Utrecht University

Antimicrobial Resistance: Is It a One Health Issue?

Writing Assignment – Literature Review
07/12/2022

Nafsika Kardomatea, MSc One Health
Student number: 4989737

Examiner: Prof. dr. Rob J. L. Willems, UMCU
Second Reviewer: dr. Anita C. Schürch, UMCU

Contents

Plain Language Summary

Antibiotics are antimicrobial medications that are used to combat infections caused by bacteria in both humans and animals. However, certain bacteria are resistant to the effects of antibiotics because of their structural or functional characteristics. Alternatively, bacteria can become resistant by spontaneous mutations that occur in their chromosomal DNA or by acquiring genes that confer resistance to certain antibiotics (i.e. antimicrobial resistance genes (ARGs)). The latter can occur by means of horizontal gene transfer (HGT) of mobile genetic elements (MGEs). MGEs are mobilizable stretches of DNA that can be transferred from a donor cell to a recipient cell. This means that ARGs can spread from one bacterium to another and crucially, even between different species of bacteria, thus resistance to these antibiotics can propagate and render previously effective antibiotics, ineffective. Currently, antimicrobial resistance (AMR) is reported to cause numerous deaths while projections estimate that these numbers will dangerously soar in the near future, threatening humanity. Indeed, without effective antibiotics, previously curable infections and diseases will become challenging to treat whereas surgical operations may even become too risky to perform because of the potential of a post-operative infection occurring. As such, efforts are concentrated to decelerate the progression of AMR. It is known that antimicrobial use (AMU) drives AMR by evolutionary selecting resistant bacterial strains. While efforts are being made to limit AMU in human medicine, it must be mentioned that high amounts of antimicrobials are also used in animals, especially in livestock. A hypothesis has been formed that resistant strains that emerge in animals, because of excessive AMU, may be able to transfer to humans. It has been proposed that this transmission event can also occur via HGT of ARG-carrying MGEs, rather than AMR bacterial strains. As such, efforts are focusing on reducing AMU on animals. However, this theory has yet to be proven with robustly generated evidence because of the complexity of such epidemiological studies. Whole-genome sequencing (WGS) technologies can provide the necessary tools in order to clarify whether bacterial strains isolated from animals and humans are genetically related, which could point to a potential transmission event occurring between these two hosts. In fact, WGS can describe the genome of a bacteria as well as potential MGEs and ARGs it may harbor. In this review, we collected 19 studies that used WGS to investigate whether inter-host transmission occurs across different countries. The results indicate that in lower-middle income countries (LMICs) where AMU is largely unmonitored and humans are in closer contacts with animals, this was observed more frequently compared to high-income countries (HICs) where there were no or very limited evidence. In upper-middle income countries (UMICs), where there is an overuse of antimicrobials in animals due to intensive farming, again more evidence were reported. These studies suggested that HGT of ARG-carrying MGEs plays an important role in inter-host transmission events. Although some evidence were found, more well-designed studies using WGS techniques are necessary to better describe the intricate pathways of an inter-host AMR transmission event. Thus, in the future, it is advisable to conduct large-sized studies with correct sampling protocols over long periods of time to offer more information on this complex issue.

Abstract

Antimicrobial resistance (AMR) constitutes a serious threat to public health by rendering bacteria, previously susceptible to antimicrobials, resistant to their effects. Recent reports indicate that a considerable amount of human deaths is associated or even directly attributable to antimicrobial-resistant bacterial infections. Bacteria can become resistant because of spontaneous mutations or horizontal acquisition of antimicrobial resistance genes (ARGs) from exogenous sources. Horizontal transfer of ARGs can occur via mobile genetic elements (MGEs), such as plasmids, transposons or bacteriophages and this way, interspecies dissemination of ARGs can occur. Use of antimicrobials constitutes a well-described driver of AMR because it applies selection pressure on bacteria. Importantly, high amounts of antimicrobials are being used in livestock, which has caused concern on potential inter-host zoonotic transmission of AMR strains or ARGs. However, there are scarce evidence indicating transmission of AMR can happen between humans and animals, especially using robust methodologies. In this review, 19 papers employing whole-genome sequencing are presented on the basis of providing robust evidence (or not) of inter-host AMR transmission. In high-income countries (HICs), no or very limited evidence were identified. Comparably, more studies reported such events in upper-middle income countries (UMICs) and lower-middle income countries (LMICs), possibly because of a higher use of antimicrobials and closer contacts between humans and animals. Horizontal transfer of ARGs via MGEs appeared to play an important role in these events. Future studies should follow longitudinal, systematic sampling across both sectors to shed more light into the frequency and speed of this occurrence globally.

Introduction

In the wake of the vast consequences of the COVID-19 pandemic, the spotlight has been cast on other looming threats to global public health, with antimicrobial resistance (AMR) having already been declared amongst the top ten by the World Health Organization (WHO) in 2019 (1,2). AMR constitutes the ability of microorganisms -in this review the focus is on bacteria- to become resistant to the effects of antimicrobial medicines to which they were previously susceptible (3). In contrast to viral pandemics, which typically occur suddenly and spread rapidly, the progress of AMR is slower and more obscure, yet its impact can be especially dire in the long term (4,5). Indeed, without effective antimicrobials the treatment of common infections would become challenging while it would be nearly unfeasible to perform both standard and major surgical operations as well as cancer treatments (4,6). In 2019, 4.95 million deaths worldwide were estimated to be associated with bacterial AMR and out of those, 1.27 million deaths were directly attributable to bacterial AMR, constituting it a leading cause of death worldwide and especially in low-income countries (3). Earlier, a report issued by O'Neill in 2016 stated an alarming projection in which by 2050 the number of deaths attributable to AMR each year is expected to rise to 10 million globally and the associated economic losses to reach USD 100 trillion (7). Others have criticized the report by O'Neill concerning the methodology employed to arrive at the reported estimations, as unreliable and crude (8). Despite this, Robinson et al. argue that the focus should be on the magnitude of the issue and its rapid progression rather than the accuracy of these estimates (9). Confronted with such predictions, official bodies have decided to adopt a collaborative One Health approach to mitigate this issue owing to the diverse elements that drive AMR (10). This approach acknowledges that the human, animal and environmental health are interconnected and advocates that strategies aiming at mitigating AMR should incorporate holistic measures factoring in these sectors (11).

Resistance to antibiotics and antibiotic resistance genes (ARGs) predate the existence of humans since many antibiotics are natural compounds, which have existed for millions (perhaps even billions) of years (12). In fact, ARGs have been discovered deep in permafrost sediments that were dated back to approximately 30,000 years and in isolated caves dated back to more than four million years (12,13). Antibiotics are produced by various species, such as soil bacteria, most likely for the purpose of limiting the expansion of nearby contestants in the environment (14). As these producers release antibiotics in the respective environment, they expose both themselves and other bacteria to the antibiotics (15). Consequently, resistance mechanisms against these antibiotics are selected evolutionarily in order for the producers to avoid self-destruction and accordingly, for other bacteria to defend themselves (15). In turn, this resistance -encoded in ARGs- can be transferred either vertically to the progeny or horizontally (i.e. horizontal gene transfer (HGT)) to close relatives or even other species, which then propagate it to their progeny (15). However, bacteria can also have an intrinsic resistance to antibiotics because of their functional and structural characteristics or become resistant through spontaneous mutations of native resident genes (16). While humans did not create AMR, they have hastened its progression through the evolutionary pressure they exert on bacteria with antimicrobial use (AMU) (17). In fact, as soon as a new antibiotic class is introduced in clinical practice, resistant strains are quick to emerge (18). This phenomenon is

further exacerbated by the misuse and overuse of antibiotics in both humans and animals (6). Humans use antibiotics to treat bacterial infections and in some cases pre or post-operatively for prophylaxis (4). In food-producing animals, antibiotics have been additionally used metaphylactically and as growth promoters, although many countries have now banned this type of antibiotic use (4,19). Despite this, Van Boeckel et al. report that 73% of all antimicrobials sold globally are used in food-producing animals with many of the antimicrobial classes being the same as the ones used in human medicine (20). In addition, the environment is considered an important reservoir of resistance that contributes to AMR dissemination (21). Besides ARGs preexisting in the environment, they can also be introduced in the water and soil through manure runoff from farms and sewage, which can then be transferred to human and animal pathogenic bacteria via HGT thereby conferring resistance to them (Figure 1) (15,21). These facts, coupled with the knowledge that many commensal and pathogenic bacterial species are common between humans and animals, have led to the hypothesis that resistant clones or resistance elements may transmit from animals to humans (and vice versa) (4). That is precisely why many efforts apply the One Health approach, focusing on decreasing and improving AMU in agriculture, besides human medicine (22). Yet, currently there is a lack of robust evidence with regards to determining whether this transmission indeed occurs -and if so- its extent, frequency, speed and, perhaps most importantly, the degree of impact that such an event could cause on humanity (4).

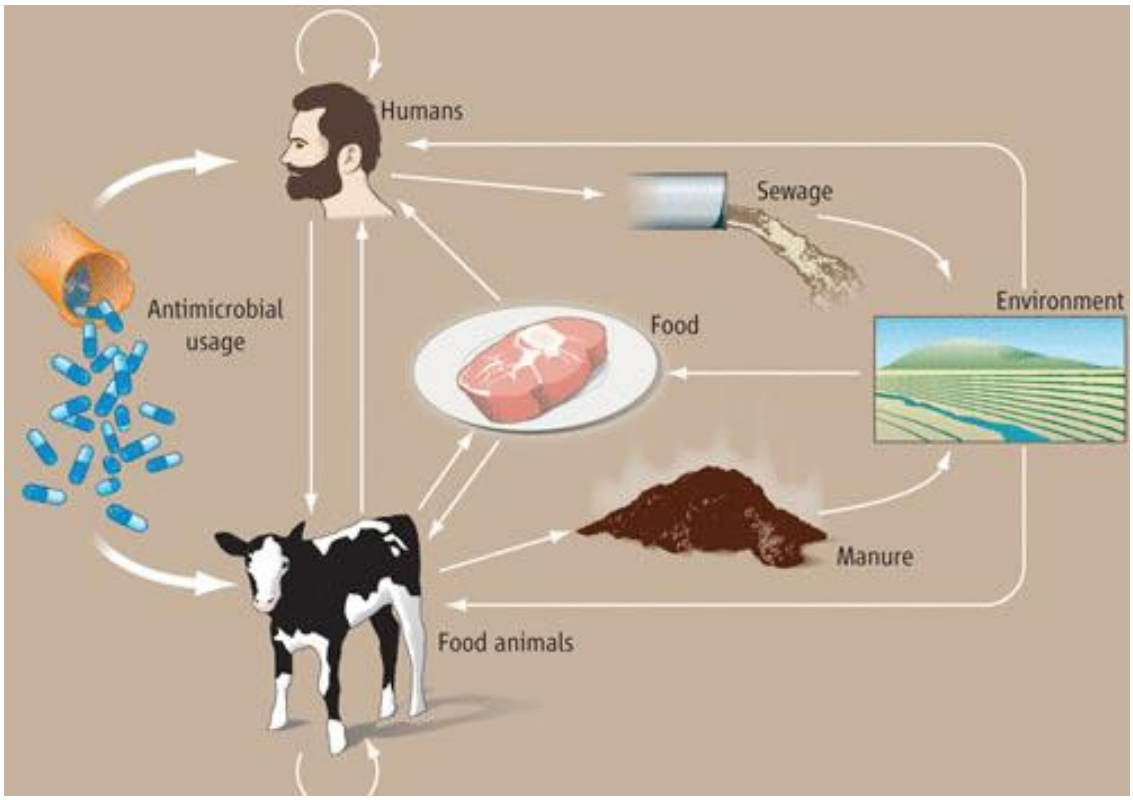

Figure 1: Routes of transmission of antimicrobial resistance amongst humans, animals and the environment (22)**.**

To control the progression of AMR, data from surveillance programs across all sectors on AMU and AMR rates are essential (10). These are traditionally collected using antimicrobial

susceptibility testing (AST) (23). However, in order to identify possible AMR transmission events and pathways between animals and humans, data at the molecular level are crucial (23). AST generally fails to provide information on the resistance mechanisms that are at play, often confounding dissimilar clones who happen to display the same resistance profiles (17). Alternatively, genetic typing methods, such as multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) can only describe a small part of a genome, thus lacking the adequacy to provide solid proof of resistance transmission and importantly, indicate the potential directionality (23,24). Whole-genome sequencing (WGS) on the other hand, provides data at a high resolution, which when combined with epidemiological data, can delineate more robustly the intricate pathways and temporospatial dynamics of interspecies AMR transmission (23,24). Besides, as Julian Parkhill mentions in a recently published article, using low-resolution techniques (e.g. MLST or PFGE) can lead to discovering presumably close similarities between isolates from different sources that when studied at a higher resolution, such as WGS, disappear (4). For instance, Leverstein-van Hall et al. employed MLST and PCR techniques to assess the relatedness of *Eschericia coli* (*E.coli*) isolates from poultry meat and humans, concluding that clonal transfer had occurred between these species (25). However, in a subsequent study, de Been et al. used WGS to analyze the same set of isolates, revealing that they were, in fact, genetically distinct (26).

Mobile genetic elements versus resistant clones

Currently, HGT of ARGs is associated with most of the troublesome AMR issues of public health concern, including multidrug resistance (MDR) (27). HGT entails primarily genes, including ARGs, that are carried on mobile genetic elements (MGEs) (28). MGEs are sections of genetic material that have the ability to move within or between genomes, such as plasmids, insertion sequences (IS), transposons and bacteriophages (phages) (29). Other important MGEs include integrons that despite lacking this ability, they contain a site-specific recombination system that can integrate, express and exchange certain DNA elements (i.e. gene cassettes), which are considered mobile (30). Also, satellite viruses can exploit bacteriophage mechanisms for transfer and induction (29). Consequently, the dissemination and accumulation of ARGs from a variety of reservoirs is largely thought to be because of HGT of MGEs, which warrants a closer look at the molecular level to delineate potential transmission events between different species and environments (30). Conversely, clinical problems arise typically because of successful resistant clones that can promote transmission amongst environments and hosts (15). Yet, which resistant clone could prove to be clinically significant depends on many factors, such as the range of environments it can adapt to and its transmissibility (15). A deeper view at the molecular level of known successful clones may additionally delineate these factors as well as potential transmission events.

How do ARGs transfer horizontally?

There are three major mechanisms of HGT; transformation, conjugation and transduction (Figure 2 (a), (b), (c), (d)) (28). Transformation occurs when a recipient cell takes up naked DNA (single or double-stranded) that exists in the extracellular space (e.g., after it has been

released by dead and lysed cells) (28). Subsequently, this DNA recombines with the chromosome of the recipient cell, thereby incorporating new genes from the incoming DNA (31). Transformation appears to have played an important role in the evolution of AMR strains in the genera *Streptococcus* and *Neisseria*, which are human pathogenic bacteria (31).

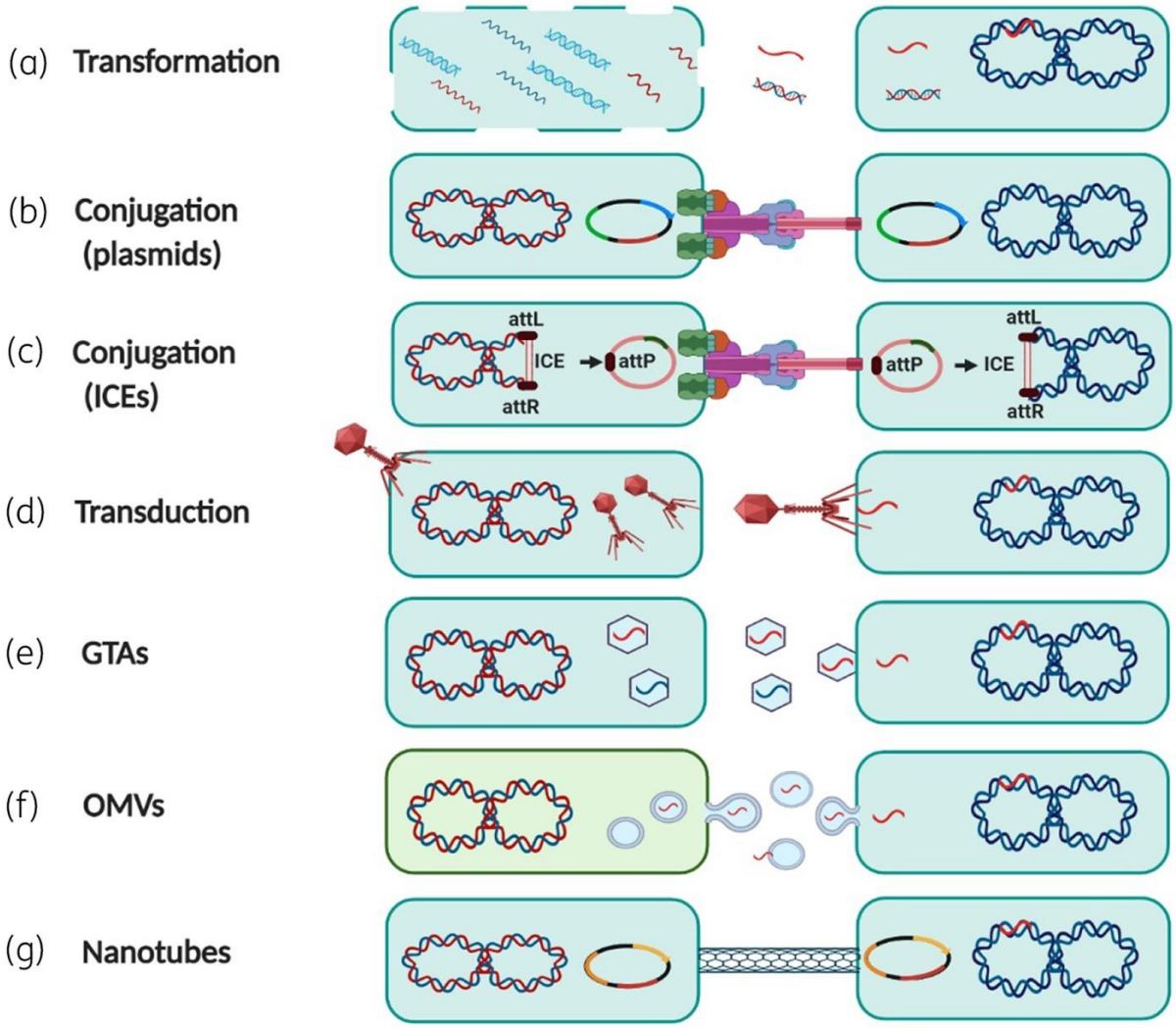

Figure 2: Main mechanisms of HGT in bacteria in a graphic representation (28)**.** (a) Transformation: naked DNA is released from lysed cells and is taken up by a competent bacterium. (b) Plasmid conjugation: plasmids are transferred between cells when they are in physical contact with each other via the mating-pore pilus. (c) ICEs conjugation: at two attachment sites (i.e. attL and attR), which are located on the chromosome of the donor cell, the ICEs are excised from it and subsequently form a circular plasmid-like shape with an attP site on the circularized ICE, which, employing the same mechanism as for plasmid, is transferred (d). After transfer, at the recipient cell, the circularized ICE re-integrates into the chromosome. (d) Transduction: genetic material is transferred from one bacterium to another with bacteriophages. (e) GTAs: DNA is encapsulated by phage-derived sequences that act as transfer vectors and be delivered into another bacterium. (f) OMVs: vesicles formed in the outer membrane of the cell encapsulate DNA and permit the transfer of genetic material into another bacterium. (g) Nanotubes: non-conjugative plasmids are transferred from one bacterium to another through nanotubes.

By contrast, direct cell-to-cell contact is necessary for conjugation to occur, in which DNA is transferred with the involvement of conjugative plasmids or conjugative transposons (also

known as integrative conjugative elements (ICEs)) (15). In particular, a bridge is formed when two bacteria are in the same environment and this permits the transfer of a plasmid from one cell to the other (31). Plasmids constitute mobile pieces of DNA of circular shape that have the ability to replicate independently from the cell's chromosome (31). Just one plasmid can carry several genes, thus coding for multiple drug resistance (31). The role of ICEs and integrons in conjugation, when linked to plasmids, is to aid in gathering, expressing and disseminating ARGs thereby hastening the development of bacterial resistance (31). However, ICEs can also be transferred themselves via conjugation (28). Both conjugation and transformation are thought to occur at increased rates when there is a high population density between donor and recipient cells (e.g. human and animal intestine) (15).

Conjugation has been recognized as the most crucial mechanism for AMR dissemination in nature since MGEs can cross species boundaries (28,31). Indicatively, diffusion of β-lactam ARG harboring plasmids by conjugation is possible between *Acinetobacter spp.*, *Pseudomonas spp.* and *Enterobacteriaceae* species (27). Additionally, the role of non-pathogenic environmental bacteria in the spread of clinically relevant ARGs has been shown to be substantial (32). In fact, the *CTX-M* extended-spectrum-β-lactamase (ESBL) gene, which confers resistance against cephalosporins and monobactams and has been discovered in pathogenic bacteria globally, was found to be nearly identical to the *CTX-M* gene found in the genome of *Kluyvera*, a non-pathogenic environmental species (33,34). Apparently, a transposable genetic element (i.e. the insertion sequence IS*Ecp1*) facilitated the selection and transfer of the progenitor resistance gene in *Kluyvera* by providing a promoter, thus permitting its expression and transposition into others species (35). Today, the enzymes CTX-M-14 and CTX-M-15 have been frequently isolated globally, with CTX-M-15-producing *Klebsiella pneumoniae* (*K. pneumoniae*) strains being mostly hospital-associated and CTX-M-15-producing *E. coli* strains being disseminated in patients in the community (36). Globally, the IncK plasmid, indicated as pCT, has spread $bla_{\text{CTX-M-14}}$ to both animals and humans (37). Other similar examples include the plasmid-encoded *qnrA* gene, which was probably transferred to several *Enterobacteriaceae* species from marine and freshwater *Shewanella algae* and the finding that *Shewanella oneidensis* constitutes a reservoir of the plasmid-encoded gene carbapenem-hydrolyzing oxacillinase-48-type b- lactamases (OXA-48), now also frequently found in *K. pneumoniae* (15). Therefore, a variety of MGEs appear to mobilize ARGs that are found widespread in the environment for transfer into human and animal pathogenic or commensal bacteria. Besides this, concerns have risen regarding MGE transfer through conjugation from resistant bacteria originating in animals to humans. In 2016, Liu et al. reported for the first time the emergence of plasmid-mediated colistin resistance (i.e. *mcr-1*) in *E. coli* isolates from raw meat, animals and humans (38). Colistin is an antibiotic of last resort for combatting MDR and carbapenem-resistant bacteria in humans (38,39). The authors speculated that the origin of *mcr-1*-mediated colistin resistance was in animals, subsequently spreading to humans, although that was not proven (38). Worryingly, PHNSHP45, which is the *mcr-1*-bearing plasmid, was shown to have a high *in vitro* transfer rate between *E. coli* strains, being able to transfer into important human pathogens (i.e., *E. coli* ST131, *K. pneumoniae* ST11 and *Pseudomonas aeruginosa*) (38). From that time, many studies have reported other novel *mcr* genes (i.e., *mcr-2* to *mcr-10*), which are widespread in *Enterobacteriaceae* (40). Clearly, HGT via conjugation of MGEs has contributed majorly to

the emergence and dissemination of clinically important pathogenic bacteria in both humans and animals.

Transduction occurs via bacteriophages that, after they lyse a bacterial cell, may occasionally incorporate bacterial DNA instead of phage DNA in their capsid, thus afterwards injecting this DNA to another bacterial cell (28). Two types of transduction are known; generalized and specialized (28). In generalized transduction, any section of the bacterial chromosome or MGEs can be incorporated by the phages whereas in specialized transduction, solely the DNA that is next to the original integrated prophages can be contained and transferred (28). This mechanism may contribute to spreading DNA between bacterial species that reside in distant environments (15).

Recently, there have been other mechanisms of HGT that have been identified, namely the transfer that is mediated by gene transfer agents (GTAs), by intercellular nanotubes and by outer membrane vesicles (OMVs) (Figure 2 (e), (f), (g)) (28). Briefly, GTAs are sequences that derive from bacteriophages, encoding phage capsid structures (28). These structures can encapsulate DNA, consequently serving as transfer vectors of a random (less than 15 kb in size) part of the bacterial genome (28). This form of "transduction" is considered more efficient than generalized transduction (28). Bacteria can communicate via molecule exchange within and between species with nanotubes (i.e. protrusions of tubular shape) (28). This way, non-hereditary and hereditary AMR can be exchanged between cells that are in close proximity (28). Finally, OMVs can take up extracellular DNA from lysed bacteria and also bacteria can store DNA into OMVs before they bud off from the bacterial cell (28). HGT via OMVs has been showcased in many species, such as *A. baumannii* and *E. coli* but also between different species (28). Overall, plasmid-mediated HGT is most efficient owing to their inbuilt mechanisms that ensure stable maintenance in the recipient cell, thereby not depending on homologous recombination contrary to transformation and transduction (15). Yet, it has become increasingly evident that studying plasmid-mediated HGT alone may not be sufficient to describe the complex transmission mechanisms and pathways of ARGs within and between species (41).

Comparing resistant clones is not enough to delineate ARG dissemination

While certain antibiotic resistances have risen because of plasmids or other MGEs and HGT, for some their spread has been perpetuated by clonal dissemination. One such case is vancomycin resistance, which appeared in *Enterococcus* species because of transposon-borne genes, such as *vanA* and *vanB*, consequently causing various threatening diseases such as urinary tract infections (UTIs), bacteraemia and meningitis (31). However, the dissemination of these ARGs is primarily dominated by clonal spread of vancomycin-resistant *Enterococcus faecium* (VRE) (42). A recent study by Arredondo-Alonso et al. in Dutch hospitals reported that the *vanA* gene cluster was mostly disseminated via clonal spread but in certain outbreaks, the dissemination occurred via HGT, which was mediated by either Tn*1546* transposition between different genomic locations and/or the involvement of plasmids (43). An interesting case is that of methicillin-resistant *Staphylococcus aureus* (MRSA) clonal complex 398 (CC398), which can colonize humans after livestock exposure (44). Price et al. demonstrated that livestock-associated MRSA CC398 originated in humans as methicillin-susceptible

Staphylococcus aureus (MSSA), subsequently spreading to livestock where it acquired the SCC*mec* cassette and *tet*(M) gene, thus acquiring methicillin and tetracycline resistance, respectively (44). This is a prime example of a bidirectional AMR transmission event between humans and animals that emerged because of MGEs but spreads via clonal dissemination (44). It must be noted though that livestock-associated MRSA CC398 strains appear to be less virulent, having lower transmission rates in humans and only occasionally resulting in infections (44,45).

Carbapenem-resistant *K. pneumoniae* is thought as the most rapid-growing AMR threat in Europe, having an increased mortality rate (23). Notably, the carbapenemase bla_{KPC} gene is also plasmid-borne and has caused several outbreaks, which are associated with the *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* ST258 clone that is found globally (36). The most widely spread carbapenemase is New Delhi metallo-β-lactamase (NDM), which is found in Gram-negative pathogens such as *Acinetobacter baumannii* (*A. baumannii*), *K. pneumoniae* and *E. coli* (36). Similarly to KPC carbapenemase, the *ndm* genes are frequently found in broad-host-range conjugative plasmids (36).

As presented, in some cases resistant clonal dissemination has contributed to the spread of clinically relevant antibiotic resistant bacterial strains thereby causing healthcare problems in both animals and humans. Many of those strains however have been the outcome of MGEs that transferred horizontally, subsequently becoming successful in their spread (28). It is questionable whether resistant clones could have the ability to transfer between hosts and, most importantly, be able to adapt, compete or dominate the microbial communities present in a different host species (46,47). Contrastingly, the hitherto evidence suggest that MGEs are capable of intra and interspecies dissemination and can readily and rapidly transmit ARGs, especially in highly populated environments (e.g. intestinal microbiome) or where close contacts occur (15,28). One could draw a parallel between the transmission of resistance and a multi-layered, Russian nesting doll-like process in which ARGs are taken up by MGEs that may potentially be acquired by certain bacterial clones via HGT (41,47). These clones, being present in gene-exchange communities, propagate ARGs in these environments and may infect animals or humans, which in turn could pass them on in other populations (47). Clearly, the emergence, dissemination and transmission of AMR within and between bacterial and host species as well as the environment is a highly complex issue, which requires a deeper investigation at the molecular level to elucidate the intricate pathways and mechanisms that are at work (23,47).

However, molecular epidemiological studies of resistance are accordingly very complicated (23,29). Conventionally, in the study of MGEs and HGT, PCR-amplicon typing methods have been used, however they are limited in resolution (24). Short-read sequencing has recently been more widely employed to determine the AMR evolution and transmission chains involving HGT, however important limitations exist with this approach as well (29). Particularly, the reads that are produced with this method are too short to permit the identification of MGEs, such as plasmids, phages and transposable elements, consequently resulting in inaccurate or incomplete *de novo* assemblies (29,41). Long-read sequencing technologies can produce reads of longer sequence and can therefore span repetitive areas -where ARGs are often located- , permitting a complete reconstruction of the structure of a genome (23,29). Nonetheless, these longer sequences often have more errors in base calling (23,29). Currently, it appears that a

combination of short- and long-read sequencing technologies can ameliorate and even complete MGEs assemblies from complex whole metagenomes (29). Still, it is important to comprehend when and how to apply these two techniques when conducting molecular epidemiological studies with the aim to trace transmission and outbreak events of resistance (41).

Current evidence of inter-host AMR transmission from genomics data

When considering the possibility of AMR transmission between animals and humans, it is essential to ponder on the potential transmission routes and transfer rates of the bacteria (4). Zoonotic foodborne bacteria, such as *Campylobacter*, are seemingly more capable of directly transmitting to humans and also be implicated in respective AMR cases causing foodborne diseases in humans but they might not be as likely to confer resistance determinants to other relevant bacterial species (4). Indicatively, *Campylobacter* has been found to be resistant to fluoroquinolones and macrolides but these resistances are the outcome of chromosomal mutations, thus there is little potential for HGT to occur and perpetuate this AMR in human clinically relevant bacteria (48). Moreover, it appears that the great majority of transmission events of ARGs happen inconspicuously amongst bacteria that are well adapted in host-associated and environmental microbial communities (e.g. *E. coli* and *Enterobacteriaceae* members), such as the intestinal microbiome (49).

In this review we aim to examine the hitherto evidence that use WGS techniques to prove (or not) the occurrence of AMR transmission between animals and humans, with a specific focus on food-producing animals. We will be mostly concentrating on interspecies AMR transmission with regards to commensal or opportunistic pathogenic bacteria that are common in both humans and animals, such as *E. coli, Enterococcus* spp., *Klebsiella* spp. and are currently responsible for many serious infections in human hospitals (50,51). Besides, in 2017 the WHO declared these bacteria of critical or high priority because of their resistance to multiple antibiotics that are crucial for human medicine (52). This is explored for low-middle income countries (LMICs), upper-middle income countries (UMICs) and high-income countries (HICs), considering the potential determinants in all settings. In addition, we seek to examine the importance of HGT compared to clonal dissemination on the issue of interspecies transmission of AMR. Ultimately, the overall risk of AMR interspecies transmission events is assessed. In Figure 3, the locations of the studies that will be reviewed are presented.

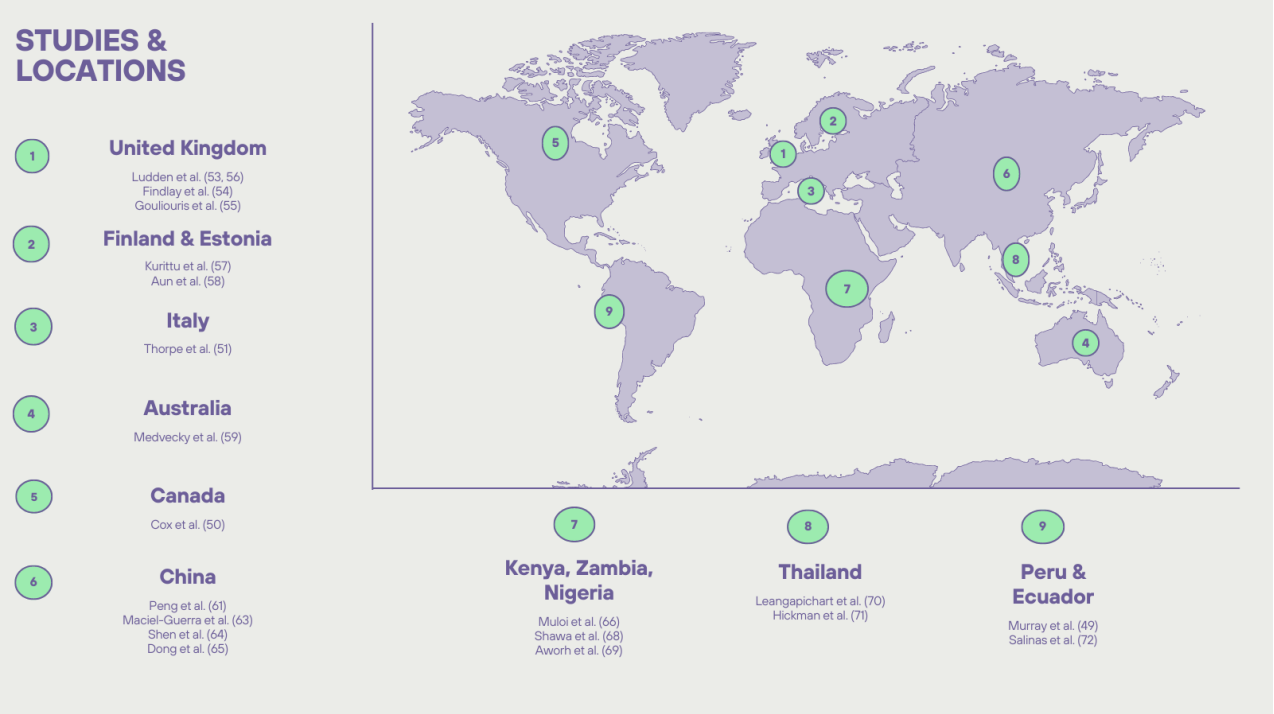

Figure 3: Schematic representation of the location of the reviewed studies.

HICs

The hitherto evidence that were identified from studies performed in HICs generally do not support the occurrence of AMR transmission events between animals and humans.

In 2019, Ludden et al. published a report exploring the possibility of drug-resistant *E. coli* associated with serious bloodstream infections in humans, which had displayed an increased prevalence in the East of England, originating from livestock or retail meat in the same geographical area (53). ESBL-*E. coli* isolates were gathered from livestock sources (i.e. cattle, pig and poultry farms) and prepackaged fresh meat products, which were subsequently sequenced, generating short-read sequences (53). The authors also retrieved open access ESBL-*E. coli* genomes that were associated with human bloodstream infections in the same area in order to compare them with the livestock isolates (53). The results of the genome analysis indicated that most multilocus sequence types (STs) were found in one source type whereas only a limited overlap of ST10, ST117 and ST95 was reported between sources (53). Phylogenetic analysis based on single nucleotide polymorphisms (SNPs) in the core genome of isolates belonging to the clonal complex (CC) CC10 and CC117 showed that while human isolates where generally distinct from the livestock ones concerning CC117, they were intermixed with regards to CC10 (53). However, with identified 85 and 96 SNPs different in CC10 and CC117 between human and livestock isolate pairs, respectively; the authors concluded that the level of genome dissimilarity in CC10 and CC117 between isolates from humans and livestock was not concordant with recent transmission between the two hosts (53). Contrastingly, the isolates from the same animal species on different farms were closely related, with a difference of zero to five SNPs (53). Seven ARGs were most frequently shared amongst the sources, conferring resistance to β-lactams ($bla_{\text{TEM-1}}$), sulfonamides (*sul2*, *sul1*), aminoglycosides (*strA*, *strB*) and tetracyclines (*tetA*, *tetB*) (53). $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-1}}$ were

the most predominant genes that confer resistance to extended-spectrum cephalosporins, which were identified in human and livestock isolates in this study (53). For each of these seven shared genes and the two most predominant ones, a long-read sequencing analysis was undertaken to examine whether these isolated ARGs were carried by the same or different MGEs in humans and livestock (53). The outcome of this analysis indicated that MGEs between livestock and humans were mostly distinct, with an estimated 5% of human isolates possibly sharing closely related AMR-related MGEs with those found in livestock (53). Overall, this study did not produce any evidence to support the claim that *E. coli* causing serious human infections in the East of England originated in livestock, whereas only limited sharing of ARGs between these two hosts was found.

In another recent study conducted by Findlay et al., third-generation cephalosporin resistant (3GC-R) *E. coli* isolates from dairy farms and human urinary *E. coli* isolates were collected in southwest England and were compared with WGS (54). The findings were similar in nature to that of the previously described study, reporting no evidence of recent sharing of 3GC-R *E. coli* between dairy farms and humans while limited evidence of recent sharing of 3GC-R *E. coli* plasmids between these hosts were identified (54). Importantly, the authors note that these plasmids isolated from humans and animals did not share a 100% identity, besides being widely spread in both humans and animals in several continents, consequently not suggesting a recent zoonotic transmission event (54). Contrastingly, the authors reported that recent farm-to-farm transmission of isolates from a variety of STs was clearly observed as well as of a newly identified epidemic plasmid (i.e. pMOO-32) (54).

Two more studies in the same country drew similar conclusions but the bacteria that were investigated were different. In particular, Gouliouris et al. explored, employing WGS, whether isolates of *Enterococcus faecium* (*E. faecium*), including vancomycin-resistant *E. faecium* (VREfm) isolates, from livestock farms, retail meat and wastewater treatment plants in the East of England were genetically related to the respective *E. faecium* isolates from human bloodstream infections (55). Notably, there was no VREfm isolated from livestock (55). The analysis further indicated that *E. faecium* strains from livestock are not causatively linked to human disease despite evidence of limited sharing of hospital-associated strains with pigs and of ARGs with livestock species (55). Specifically, the most closely related isolate pair between livestock and humans differed by 129 SNPs, which translates to around 18 years of evolution (55). Some hospital-associated strains isolated from humans were distantly related to certain pig isolates, differing by 50 to 91 SNPs whereas there was no close relation of human isolates to those of cattle, turkey or chickens (55). Interestingly, isolate pairs of livestock and wastewater and of humans and wastewater were found to be closely related, with an eight and two SNPs difference, respectively, which indicated sharing between these niches within the past year (55). *E. faecium* relatedness was discovered between turkeys and chickens but not between any other livestock species, while links between livestock farms and wastewater isolates were discovered as well (55). Twenty-six ARGs were found in both human and livestock isolates, with macrolide, aminoglycoside and tetracycline resistance genes being frequent in both niches (55). Proof of gene sharing between livestock and humans regarding these ARGs was also discovered but with variable proportions (55). The second study conducted in the same area, examined the genetic relatedness of *K. pneumoniae* isolates using WGS, from hematology wards, the hospital environment, livestock farms, meat products,

hospital sewer and municipal wastewater treatment plants (56). However, no evidence were discovered that indicate livestock or wastewater were recent reservoirs of *K. pneumoniae* isolates from patients (56). Transmission of *K. pneumoniae* was, contrastingly, identified between poultry farms (56). Despite identifying highly related plasmids harboring $bla_{\text{CTX-M-15}}$ from healthcare and non-healthcare settings, there was no proof of sharing the same plasmid in isolates from these two settings (56). Consequently, the evidence collected from the four aforementioned studies in the United Kingdom (UK) do not support the occurrence of a zoonotic transmission event across these three bacterial species, although there were (very) limited indications of ARG and MGE sharing in some of them.

The observations made in these studies were further substantiated by a recent study by Kurittu et al., which explored the potential zoonotic transmission routes of ESBL-producing *E. coli* in Finland (57). The authors employed WGS to compare isolates from different sources in Finland, including animal (i.e. broiler, barnacle geese, cattle), food (i.e. broiler meat, eggs, imported food) and the environment (i.e. wastewater) (57). However, isolates from non-human sources were found to be genetically distinct from the respective ones from human sources (57). Only two isolates collected previously in the same area; one from a healthy veterinarian and one from a human volunteer and a clinical isolate were found to be closely related, with a distance of 24 allelic difference (57). Nevertheless, this study had the limitation of being small-scale, with only limited number of human isolates analyzed in a highly restricted geographical area (57). Furthermore, plasmid MLST (pMLST) analysis was employed to characterize and compare identified plasmids, which is not as robust as long-sequencing methods (57).

A newly published large-scale One Health study by Thorpe et al. in Northern Italy provided a more comprehensive analysis of AMR transmission dynamics concerning *Klebsiella* species by collecting samples over a 17-month period from various sources (i.e. clinic, community, veterinary, agricultural and environmental) and employing WGS and bioinformatics tools (51). It is important to note that this restricted geographical area is known to have an increased rate of MDR *K. pneumoniae* associated with healthcare cases, especially carbapenem non-susceptible *Klebsiella* (51). The authors, however report no evidence, genotypic or phenotypic, of non-susceptibility to carbapenems outside the clinical environment (51). The most dominant ESBL genes that were identified were $bla_{\text{CTX-M-15}}$ and variants of $bla_{\text{SHV-27}}$, which were non-randomly distributed amongst the different sources (51). In fact, isolates of *K. pneumoniae* carrying the $bla_{\text{CTX-M-15}}$ gene were majorly from humans while exceptions consisted of isolates from companion animals and the hospital environment (51). By contrast, 30% of *K. pneumoniae* isolates bearing the $bla_{\text{SHV-27}}$ gene were from humans whereas 51% were from cows (51). However, there was a very limited transmission observed between humans and animals, especially cows, but there was some evidence suggesting that zoonotic transmission events happen relatively commonly between humans and companion animals and more frequently between humans and other sources, such as river water and invertebrates (51). Conversely, most of the transmission appeared to occur within sources, thus acquisition by humans in almost all cases originated from other humans rather than animals or the environment (51). It is the authors' belief that novel resistant lineages or of high virulence are more likely to emerge within hospital settings rather than in the environment or in animals but did not exclude the latter being possible, especially since this study did not focus specifically on MGEs (51).

In a Canadian study, Cox et al. investigated, using WGS, whether there was a relatedness between gentamicin resistant (Gen^r) *E. coli* isolated from human infections and chicken between 2014 and 2017 (50). Similarly to the previously discussed studies, a high diversity of Gen^r isolates was discovered through phylogenetic analysis, although there was a small number of clustering between human and chicken isolates, which indicated that potentially chickens may have been a minor reservoir of Gen^r *E. coli* causing human infections (50). On par with the findings of the other studies, within sources, there was a closely related similarity of less than 10 single nucleotide variants (SNVs) (50). An important observation was the co-occurrence of Gen^r and the spectinomycin resistant ($Spec^r$) genes, which was more commonly found in chicken isolates than in human ones (50). Through long-read sequencing, these two genes were found to be often linked to the same plasmid, thereby suggesting that spectinomycin use in chicken farms may result in co-selection of Gen^r (50). Nevertheless, plasmids form human and chicken sources were discovered to be very dissimilar, with little homology between Gen^r plasmids from human and chicken sources (50).

In Estonia, Aun et al. applied phylogenetic and *in silico* MLST methods to characterize the *Enterococci* strains isolated from humans (i.e. clinical samples and volunteers), farm animals (i.e. poultry, cattle, pig), the environment and wild birds (58). The findings of this study indicated that a few *Enterococcus faecalis* (*E. faecalis*) strains that colonize different farm animal species and humans may be closely related and may possibly share MGEs carrying ARGs (58). Yet, no *Enterococcus* strains that were isolated from wild birds carried acquired ARGs, suggesting that in Estonia contamination of the environment with ARGs and antibiotics is low and transfer of these genes cannot occur via wild birds (58).

Contrary to the Estonian study, Medvecky et al. identified evidence of zoonotic (or zooanthropogenic) transmission of a bla_{CMY-2}-carrying *E. coli* ST963 clone between silver gulls and humans in Australia (59). In particular, the phylogenetic analysis indicated a specific clade of ST963 *E. coli* isolates that carried a chromosomal copy of the cephalosporinase gene bla_{CMY-2}, which included close clusters of less than 20 SNPs differences of animal and human isolates (59). Therefore, the authors suggested that in Australia this specific clone is widely spread by humans and wild birds (i.e. silver gulls) (59).

Overall, these nine studies, employing WGS techniques to analyze the proposed potential of AMR transmission events occurring between animals and humans that is often put forward by the One Health approach, did not uncover substantial evidence to support this claim. Limited instances of clones sharing phylogenetic close relations were reported between animals and humans in these studies, although in some cases the environment did appear to be a more important AMR source for humans (51,53). Interestingly, there appears to be a higher possibility of sharing AMR clones between humans and companion animals while there is an indication of wild birds contributing to the spread of AMR clones, as suggested by the study by Medvecky et al. in Australia (51,59). Alternatively, sharing of MGEs harboring ARGs between humans and animals was also observed in low proportions of isolates (53–55). Nonetheless, these studies were conducted in HICs where AMU policies have been widely enforced to limit the emergence of AMR strains in livestock while typically, the livestock production system is industrialized thereby restricting close contacts between humans and production animals, which could be an important factor in the spread of AMR. By contrast, the less restricted and monitored AMU in UMICs and especially in LMICs coupled with the closer

contacts between humans and animals in these countries may facilitate dissemination of AMR between them. Potential evidence for this is being discussed in the following paragraphs.

UMICs

China is currently considered an UMIC and its AMU has been considerably larger than HICs, having comparably a more intensive livestock production system (60–62). These unique factors may promote AMR dissemination between animals and humans, which has motivated researchers to conduct pertinent molecular epidemiological studies to delineate whether inter-host spread occurs in this setting (61). One such study was conducted by Peng et al. in which *E. coli* isolates were collected longitudinally from farm animals (i.e. chickens) and abattoirs, including samples from carcasses and workers, as well as the workers' households and environments, which were then analyzed with WGS and machine learning techniques (61). The authors reported phylogenetic mixing amongst human, environmental and animal isolates of both non-pathogenic and pathogenic *E. coli* strains as well as within hosts (Figure 4) (61). Particularly, there was a genetic relatedness of zero to eight SNPs difference amongst certain chicken and human isolates, human and environmental isolates and amongst isolates from all three niches intermixed, which indicated the potential for transmission of both pathogenic and non-pathogenic *E. coli* strains across these niches (61). An examination of Figure 4 indicates that isolates of the CC469 and CC101 from chickens, farm workers and the farm environment were genetically related. Additionally, CC205 isolates from farm workers and chickens were also related and the same was true for isolates belonging to CC648 collected from chickens and the farm environment. The isolates belonging to CC23 were genetically related between abattoir workers and chicken samples. Interestingly, isolates of CC86 from chickens and abattoir worker household members were also found to be genetically related. Genetic relatedness was also indicated amongst isolates belonging to CC206 that were collected from chickens, farm workers and abattoir household members. Of note is the observation that an extensive network of ARGs was apparently shared amongst humans, livestock and abattoir environments whereas isolates amongst these sources were found to share closely related MGEs that harbored ARGs (61). Specifically, 153/154 isolates carried at least one plasmid, with 147 of those bearing a mobile or conjugative plasmid, with a higher proportion of genes carried on plasmids found in chicken isolates compared to human isolates (61). It was reported that 29.3% of human isolates shared closely related AMR-associated MGEs with those found in livestock, which is a much larger proportion than that identified by Ludden et al. in the East of England (i.e. 5%) (53,61). A hypothesis was drawn by the authors that plasmids that clustered with marginal differences indicated a relatively recent transmission of those plasmids between isolates via HGT (61). Additionally, the authors were able to use these shared plasmids to identify potential hotspots for transmission of *E. coli*, identifying the following; in the abattoir, the carcasses, water and hands of abattoir workers and household members constituted hotspots whereas in the farm environment, isolates from the nose of farm workers displayed similarity to the environmental and chicken isolates (61). Maciel-Guerra et al. reported similar findings in an analogous study conducted in China, with human and broiler chicken isolates of *E. coli* being intermixed and several ARGs and MGEs being found in both human and broiler chicken samples (63). Regarding these human samples, predominately they were from farm

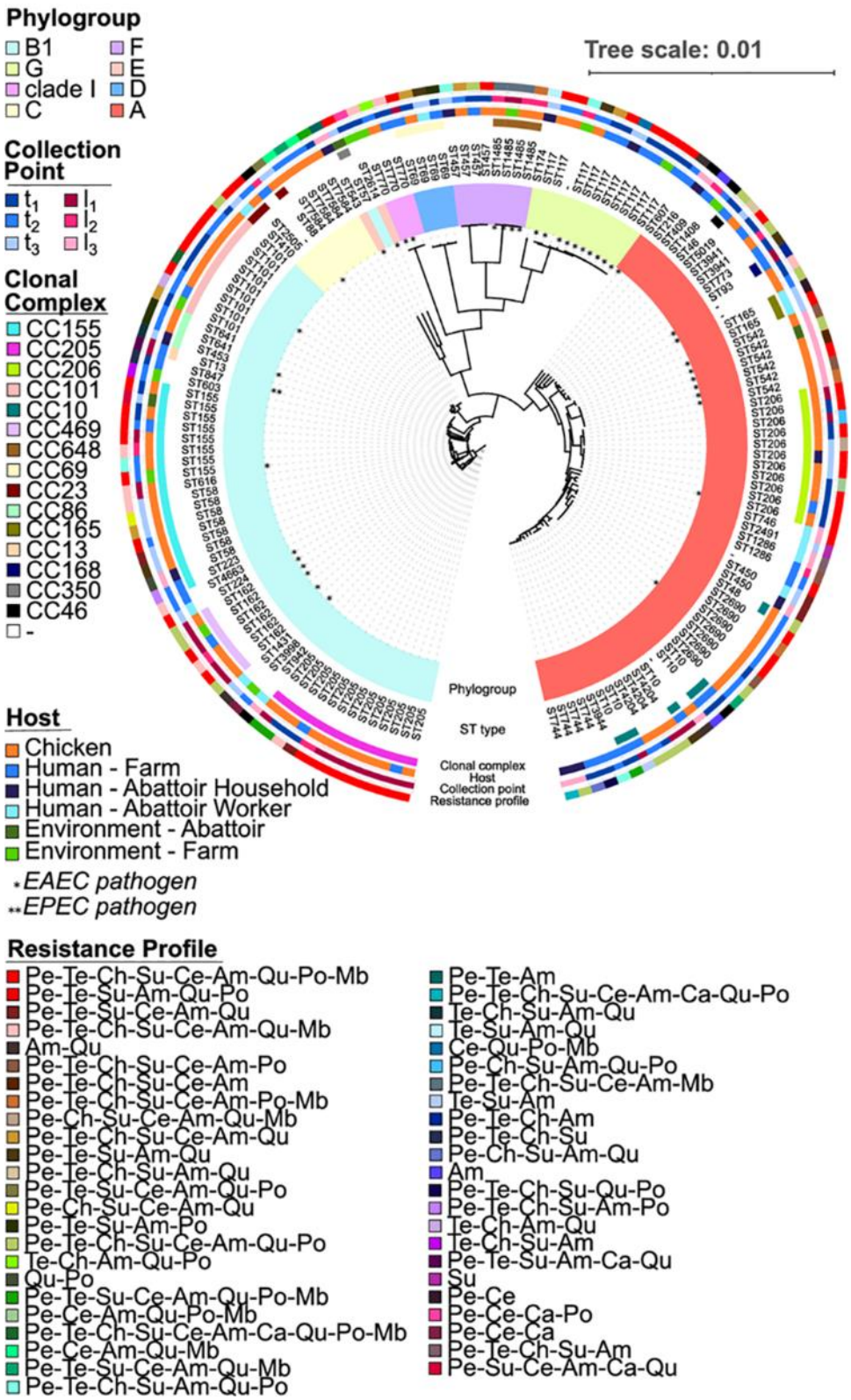

Figure 4: Maximum likelihood phylogenetic tree of the whole cohort by Peng et al. (61)**.** The tree was drafted using the core genome of 154 isolates with recombination correction, which were cultured from human, animal, meat and environmental samples gathered from the respective farm and slaughterhouse. Inner ring: phylogroups, sequence types, clonal complex, collection time points and host type. Outer ring: resistance profile. Enteroaggregative *Escherichia coli* (EAEC) and Enteropathogenic *Escherichia coli* (EPEC) isolates indicated by * and ** respectively.

workers, one was from a slaughterhouse worker and one from a household member (63). In one human household sample, the *CTX-M-55* β-lactamase gene and the MGE aligned with broiler chicken samples (63). Additionally the *QnrS1* gene, which is clinically relevant as it confers transferable resistance to quinolones, was widely present in both human and chicken isolates and included two distinct mobile ARG patterns found in both hosts; the transposase *ISKpn19* and secondly, the transposase *ISCc36* (63). Similar observations, albeit with different ARG patterns, were made with regards to the genes $bla_{\text{TEM-1}}$, *tetM* and *mphA* (63). These two studies hint at a potential occupational risk of inter-host transmission of both clones and ARG-carrying MGEs.

In a large retrospective cross-sectional study spanning 22 Chinese provinces and municipalities carried out by Shen et al., the inter-host transmission potential was explored for carbapenem-resistant *E. coli* (CREC), particularly NDM-producing *E. coli*, which causes an increased burden in healthcare as well as NDM-producing *E. coli* strains (64). The findings of this study suggest a close genetic relationship amongst isolates from humans, pigs and chickens (64). In more detail, 27.3% of NDM-positive *E. coli* isolates from pigs were predicted to originate from humans, pigs and flies whereas 19.3% of NDM-positive *E. coli* from chickens were predicted to originate from humans and another 8.1% and 1.6% from pigs and flies, respectively (64). Additionally, 53.8% and 14.9% of the human isolates were predicted to have originated from chickens and pigs, respectively (64). Fly-derived isolates were predicted to have originated from humans at a percentage of 10.9%, chickens (47.8%) and pigs (41.3%) (64). Furthermore, isolates were identified from humans, chickens and flies that had similar bla_{NDM}-carrying IncX3 plasmids (64). Consequently, the authors conclude that NDM-positive CREC isolates may constitute a significant transmission risk between animals and humans and hypothesize that initially CREC emerged in clinical settings, subsequently entering the livestock sector, which favored its persistence and led to its circulation between animals and humans via the food chain or environmental vectors (64). Contradictory to the aforementioned studies that did discover evidence of AMR transmission between animals and humans in China, Dong et al. did not observe inter-host transmission of *tet(X)*-positive *E. coli* between these hosts (65).

It appears that more evidence of AMR transmission were reported in the studies from China compared to those conducted in HICs. The two studies by Peng et al. and Maciel-Guerra et al. additionally point to an occupational risk of AMR inter-host transmission (61,63). Specifically, humans working at either a farm or slaughterhouse environment appear to share some isolates, which are genetically related to the animal isolates whereas similar evidence for household members that do not work in these environments were fewer. Similar observations were made regarding ARGs and MGEs. Taken together, these results could indicate that perhaps there is not a sustain colonization occurring in farm and slaughterhouse workers but rather occupational exposure leads to frequent contamination with AMR clones or ARGs that subsequently dissipate, thereby not propagating these AMR determinants further into the human population.

LMICs

To provide a complete picture on the issue however, the situation on LMICs should also be explored. Muloi et al. sampled *E. coli* from humans, livestock and peri-domestic wildlife in 99 households and their environments in Nairobi, Kenya and explored whether transmission occurred between these settings via WGS (66). Typically, households in that area practice livestock keeping, which has been indicated as a potential risk factor of zoonotic transmission (66). In this study, it was revealed that the households substantially influenced the diversity and sharing patterns of *E. coli* and that those were also heavily shaped by host type (66). The authors revealed that within households, *E. coli* sharing was much higher within the same host category whereas no substantial pattern was observed among households (66). Concurrently, inter-household and inter-host sharing of *E. coli* was discovered, including between humans and animals (most of which concerned poultry) but that was found to occur much less often (66). Particularly, analysis of core-genome SNPs from isolates within the same household showed that most of the sharing pairs were separated by a range of less than four to a maximum of ten SNPs, corresponding to several months or several years of evolutionary time (66). Most isolate pairs were from the same host but a 36% was between host categories, with 25% being between wildlife and livestock, 6% between humans and livestock and 3% between humans and wildlife (66). Moreover, ten sharing pairs of human, which were all males and did not all keep livestock, and livestock isolates belonged to STs that were not host restricted (66). Six of those concerned the same household whereas the remaining four did not keep livestock but did share bacteria with livestock from other households (66). In those ten sharing events, the majority of the persons that did not keep livestock reported to have direct contact with livestock via collection of eggs, milking or handling (66). The resistome (i.e. collection of all ARGs and their precursors in pathogenic and non-pathogenic bacteria (67)) similarity was found to be driven by shared exposure to antimicrobials since it was differently distributed across household and host (66). Also, humans and wildlife, including mammals and birds, were found to have a higher than expected resistome similarity (66). The authors hypothesize that this was due to AMU selective pressure rather than dissemination from a common source (66).

An interesting study carried out in Zambia by Shawa et al., provided evidence of direct transmission of the MDR *E. coli* O17:H18-ST69 clone between poultry to humans, although its directionality could not be ascertained (68). In support of this, a high degree of genetic relatedness was displayed between *E. coli* O17:H18-ST69 from humans and poultry by the phylogenetic analysis and hierarchical clustering (68). Importantly, 20% of poultry and 24% of human-related isolates contained the *E. coli* O17:H18-ST69 clone together with two plasmids carrying 14 ARGs (68). These plasmids belonged to the IncFI and IncI-complex incompatibility group and were shared between these host isolates (68). However, the isolates also contained other niche-specific AMR plasmids, harboring $bla_{\text{CTX-M}}$ genes, which indicated independent ESBL acquisition pathways (68). Considering these results, the authors suggested that the spread of MDR between the two niches happened via direct transmission of this specific clone together with the two plasmids (i.e. IncFI and IncI complex) (68). They further hypothesize that the clones subsequently diverged, following different evolutionary pathways because of various selection pressures exerted on them based on each host's environment that led to the acquisition of distinct ESBL-producing plasmids via HGT (68).

In another study, this time conducted in Nigeria, Aworh et al. investigated the zoonotic transmission of ESBL-producing *E. coli* among beef cattle, humans and the abattoir environment (69). In a little over 90% of the ESBL-producing *E. coli* isolates from all sources the $bla_{\text{CTX-M-15}}$ gene was observed while in around 5% and 2% of the isolates the $bla_{\text{CTX-M-14}}$ and the $bla_{\text{CTX-M-55}}$ genes were observed, respectively (69). Approximately 84% of the isolates harbored a plasmid-mediated quinolone-resistant gene (i.e. *qnrS1*) and intriguingly, one human isolate carried a plasmid-mediated colistin-resistant gene (i.e. *mcr 1.1*) (69). Quite a few isolates from humans, cattle and the abattoir environment had pairwise differences that ranged from zero to 30 SNPs, thereby displaying clonal relationships (69). One human and one cattle isolate (ST46) had a pairwise difference of two SNPs, constituting them closely related and suggesting that potentially resistance horizontally transferred between the two hosts (69). Notably, these isolates originated from the same abattoir location and had similar ARGs, which were carried by identical ISs, miniature inverted sequences (MITEs) and transposons whereas no ESBL gene was harbored on plasmid replicons (69). As such, Aworh et al. speculate that ARGs might transfer from humans to cattle or vice versa via HGT of MGEs (69).

Two studies in Thailand report results based on WGS regarding zoonotic AMR transmission, although the bacterial species that were studied were different (i.e. *K. pneumoniae* and *E. coli*) (70,71). In particular, Leangapichart et al. reported that transmission of *K. pneumoniae* between animals and humans was detected concerning solely one human-pig pair, which belonged to ST29 and differed by three SNPs, suggesting limited zoonotic transmission (70). Conversely, Hickman et al. report extensive zoonotic sharing between pigs and humans of ARGs via HGT, which was suggested HGT as the main mechanism of zoonotic ARG spread in this study (71). In agreement with the previous study, limited evidence suggesting clonal transfer between pigs and humans was found and those concerned humans in close contact with the animals (71).

A study in Peru explored the potential of market chickens being a reservoir of antimicrobial-resistant *E. coli* that could colonize market vendors and other humans (49). An interesting finding of this study was that resistance to florfenicol was higher in chicken vendors than other groups of humans (49). It is important to mention that florfenicol is often used as a growth promoter in poultry farms, yet it is not approved for use in humans (49). The florfenicol resistance gene *floR* was identified in isolates from both chickens and humans and was associated with conjugative plasmids that shared a high degree of similarity between these two hosts (49). Consequently, the authors speculated that florfenicol resistance in humans may happen through the colonization of strains that carry the *floR* gene or through plasmid conjugation from animal strains into human commensals, facilitated by improper handling of poultry meat from vendors and customers (49). Critically, the authors propose that the *floR* gene might represent a potential marker of AMR in humans that can be traced back to animals (49). However, this finding was the outcome of comparing plasmid replicon types using a relevant database (i.e. PlasmidFinder), which is not a high-resolution method (49). Furthermore, the authors investigated whether *E. coli* isolates from human babies, adults, chicken vendors, organic and non-organic chickens were genetically related using a maximum-likelihood phylogenetic tree (49). Only within host isolate groups were found to be similar, differing by less than 100 SNPs (49). By contrast, although there were some shared STs between chicken vendors and market chickens, those differed by more than 900 SNPs,

suggesting that direct transmission did not occur between hosts and that those STs were more distantly related (49). Likewise, STs that were shared between babies and organic chickens as well as non-vendors and market chickens were found to be similarly distinct (49).

Salinas et al. investigated whether *E. coli* strains from both children and domestic animals in the same community in Ecuador with corresponding phenotypic AMR patterns had clonal relationship or shared the same plasmids via WGS (72). The outcome of this analysis suggested that none of the isolated strains were clonally transferred between children and domesticated animals since the entirety of the MDR *E. coli* isolates had minimally 90 whole-genome SNP differences between them (72). Additionally, despite most of the isolates sharing the same ARGs and replicons, long-read sequencing implied that these were located on different plasmid structures (72). Yet, Salinas et al. theorize that a common pool of ARGs may be concurrently circulating in the same community but on different plasmids and on different *E. coli* clones, a phenomenon most likely promoted by transposons, integrons or gene cassettes (72). The authors of this paper additionally emphasize the importance of using whole-genome techniques to investigate the relatedness of clones and plasmids instead of MLST methods as the latter could lead to erroneous conclusions (72). For instance, whole-genome sequencing identified three human isolates belonged to ST226 but extended MLST indicated that they belonged to ST681 (72). Likewise, three human and one cat isolates belonged to ST10 but extended MLST suggested that two of the human isolates and the one from the cat belonged to ST2 and the remaining human isolate belonged to ST767 (72). However, WGS demonstrated that the two human isolates belonging to ST2 were not, in fact, related, differing by 5,392 SNPs and that these two human isolates further differed from the cat isolate by 6,138 and 7,409 SNPs, respectively (72).

Clearly, there are more instances of AMR dissemination occurring between animals and humans in UMICs and LMICs compared to HICs. Three out of the four studies conducted in China presented evidence regarding the inter-host transmission through both HGT and clonal spread, although clonal spread appeared to concern limited cases of mostly farm and abattoir workers (61,63,64). Likewise, five of the seven LMIC studies reported evidence of clonal spread between animals and humans, albeit those were very limited in nature in most reports (66,68–71). HGT of ARGs was suggested to have occurred between humans and animals in four out of the seven LMIC studies, with some characterizing it as extensive (49,66,69,71). Therefore, HGT of ARG-carrying MGEs might be a more important dissemination pathway between humans and animals. However, other studies from UMICs and LMICs report contradictory results. Indeed, one study in China and one in Ecuador did not find any evidence of inter-host AMR clonal spread nor via HGT (65,72). In addition, although one study in Peru suggested that an ARG had horizontally transferred to humans from animals, albeit employing lower resolution techniques, they did not discover any evidence of clonal dissemination between these hosts (49). Clearly, there is still uncertainty on the extent and via which pathways zoonotic AMR spread occurs even in UMICs and LMICs despite certain studies reporting more evidence than HICs. Certain determinants, such as increased AMU but also livelihood factors and different practices in keeping animals could potentially be causing the observed differences.

Discussion

The threat that AMR poses on humanity is undoubtedly grave. Already, an alarming amount of deaths have been associated or directly attributed to AMR and predictions are even more somber (3,7,73). Particularly worrisome is the rapid increase of infections in humans caused by resistant bacteria, especially those resistant to last resort antimicrobials (73). As AMU has been identified as an important driver of AMR, concerns have been reasonably voiced regarding AMU in livestock and the possibility of AMR spreading from animal sources to humans and the environment (4,22). As a result, the One Health approach has been adopted to guide both preventative and surveillance actions across all three interfaces (47). Yet, so far there has been little evidence that can safely conclude the occurrence of zoonotic transmission of AMR (4,24,74). Often, studies that in the past attributed certain clinically relevant AMR clone emergence to livestock were subsequently disputed by use of higher-resolution methodologies (19,24). Today, the advent of WGS and its wider accessibility provides a unique opportunity to study at a higher resolution the intricate pathways and mechanisms of AMR transmission and emergence (23). We reviewed 19 molecular epidemiological studies that employed WGS and bioinformatics tools to assess the possibility and the extent to which such transmission events between hosts and the environment may be occurring. Out of the 19 papers reviewed, nine concerned HICs, four were conducted in UMICs (i.e. China) and seven were carried out in LMICs. It has been theorized that the risk of a zoonotic AMR transmission event happening is bigger in LMICs and UMICs because there is higher AMU, which is, additionally, less monitored (47). Furthermore, in LMICs, typically farming practices are at the household-level, thus humans are in closer contacts with animals, which may constitute an additional risk.

In this review, we observed that HICs did not report sharing of AMR clones and failed to identify any transmission event, mostly suggesting that emergence of new clinically relevant AMR strains is more likely to occur in hospital settings and amongst humans. Nevertheless, some limited sharing of ARGs via HGT of MGEs was indicated by those studies (50,51,53–56). In addition, wild birds may be playing an important role in the dissemination of clinically relevant AMR clones, as reported by Medvecky et al. in Australia although another study by Aun et al. in Estonia, failed to detect this (58,59). The situation in China (UMIC) was quite different in that most studies did indicate zoonotic transmission of clones and/or ARG-carrying MGEs (63,64). Of note is that this appeared to concern mostly farm and abattoir workers, which could suggest that exposure to these environments may lead to an occupational risk of more frequent AMR determinant dissemination from animals. A study conducted in The Netherlands by Van Gompel et al. seem to indicate that ARG carriage in people exposed to pigs and pork is higher than in people who are not (75). The evidence from these studies could point to contamination with AMR determinants rather than sustained colonization since workers' household members were infrequently found to share ARGs or AMR clones, although more studies would be necessary to arrive at this conclusion. Crucially, HGT of MGEs was speculated to be quite important in the propagation of resistance amongst hosts and specifically, MGEs other than plasmids, such as insertion sequences and transposons were identified to be central to inter-host ARG dissemination (61,63). Only one study by Dong et al. did not find evidence of zoonotic AMR transmission (65). These findings could be potentially explained by the fact that China has an intensive livestock production system, using many antimicrobials

(61). The studies from LMICs again provided more evidence of genetic relatedness between human and animal AMR isolates compared to HICs, especially for people in close contacts with animals, having similar observations as in the Chinese studies regarding the importance of HGT of MGEs in the issue (49,66,68,69,71). Two studies, found limited or no evidence of inter-host AMR clonal spread (70,72). An interesting aspect was the one presented by Muloi et al. that reported that although there was often sharing of ARGs and bacteria between households, within each household there was a different resistome and bacterial persistence (66). The authors conclude that this empirically underpins the hypothesis by Lourens Baas Becking that "Everything is everywhere, but, the environment selects" (66). In the great majority of the studies that included environmental isolates, genetic relatedness amongst human and/or animal isolates of AMR strains was discovered, indicating its potentially substantial role in the AMR transmission pathways. Although WGS techniques combined with other tools, have the potential to delineate these pathways by providing a high-resolution view of the events at the molecular level, Salinas et al. stress that researchers should be cautious when interpreting results that are based on MLST techniques rather than solely on whole-genome sequencing due to the possibility of errors (72). Additionally, when exploring MGEs, it is preferable to use a combination of short- and long-read sequencing (41,72).

Hitherto, the evidence suggest that it is probably more likely for such transmission events to be observed in LMICs or UMICs; however those are mostly limited in nature and do not appear to have caused serious healthcare issues in humans. The directionality of these transmissions nevertheless is still unknown. Furthermore, ARG-carrying MGEs are proposed to be most crucial in the issue of inter-host dissemination of AMR by the majority of these studies, based on their findings.

Still, the question remains whether these transmission events that appear to happen mostly infrequently and in comparably small proportions of the population could prove to cause significant problems in healthcare. A study by Mughini-Gras et al. argues that human-to-human transmission of ESBL-producing and pAmpC-producing *E. coli* within a community may not be enough to sustain their carriage in humans without transmission occurring to and from non-human sources (76). Therefore, it is essential to continue investigating the potential scenarios and pathways of inter-host transmission of AMR in a One Health manner, using high-resolution techniques and well-designed studies. Although there are quite possibly a multitude of possible scenarios that AMR inter-host transmission might occur, in Figure 5 four possibilities are depicted, based on the studies that were discussed in this review. One possibility is that AMR clones or MGEs that carry ARGs may be transferred directly from animals to humans (or vice versa) (Figure 5A). The second scenario is indirect, thus these AMR elements may be transferred via a vector (either biological or non-biological) as also proposed by Shen et al. (Figure 5B)(64). Thirdly, these AMR elements may be disseminated from either humans or animals to the environment and subsequently transmitting to animals or humans, respectively (Figure 5C). Finally, as proposed by Shawa et al. (68), a AMR strain may be transferred from an unknown source to either animals or humans and subsequently transmitting to humans or animals, respectively. Then, distinct ARG-carrying MGEs might be transferred to each host, consequently being integrated to the strain resulting in a divergent strain in each host that nevertheless had the same origin (Figure 5D).

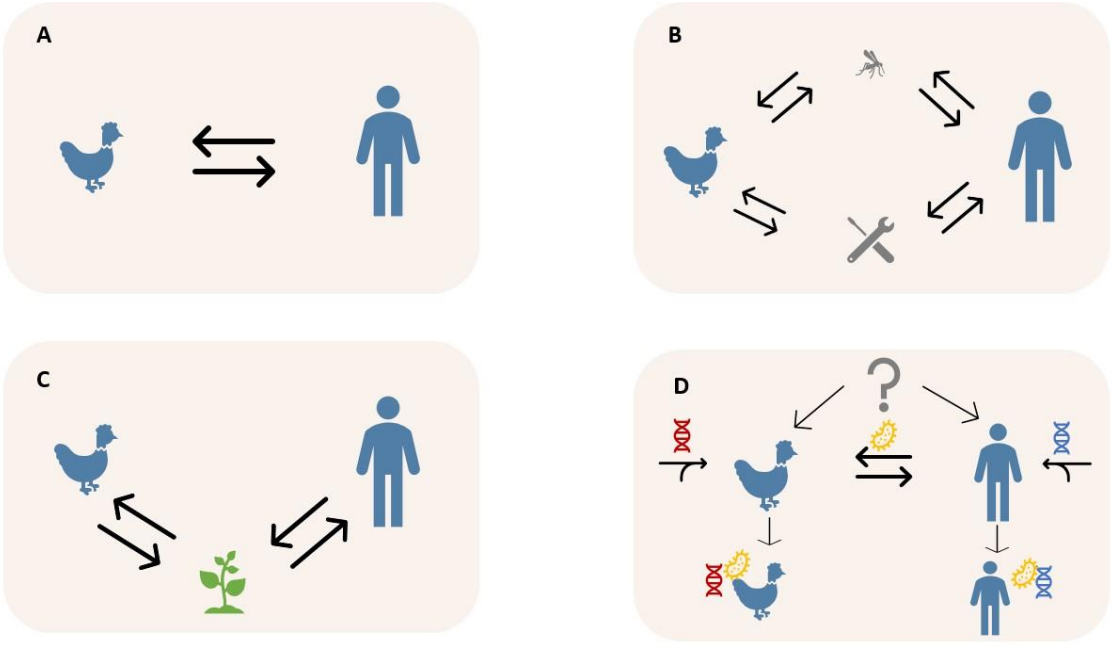

Figure 5: Schematic representation of four potential scenarios of AMR transmission between animals and humans. The arrows indicate transmission of either AMR clones or MGEs carrying ARGs. (A) Direct transmission from animals to humans and vice versa. (B) Indirect transmission from animals to humans (or vice versa) through biological (e.g. flies) or non-biological (e.g. equipment) vectors. (C) Dissemination from animals (or humans) to and from the environment. (D) As described by Shawa et al. (68), from an unknown source AMR clones with their ARG-carrying MGEs may be transferred to either animals or human and subsequently spreading between them. Later, each host might acquire different ARG-carrying MGE and the clone goes on to evolve separately in each host. The question mark indicates an unknown source of AMR. The yellow moon-like object represents the bacterial clone. The red and blue DNA molecules represent distinct ARG-carrying MGEs.

Understandably, a lot is still unknown and well-designed molecular epidemiological studies, employing WGS methodologies are sorely needed. In the future, it would be advantageous if large-scale longitudinal studies were conducted, with an appropriate sampling framework at both the animal and human front (4). These should, ideally span broad geographical areas and be unbiased (4). Essentially, they must consider the microorganisms that are being studied, their respective niches and AMR determinants in each area that is being investigated (4). This could provide more information that could guide future policies with the aim to limit the spread and progression of AMR at a global scale. There were two examples of studies that approached this ideal design in the literature that this review covered, namely the study by Thorpe et al. and Salinas et al., which could constitute a guide for future studies exploring inter-host transmission of AMR (51,72).

Currently, the evidence suggest that under certain conditions, inter-host transmission of AMR can occur, albeit rarely and with varying pathways. Whether this is a slow or a fast process is so far unknown, although a very recent study implies that plasmid movement across different niches may be happening at more increased rates than previously thought at least concerning *Enterobacterales* (77). However, it is important to mention that this study, at the time that this review was written, had not been reviewed. Regardless, the fact that this occurrence might be rare should not minimize the potential risk that it could pose in the event

that a highly virulent AMR strain emerges. Thus, efforts should continue to incorporate the One Health approach, in a more targeted way to elucidate these unknown aspects.

References

1. World Health Organization (WHO). Ten threats to global health in 2019 [Internet]. [cited 2022 Nov 9]. Available from: https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019

2. WHO. Thirteenth General Programme of Work 2019–2023. WHO Press. 2018;(April 2018):50.

3. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55.

4. Parkhill J. Antimicrobial Resistance Exchange Between Humans and Animals: Why We Need to Know More. Engineering [Internet]. 2022;15:11–2. Available from: https://doi.org/10.1016/j.eng.2022.04.007

5. EClinicalMedicine. Antimicrobial resistance: a top ten global public health threat. eClinicalMedicine. 2021;41:101221.

6. OECD. Antimicrobial Resistance in the EU/EEA: A One Health Response Interventions. 2022;

7. O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations. Arch Pharm Pract. 2016;7(3):110.

8. de Kraker MEA, Stewardson AJ, Harbarth S. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? PLoS Med. 2016;13(11):1–6.

9. Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, et al. Antibiotic resistance is the quintessential One Health issue. Trans R Soc Trop Med Hyg. 2016;110(7):377–80.

10. WHO, FAO, OIE, UNE. Strategic Framework for collaboration on antimicrobial resistance – together for One Health. 2022; Available from: www.who.int

11. FAO, UNEP, WHO, WHOAH. Global Plan of Action on One Health. Towards a more comprehensive One Health, approach to global health threats at the human-animal-environment interface. [Internet]. Rome: FAO; UNEP; WHO; World Organisation for Animal Health (WOAH) (founded as OIE); 2022. Available from: http://www.fao.org/documents/card/en/c/cc2289en

12. D'Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, et al. Antibiotic resistance is ancient. Nature [Internet]. 2011;477(7365):457–61. Available from: http://dx.doi.org/10.1038/nature10388

13. Bhullar K, Waglechner N, Pawlowski A, Koteva K, Banks ED, Johnston MD, et al. Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One. 2012;7(4):1–11.

14. Von Wintersdorff CJH, Penders J, Van Niekerk JM, Mills ND, Majumder S, Van Alphen LB, et al. Dissemination of antimicrobial resistance in microbial ecosystems

through horizontal gene transfer. Front Microbiol. 2016;7(FEB):1–10.

15. Andersson DI, Hughes D. Selection and transmission of antibiotic-resistant bacteria. Microb Transm. 2019;117–37.

16. Christaki E, Marcou M, Tofarides A. Antimicrobial Resistance in Bacteria: Mechanisms, Evolution, and Persistence. J Mol Evol [Internet]. 2020;88(1):26–40. Available from: https://doi.org/10.1007/s00239-019-09914-3

17. Baker S, Thomson N, Weill F-X, Holt KE. Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens. Science (80-) [Internet]. 2018 May 18;360(6390):733–8. Available from: https://www.science.org/doi/10.1126/science.aar3777

18. Hutchings M, Truman A, Wilkinson B. Antibiotics: past, present and future. Curr Opin Microbiol [Internet]. 2019;51(Figure 1):72–80. Available from: https://doi.org/10.1016/j.mib.2019.10.008

19. Kahn LH. Antimicrobial resistance: A One Health perspective. Trans R Soc Trop Med Hyg. 2017;111(6):255–60.

20. Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, et al. Global trends in antimicrobial resistance in animals in low- and middle-income countries. Science (80-) [Internet]. 2019 Sep 20 [cited 2022 Nov 10];365(6459). Available from: https://www-science-org.proxy.library.uu.nl/doi/10.1126/science.aaw1944

21. McEwen SA, Collignon PJ. Antimicrobial Resistance: a One Health Perspective. Aarestrup FM, Schwarz S, Shen J, Cavaco L, editors. Microbiol Spectr [Internet]. 2018 Apr 6;6(2):255–60. Available from: http://academic.oup.com/trstmh/article/111/6/255/4554993/Antimicrobial-resistance-a-One-Health-perspective

22. McEwen SA, Collignon PJ. Antimicrobial resistance: A One Health perspective. Microbiol Spectr 6(2)ARBA-0009-2017. 2017;111(6):255–60.

23. Waddington C, Carey ME, Boinett CJ, Higginson E, Veeraraghavan B, Baker S. Exploiting genomics to mitigate the public health impact of antimicrobial resistance. Genome Med [Internet]. 2022;14(1):1–14. Available from: https://doi.org/10.1186/s13073-022-01020-2

24. Muloi D, Ward MJ, Pedersen AB, Fèvre EM, Woolhouse MEJ, Van Bunnik BAD. Are Food Animals Responsible for Transfer of Antimicrobial-Resistant Escherichia coli or Their Resistance Determinants to Human Populations? A Systematic Review. Foodborne Pathog Dis. 2018;15(8):467–74.

25. Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, et al. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect [Internet]. 2011;17(6):873–80. Available from: http://dx.doi.org/10.1111/j.1469-0691.2011.03497.x

26. de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, et al. Dissemination of Cephalosporin Resistance Genes between Escherichia coli Strains

from Farm Animals and Humans by Specific Plasmid Lineages. PLoS Genet. 2014;10(12).

27. Lerminiaux NA, Cameron ADS. Horizontal transfer of antibiotic resistance genes in clinical environments. Can J Microbiol. 2019;65(1):34–44.

28. Liu G, Thomsen LE, Olsen JE. Antimicrobial-induced horizontal transfer of antimicrobial resistance genes in bacteria: A mini-review. J Antimicrob Chemother. 2022;77(3):556–67.

29. Carr VR, Shkoporov A, Hill C, Mullany P, Moyes DL. Probing the Mobilome: Discoveries in the Dynamic Microbiome. Trends Microbiol [Internet]. 2021;29(2):158–70. Available from: https://doi.org/10.1016/j.tim.2020.05.003

30. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev. 2018;31(4).

31. Nadeem SF, Gohar UF, Tahir SF, Mukhtar H, Pornpukdeewattana S, Nukthamna P, et al. Antimicrobial resistance: more than 70 years of war between humans and bacteria. Crit Rev Microbiol [Internet]. 2020;46(5):578–99. Available from: https://doi.org/10.1080/1040841X.2020.1813687

32. Peterson E, Kaur P. Antibiotic resistance mechanisms in bacteria: Relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol. 2018;9(NOV):1–21.

33. Cantón R, Coque TM. The CTX-M β-lactamase pandemic. Curr Opin Microbiol. 2006;9(5):466–75.

34. Humeniuk C, Arlet G, Gautier V, Grimont P, Labia R, Philippon A. β-lactamases of Kluyvera ascorbata, probable progenitors of some plasmid-encoded CTX-M types. Antimicrob Agents Chemother. 2002;46(9):3045–9.

35. Poirel L, Decousser J, Nordmann P. IS Ecp1B -Mediated Transposition of bla CTX-M in Escherichia coli. 2005;49(1):1–4.

36. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13(1):42–51.

37. Cottell JL, Webber MA, Coldham NG, Taylor DL, Cerdeño-Tárraga AM, Hauser H, et al. Complete sequence and molecular epidemiology of IncK epidemic plasmid encoding blaCTX-M-14. Emerg Infect Dis. 2011;17(4):645–52.

38. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infect Dis [Internet]. 2016;16(2):161–8. Available from: http://dx.doi.org/10.1016/S1473-3099(15)00424-7

39. Luo Q, Wang Y, Xiao Y. Prevalence and transmission of mobilized colistin resistance (mcr) gene in bacteria common to animals and humans. Biosaf Heal [Internet]. 2020;2(2):71–8. Available from: https://doi.org/10.1016/j.bsheal.2020.05.001

40. Mmatli M, Mbelle NM, Osei Sekyere J. Global epidemiology, genetic environment,

risk factors and therapeutic prospects of mcr genes: A current and emerging update. Front Cell Infect Microbiol. 2022;12(August):1–30.

41. Sheppard AE, Stoesser N, Wilson DJ, Sebra R, Kasarskis A, Anson LW, et al. Nested Russian doll-like genetic mobility drives rapid dissemination of the carbapenem resistance gene blakpc. Antimicrob Agents Chemother. 2016;60(6):3767–78.

42. Raven KE, Gouliouris T, Brodrick H, Coll F, Brown NM, Reynolds R, et al. Complex routes of nosocomial vancomycin-resistant Enterococcus faecium transmission revealed by genome sequencing. Clin Infect Dis. 2017;64(7):886–93.

43. Arredondo-Alonso AS, Top J, Corander J, Willems RJL, Anita C. Mode and dynamics of vanA-type vancomycin-resistance dissemination in Dutch hospitals. 2020;

44. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. Staphylococcus aureus CC398: Host adaptation and emergence of methicillin resistance in livestock. MBio. 2012;3(1):1–6.

45. Crespo-Piazuelo D, Lawlor PG. Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) prevalence in humans in close contact with animals and measures to reduce on-farm colonisation. Ir Vet J [Internet]. 2021;74(1):1–12. Available from: https://doi.org/10.1186/s13620-021-00200-7

46. Ott LC, Mellata M. Models for Gut-Mediated Horizontal Gene Transfer by Bacterial Plasmid Conjugation. Front Microbiol. 2022;13(June).

47. Hernando-Amado S, Coque TM, Baquero F, Martínez JL. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nat Microbiol [Internet]. 2019;4(9):1432–42. Available from: http://dx.doi.org/10.1038/s41564-019-0503-9

48. Tang Y, Fang L, Xu C, Zhang Q. Antibiotic resistance trends and mechanisms in the foodborne pathogen, Campylobacter. Anim Heal Res Rev. 2017;18(2):87–98.

49. Murray M, Salvatierra G, Dávila-Barclay A, Ayzanoa B, Castillo-Vilcahuaman C, Huang M, et al. Market Chickens as a Source of Antibiotic-Resistant Escherichia coli in a Peri-Urban Community in Lima, Peru. Front Microbiol. 2021;12(March).

50. Cox GW, Avery BP, Parmley EJ, Irwin RJ, Reid-Smith RJ, Deckert AE, et al. A One Health Genomic Investigation of Gentamicin Resistance in Escherichia coli from Human and Chicken Sources in Canada, 2014 to 2017. Antimicrob Agents Chemother. 2022;205.

51. Thorpe HA, Booton R, Kallonen T, Gibbon MJ, Couto N, Passet V, 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 [Internet]. 2022 Nov 21; Available from: https://www.nature.com/articles/s41564-022-01263-0

52. World Health Organization (WHO)(WHO/EMP/IAU/2017.12). (Licence: CC BY-NC-SA 3.0 IGO.. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. Geneva: WHO/EMP/IAU/2017.12 ©; 2017.

53. Ludden C, Raven KE, Jamrozy D, Gouliouris T, Blane B, Coll F, et al. One Health

Genomic Surveillance of Escherichia coli Demonstrates Distinct Lineages and Mobile Genetic Elements in Isolates from Humans versus Livestock. Sansonetti PJ, editor. MBio [Internet]. 2019 Feb 26;10(1):1–12. Available from: https://journals.asm.org/doi/10.1128/mBio.02693-18

54. Findlay J, Mounsey O, Lee WWY, Newbold N, Morley K, Schubert H, et al. Molecular Epidemiology of Escherichia coli Producing CTX-M and pAmpC β-Lactamases from Dairy Farms Identifies a Dominant Plasmid Encoding CTX-M-32 but No Evidence for Transmission to Humans in the Same Geographical Region. Appl Environ Microbiol. 2020;87(1):1–9.

55. Gouliouris T, Raven KE, Ludden C, Blane B, Corander J, Horner CS, et al. Genomic surveillance of Enterococcus faecium reveals limited sharing of strains and resistance genes between livestock and humans in the United Kingdom. MBio. 2018;9(6).

56. Ludden C, Moradigaravand D, Jamrozy D, Gouliouris T, Blane B, Naydenova P, et al. A one health study of the genetic relatedness of Klebsiella pneumoniae and their mobile elements in the east of England. Clin Infect Dis. 2020;70(2):219–26.

57. Kurittu P, Khakipoor B, Jalava J, Karhukorpi J, Heikinheimo A. Whole-Genome Sequencing of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli From Human Infections in Finland Revealed Isolates Belonging to Internationally Successful ST131-C1-M27 Subclade but Distinct From Non-human Sources. Front Microbiol. 2022;12(January).

58. Aun E, Kisand V, Laht M, Telling K, Kalmus P, Väli Ü, et al. Molecular Characterization of Enterococcus Isolates From Different Sources in Estonia Reveals Potential Transmission of Resistance Genes Among Different Reservoirs. Front Microbiol. 2021;12(March):1–13.

59. Medvecky M, Papagiannitsis CC, Wyrsch ER, Bitar I, Cummins ML, Djordjevic SP, et al. Interspecies Transmission of CMY-2-Producing Escherichia coli Sequence Type 963 Isolates between Humans and Gulls in Australia. mSphere. 2022;7(4).

60. Zhou S, Hu A. China: Surpassing the “Middle Income Trap.” China: Surpassing the “Middle Income Trap.” 2021.

61. Peng Z, Maciel-Guerra A, Baker M, Zhang X, Hu Y, Wang W, et al. Whole-genome sequencing and gene sharing network analysis powered by machine learning identifies antibiotic resistance sharing between animals, humans and environment in livestock farming [Internet]. Vol. 18, PLoS Computational Biology. 2022. 1–38 p. Available from: http://dx.doi.org/10.1371/journal.pcbi.1010018

62. Cui D, Liu X, Hawkey P, Li H, Wang Q, Mao Z, et al. Use of and microbial resistance to antibiotics in China: a path to reducing antimicrobial resistance. J Int Med Res. 2017;45(6):1768–78.

63. Maciel-Guerra A, Baker M, Hu Y, Wang W, Zhang X, Rong J, et al. Dissecting microbial communities and resistomes for interconnected humans, soil, and livestock. ISME J. 2022;(September):1–15.

64. Shen Y, Hu F, Wang Y, Yin D, Yang L, Chen Y, et al. Transmission of Carbapenem Resistance Between Human and Animal NDM-Positive Escherichia coli Strains.

Engineering [Internet]. 2022;15:24–33. Available from: https://doi.org/10.1016/j.eng.2021.07.030

65. Dong N, Zeng Y, Cai C, Sun C, Lu J, Liu C, et al. Prevalence, transmission, and molecular epidemiology of tet(X)-positive bacteria among humans, animals, and environmental niches in China: An epidemiological, and genomic-based study. Sci Total Environ [Internet]. 2022;818:151767. Available from: https://doi.org/10.1016/j.scitotenv.2021.151767

66. Muloi DM, Wee BA, McClean DMH, Ward MJ, Pankhurst L, Phan H, et al. Population genomics of Escherichia coli in livestock-keeping households across a rapidly developing urban landscape. Nat Microbiol. 2022;7(4):581–9.

67. Kim DW, Cha CJ. Antibiotic resistome from the One-Health perspective: understanding and controlling antimicrobial resistance transmission. Exp Mol Med [Internet]. 2021;53(3):301–9. Available from: http://dx.doi.org/10.1038/s12276-021-00569-z

68. Shawa M, Furuta Y, Paudel A, Kabunda O, Mulenga E, Mubanga M, et al. Clonal relationship between multidrug-resistant Escherichia coli ST69 from poultry and humans in Lusaka, Zambia. FEMS Microbiol Lett. 2021;368(21–24):1–11.

69. Aworh MK, Ekeng E, Nilsson P, Egyir B, Owusu-Nyantakyi C, Hendriksen RS. Extended-Spectrum ß-Lactamase-Producing Escherichia coli Among Humans, Beef Cattle, and Abattoir Environments in Nigeria. Front Cell Infect Microbiol. 2022;12(April):1–11.

70. Leangapichart T, Lunha K, Jiwakanon J, Angkititrakul S, Järhult JD, Magnusson U, et al. Characterization of Klebsiella pneumoniae complex isolates from pigs and humans in farms in Thailand: Population genomic structure, antibiotic resistance and virulence genes. J Antimicrob Chemother. 2021;76(8):2012–6.

71. Hickman RA, Leangapichart T, Lunha K, Jiwakanon J, Angkititrakul S, Magnusson U, et al. Exploring the Antibiotic Resistance Burden in Livestock, Livestock Handlers and Their Non-Livestock Handling Contacts: A One Health Perspective. Front Microbiol. 2021;12(April):1–12.

72. Salinas L, Cárdenas P, Johnson TJ, Vasco K, Graham J, Trueba G. Diverse Commensal Escherichia coli Clones and Plasmids Disseminate Antimicrobial Resistance Genes in Domestic Animals and Children in a Semirural Community in Ecuador. mSphere. 2019;4(3):1–10.

73. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, 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. 2019;19(1):56–66.

74. Wee BA, Muloi DM, van Bunnik BAD. Quantifying the transmission of antimicrobial resistance at the human and livestock interface with genomics. Clin Microbiol Infect [Internet]. 2020;26(12):1612–6. Available from: https://doi.org/10.1016/j.cmi.2020.09.019

75. Gompel L Van, Luiken REC, Hansen RB, Munk P, Bouwknegt M, Heres L, et al.

Description and determinants of the faecal resistome and microbiome of farmers and slaughterhouse workers : A metagenome-wide cross-sectional study. Environ Int [Internet]. 2020;143(July):105939. Available from: https://doi.org/10.1016/j.envint.2020.105939

76. Mughini-Gras L, Dorado-García A, van Duijkeren E, van den Bunt G, Dierikx CM, Bonten MJM, et al. Attributable sources of community-acquired carriage of Escherichia coli containing β-lactam antibiotic resistance genes: a population-based modelling study. Lancet Planet Heal. 2019;3(8):e357–69.

77. Matlock W, Lipworth S, Chau KK, Oun MA, Kavanagh J, Andersson M, et al. Enterobacterales-associated plasmid sharing amongst human bloodstream infections, livestock, wastewater, and waterway niches: a genomic surveillance study in Oxfordshire, UK. BioRxiv. 2022;1–36.