrastructure and inform epidemiological and human health risk assessment models[42]. Benchmarking and threshold data (that is, data to support security protocols) are needed to inform evolutionary, epidemiological and other risk modelling efforts and to provide environmental regulators and managers with environmental quality standards and compliance guidelines to support informed management[40,43,59].

One Health AMR surveillance

In 2019, global annual deaths involving AMR were estimated at 4.95 million, with low and middle-income countries (LMICs) bearing the highest burden[44,60,61]. There is an urgent need to reduce the drivers (that is, selection pressures) of AMR and improve sanitation and stewardship practices at a global level, while ensuring that all countries have adequate access to these essential drugs. Diverse mitigation strategies are needed and will require greater collaboration and engagement across diverse disciplines including skills within and external to medical, veterinary and pharmaceutical professions, as well as a willingness from all nations to tackle AMR and enhance the longevity of current and future antibiotics[62–67] (Box 2). Curtailing AMR is considered essential to meet the UN Sustainable Development Goals, which aim to address poverty, hunger and inequality and prioritize healthy lives, by 2030 (ref. 68).

Tackling AMR in LMICs is especially challenging, as human and animal proximity, coupled with less stringent antibiotic-prescribing practices and insufficient sanitation and biosecurity infrastructure, provide favourable conditions for the selection and spread of resistant bacteria[60,69]. Many LMICs have action plans to tackle AMR, but studies have highlighted poor governance and accountability, inadequate funding and corruption as major impediments to implementing AMR mitigation strategies[70,71]. Access to clean water, improved sanitation and hygiene in healthcare facilities and in the community at large, particularly in LMICs, are a priority[72] but will be limited in impact without implementation of internationally coordinated efforts among public health agencies, veterinary agencies, environmental agencies and other relevant stakeholders to collect and analyse data on AMR and its drivers across different sectors, that is, One Health AMR surveillance. The utility of such an approach, albeit on a small scale, is highlighted in a recent WGS study of *E. coli* in livestock-keeping households in Nairobi, Kenya. The study showed that it is possible to draw insight into strain-sharing events and identify key transmission pathways across households and hosts[73]. LMICs frequently encounter limitations regarding financial resources, a scarcity of trained professionals, and inadequate sequencing and bioinformatics capabilities, all of which are crucial for analysing genomic data. Moreover, the absence of necessary frameworks and guidelines to address ethical considerations adds further complexity to the implementation of genomics-based surveillance in LMICs. To address these challenges, there is an immediate necessity to standardize and modularize methodologies for tracking AMR to ensure their accessibility and applicability in LMICs. Additionally, the establishment of robust systems for sample archiving and data sharing plays a pivotal role in enhancing AMR surveillance and facilitating effective interventions. Large-scale amendments in waste management infrastructure (municipal, medical, veterinary and agriculture), the promotion of circular economic strategies and other sustainable agricultural practices[74] at all scales will likely play a key role in mitigating AMR[75].

Genomics needs to be at the heart of surveillance as it is required to unravel the complexities of AMR by identifying emerging resistant microorganisms and the MGEs that purvey AMR, hot spots for HGT and host-associated MGEs, to inform stewardship practices, to define baseline resistance data that inform the impact of implemented mitigation strategies and for tracking pathogen transmission in hospital and veterinary clinical environments and across hosts and environments[14,39] (Box 2).

Study design and metadata for One Health AMR surveillance

Depending on the specific research or management objective, the study design for One Health AMR surveillance may require a combination of molecular and culture-based methods and/or integration and analysis of multiple data streams[40]. The integration of multiple data streams for analysis and insight generation is a major challenge for multi-sectoral surveillance of AMR and demands that data

Box 2

Practical recommendations to implement genomics-enabled surveillance and mitigation strategies

Establishing a national One Health antimicrobial resistance (AMR) surveillance programme incorporating genomics

- Develop an integrated national AMR surveillance programme encompassing human health, animal health, agriculture, food and environmental management sectors.
- Establish standardized protocols and guidelines for consistent data collection, analysis and reporting linked to genomic data.
- Foster collaboration and data sharing among stakeholders, including healthcare facilities, veterinary services, agricultural industries, environmental agencies and research institutions.
- Use advanced genomic data analytics tools and techniques to identify patterns, trends and emerging resistance profiles to inform policy decisions, identify risks and evaluate mitigation strategies.

Increase AMR awareness and education and foster collaboration

- Raise awareness of the links between antimicrobial use and AMR.
- Promote interdisciplinary genomics research and surveillance programmes that consider human, animal, plant, food and environmental health interconnectedness.
- Implement public awareness campaigns to educate people about the risks of AMR and the importance of responsible antibiotic use.
- Increase public awareness about the links between climate change, water, sanitation, hygiene and disease risks.
- Provide waste and residue management education and training.
- Promote knowledge exchange and sharing of best practices.
- Deliver targeted education and training programmes for healthcare professionals, veterinarians, farmers and other stakeholders on AMR surveillance and mitigation strategies.
- Facilitate collaboration across industries and sectors.

Enhancing laboratory capacity in lower and middle-income countries (LMICs)

- Invest in strengthening laboratory capacity for AMR surveillance, ensuring adequate resources, equipment and skilled personnel, including appropriate implementation of genomics tools.
- Implement quality assurance measures, proficiency testing and accreditation programmes to ensure reliable and accurate laboratory results.
- Foster collaborations between national and international laboratories.
- Develop investment cases for appropriate deployment of genomics for AMR surveillance in LMICs.

Encouraging research and innovation

- Invest in research on the environmental impact of faecal contamination and antibiotic usage in agriculture related to AMR dissemination.
- Develop innovative solutions for alternative farming practices and waste management technologies.
- Foster research on the interactions between climate change, pathogens and AMR.
- Invest in genomics and surveillance tools for effective AMR surveillance and management.
- Promote standardization of genomics tools for consistent and reliable results.
- Allocate resources for AMR surveillance and mitigation research, including studies on resistance mechanisms, transmission dynamics and intervention strategies.
- Encourage innovation in diagnostic tools, technologies and surveillance methodologies for improved AMR detection and tracking.
- Facilitate collaboration between researchers, industry and policymakers to translate research findings into practical solutions and evidence-based policies.
- Develop surveillance systems to track water-borne and foodborne diseases, as well as AMR patterns.

Strengthening regulation and oversight in agriculture

- Implement regulations that ensure responsible antibiotic use in agriculture.
- Develop guidelines for management of water, waste and organic fertilizers to prevent the release of antibiotic residues and heavy metals.
- Enhance collaboration between animal industries, regulators and researchers to address antibiotic usage in agriculture.

Improving antibiotic stewardship

- Implement strategies to reduce unnecessary antibiotic prescriptions and promote appropriate use incorporating genomic surveillance data.
- Enhance education and awareness among healthcare professionals and the public about AMR and responsible antibiotic use.
- Establish antimicrobial stewardship programmes in healthcare facilities to monitor and optimize antibiotic usage.
- Promote alternative approaches in agribusiness, such as agroecology and responsible antibiotic use in animal husbandry.
- Implement regulations that ensure responsible antibiotic use in agriculture. Develop guidelines for proper management of organic fertilizers, preventing the release of antibiotic residues and heavy metals.
- Enhance collaboration between animal industries, regulators and researchers to address antibiotic use in agriculture.

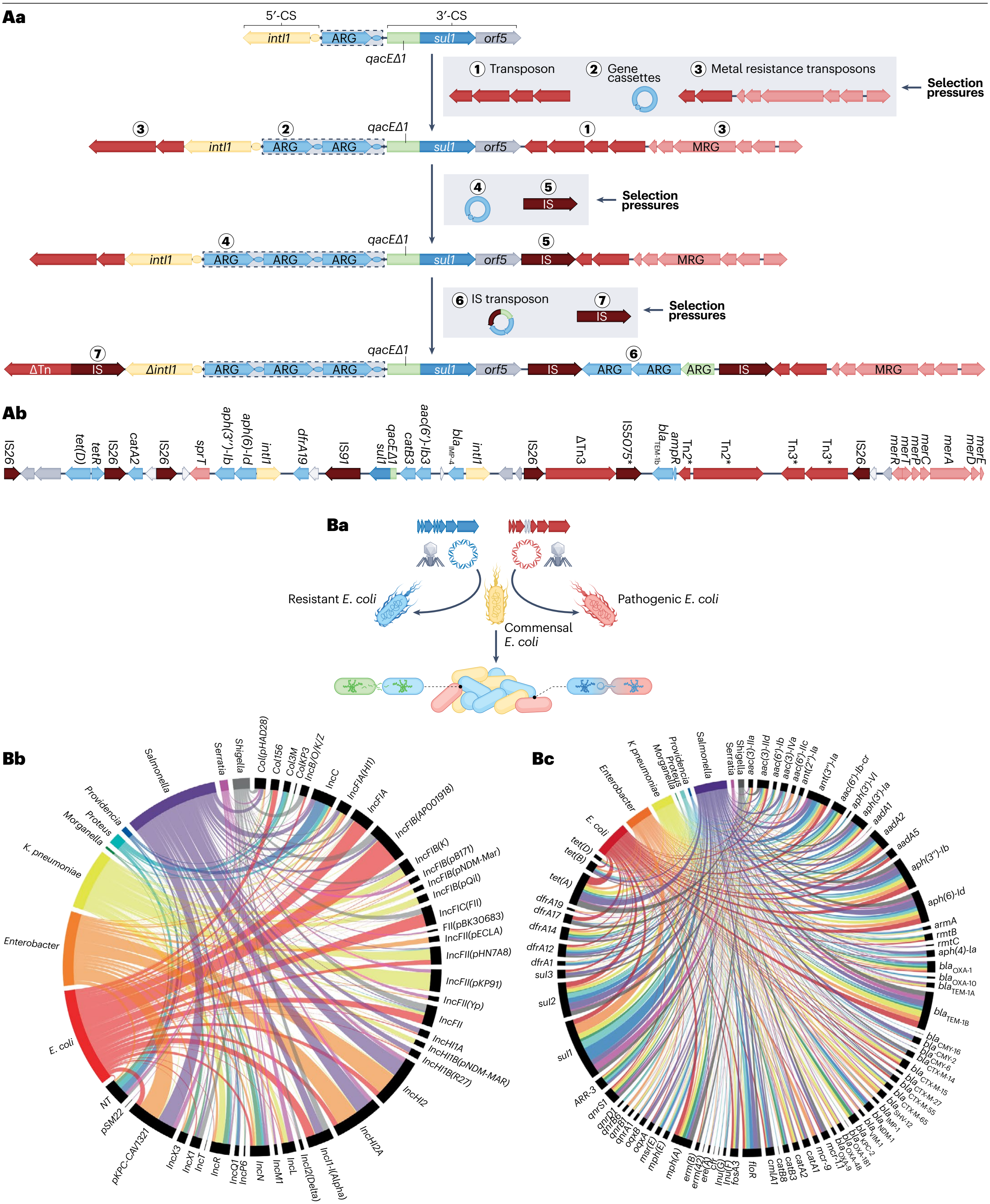
Aa
5′-CS
3′-CS
intI1
ARG
sul1
orf5
qacEΔ1
1 Transposon
2 Gene cassettes
3 Metal resistance transposons
Selection pressures
MRG
4
5 IS
6 IS transposon
7 IS
ΔTn
ΔintI1
Ab
Ba
Resistant E. coli
Pathogenic E. coli
Commensal E. coli
Bb
Bc

Fig. 4 | Plasmids as mobile genetic elements that drive antimicrobial resistance. A, Class 1 integrons and complex resistance regions (CRRs). Illustration of how selection pressures on class 1 integrons can lead to the formation of CRRs (panel **Aa**). An example of a CRR identified in an HI2 plasmid (pCE1537-A, accession number MT232840) from an *Escherichia coli* isolate sourced from a silver gull (panel **Ab**). **B**, Antimicrobial resistance (AMR) plasmids in Enterobacteriaceae. The acquisition of mobile genetic elements (MGEs) by commensal *E. coli* drives the evolution of pathotypes and/or AMR — the determinants of AMR are shared with other bacterial species in proximity, for example, within biofilms (panel **Ba**). Chord diagram depicting the plasmid reps (identified using PlasmidFinder) found in 7,634 completed plasmid sequences from the NCBI (National Center for Biotechnology Information) database harbouring antibiotic resistance genes (ARGs) (identified using ResFinder) (*E. coli* = 2,027 plasmids; *Enterobacter* spp. = 1,520; *Klebsiella pneumoniae* = 1,254; *Morganella* spp. = 46; *Proteus* spp. = 235; *Providencia* spp. = 114; *Salmonella* spp. = 1923; *Serratia* spp. = 166; *Shigella* spp. = 349). The bandwidth is proportional to the number of plasmids per bacteria group and the number of total individual plasmid replicons, and plasmid replicons that were present in <5% of any bacterial group were excluded (panel **Bb**). Chord diagram representing shared ARGs in the same plasmid sequences from panel **Bb**. Band widths are proportional to number of plasmid sequences for each bacterium and the total individual ARG counts, and ARGs that were present in <5% in any bacterial group were excluded (panel **Bc**). MRG, metal resistance gene.

management (storage, sharing, governance, security, and national and global data aggregation) be prioritized and end users be considered in the study design.

Mixed-method approaches typically generate data streams with different characteristics and structures that need to be integrated to derive informed insights. To ensure interoperability of data infrastructure and assets, standardized metadata collection and suitable quality controls need to underpin data storage and analysis frameworks. Additionally, those frameworks must be flexible. Stakeholders and end users vary in digital readiness and informational needs, and monitoring preferences vary and require alignment to support One Health insights. To transform data into knowledge, both supervised and unsupervised learning approaches are needed, with relevant data streams including quantitative PCR and high-throughput targeted and untargeted genomics.

Developing thresholds for One Health AMR transmission

A concept that is yet to be defined for a One Health context is the concept of phylogenetic 'relatedness'. The value to surveillance of bacterial phylogenetic approaches has been demonstrated through its use in restricted epidemiological environments to detect bacterial species outbreaks and transmission events. For example, the transmission of drug-resistant *Klebsiella* spp. in hospitals across Europe found clear phylogenetic clustering at the facility level, and identified a putative 'SNP cut-off' ($n = 21$) for identifying transmission[76]. Establishing this type of SNP threshold can be used to better identify transmission events and track outbreaks in the right context. However, mutation rates can differ between commensal bacteria and pathogens[77,78], and certain mutations may be directly related to a change in host species but, possibly, be transient[79], adding complexity in defining thresholds across sectors[80]. An important difference between tracking transmission in outbreaks within restricted epidemiological settings and multi-sectorial AMR surveillance is the likelihood that there will be the opportunity for a high enough sampling density to identify closely linked genomic–epidemiological clusters and transmission events. Such a level of sampling intensity during One Health surveillance is much less likely than in the example of hospital-level outbreak surveillance. In contrast to the earlier example of the *Klebsiella* spp. outbreak in European hospitals[76], the investigation of *Klebsiella* spp. in a One Health context in northern Italy did not identify phylogenetic evidence of significant transmission between sectors[81], suggesting that, in this context at least, there was little evidence of species transmission between human and non-human sectors. Nonetheless, developing phylogenetic 'thresholds' for multi-sectoral genomic surveillance will assist in implementing genomics-enabled surveillance approaches for identifying cross-sectoral species transmission and assessing and/or developing interventions. To take wastewater management as an example: assessment and choice of appropriate engineering and other interventions will require careful sampling and laboratory-scale or pilot-scale experiments to determine the requirements to break transmission chains. If critical control points, such as wastewater treatment plants, are used as a target for intensive sampling, it is likely that sufficient power will be achieved to enable meaningful comparison of the effects of different interventions on transmission.

The ecology of AMR

Much of our understanding of AMR is derived from detailed genomic studies of pathogen populations derived from clinical settings. In healthcare settings, the ESKAPE-E pathogens — so named for the Gram-positive species *Enterococcus faecium* and *S. aureus*, and the Gram-negative species *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *E. coli* — are responsible for most antibiotic-resistant infections[82–85]. Although antibiotic-resistant Gram-positive ESKAPE-E pathogens remain important, the number of MRSA infections has stabilized or fallen in recent years[86,87], in part because they respond to clinical control strategies better than Gram-negative ESKAPE-E members[83,85]. Infections caused by MDR Gram-negative ESKAPE-E pathogens continue to escalate globally, and are considered a greater AMR threat owing to their ability to occupy a broad range of environments, disseminate widely by the faecal–oral route and acquire resistance to nearly all antibiotic classes by HGT[14,88–91].

ARGs are ancient[92], predating the rapid rise in the use of antimicrobial drugs in clinical and agricultural settings over the past century[14]. Many ARGs have their origins in environmental bacterial species, with evidence showing clinically important ARGs being introduced into key pathogens from *Leclercia* (fosfomycin resistance gene *fosA8*)[93], environmental *Shewanella* spp. (fluoroquinolone resistance gene *qnrA*[94] and carbapenemase $bla_{\text{OXA-48}}$ (ref. 95)), *Kluveria* (ESBL $bla_{\text{CTX-M}}$)[96,97] and plant pathogens (carbapenemase $bla_{\text{NDM-1}}$ (ref. 98)), whereas *mcr-1* (colistin resistance) may have its origins in non-human pathogenic bacteria that circulate in intensive animal production[99]. *K. pneumoniae* may play an important role in this regard as it may act as a conduit for ARGs from environmental to clinically important bacteria[88]. AMR in *A. baumannii* is also likely to have important links to the environment[100,101]. Although the initial mobilization of ARGs is a rare event, it need only happen once in a single location, after which antimicrobial selection pressures may ensure rapid spread within a locality and, depending on their fitness cost, across bacterial species and wider geographic locations[3,14].

The application of genomics across the One Health spectrum has already yielded important insights into transmission dynamics between

humans, companion and food-producing animals, the environment and wildlife in developed countries[13,102,103] and in LMICs[73,104]. The role of colonizing opportunistic pathogens is substantial in this regard and has important ramifications for addressing AMR[105]. These, and many other observations[106], underscore the importance of understanding the ecology of AMR from a One Health perspective. Nonetheless, considerable knowledge gaps remain in understanding the ecology of AMR in commensal and environmental microbial populations.

A One Health indicator organism for monitoring AMR

Although no one bacterial species can fully represent AMR patterns in other bacterial species or specific settings, *E. coli* surveillance through a One Health lens offers several advantages for monitoring AMR.

Genomic surveillance of *E. coli*

A major advantage of *E. coli* is that both healthy and diseased populations can be monitored, on account of *E. coli* being a natural component of gut microbiota (that is, commensal). Another advantage is that *E. coli* is a multi-sectoral organism. It can survive and thrive in soil, plants, wastewater and surface waters, and even establish an existence within free-living protozoa[107]. Treatment for *E. coli* infections often crosses over humans and animals; depending on the geographic location and antimicrobial stewardship guidelines, the same antibiotics used to treat human *E. coli* infections are used in veterinary medicine. Multiple antibiotic-resistant *E. coli* populations encoding resistance to clinically important antibiotics can be found in retail meats, fresh produce, companion animals and synanthropic wildlife populations[10,108–122].

In humans, *E. coli* is one of the first organisms to colonize the gastrointestinal tract, where it remains for life and becomes the most prevalent member of the Enterobacteriaceae in the healthy human gut[123,124]. *E. coli* becomes pathogenic through the acquisition of virulence genes, generally carried on MGEs, such as plasmids, phages and genomic islands. Pathogenic *E. coli* that causes gastrointestinal foodborne illnesses is known as intestinal pathogenic *E. coli*. *E. coli* that causes disease outside the intestine (for example, urinary tract infections (UTIs), bloodstream infections, wound infections, meningitis and ventilator-associated pneumonia) is known as extraintestinal pathogenic *E. coli* (ExPEC). *E. coli* navigates gut colonization with frequent expulsion into the environment, where it becomes exposed to diverse selection pressures (Fig. 1), colonizes new hosts and exchanges genetic information by HGT. In wastewater, sanitation-resistant *E. coli* lineages that have acquired a locus of heat resistance, a chromosomally harboured MGE, bestowing resistance to extreme heat, chlorine and oxidizing agents have been described[125]. A similar scenario is unfolding in the food industry where *E. coli* carrying the locus of heat resistance has been identified, posing food safety concerns[126].

Interest in *E. coli* as a One Health indicator organism for AMR is increasing, driven by a growing avalanche of observations. For example, from 1980 to 2010 there was a demonstrable increase in the frequency of virulence gene carriage in *E. coli* in the commensal flora of healthy humans, correlated with the increase in carriage of a particular clonal type of *E. coli* — phylogroup B2 (refs. 127,128). Over the same period, *E. coli* has continued to acquire an impressive arsenal of ARGs[128,129]. ExPEC strains are the most frequently isolated Gram-negative bacteria, with resistance to diverse antibiotic classes rising[130–132]. ExPEC strains are the leading cause of UTIs, and approximately 400 million annual UTI cases occur globally with an estimated economic cost of approximately US $2 billion in the United States alone[133] and more than US $6 billion globally. Third-generation cephalosporins are used to treat various bacterial infections, including UTIs. In *E. coli*, current rates of resistance to third-generation cephalosporins (for example, cefotaxime) vary significantly between countries, but according to 2020 World Health Organization (WHO) Global Antimicrobial Resistance and Use Surveillance System (GLASS) data the median rates of cefotaxime resistance in *E. coli* causing UTIs was 44%, ranging from 3.5% in Norway to 93.8% in Burkina Faso. For *E. coli* causing bloodstream infections, the rates of cefotaxime resistance were similar, with a median of 38.5% and ranging from 5.3% in Norway to 90% in Côte d'Ivoire. *E. coli* is now recognized as one of the seven top global nosocomial MDR pathogens and extended spectrum β-lactamase (ESBL)-producing *E. coli* strains have been placed on the WHO list of critical-priority pathogens[134].

ESBL-producing *E. coli* in WHO global genomic surveillance programmes

To begin addressing the AMR crisis using a One Health approach, the WHO, the Food and Agriculture Organization (FAO), the World Organisation for Animal Health (OIE) and the United Nations Environment Programme (UNEP) joined forces by way of the Quadripartite agreement[135]. Although a global, robust, multi-sectoral AMR surveillance programme is yet to be realized, in 2021 the WHO, in conjunction with the Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), developed the Tricycle protocol[136]. The Tricycle protocol describes a foundational AMR surveillance system that traverses the human, animal and environmental sectors. As a starting point, the protocol has opted to use a single indicator organism for AMR — ESBL-producing *E. coli*. ESBLs hydrolyse oxyimino-cephalosporins, drugs developed and introduced to overcome the earlier spread of $bla_{\text{TEM-1}}$, which encodes resistance to broad-spectrum penicillins[137,138]. ESBL *E. coli* is thus resistant to most β-lactam antibiotics, necessitating that ESBL *E. coli* infections be treated with last-resort antibiotics. Prevalence of ESBL *E. coli* colonization in humans and animals varies with time and place, and links have been shown between ESBL *E. coli* in the environment and food chain and human morbidity[119].

Challenges of restricting genomic surveillance for AMR

Although the Tricycle protocol represents an important advance in undertaking systematic genomic surveillance across sectors, the restriction of genomic surveillance to ESBL-producing *E. coli* raises concerns[139–141]. The main concern lies with the risk that a selection criterion for surveillance (ESBL *E. coli*) will bias the interpretation of genomic data used to identify *E. coli* phylogenetic lineages and plasmids dominantly causing disease in humans. *E. coli* is a genetically complex organism, and its clinical relevance is site-specific. Currently, regulatory trends are moving to more precise markers, rather than indicators[40]. The potential for bias arises because *E. coli* causing UTIs is often a non-MDR isolate and does not show reduced sensitivity to extended spectrum β-lactams[139,141]. A recent study indicated that broad and unselected sampling regimes are needed to effectively evaluate plasmid sharing, diversity and evolution across human, animal and environmental niches[103]. Finally, failure to accurately identify phylogenetic lineages causing disease has ramifications for understanding the frequency of emergence of new bacterial threats (AMR or virulence), reservoirs and transmission vectors.

Another limitation in restricting AMR genomic surveillance to ESBL-producing *E. coli* is that it does not account sufficiently for the complex biological interplay between laterally acquired and resident gene networks, which influences the fate of laterally acquired

genetic cargo. This concept lies at the heart of pathogen emergence. Clonal expansion and gene/mobile element acquisition are different evolutionary mechanisms at play during pathogen emergence[127]. Plasmid acquisition can influence host range and horizontal gene plasmid behaviour[12,142], shape chromosomal evolution[143] and play an important role in the emergence of new pathogens[144].

Plasmid mobility is currently underappreciated in One Health analyses[103]. Plasmids are critical MGEs to observe in the context of One Health AMR because CRRs are frequently located on plasmids that circulate widely in the Enterobacteriaceae[145–148]. As the literature on AMR plasmids tends to focus on ESKAPE-E pathogens, other WHO priority pathogens have been somewhat overlooked, including those in the critical category such as *Morganella*, *Proteus*, *Providencia* and *Serratia* spp. Nevertheless, members of Enterobacteriaceae often reside within bacterial communities and biofilms in which genetic material is readily shared.

The mechanisms behind the acquisition, assembly and mobilization of ARGs are vast and have been recently reviewed[2]. CRRs often carry class 1 integrons (Fig. 4A), genetic elements that play a central role in the purveyance of MDR because of their ability to capture and express diverse resistance gene cassettes from widely divergent sources. Insertion elements such as IS*26* form part of the IS*6*/IS*26* family of insertion elements, and are common in CRRs where their presence can facilitate the creation of larger and varied CRRs[2]. The formation of hybrid plasmids, generated by the fusion of two unrelated plasmids by the action of insertion elements, such as IS*26*, is increasingly documented[149–151]. Moreover, the activity of IS*26* is thought to influence plasmid long-term persistence and molecular integrity (plasmid fitness); a growing body of evidence has linked the activity of insertion elements to the creation of mutations that alleviate the fitness costs imposed by the capture of CRRs and the MGEs that carry them[152]. IS*26* plays a significant role in the formation and spread of CRRs[2,153]; is involved in the capture and mobilization of ARGs[154]; alters the conserved structure of the class 1 integron via the formation of deletions in the class 1 integrase and in the 3′ conserved segment[155]; and is widely disseminated in *E. coli*[10] and the Proteobacteriaceae more broadly. Notably, IS*257*/IS*431* elements found in Gram-positive bacteria that are functionally similar also belong to the IS*6*/IS*26* family[156]. A snapshot of the distribution of ARGs and the plasmid types that carry them between nine Enterobacteriaceae is provided in Fig. 4B.

If sequence data are too restricted by the choice of monitoring target, they may not allow for distinguishing selection pressures that cause AMR to evolve[157] and those that influence how *E. coli* acquire virulence and ARGs. For example, wildlife may not be the recipients of antibiotics but they have an increasingly important role in the carriage and, potentially, the evolution of highly drug-resistant enterobacterial lineages[10,158]. For the biological reasons presented above, we believe that all *E. coli* strains (commensal and pathogenic) should be included in One Health genomic surveillance of AMR, as is already occurring in some surveillance programmes[159].

Conclusions

The evolutionary nature of AMR makes it a constantly changing and evolving threat. There is no easy solution to AMR, but a combination of approaches is required, including ongoing surveillance. Genomic technologies have become indispensable tools for understanding AMR spread and evolution, enabling the tracing of clonal expansion and transmission of existing and emerging pathogens as well as MGEs that capture and spread AMR. Genomic technologies are revolutionizing how we conduct the surveillance of microorganisms that pose a threat to humans, food production systems, soil and water quality, and wildlife. They allow us to garner a deeper understanding of the role of the environment, human and commercial activities in the evolution and spread of AMR and pathogens, and can thus support the often called for but so far unimplemented One Health approach to national and global AMR surveillance and management. This One Health approach is central to understanding AMR and pathogen evolution and, most importantly, mitigating it.

Published online: 25 September 2023

References

1. Djordjevic, S. P., Stokes, H. W. & Chowdhury, P. R. Mobile elements, zoonotic pathogens and commensal bacteria: conduits for the delivery of resistance genes into humans, production animals and soil microbiota. *Front. Microbiol.* **4**, 86 (2013).
This study addresses the importance of understanding how resistance genes and the genetic scaffolds that mobilize them into clinically important bacteria are likely to have their origins in completely unrelated parts of the microbial biosphere.
2. Partridge, S. R., Kwong, S. M., Firth, N. & Jensen, S. O. Mobile genetic elements associated with antimicrobial resistance. *Clin. Microbiol. Rev.* **31**, 00088 (2018).
This study identifies the discerning features of MGEs that have the ability to move ARG cargo within or between DNA molecules and those that drive dissemination between bacterial cells.
3. Gillings, M. R. Lateral gene transfer, bacterial genome evolution, and the Anthropocene. *Ann. N. Y. Acad. Sci.* **1389**, 20–36 (2017).
4. Christaki, E., Marcou, M. & Tofarides, A. Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. *J. Mol. Evol.* **88**, 26–40 (2020).
5. Aronin, S. I., Dunne, M. W., Yu, K. C., Watts, J. A. & Gupta, V. Increased rates of extended-spectrum β-lactamase isolates in patients hospitalized with culture-positive urinary Enterobacterales in the United States: 2011–2020. *Diagn. Microbiol. Infect. Dis.* **103**, 115717 (2022).
6. Dejonckheere, Y., Desmet, S. & Knops, N. A study of the 20-year evolution of antimicrobial resistance patterns of pediatric urinary tract infections in a single center. *Eur. J. Pediatr.* https://doi.org/10.1007/s00431-022-04538-0 (2022).
This study traces the evolution of drug resistance in paediatric patients with UTIs over a considerable time period.
7. Pires, J., Huisman, J. S., Bonhoeffer, S. & Van Boeckel, T. P. Increase in antimicrobial resistance in *Escherichia coli* in food animals between 1980 and 2018 assessed using genomes from public databases. *J. Antimicrob. Chemother.* **77**, 646–655 (2022).
8. Schar, D. et al. Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia. *Nat. Commun.* **12**, 5384 (2021).
This large meta-analysis reports antibiotic-resistant bacteria from aquatic food animals in Asia from 2000 and highlights the need to study resistance to medically important antimicrobials in foodborne pathogens.
9. Turnidge, J. D., Meleady, K. T., Turnidge, J. D. & Meleady, K. T. Antimicrobial Use and Resistance in Australia (AURA) surveillance system: coordinating national data on antimicrobial use and resistance for Australia. *Aust. Health Rev.* **42**, 272–276 (2017).
10. Wyrsch, E. R. et al. Urban wildlife crisis: Australian silver gull is a bystander host to widespread clinical antibiotic resistance. *mSystems* **7**, e0015822 (2022).
This comprehensive WGS study of *E. coli* from an urban-adapted bird species highlights carriage of emerging and novel multiple drug-resistant lineages carrying genes encoding resistance to clinically important antibiotics.
11. Medvecky, M. et al. Interspecies transmission of CMY-2-producing *Escherichia coli* sequence type 963 isolates between humans and gulls in Australia. *mSphere* **7**, e00238-22 (2022).
12. Cummins, M. L., Reid, C. J. & Djordjevic, S. P. F Plasmid lineages in *Escherichia coli* ST95: implications for host range, antibiotic resistance, and zoonoses. *mSystems* **7**, e01212–e01221 (2022).
This study performs a phylogenomic analysis of ST95 and identifies lineages that carry different F virulence plasmids with implications for host colonization and zoonosis.
13. Inda-Díaz, J. S. et al. Latent antibiotic resistance genes are abundant, diverse, and mobile in human, animal, and environmental microbiomes. *Microbiome* **11**, 44 (2023).
This work highlights knowledge gaps in defining the resistome and describes the creation of a reference database for existing and latent antimicrobial resistance genes.
14. Baker, S., Thomson, N., Weill, F.-X. & Holt, K. E. Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens. *Science* **360**, 733–738 (2018).
15. Lane, C. R. et al. Search and contain: impact of an integrated genomic and epidemiological surveillance and response program for control of carbapenemase-producing Enterobacterales. *Clin. Infect. Dis.* **73**, e3912–e3920 (2021).
16. Sia, C. M. et al. Genomic diversity of antimicrobial resistance in non-typhoidal *Salmonella* in Victoria, Australia. *Microb. Genom.* **7**, 000725 (2021).
17. Bharat, A. et al. Correlation between phenotypic and in silico detection of antimicrobial resistance in *Salmonella enterica* in Canada using Staramr. *Microorganisms* **10**, 292 (2022).

18. Rebelo, A. R. et al. One day in Denmark: comparison of phenotypic and genotypic antimicrobial susceptibility testing in bacterial isolates from clinical settings. *Front. Microbiol.* **13**, 804627 (2022).
19. Sherry, N. L. et al. An ISO-certified genomics workflow for identification and surveillance of antimicrobial resistance. *Nat. Commun.* **14**, 60 (2023).
This paper is one of the first to demonstrate the certification of genomic interpretation of AMR to ISO standards, providing a framework for implementation into public health surveillance.
20. Hendriksen, R. S. et al. Using genomics to track global antimicrobial resistance. *Front. Public Health* **7**, 00242 (2019).
21. Papp, M. & Solymosi, N. Review and comparison of antimicrobial resistance gene databases. *Antibiotics* **11**, 339 (2022).
22. Kim, J. I. et al. Machine learning for antimicrobial resistance prediction: current practice, limitations, and clinical perspective. *Clin. Microbiol. Rev.* **35**, e00179-21 (2022).
23. Meyer, F. et al. Critical assessment of metagenome interpretation: the second round of challenges. *Nat. Methods* **19**, 429–440 (2022).
24. DeMaere, M. Z. & Darling, A. E. bin3C: exploiting Hi-C sequencing data to accurately resolve metagenome-assembled genomes. *Genome Biol.* **20**, 46 (2019).
25. Zankari, E. et al. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *J. Antimicrob. Chemother.* **72**, 2764–2768 (2017).
26. Zankari, E. et al. Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother.* **67**, 2640–2644 (2012).
27. McArthur, A. G. et al. The Comprehensive Antibiotic Resistance Database. *Antimicrob. Agents Chemother.* **57**, 3348–3357 (2013).
28. Feldgarden, M. et al. Curation of the AMRFinderPlus databases: applications, functionality and impact. *Microb. Genom.* **8**, mgen000832 (2022).
29. Arango-Argoty, G. A. et al. ARGminer: a web platform for the crowdsourcing-based curation of antibiotic resistance genes. *Bioinformatics* **36**, 2966–2973 (2020).
30. Gibson, M. K., Forsberg, K. J. & Dantas, G. Improved annotation of antibiotic resistance determinants reveals microbial resistomes cluster by ecology. *ISME J.* **9**, 207–216 (2015).
31. Hunt, M. et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. *Micro. Genom.* **3**, e000131 (2017).
32. Clausen, P. T. L. C., Aarestrup, F. M. & Lund, O. Rapid and precise alignment of raw reads against redundant databases with KMA. *BMC Bioinforma.* **19**, 307 (2018).
33. Steinig, E. et al. Phylodynamic signatures in the emergence of community-associated MRSA. *Proc. Natl Acad. Sci. USA* **119**, e2204993119 (2022).
34. Steinig, E. et al. Phylodynamic inference of bacterial outbreak parameters using nanopore sequencing. *Mol. Biol. Evol.* **39**, msac040 (2022).
35. Rife, B. D. et al. Phylodynamic applications in 21st century global infectious disease research. *Glob. Health Res. Policy* **2**, 13 (2017).
36. Dawson, D., Rasmussen, D., Peng, X. & Lanzas, C. Inferring environmental transmission using phylodynamics: a case-study using simulated evolution of an enteric pathogen. *J. R. Soc. Interface* **18**, 20210041 (2021).
37. Ingle, D. J., Howden, B. P. & Duchene, S. Development of phylodynamic methods for bacterial pathogens. *Trends Microbiol.* **29**, 788–797 (2021).
This important review highlights the potential utility of phylodynamic analyses to enhance understanding of bacterial evolution and transmission.
38. Miłobedzka, A. et al. Monitoring antibiotic resistance genes in wastewater environments: the challenges of filling a gap in the One-Health cycle. *J. Hazard. Mater.* **424**, 127407 (2022).
39. Munk, P. et al. Genomic analysis of sewage from 101 countries reveals global landscape of antimicrobial resistance. *Nat. Commun.* **13**, 7251 (2022).
40. Liguori, K. et al. Antimicrobial resistance monitoring of water environments: a framework for standardized methods and quality control. *Environ. Sci. Technol.* **56**, 9149–9160 (2022).
This work presents a framework developed in consultation with experts in academia, government and water utility management, and through analyses of the literature, describes standardized methods for monitoring AMR in water.
41. Hendriksen, R. S. et al. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nat. Commun.* **10**, 1124 (2019).
This work presents metagenomic sequencing of sewage as an economically and ethically acceptable approach to global AMR surveillance, providing important insights into AMR carriage in the healthy human gut in a region-specific manner.
42. Banerjee, S. & van der Heijden, M. G. A. Soil microbiomes and one health. *Nat. Rev. Microbiol.* **21**, 6–20 (2023).
43. Larsson, D. G. J. & Flach, C.-F. Antibiotic resistance in the environment. *Nat. Rev. Microbiol.* **20**, 257–269 (2022).
This review examines risk scenarios, surveillance methods and potential factors driving antibiotic resistance, and identifies actionable measures to mitigate the risks associated with antibiotic resistance in the environment.
44. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **399**, 629–655 (2022).
45. Kim, D.-W. & Cha, C.-J. Antibiotic resistome from the One-Health perspective: understanding and controlling antimicrobial resistance transmission. *Exp. Mol. Med.* **53**, 301–309 (2021).
46. Lamberte, L. E. & van Schaik, W. Antibiotic resistance in the commensal human gut microbiota. *Curr. Opin. Microbiol.* **68**, 102150 (2022).
47. Crits-Christoph, A., Hallowell, H. A., Koutouvalis, K. & Suez, J. Good microbes, bad genes? The dissemination of antimicrobial resistance in the human microbiome. *Gut Microbes* **14**, 2055944 (2022).
48. Arumugam, K. et al. Recovery of complete genomes and non-chromosomal replicons from activated sludge enrichment microbial communities with long read metagenome sequencing. *npj Biofilms Microbiomes* **7**, 1–13 (2021).
49. Bertrand, D. et al. Hybrid metagenomic assembly enables high-resolution analysis of resistance determinants and mobile elements in human microbiomes. *Nat. Biotechnol.* **37**, 937–944 (2019).
50. Kolmogorov, M. et al. metaFlye: scalable long-read metagenome assembly using repeat graphs. *Nat. Methods* **17**, 1103–1110 (2020).
51. Pellow, D. et al. SCAPP: an algorithm for improved plasmid assembly in metagenomes. *Microbiome* **9**, 144 (2021).
52. Somerville, V. et al. Long-read based de novo assembly of low-complexity metagenome samples results in finished genomes and reveals insights into strain diversity and an active phage system. *BMC Microbiol.* **19**, 143 (2019).
53. Fitzpatrick, F., Doherty, A. & Lacey, G. Using artificial intelligence in infection prevention. *Curr. Treat. Options Infect. Dis.* **12**, 135–144 (2020).
54. Wheeler, N. E., Gardner, P. P. & Barquist, L. Machine learning identifies signatures of host adaptation in the bacterial pathogen *Salmonella enterica*. *PLoS Genet.* **14**, e1007333 (2018).
55. Lupolova, N., Dallman, T. J., Holden, N. J. & Gally, D. L. Patchy promiscuity: machine learning applied to predict the host specificity of *Salmonella enterica* and *Escherichia coli*. *Microb. Genom.* **3**, e000135 (2017).
56. Munck, N., Njage, P. M. K., Leekitcharoenphon, P., Litrup, E. & Hald, T. Application of whole-genome sequences and machine learning in source attribution of *Salmonella typhimurium*. *Risk Anal.* **40**, 1693–1705 (2020).
57. Tanui, C. K., Benefo, E. O., Karanth, S. & Pradhan, A. K. A machine learning model for food source attribution of *Listeria monocytogenes*. *Pathogens* **11**, 691 (2022).
58. Li, L.-G., Yin, X. & Zhang, T. Tracking antibiotic resistance gene pollution from different sources using machine-learning classification. *Microbiome* **6**, 93 (2018).
59. Vassallo, A., Kett, S., Purchase, D. & Marvasi, M. Antibiotic-resistant genes and bacteria as evolving contaminants of emerging concerns (e-CEC): is it time to include evolution in risk assessment? *Antibiotics* **10**, 1066 (2021).
60. Ikhimiukor, O. O., Odih, E. E., Donado-Godoy, P. & Okeke, I. N. A bottom-up view of antimicrobial resistance transmission in developing countries. *Nat. Microbiol.* **7**, 757–765 (2022).
61. O'Neill, J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. *Wellcome Collection* https://wellcomecollection.org/works/rdpck35v/items (2014).
62. Flowers, P. Antimicrobial resistance: a biopsychosocial problem requiring innovative interdisciplinary and imaginative interventions. *J. Infect. Prev.* **19**, 195–199 (2018).
63. Raboisson, D., Ferchiou, A., Sans, P., Lhermie, G. & Dervillé, M. The economics of antimicrobial resistance in veterinary medicine: optimizing societal benefits through mesoeconomic approaches from public and private perspectives. *One Health* **10**, 100145 (2020).
64. George, A. Antimicrobial resistance (AMR) in the food chain: trade, One Health and Codex. *Trop. Med. Infect. Dis.* **4**, 54 (2019).
65. Queenan, K., Häsler, B. & Rushton, J. A One Health approach to antimicrobial resistance surveillance: is there a business case for it? *Int. J. Antimicrob. Agents* **48**, 422–427 (2016).
66. Collignon, P. J. & McEwen, S. A. One Health—its importance in helping to better control antimicrobial resistance. *Trop. Med. Infect. Dis.* **4**, 22 (2019).
67. McEwen, S. A. & Collignon, P. J. Antimicrobial resistance: a One Health perspective. *Microbiol. Spectr.* https://doi.org/10.1128/microbiolspec.arba-0009-2017 (2018).
68. World Health Organization. The fight against antimicrobial resistance is closely linked to the sustainable development goals. https://apps.who.int/iris/handle/10665/337519. (WHO, 2020).
69. Sartelli, M. et al. Antibiotic use in low and middle-income countries and the challenges of antimicrobial resistance in surgery. *Antibiotics* **9**, 497 (2020).
70. Collignon, P., Athukorala, P., Senanayake, S. & Khan, F. Antimicrobial resistance: the major contribution of poor governance and corruption to this growing problem. *PLoS ONE* **10**, e0116746 (2015).
71. Harant, A. Assessing transparency and accountability of national action plans on antimicrobial resistance in 15 African countries. *Antimicrob. Resist. Infect. Control* **11**, 15 (2022).
72. Musoke, D. et al. The role of environmental health in preventing antimicrobial resistance in low- and middle-income countries. *Environ. Health Prev. Med.* **26**, 100 (2021).
73. Muloi, D. M. et al. Population genomics of *Escherichia coli* in livestock-keeping households across a rapidly developing urban landscape. *Nat. Microbiol.* **7**, 581–589 (2022).
This WGS analysis of *E. coli* from humans, livestock and wildlife across households in Nairobi, Kenya shows evidence of interhost and interhousehold transmission, with implications for the emergence of zoonoses and the spread of AMR.
74. Horrigan, L., Lawrence, R. S. & Walker, P. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environ. Health Perspect.* **110**, 445–456 (2002).
75. Sanz-García, F. et al. Translating eco-evolutionary biology into therapy to tackle antibiotic resistance. *Nat. Rev. Microbiol.* https://doi.org/10.1038/s41579-023-00902-5 (2023).
76. David, S. et al. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nat. Microbiol.* **4**, 1919–1929 (2019).

77. Denamur, E. et al. High frequency of mutator strains among human uropathogenic *Escherichia coli* isolates. *J. Bacteriol.* **184**, 605–609 (2002).
78. Denamur, E. & Matic, I. Evolution of mutation rates in bacteria. *Mol. Microbiol.* **60**, 820–827 (2006).
79. Reeves, P. R. et al. Rates of mutation and host transmission for an *Escherichia coli* clone over 3 years. *PLoS ONE* **6**, e26907 (2011).
80. Duval, A., Opatowski, L. & Brisse, S. Defining genomic epidemiology thresholds for common-source bacterial outbreaks: a modelling study. *Lancet Microbe* **4**, e349–e357 (2023).
81. Thorpe, H. A. et al. A large-scale genomic snapshot of *Klebsiella* spp. isolates in northern Italy reveals limited transmission between clinical and non-clinical settings. *Nat. Microbiol.* **7**, 2054–2067 (2022).
82. Rice, L. B. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J. Infect. Dis.* **197**, 1079–1081 (2008).
83. Rice, L. B. Progress and challenges in implementing the research on ESKAPE pathogens. *Infect. Control. Hosp. Epidemiol.* **31** (Suppl. 1), S7–S10 (2010).
84. Boucher, H. W. et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **48**, 1–12 (2009).
85. Pendleton, J. N., Gorman, S. P. & Gilmore, B. F. Clinical relevance of the ESKAPE pathogens. *Expert. Rev. Anti Infect. Ther.* **11**, 297–308 (2013).
86. Diekema, D. J. et al. The microbiology of bloodstream infection: 20-year trends from the SENTRY antimicrobial surveillance program. *Antimicrob. Agents Chemother.* **63**, e00355-19 (2019).
87. Kajihara, T., Yahara, K., Hirabayashi, A., Shibayama, K. & Sugai, M. Japan Nosocomial Infections Surveillance (JANIS): current status, international collaboration, and future directions for a comprehensive antimicrobial resistance surveillance system. *Jpn. J. Infect. Dis.* **74**, 87–96 (2021).
88. Wyres, K. L. & Holt, K. E. *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Curr. Opin. Microbiol.* **45**, 131–139 (2018).
This paper presents data to support the contention that *Klebsiella* spp. as a genus may play a seminal role in capturing and spreading AMR genes from environmental microbial populations into the ESKAPE and other clinically important pathogens.
89. von Wintersdorff, C. J. H. et al. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front. Microbiol.* **7**, 173 (2016).
90. O'Neal, L., Alvarez, D., Mendizábal-Cabrera, R., Ramay, B. M. & Graham, J. Community-acquired antimicrobial resistant Enterobacteriaceae in Central America: a One Health systematic review. *Int. J. Environ. Res. Public Health* **17**, 7622 (2020).
91. Campos-Madueno, E. I. et al. Carbapenemase-producing *Klebsiella pneumoniae* strains in Switzerland: human and non-human settings may share high-risk clones. *J. Glob. Antimicrob. Resist.* **28**, 206–215 (2022).
92. D'Costa, V. M. et al. Antibiotic resistance is ancient. *Nature* **477**, 457–461 (2011).
93. Poirel, L. et al. Identification of FosA8, a plasmid-encoded fosfomycin resistance determinant from *Escherichia coli*, and its origin in *Leclercia adecarboxylata*. *Antimicrob. Agents Chemother.* https://doi.org/10.1128/aac.01403-19 (2019).
94. Poirel, L., Rodriguez-Martinez, J.-M., Mammeri, H., Liard, A. & Nordmann, P. Origin of plasmid-mediated quinolone resistance determinant QnrA. *Antimicrob. Agents Chemother.* **49**, 3523–3525 (2005).
95. Tacão, M., Araújo, S., Vendas, M., Alves, A. & Henriques, I. *Shewanella* species as the origin of bla_{OXA-48} genes: insights into gene diversity, associated phenotypes and possible transfer mechanisms. *Int. J. Antimicrob. Agents* **51**, 340–348 (2018).
96. Canton, R., Gonzalez-Alba, J. M. & Galán, J. C. CTX-M enzymes: origin and diffusion. *Front. Microbiol.* **3**, 110 (2012).
97. Rodríguez, M. M. et al. Chromosome-encoded CTX-M-3 from *Kluyvera ascorbata*: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. *Antimicrob. Agents Chemother.* **48**, 4895–4897 (2004).
98. Sekizuka, T. et al. Complete sequencing of the blaNDM-1-positive IncA/C plasmid from *Escherichia coli* ST38 isolate suggests a possible origin from plant pathogens. *PLoS ONE* **6**, e25334 (2011).
99. Wang, R. et al. The global distribution and spread of the mobilized colistin resistance gene mcr-1. *Nat. Commun.* **9**, 1179 (2018).
100. Castillo-Ramírez, S. Zoonotic *Acinetobacter baumannii*: the need for genomic epidemiology in a One Health context. *Lancet Microbe* **3**, e895–e896 (2022).
101. Prity, F. T. et al. The evolutionary tale of eight novel plasmids in a colistin-resistant environmental *Acinetobacter baumannii* isolate. *Microb. Genom.* **9**, mgen001010 (2023).
102. Liu, C. M. et al. Using source-associated mobile genetic elements to identify zoonotic extraintestinal *E. coli* infections. *One Health* https://doi.org/10.1016/j.onehlt.2023.100518 (2023).
This large, geographically matched, comparative genomic analysis of contemporaneous clinical and meat-source *E. coli* isolates identifies source-associated MGEs and estimates that approximately 8% of human extraintestinal *E. coli* infections are potentially attributable to foodborne zoonotic *E. coli*.
103. Matlock, W. et al. Enterobacterales plasmid sharing amongst human bloodstream infections, livestock, wastewater, and waterway niches in Oxfordshire, UK. *eLife* **12**, e85302 (2023).
This pan-genome analysis of plasmid clusters in a geographically and temporally selected subset of isolates shows evidence of widespread plasmid sharing across species and niches and accessory cargo exchange.
104. Swarthout, J. M., Chan, E. M. G., Garcia, D., Nadimpalli, M. L. & Pickering, A. J. Human colonization with antibiotic-resistant bacteria from nonoccupational exposure to domesticated animals in low- and middle-income countries: a critical review. *Environ. Sci. Technol.* **56**, 14875–14890 (2022).
105. Price, L. B., Hungate, B. A., Koch, B. J., Davis, G. S. & Liu, C. M. Colonizing opportunistic pathogens (COPs): the beasts in all of us. *PLoS Pathog.* **13**, e1006369 (2017).
***E. coli* (ExPEC), *K. pneumoniae* and *Streptococcus pneumoniae* are important examples of colonizing opportunistic pathogens with a benign existence in the human body, but when conditions favour their transition to a pathogenic state, often in a different body site, they exact a horrendous toll on human health.**
106. Castillo-Ramírez, S., Ghaly, T. & Gillings, M. Non-clinical settings—the understudied facet of antimicrobial drug resistance. *Environ. Microbiol.* **23**, 7271–7274 (2021).
107. Montalbano Di Filippo, M. et al. Exploring the nature of interaction between shiga toxin producing *Escherichia coli* (STEC) and free-living amoeba—*Acanthamoeba* sp. *Front. Cell. Infect. Microbiol.* **12**, 926127 (2022).
108. Loest, D. et al. Carbapenem-resistant *Escherichia coli* from shrimp and salmon available for purchase by consumers in Canada: a risk profile using the Codex framework. *Epidemiol. Infect.* **150**, e148 (2022).
109. Zhang, Q. et al. Rapid increase in carbapenemase-producing Enterobacteriaceae in retail meat driven by the spread of the bla_{NDM-5}-carrying IncX3 plasmid in China from 2016 to 2018. *Antimicrob. Agents Chemother.* **63**, e00573-19 (2019).
110. Jamin, C. et al. Genetic analysis of plasmid-encoded mcr-1 resistance in Enterobacteriaceae derived from poultry meat in the Netherlands. *JAC Antimicrob. Resist.* **3**, dlab156 (2021).
111. Feng, J. et al. Characterization of carbapenem-resistant enterobacteriaceae cultured from retail meat products, patients, and porcine excrement in China. *Front. Microbiol.* **12**, 743468 (2021).
112. Reid, C. J., Blau, K., Jechalke, S., Smalla, K. & Djordjevic, S. P. Whole genome sequencing of *Escherichia coli* from store-bought produce. *Front. Microbiol.* **10**, 3050 (2020).
113. Igbinosa, E. O., Beshiru, A., Igbinosa, I. H., Cho, G.-S. & Franz, C. M. A. P. Multidrug-resistant extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* from farm produce and agricultural environments in Edo State, Nigeria. *PLoS ONE* **18**, e0282835 (2023).
114. Chelaghma, W. et al. Occurrence of extended spectrum cephalosporin-, carbapenem- and colistin-resistant Gram-negative bacteria in fresh vegetables, an increasing human health concern in Algeria. *Antibiotics* **11**, 988 (2022).
115. Manageiro, V., Jones-Dias, D., Ferreira, E. & Caniça, M. Plasmid-mediated colistin resistance (mcr-1) in *Escherichia coli* from non-imported fresh vegetables for human consumption in Portugal. *Microorganisms* **8**, 429 (2020).
116. Teng, L. et al. A cross-sectional study of companion animal-derived multidrug-resistant *Escherichia coli* in Hangzhou, China. *Microbiol. Spectr.* **11**, e02113–e02122 (2023).
117. Marques, C. et al. Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16 year retrospective study. *J. Antimicrob. Chemother.* **73**, 377–384 (2018).
118. Garcês, A. et al. Bacterial isolates from urinary tract infection in dogs and cats in Portugal, and their antibiotic susceptibility pattern: a retrospective study of 5 years (2017–2021). *Antibiotics* **11**, 1520 (2022).
119. Sano, E. et al. One Health clones of multidrug-resistant *Escherichia coli* carried by synanthropic animals in Brazil. *One Health* **16**, 100476 (2023).
120. Devnath, P., Karah, N., Graham, J. P., Rose, E. S. & Asaduzzaman, M. Evidence of antimicrobial resistance in bats and its planetary health impact for surveillance of zoonotic spillover events: a scoping review. *Int. J. Environ. Res. Public Health* **20**, 243 (2023).
121. Martín-Maldonado, B. et al. Urban birds as antimicrobial resistance sentinels: white storks showed higher multidrug-resistant *Escherichia coli* levels than seagulls in Central Spain. *Animals* **12**, 2714 (2022).
122. Torres, R. T. et al. A walk on the wild side: wild ungulates as potential reservoirs of multi-drug resistant bacteria and genes, including *Escherichia coli* harbouring CTX-M β-lactamases. *Environ. Pollut.* **306**, 119367 (2022).
123. Martinson, J. N. V. et al. Rethinking gut microbiome residency and the Enterobacteriaceae in healthy human adults. *ISME J.* **13**, 2306–2318 (2019).
124. Martinson, J. N. V. & Walk, S. T. *Escherichia coli* residency in the gut of healthy human adults. *EcoSal* **9**, ESP0003 (2020).
125. Yu, D., Ryu, K., Zhi, S., Otto, S. J. G. & Neumann, N. F. Naturalized *Escherichia coli* in wastewater and the co-evolution of bacterial resistance to water treatment and antibiotics. *Front. Microbiol.* **13**, 810312 (2022).
126. Guragain, M., Brichta-Harhay, D. M., Bono, J. L. & Bosilevac, J. M. Locus of heat resistance (LHR) in meat-borne Escherichia coli: screening and genetic characterization. *Appl. Environ. Microbiol.* **87**, e02343-20 (2021).
127. Marin, J. et al. The population genomics of increased virulence and antibiotic resistance in human commensal *Escherichia coli* over 30 years in France. *Appl. Environ. Microbiol.* **88**, e00664-22 (2022).
128. Massot, M. et al. Phylogenetic, virulence and antibiotic resistance characteristics of commensal strain populations of *Escherichia coli* from community subjects in the Paris area in 2010 and evolution over 30 years. *Microbiology* **162**, 642–650 (2016).
129. Flannery, D. D. et al. Antibiotic susceptibility of *Escherichia coli* among infants admitted to neonatal intensive care units across the US from 2009 to 2017. *JAMA Pediatr.* **175**, 168–175 (2021).

130. Cassini, A. et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect. Dis.* **19**, 56–66 (2019).
131. Poolman, J. T. & Wacker, M. Extraintestinal pathogenic *Escherichia coli*, a common human pathogen: challenges for vaccine development and progress in the field. *J. Infect. Dis.* **213**, 6–13 (2016).
132. Weiner-Lastinger, L. M. et al. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015–2017. *Infect. Control. Hospital Epidemiol.* **41**, 1–18 (2020).
133. Foxman, B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect. Dis. Clin. North Am.* **28**, 1–13 (2014).
134. Tacconelli, E. et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* **18**, 318–327 (2018).
135. United Nations Environment Programme. Bracing for superbugs: strengthening environmental action in the One Health response to antimicrobial resistance https://www.unep.org/resources/superbugs/environmental-action (2023).
136. World Health Organization. WHO integrated global surveillance on ESBL-producing *E. coli* using a "One Health" approach: implementation and opportunities. (WHO, 2021).
137. Livermore, D. M. Defining an extended-spectrum β-lactamase. *Clin. Microbiol. Infect.* **14**, 3–10 (2008).
138. Livermore, D. M. & Hawkey, P. M. CTX-M: changing the face of ESBLs in the UK. *J. Antimicrob. Chemother.* **56**, 451–454 (2005).
139. Ludden, C. et al. Defining nosocomial transmission of *Escherichia coli* and antimicrobial resistance genes: a genomic surveillance study. *Lancet Microbe* **2**, e472–e480 (2021).
140. Manges, A. R. *Escherichia coli* causing bloodstream and other extraintestinal infections: tracking the next pandemic. *Lancet Infect. Dis.* **19**, 1269–1270 (2019).
141. Mills, E. G. et al. A one-year genomic investigation of *Escherichia coli* epidemiology and nosocomial spread at a large US healthcare network. *Genome Med.* **14**, 147 (2022).
142. Stephens, C. M., Adams-Sapper, S., Sekhon, M., Johnson, J. R. & Riley, L. W. Genomic analysis of factors associated with low prevalence of antibiotic resistance in extraintestinal pathogenic *Escherichia coli* sequence type 95 strains. *mSphere* **2**, e00390 (2017).
143. Carrilero, L., Dunn, S. J., Moran, R. A., McNally, A. & Brockhurst, M. A. Evolutionary responses to acquiring a multidrug resistance plasmid are dominated by metabolic functions across diverse *Escherichia coli* lineages. *mSystems* **8**, e0071322 (2023).
144. Reid, C. J. et al. A role for ColV plasmids in the evolution of pathogenic *Escherichia coli* ST58. *Nat. Commun.* **13**, 1–15 (2022).
This report explores important concepts that underpin the emergence of a pathogenic lineage of *E. coli* with emphasis on the role played by the stable co-acquisition of key virulence-associated genes.
145. Li, L. et al. Genomic characterization of mcr-1-carrying foodborne *Salmonella enterica* serovar Typhimurium and identification of a transferable plasmid carrying mcr-1, $bla_{CTX\text{-}M\text{-}14}$, qnrS2, and oqxAB genes from ready-to-eat pork product in China. *Front. Microbiol.* **13**, 903268 (2022).
146. Macori, G. et al. Characterisation of early positive mcr-1 resistance gene and plasmidome in *Escherichia coli* pathogenic strains associated with variable phylogroups under colistin selection. *Antibiotics* **10**, 1041 (2021).
147. Zhang, X. et al. Spread and molecular characteristics of enterobacteriaceae carrying fosA-like genes from farms in China. *Microbiol. Spectr.* **10**, e005422 (2022).
148. Zhao, W.-H. & Hu, Z.-Q. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Crit. Rev. Microbiol.* **39**, 79–101 (2013).
149. Du, P. et al. Novel IS*26*-mediated hybrid plasmid harbouring tet_{X4} in *Escherichia coli*. *J. Glob. Antimicrob. Resist.* **21**, 162–168 (2020).
150. He, D. et al. Emergence of a hybrid plasmid derived from IncN1-F33:A–:B– and *mcr-1*-bearing plasmids mediated by IS*26*. *J. Antimicrob. Chemother.* **74**, 3184–3189 (2019).
151. Vinué, L. et al. Plasmids and genes contributing to high-level quinolone resistance in *Escherichia coli*. *Int. J. Antimicrob. Agents* **56**, 105987 (2020).
152. Porse, A., Schønning, K., Munck, C. & Sommer, M. O. A. Survival and evolution of a large multidrug resistance plasmid in new clinical bacterial hosts. *Mol. Biol. Evol.* **33**, 2860–2873 (2016).
153. Venturini, C., Beatson, S. A., Djordjevic, S. P. & Walker, M. J. Multiple antibiotic resistance gene recruitment onto the enterohemorrhagic *Escherichia coli* virulence plasmid. *FASEB J.* **24**, 1160–1166 (2010).
154. Harmer, C. J. & Hall, R. M. IS*26* cannot move alone. *J. Antimicrob. Chemother.* **76**, 1428–1432 (2021).
155. Dawes, F. E. et al. Distribution of class 1 integrons with IS*26*-mediated deletions in their 3′-conserved segments in *Escherichia coli* of human and animal origin. *PLoS ONE* **5**, e12754 (2010).
156. Harmer, C. J. & Hall, R. M. An analysis of the IS6/IS*26* family of insertion sequences: is it a single family? *Microb. Genom.* **5**, e000291 (2019).
157. Tedijanto, C., Olesen, S. W., Grad, Y. H. & Lipsitch, M. Estimating the proportion of bystander selection for antibiotic resistance among potentially pathogenic bacterial flora. *Proc. Natl Acad. Sci. USA* **115**, E11988–E11995 (2018).
158. Cummins, M. L. et al. Whole-genome sequence analysis of an extensively drug-resistant *Salmonella enterica* serovar Agona isolate from an Australian silver gull (*Chroicocephalus novaehollandiae*) reveals the acquisition of multidrug resistance plasmids. *mSphere* **5**, e00743-20 (2020).
159. Nyirabahizi, E. et al. Evaluation of *Escherichia coli* as an indicator for antimicrobial resistance in *Salmonella* recovered from the same food or animal ceca samples. *Food Control.* **115**, 107280 (2020).
160. United Nations Environment Programme. Environmental dimensions of antimicrobial resistance: summary for policymakers. https://wedocs.unep.org/bitstream/handle/20.500.11822/38373/antimicrobial_R.pdf (2022).
161. Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A. & Collins, J. J. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**, 797–810 (2007).
162. Miller, C. et al. SOS response induction by β-lactams and bacterial defense against antibiotic lethality. *Science* **305**, 1629–1631 (2004).
163. Shapiro, R. S. Antimicrobial-induced DNA damage and genomic instability in microbial pathogens. *PLoS Pathog.* **11**, e1004678 (2015).
164. Cheng, Y.-Y. et al. Efficient plasmid transfer via natural competence in a microbial co-culture. *Mol. Syst. Biol.* **19**, e11406 (2023).
165. Fornelos, N., Browning, D. F. & Butala, M. The use and abuse of LexA by mobile genetic elements. *Trends Microbiol.* **24**, 391–401 (2016).
166. Baharoglu, Z., Bikard, D. & Mazel, D. Conjugative DNA transfer induces the bacterial SOS response and promotes antibiotic resistance development through integron activation. *PLoS Genet.* **6**, e1001165 (2010).
167. Ginn, O. et al. Open waste canals as potential sources of antimicrobial resistance genes in aerosols in urban Kanpur, India. *Am. J. Trop. Med. Hyg.* **104**, 1761–1767 (2021).
168. Karkman, A., Pärnänen, K. & Larsson, D. G. J. Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. *Nat. Commun.* **10**, 80 (2019).
169. Dolejska, M. & Papagiannitsis, C. C. Plasmid-mediated resistance is going wild. *Plasmid* **99**, 99–111 (2018).
170. Snaith, A. E. et al. The highly diverse plasmid population found in *Escherichia coli* colonizing travellers to Laos and its role in antimicrobial resistance gene carriage. *Microb. Genom.* **9**, 001000 (2023).
171. Rodríguez-Molina, D. et al. International travel as a risk factor for carriage of extended-spectrum β-lactamase-producing *Escherichia coli* in a large sample of European individuals—The AWARE Study. *Int. J. Environ. Res. Public Health* **19**, 4758 (2022).
172. Ginn, O. et al. Detection and quantification of enteric pathogens in aerosols near open wastewater canals in cities with poor sanitation. *Environ. Sci. Technol.* **55**, 14758–14771 (2021).
This study emphasizes that aerosols generated in densely populated regions with comparatively poor sanitation practices are under-recognized as a mechanism of transmission of ARGs and enteric pathogens.
173. Xin, H. et al. Animal farms are hot spots for airborne antimicrobial resistance. *Sci. Total Environ.* **851**, 158050 (2022).
174. Lv, B. et al. Abundances and profiles of antibiotic resistance genes as well as co-occurrences with human bacterial pathogens in ship ballast tank sediments from a shipyard in Jiangsu Province, China. *Ecotoxicol. Environ. Saf.* **157**, 169–175 (2018).
175. Lv, B. et al. Vessel transport of antibiotic resistance genes across oceans and its implications for ballast water management. *Chemosphere* **253**, 126697 (2020).
176. Elankumaran, P., Browning, G. F., Marenda, M. S., Reid, C. J. & Djordjevic, S. P. Close genetic linkage between human and companion animal extraintestinal pathogenic *Escherichia coli* ST127. *Curr. Res. Microb. Sci.* **3**, 100106 (2022).
177. Abdullahi, I. N. et al. Clonal relatedness of coagulase-positive staphylococci among healthy dogs and dog-owners in Spain. Detection of multidrug-resistant-MSSA-CC398 and novel linezolid-resistant-MRSA-CC5. *Front. Microbiol.* **14**, 1121564 (2023).
178. Yang, Q. E. et al. Environmental dissemination of mcr-1 positive Enterobacteriaceae by *Chrysomya* spp. (common blowfly): an increasing public health risk. *Environ. Int.* **122**, 281–290 (2019).
This study sheds light on the role of blow flies in disseminating clinically important ARGs, particularly in resource-poor environments.
179. Tyrrell, C. et al. Differential impact of swine, bovine and poultry manure on the microbiome and resistome of agricultural grassland. *Sci. Total Environ.* **886**, 163926 (2023).
180. Marutescu, L. G. et al. Insights into the impact of manure on the environmental antibiotic residues and resistance pool. *Front. Microbiol.* **13**, 965132 (2022).
181. Klein, E. Y. et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl Acad. Sci. USA* **115**, E3463–E3470 (2018).
182. Kuppusamy, S. et al. Veterinary antibiotics (VAs) contamination as a global agro-ecological issue: a critical view. *Agric. Ecosyst. Environ.* **257**, 47–59 (2018).
183. Ma, F., Xu, S., Tang, Z., Li, Z. & Zhang, L. Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosaf. Health* **3**, 32–38 (2021).
184. Tiseo, K., Huber, L., Gilbert, M., Robinson, T. P. & Van Boeckel, T. P. Global trends in antimicrobial use in food animals from 2017 to 2030. *Antibiotics* **9**, E918 (2020).
185. Van Boeckel, T. P. et al. Global trends in antimicrobial use in food animals. *Proc. Natl Acad. Sci. USA* **112**, 5649–5654 (2015).

186. Kemper, N. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol. Indic.* **8**, 1–13 (2008).
187. Zhang, N. et al. Coexistence between antibiotic resistance genes and metal resistance genes in manure-fertilized soils. *Geoderma* **382**, 114760 (2021).
188. Berendes, D. M., Yang, P. J., Lai, A., Hu, D. & Brown, J. Estimation of global recoverable human and animal faecal biomass. *Nat. Sustain.* **1**, 679–685 (2018).
This study estimates the global production of human and animal faeces, emphasizing the increasing animal to human ratio with time (6:1 by 2050), and highlights the importance of managing the persistent threats to global public health particularly in LMICs as well as the opportunities for recovery of resources via circular economies, with implications for zoonosis, One Health and AMR.
189. Cavicchioli, R. et al. Scientists' warning to humanity: microorganisms and climate change. *Nat. Rev. Microbiol.* **17**, 569–586 (2019).
190. MacFadden, D. R., McGough, S. F., Fisman, D., Santillana, M. & Brownstein, J. S. Antibiotic resistance increases with local temperature. *Nat. Clim. Change* **8**, 510–514 (2018).
191. McGough, S. F., MacFadden, D. R., Hattab, M. W., Mølbak, K. & Santillana, M. Rates of increase of antibiotic resistance and ambient temperature in Europe: a cross-national analysis of 28 countries between 2000 and 2016. *Eurosurveillance* **25**, 1900414 (2020).
192. Walsh, T. R., Weeks, J., Livermore, D. M. & Toleman, M. A. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect. Dis.* **11**, 355–362 (2011).
193. Reckien, D. & Aalst, M. K. van. in *Climate Change 2022: Impacts, Adaptation and Vulnerability: Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.* Pörtner, H.O, Roberts, D.C, Tignor, M., Poloczanska, E.S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., Rama, B. (eds.), 3–33 (Cambridge Univ. Press, 2022).
194. Fouladkhah, A. C., Thompson, B. & Camp, J. S. The threat of antibiotic resistance in changing climate. *Microorganisms* **8**, E748 (2020).
195. Delgado-Baquerizo, M. et al. The proportion of soil-borne pathogens increases with warming at the global scale. *Nat. Clim. Chang.* **10**, 550–554 (2020).
196. Escobar, L. E. et al. A global map of suitability for coastal *Vibrio cholerae* under current and future climate conditions. *Acta Trop.* **149**, 202–211 (2015).
197. Mora, C. et al. Over half of known human pathogenic diseases can be aggravated by climate change. *Nat. Clim. Chang.* **12**, 869–875 (2022).

Acknowledgements
The authors thank F. MacIver from the University of Technology Sydney for assistance with preparing and editing the manuscript and S. Zufan from the University of Melbourne for assistance with preparation of Fig. 3. This work was supported by the Medical Research Future Fund (MRFF)-supported AusPathoGen Program (FSPGN000049) and by the Australian Centre for Genomic Epidemiological Microbiology (Ausgem), a collaborative research initiative between the New South Wales Department of Primary Industries and the University of Technology Sydney. B.P.H. is supported by a National Health and Medical Research Council (NHMRC) Fellowship (GNT1196103).

Author contributions
S.P.D., V.M.J., T.S., M.L.C., A.E.W., B.D., E.D. and B.P.H. researched the literature. S.P.D., V.M.J., T.S., M.L.C., A.E.W., E.R.W., C.J.R., E.D. and B.P.H. contributed substantially to discussions of the content. S.P.D., V.M.J., T.S., M.L.C., E.D. and B.P.H. wrote the article. S.P.D., V.M.J., T.S., A.E.W., B.D., C.J.R., E.D. and B.P.H. reviewed and/or edited the manuscript before submission.

Competing interests
The authors declare no competing interests.

Additional information
Peer review information *Nature Reviews Genetics* thanks Erick Denamur and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Related links
hAMRonization: https://github.com/pha4ge/hAMRonization
PHA4GE Consortium: https://pha4ge.org/