



# Role of horizontal gene transfer in the dissemination of antimicrobial resistance in food animal production

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Horizontal gene transfer (HGT) plays a major role in the dissemination of antimicrobial resistance genes (ARG) among diverse bacterial populations. Several studies have shown the presence of mobile genetic elements such as plasmids and integrons carrying ARG among bacteria from livestock and related environments. This is a potential concern as these resistant determinants can be exchanged between human and animal pathogenic microorganisms through direct and indirect contact. Although knowledge on HGT and development of multidrug resistance in microbes has been substantially elucidated, our knowledge on how and to what extent HGT propels antimicrobial resistance transmission from livestock to humans remains limited. This review article highlights the different pathways by which HGT propagates antimicrobial resistance transmission from farm animals to humans and propose strategies that can be implemented to control antimicrobial resistance dissemination in food animal production.

## Addresses

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## Introduction

The discovery of penicillin in 1928 marked the beginning of an important era in public health, which triggered the availability of an array of natural and synthetic antibiotics. Subsequently, in the 1940s, it was demonstrated that feeding sub-therapeutic levels of antibiotics improved feed efficiency and accelerated animal growth,

which was followed by a large-scale application of antibiotics in food animal production. In food animals, antibiotics have been used at therapeutic concentrations for treating diseases, prophylactic levels for preventing diseases, and at ‘metaphylaxis’ levels to treat sick animals while preventing disease in non-infected animals [1]. The widespread and long-term use of antibiotics in human medicine, veterinary medicine, and animal agriculture and potentially their misuse led to the emergence of antimicrobial-resistant bacteria, creating a major health hazard to humans and animals globally [2–4].

Antimicrobial-resistant (AMR) bacteria exist across animal, human and environment triad. The knowledge regarding evolution of AMR and the dynamics of AMR gene antimicrobial resistance genes (ARG) spread across this triad is critical for predicting emerging pathogens and controlling AMR dissemination [4]. AMR can be established either vertically by point mutations or horizontally via acquisition of mobile genetic elements (MGE) such as plasmids and transposons [5]. The global presence of ‘superbugs’ carrying multidrug resistant (MDR) plasmids indicates the rapid propagation of MDR bacteria via horizontal gene transfer (HGT) [6]. Considerable advances have been made in understanding the drivers for AMR transmission in food animal production, and the mechanisms by which AMR pathogens evolve and spread from food animals to humans. Therefore, the goal of this review is to discuss key concepts by which HGT promotes AMR evolution in food animal production systems, summarize current knowledge of AMR transmission from food animals to humans and propose strategies to control HGT in food animal production.

## Modes of horizontal gene transfer

HGT is the movement of genetic material between bacteria. HGT is primarily responsible for AMR spread in bacteria, resulting in rapid and broad dissemination of AMR determinants between bacteria and across species boundaries [7–9]. ARGs are often carried in MGE such as plasmids, integrons or transposons, which act as vectors for transferring genetic information between bacterial cells. The three classical pathways of HGT are conjugation, transduction, and transformation, of which conjugation constitutes the primary mechanism by which HGT occurs in bacteria involving direct cell-to-

cell contact for DNA transfer [8]. During transformation, short fragments of naked DNA are taken up by naturally competent bacteria. Transduction involves transfer of DNA via bacteriophages [8]. Essentially all these mechanisms can lead to rapid evolution of bacteria as HGT dramatically increases their fitness, especially for their survival in the presence of antimicrobials [10].

### Risk factors for horizontal gene transfer

Gastrointestinal tract (GIT) of all living beings is a complex ecosystem containing all the essential elements for successful HGT. The food animal GIT is inhabited by  $\sim 10^{10}$ – $10^{11}$  bacterial cells/ml making it an optimal environment for genetic exchange. The GIT is under constant stress which provides a hostile environment for intestinal bacteria. Antimicrobial administration is a major stress factor that exerts transient and long-lasting pressure to the GIT bacteria. It is estimated that  $\sim 73\%$  of antimicrobials sold globally are used in food animals [11]. Antibiotics such as fluoroquinolones and beta-lactams are known to induce SOS response by activating RecA and LexA proteins, the two master regulators of SOS response during DNA damage in bacterial cells [12]. Further, DNA damage could promote HGT by a) increasing expression of genes required for integrative conjugative elements (ICE) (by inactivating repressor SetR, which in turn derepress *setC* and *setD* genes that encode over 40 genes involved in conjugal transfer) [13], b) by triggering competence by antibiotics such as fluoroquinolones (inhibits DNA gyrase and break DNA double strand) and enhancing transformation [14], or c) by triggering bacterial prophage induction by antibiotic such as ciprofloxacin enhancing transduction frequency [15,16]. Finally, the animal GIT also serves as an immense reservoir of ARGs, which can be transferred to other bacteria via HGT. For example, a metagenomic study involving healthy pigs under no antibiotic selective pressure identified 257 ARGs from fecal samples [17]. Interestingly, a significantly higher number of ARGs were located in MGE, mostly plasmids and integrons, where animals were raised more intensively under antibiotic pressure [18]. Similarly, extended-spectrum cephalosporin resistance often with other ARGs and sharing similar resistance plasmids was observed among diverse *Escherichia coli* (*E. coli*) isolated from free-range broiler flocks from two different hatcheries [19].

Disinfectants are commonly used to rapidly kill or inactivate undesired microorganisms in farm environments. However, the increased use of disinfectants can also have an impact on AMR transmission. Apart from the issue of resistance development against disinfectants, the greatest concern is for disinfectants potential to coselect for bacterial resistance to antibiotics. A metagenomic study investigating the patterns of

coselection by the non-antibiotic factors on AMR showed the co-occurrence of ARGs, metal resistance, and disinfectant resistance genes in pig farms [20]. Several disinfectants including widely used chlorhexidine digluconate and triclosan were shown to promote conjugal transfer of AMR at concentrations well below the minimum inhibitory concentrations of the recipient *E. coli* [20]. Hence, caution should be maintained to ensure the judicious use of disinfectants to avoid HGT events leading to antibiotic gene coselection.

### Prevalence and transmission of antimicrobial-resistant bacteria from livestock production systems

In natural settings, microbes harboring ARGs are part of a complex community, where they interact with each other. In such a setting, HGT acts as a gene-sharing network, where species composition and diversity play a major role in the spread of ARGs between and within species [21,22]. In the case of food animals, different livestock species house unique microbiomes, with some species becoming pivotal hub species that promote the spread of ARGs [23,24]. Analysis of pangenomes of different livestock species showed that *E. coli* belonging to phylogroup A (that includes sequence type 10 often harboring CTX-M-1 (active on CefoTaXime, first isolated from Munich) group of extended-spectrum  $\beta$ -lactamases (ESBLs)) was mostly recovered from pigs [25]. Moreover, a higher number of HGT events involving ARGs between pig isolates of different genera of *Enterobacteriaceae* family was observed in comparison to cattle and sheep [25]. Bacteria belonging to such phylogroup can potentially spread AMR clones across the food chain and understanding the prevalence and HGT capacity of those organisms can be vital in reducing the risk of transmission of AMR bacteria and ARGs from food animals to humans.

Meat-producing animals are also a major source of AMR foodborne pathogens, especially those belonging to the *Enterobacteriaceae* family (*E. coli*, non-typhoidal *Salmonella spp.* and *Campylobacter spp.*) [26]. Resistance against colistin (*mcr-8* gene), which is a last resort antibiotic to treat clinical infections caused by MDR Gram negative bacteria was found to be carried in a transferable IncFII-type conjugative plasmid in multiple animal species (pigs, chickens) and humans [27,28]. Several other *mcr* homologs were also identified in *Enterobacteriaceae* from different animal origins and were shown to be carried in conjugative plasmids [29–31]. These findings represent a paradigm shift in the colistin-resistant mechanism, which was previously dominated by chromosomal mutations. Interestingly, isolates that carried *mcr-8* gene also coharbored carbapenemase encoding *bla<sub>NDM</sub>* gene and were isolated in both humans and animals [27]. Among *Campylobacter spp.*, horizontal

transfer of chromosomal *tet(O)* gene between different *C. coli* strains was reported in the turkey intestine. They observed that certain strains of *Campylobacter* to be more prone to the acquisition or the transfer of ARGs. Hence, identifying such strains would be critical for the prevention of AMR transmission [32]. A recent in-vivo study in chickens demonstrated that *Salmonella* Heidelberg was able to acquire IncK2 plasmid carrying ESBL gene (*bla<sub>CMY-2</sub>*) via conjugation from the donor *E. coli*. Acquisition of new genetic material by pathogens such as *S. Heidelberg* is likely to increase fitness and increase the level of antibiotic tolerance [33].

Acquiring AMR pathogens via food consumption can risk human health and lead to treatment failure in potentially infected human patients. In 2018, the U.S. national surveillance programs to monitor AMR bacteria reported an increase in the isolation of MDR *Salmonella* from chicken samples [34]. Furthermore, *S. Infantis* and *S. Enteritidis* carrying transmissible quinolone resistance genes were identified from retail meat products [34]. Similarly, a study investigating the presence of common foodborne pathogens and AMR bacteria in retail ground beef and pork recovered carbapenamase-resistant *Raoultella ornithinolytica* coharbouring *bla<sub>KPC-2</sub>* and *bla<sub>NDM-5</sub>* on IncN and IncX3 plasmids, respectively [35••]. Recent emergence of MDR *S. Infantis* with reduced susceptibility to fluoroquinolones and carrying an MDR megaplasmid conferring resistance up to ten ARG (including *bla<sub>CTX-M-65</sub>*) from retail poultry, food animal cecal samples and humans has driven important changes in *Salmonella* trends, supplanting other serotypes in U.S. poultry [36]. In-depth analysis of milk samples from public markets revealed the presence of several ARGs (including PC1 beta-lactamase (from *Staphylococcus aureus* strain PC1) in the plasmid) in the milk metagenome [37,38•]. Moreover, *bla<sub>CMY-2</sub>* gene capable for HGT was demonstrated in raw milk making it a clear source of ARGs [38•]. These findings highlight the increased ARG transmission risk from animals to humans via meat and milk consumption, thereby signifying the need for robust surveillance measures for animal-derived products.

### Indirect transmission of antimicrobial resistance genes from livestock to human

A significant portion of the antimicrobials used in animal husbandry operations can be found in the GIT of animals at low and sub-lethal concentrations, which inhibit the growth of susceptible bacterial populations [39,40]. This potentially exerts selective pressure on the gut bacteria to acquire ARGs, thereby leading to the evolution of resistant populations [41]. When the AMR bacteria and ARGs disseminate to surrounding environments, it leads to environmental pollution and

subsequently leads to human exposure, particularly for agricultural workers and those living in neighboring areas. Systematic analysis of microbial and ARG composition among farm workers, surrounding villagers, animal feces and air showed that vast amounts of bacteria were shared among farm workers, animals and air with a reduction in microbial diversity and an increase in ARG abundance in farm workers [42••]. Mourkas et al. in 2019 [43] reported that *C. jejuni* and *C. coli* strains isolated from livestock, sewage and humans shared similar resistance profiles and attributed their occurrence due to the HGT of plasmids and MDR genomic islands.

Soil is another major environmental reservoir for ARGs through interaction with human and/or animal-associated microbial species [44,45]. ARGs can also enter through amendment of soil with manure from antibiotic-treated animals, which are considered a reservoir of such genes [45–47]. Manure provides soil a unique environment for the spread of ARG by HGT. Manure is rich in nutrients, with diverse bacterial population that accumulates antibiotic residues capable of inducing selection pressure that potentially triggers the exchange of bacterial DNA. The abundance of ARGs in untreated livestock waste varied from  $10^6$ – $10^{11}$  copies/g dry weight. Additionally, a strong correlation has been found between the heavy use of certain antibiotics in livestock and the presence of ARGs and MGEs, such as plasmids, ICE, transposons and integrons, involved in the spread of ARGs via HGT pathways from manure to soil microbes [48]. A study from North Carolina reported that land manure application resulted in long-term persistence of *Salmonella* plasmids in commercial swine farms [49]. Moreover, the *Salmonella* strains belonging to six serotypes were able to transfer ARGs by conjugation not only to *E. coli* but also among different serotypes of *Salmonella* [49]. Therefore, animal manure amendment in agriculture poses a significant public health concern for AMR bacterial transmission to humans.

Several studies have reported the adverse impact of soil fertilization with pig, chicken, and cow manure on the soil resistome and the transfer frequency onto vegetables and fruits [50–52]. *Acinetobacter baumannii*, which is considered the most antibiotic-resistant bacterium was recovered from fresh vegetables (lettuce, carrot, and potatoes) in Connecticut [53]. It was found that all the recovered *A. baumannii* isolates were resistant to multiple antibiotics including imipenem, ceftriaxone, erythromycin and streptomycin [53]. Since fresh produce is consumed raw, their intake can result in the ingestion of AMR bacteria which may colonize the human gut or pass transiently posing a significant threat to public health. Therefore, there is a potential concern regarding the use of manure as an agricultural amendment due to its potential contribution of the pool of ARGs to resident soil bacteria, crops, and human pathogens.

## Strategies to control antimicrobial resistance in food animals

### Surveillance efforts focused on next-generation sequencing

Whole-genome sequencing (WGS) of bacterial isolates phenotypically resistant to antibiotics provides reliable information through *in silico*-based ARG profiling to identify their localization in foodborne pathogens. As such, the sequence data can be used to provide reliable microbial identification, their phylogenetic relationships, and traits relevant for epidemiological studies [54]. Further, WGS can help to screen unknown or novel ARGs or mutations and potential genetic elements related to HGT mechanisms that aid in the persistence of AMR in food animals [55,56]. In principle, *in-silico* AMR detection involves using a search algorithm to query input sequence data (either as reads or as assemblies) for the detection of predetermined set of AMR determinants contained within the selected reference database [54]. At present there are at least, 47 online available pipelines for identifying ARGs in WGS. Resfinder, Comprehensive Antibiotic Resistance Database, MEGARes (Microbial Ecology Group Antimicrobial Resistance), ARG-ANNOT (Antibiotic Resistance Gene - Annotation), Resfams among others, are actively curated and employed to identify genetic elements involved in AMR [54,57]. Additionally, recent bioinformatic applications that combine metagenome assembled genomes and Hi-C (High-throughput Chromosome conformation technique) proximity sequence analysis in tandem may possibly help to evaluate the host-plasmid linkage and track the extent of transmission of plasmid related ARG/integron alleles across bacterial communities with increased specificity [58,59]. This approach can help strategize novel microbial risk assessment tools for the food animal production continuum. In addition, curation and referencing of whole-genome sequences of AMR bacterial species and their related plasmids can help complement the aforesaid efforts by enhancing the discriminatory potential and host-plasmid linkage specificity.

### Judicious antimicrobial usage

The ecological persistence of ARGs in the farm environment is a critical factor that favors persistence and cross-transmission of AMR microbes between food animals and the environment. The cessation of beef cattle backgrounding operation for a period of two years resulted in a statistically significant reduction in the concentrations of ARG in the soil environment, however, it is not clear how many more years it would take for an ARG decline in the farm environment comparable to the preanimal production level [60]. Alternatively, researchers have also indicated that prudent antimicrobial usage in food animal production can potentially help to reduce ARG persistence in the farm. Implementation of quarantine practice in beef production in Italy was

shown to be a feasible strategy to reduce antimicrobial usage in male calves compared to the ones that received non-quarantine based fattening cycle [61]. A recent study conducted in Canada in commercial broiler chickens revealed that short-term antibiotic withdrawal period (15 month period) was ineffective, but the judicious use of antibiotics for over a period of six years (long-term) was capable of reducing the ARG abundance [62]. Some of these research findings shed light on the fact that the judicious use of antibiotics could possibly augment the efforts in reducing ARG persistence and indirectly impact HGT in the farming environment.

### Antibiotic alternatives

Several researchers have identified plasmid transfer inhibitors to reduce HGT activity between bacteria. Flavophospholipol, an antimicrobial feed additive for poultry and piglets, was capable of inhibiting the conjugal transfer of plasmid coding ESBL and *vanA* genes in *E. coli* and *Enterococcus faecalis*, respectively [63]. Similarly, synthetic fatty acids (2-hexadecynoic acid) nanoparticles, bacteriophage-and microbiota-based therapies and CRISPR system were also identified as potential inhibitors of bacterial conjugation mostly by targeting plasmid removal [64,65]. Also, Carica papaya seed macerate was found to decrease the conjugal transfer of R plasmid from *S. Typhimurium* to *E. coli* in vitro and in vivo [66]. Recent studies conducted in our laboratory identified the efficacy of four Generally Recognized as Safe phytochemicals namely, *trans*-cinnamaldehyde, carvacrol, caprylic acid and beta-resorcyate, in significantly reducing the HGT frequency of *bla*<sub>TEM</sub> from MDR *Salmonella* serotypes to *E. coli* strain an *Escherichia coli* strain (OP50) in broth, water and manure (Poonam Vinayamohan, PhD thesis, University of Connecticut, 2020). The aforesaid research findings collectively suggest the possibility of reducing HGT spread of ARGs by screening drug targets that specifically affect the bacterial conjugative machinery.

### Conclusions

A large amount of research has been conducted to better understand the role played by HGT in the transmission of ARGs from food animals to humans. The dissemination of ARGs by HGT could potentially lead to the rapid selection of resistant bacteria in a wide variety of environments, including soil, water, manure, and human and animal guts. Research indicates that despite restricting antimicrobial use, AMR persists due to the dissemination of ARGs among microbial communities. Thus, unifying key concepts in the evolution of HGT and resistance transmission from food animals to humans will be critical for devising interventions to prevent emergence and risk hazards of MDR pathogens in humans.

## CRedit authorship contribution statement

**Poonam Vinayamohan:** Writing – original draft, Writing – review & editing. **Abraham Pellissery:** Writing – original draft, Writing – review & editing. **Kumar Venkitanarayanan:** Concept development, Supervision, Writing – review & editing.

## Conflict of interest statement

None.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest.

1. Viola C, DeVincent SJ: **Overview of issues pertaining to the manufacture, distribution, and use of antimicrobials in animals and other information relevant to animal antimicrobial use data collection in the United States.** *Prev Vet Med* 2006, **73**:111-131.
  2. Ma F, Xu S, Tang Z, Li Z, Zhang L: **Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans.** *Biosaf Health* 2021, **3**:32-38.
  3. Kardos N: **Overuse of antibiotics and antibiotic resistance in medical applications featuring carbapenemase resistant enterobacteriaceae (CRE).** *SOJ Microbiol Infect Dis* 2017, **5**:1-21.
  4. Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, Hay SI, Jiwakanon J, Kakkar M, Kariuki S, et al.: **Antibiotic resistance is the quintessential One Health issue.** *Trans R Soc Trop Med Hyg* 2016, **110**:377-380.
  5. Yelin I, Kishony R: **Antibiotic resistance.** *Cell* 2018, **172**:1136.
  6. Sun D, Jeannot K, Xiao Y, Knapp CW: **Editorial: horizontal gene transfer mediated bacterial antibiotic resistance.** *Front Microbiol* 2019, **10**:1933.
  7. Gyles C, Boerlin P: **Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease.** *Vet Pathol* 2014, **51**:328-340.
  8. Barlow M: **What antimicrobial resistance has taught us about horizontal gene transfer.** *Methods Mol Biol* 2009, **532**:397-411.
  9. Koonin E v, Makarova KS, Aravind L: **Horizontal gene transfer in prokaryotes: quantification and classification.** *Ann Rev Microbiol* 2001, **55**:709-742.
  10. Hiltunen T, Virta M, Anna-Liisa L: **Antibiotic resistance in the wild: an eco-evolutionary perspective.** *Philos Trans R Soc B Biol Sci* 2017, **372**:20160039.
  11. van Boeckel TP, Glennon EE, Chen D, Gilbert M, Robinson TP, Grenfell BT, Levin SA, Bonhoeffer S, Laxminarayan R: **Reducing antimicrobial use in food animals.** *Science* 2017, **357**:1350-1352 (1979).
  12. Zeng X, Lin J: **Factors influencing horizontal gene transfer in the intestine.** *Anim Health Res Rev* 2017, **18**:153-159.
  13. Mohanraj RS, Mandal J: **Azithromycin can induce SOS response and horizontal gene transfer of SXT element in Vibrio cholerae.** *Mol Biol Rep* 2022, **1**:1-12.
  14. Charpentier X, Polard P, Claverys JP: **Induction of competence for genetic transformation by antibiotics: convergent evolution of stress responses in distant bacterial species lacking SOS?** *Curr Opin Microbiol* 2012, **15**:570-576.
  15. Modi SR, Lee HH, Spina CS, Collins JJ: **Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome.** *Nature* 2013, **499**:219-222 (2013 499:7457).
  16. Ulrich RL, DeShazer D, Kenny TA, Ulrich MP, Moravusova A, Opperman T, Bavari S, Bowlin TL, Moir DT, Panchal RG: **Characterization of the burkholderia thailandensis sos response by using whole-transcriptome shotgun sequencing.** *Appl Environ Microbiol* 2013, **79**:5830-5843.
  17. Joyce A, McCarthy CGP, Murphy S, Walsh F: **Antibiotic resistomes of healthy pig faecal metagenomes.** *Microb Genom* 2019, **5**:e000272.
  18. Mencia-Ares O, Cabrera-Rubio R, Cobo-Díaz JF, Álvarez-Ordóñez A, Gómez-García M, Puente H, Cotter PD, Crispie F, Carvajal A, Rubio P, et al.: **Antimicrobial use and production system shape the fecal, environmental, and slurry resistomes of pig farms.** *Microbiome* 2020, **8**:1-17.
- The authors demonstrated that a higher number of antimicrobial resistance genes were located in mobile genetic elements in animals that are intensively raised.
19. Baron S, le Devendec L, Touzain F, Jouy E, Lucas P, de Boissésion C, Larvor E, Kempf I: **Longitudinal study of Escherichia coli plasmid resistance to extended-spectrum cephalosporins in free-range broilers.** *Vet Microbiol* 2018, **216**:20-24.
  20. Jutkina J, Marathe NP, Flach CF, Larsson DGJ: **Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations.** *Sci Total Environ* 2018, **616-617**:172-178.
  21. Hu Y, Yang X, Li J, Lv N, Liu F, Wu J, Lin IYC, Wu N, Weimer BC, Gao GF, et al.: **The bacterial mobile resistome transfer network connecting the animal and human microbiomes.** *Appl Environ Microbiol* 2016, **82**:6672-6681.
  22. Tamminen M, Virta M, Fani R, Fondi M: **Large-scale analysis of plasmid relationships through gene-sharing networks.** *Mol Biol Evol* 2012, **29**:1225-1240.
  23. Forsberg KJ, Patel S, Gibson MK, Lauber CL, Knight R, Fierer N, Dantas G: **Bacterial phylogeny structures soil resistomes across habitats.** *Nature* 2014, **509**:612-616 (2014 509:7502).
  24. Popa O, Dagan T: **Trends and barriers to lateral gene transfer in prokaryotes.** *Curr Opin Microbiol* 2011, **14**:615-623.
  25. Shaw LP, Chau KK, Kavanagh J, AbuOun M, Stubberfield E, Gweon HS, Barker L, Rodger G, Bowes MJ, Hubbard ATM, et al.: **Niche and local geography shape the pangenome of wastewater-and livestock-associated Enterobacteriaceae.** *Sci Adv* 2021, **7**:eabe3868.
- Pangenome analysis of the samples from different livestock species demonstrated that a higher number of horizontal gene events involving antimicrobial resistance genes in pig isolates of *Enterobacteriaceae* family.
26. Heredia N, Garcia S: **Animals as sources of food-borne pathogens: a review.** *Anim Nutr* 2018, **4**:250-255.
  27. Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, Zhang S, Shen J, Shen Z, Wang Y: **Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae article.** *Emerg Microb Infect* 2018, **7**:1-9.
  28. Carroll LM, Gaballa A, Guldemann C, Sullivan G, Henderson LO, Wiedmann M: **Identification of novel mobilized colistin resistance gene mcr-9 in a multidrug-resistant, colistin-susceptible salmonella enterica serotype typhimurium isolate.** *mBio* 2019, **10**:e00853-19.
  29. Yi L, Wang J, Gao Y, Liu Y, Doi Y, Wu R, Zeng Z, Liang Z, Liu JH: **mcr-1-harboring salmonella enterica serovar typhimurium sequence type 34 in pigs, China.** *Emerg Infect Dis* 2017, **23**:291-295.
  30. Hadjadj L, Baron SA, Olaitan AO, Morand S, Rolain JM: **Co-occurrence of variants of mcr-3 and mcr-8 genes in a Klebsiella pneumoniae isolate from Laos.** *Front Microbiol* 2019, **10**:2720.
  31. Garcia-Graells C, de Keersmaecker SCJ, Vanneste K, Pochet B, Vermeersch K, Roosens N, Dierick K, Botteldoorn N: **Detection of plasmid-mediated colistin resistance, mcr-1 and mcr-2 genes, in Salmonella spp. isolated from food at retail in Belgium from 2012 to 2015.** *Foodborne Pathog Dis* 2018, **15**:114-117 (<https://home.leafertpub.com/fpd>).
  32. Guernier-Cambert V, Trachsel J, Maki J, Qi J, Sylte MJ, Hanafy Z, Kathariou S, Looft T: **Natural horizontal gene transfer of antimicrobial resistance genes in Campylobacter spp. from Turkeys and Swine.** *Front Microbiol* 2021, **12**:2559.
  33. Oladeinde A, Cook K, Lakin SM, Woyda R, Abdo Z, Looft T, Herrington K, Zock G, Lawrence JP, Thomas JC, et al.: **Horizontal gene transfer and acquired antibiotic resistance in Salmonella enterica serovar heidelberg following in vitro incubation in broiler ceca.** *Appl Environ Microbiol* 2019, **85**:e01903-19.
  34. **2018 NARMS Update: Integrated Report Summary | FDA ; 2018.**
  35. Ballash GA, Albers AL, Mollenkopf DF, Sechrist E, Adams RJ, Wittum TE: **Antimicrobial resistant bacteria recovered from retail ground meat products in the US include a Raoultella ornithinolytica co-harboring blaKPC-2 and blaNDM-5.** *Sci Rep* 2021, **11**:14041.

The authors recovered carbapenamase-resistant *Raoultella ornithinolytica* cohabouring *bla*<sub>KPC-2</sub> and *bla*<sub>NDM-5</sub> on IncN and IncX3 plasmids from retail meat samples.

36. Tate H, Folster JP, Hsu CH, Chen J, Hoffmann M, Li C, Morales C, Tyson GH, Mukherjee S, Brown AC, *et al.*: **Comparative analysis of extended-spectrum- $\beta$ -lactamase CTX-M-65-producing *Salmonella enterica* serovar Infantis isolates from humans, food animals, and retail chickens in the United States.** *Antimicrob Agents Chemother* 2017, **61**:e00488-17.

37. Tóth AG, Csabai I, Krikó E, Tózsér D, Maróti G, Patai Á v, Makrai L, Szita G, Solymsi N: **Antimicrobial resistance genes in raw milk for human consumption.** *Sci Rep* 2020, **10**:1-7 (2020 10:1).

38. Liu J, Liu J, Zhu Y, Zhu Y, Jay-Russell M, Lemay DG, Lemay DG, Mills DA, Mills DA, *et al.*: **Reservoirs of antimicrobial resistance genes in retail raw milk.** *Microbiome* 2020, **8**:1-15.

The authors demonstrated the presence of *bla*<sub>CMY-2</sub> gene capable for horizontal gene transfer in raw milk samples from public markets.

39. Zhu YG, Zhao Y, Li B, Huang CL, Zhang SY, Yu S, Chen YS, Zhang T, Gillings MR, Su JQ: **Continental-scale pollution of estuaries with antibiotic resistance genes.** *Nat Microbiol* 2017, **2**:1-7 (2017 2:4).

40. Woolhouse MEJ, Ward MJ: **Sources of antimicrobial resistance.** *Science* 2013, **341**:1460-1461 (1979).

41. Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, Andersson DI: **Selection of resistant bacteria at very low antibiotic concentrations.** *PLoS Pathog* 2011, **7**:e1002158.

42. Ding D, Zhu J, Gao Y, Yang F, Ma Y, Cheng X, Li J, Dong P, Yang H, Chen S: **Effect of cattle farm exposure on oropharyngeal and gut microbial communities and antibiotic resistance genes in workers.** *Sci Total Environ* 2022, **806**:150685.

Metagenomic analysis of samples from animals, farm workers demonstrated that vast amounts of bacteria were shared among farm workers, animals and air with a reduction in microbial diversity and an increase in antimicrobial gene abundance in farm workers.

43. Mourkas E, Florez-Cuadrado D, Pascoe B, Calland JK, Bayliss SC, Mageiros L, Méric G, Hitchings MD, Quesada A, Porrero C, *et al.*: **Gene pool transmission of multidrug resistance among *Campylobacter* from livestock, sewage and human disease.** *Environ Microbiol* 2019, **21**:4597-4613.

44. Martínez JL: **Antibiotics and antibiotic resistance genes in natural environments.** *Science* 2008, **321**:365-367 (1979).

45. Fletcher S: **Understanding the contribution of environmental factors in the spread of antimicrobial resistance.** *Environ Health Prev Med* 2015, **20**:243-252.

46. Udikovic-Kolic N, Wichmann F, Broderick NA, Handelsman J: **Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization.** *Proc Natl Acad Sci U S A* 2014, **111**:15202-15207.

47. Marti R, Scott A, Tien YC, Murray R, Sabourin L, Zhang Y, Topp E: **Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest.** *Appl Environ Microbiol* 2013, **79**:5701-5709.

48. Lima T, Domingues S, Silva GJ da: **Manure as a potential hotspot for antibiotic resistance dissemination by horizontal gene transfer events.** *Vet Sci (3)* 2020, **7**:110.

49. Pornsukarom S, Thakur S: **Horizontal dissemination of antimicrobial resistance determinants in multiple *Salmonella* serotypes following isolation from the commercial swine operation environment after manure application.** *Appl Environ Microbiol* 2017, **83**:e01503-17, <https://doi.org/10.1128/AEM>

50. Heuer H, Smalla K: **Plasmids foster diversification and adaptation of bacterial populations in soil.** *FEMS Microbiol Rev* 2012, **36**:1083-1104.

51. Jechalke S, Kopmann C, Rosendahl I, Groeneweg J, Weichelt V, Krögerreclenfort E, Brandes N, Nordwig M, Ding G-C, Siemens J, *et al.*: **Increased abundance and transferability of resistance genes after**

**field application of manure from sulfadiazine-treated pigs.** *Am Soc Microbiol* 2013, **79**:1704-1711.

52. Marti R, Scott A, Tien YC, Murray R, Sabourin L, Zhang Y, Topp E: **Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest.** *Appl Environ Microbiol* 2013, **79**:5701-5709.

53. Karumathil DP, Yin HB, Kollanoor-Johny A, Venkitanarayanan K: **Prevalence of multidrug-resistant bacteria on fresh vegetables collected from farmers' markets in Connecticut.** *J Food Prot* 2016, **79**:1446-1451.

54. Hendriksen RS, Bortolaia V, Tate H, Tyson GH, Aarestrup FM, McDermott PF: **Using genomics to track global antimicrobial resistance.** *Front Public Health* 2019, **7**:242.

55. Aerts M, Battisti A, Hendriksen R, Kempf I, Teale C, Tenhagen BA, Veldman K, Wasyl D, Guerra B, Liébana E, *et al.*: **Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food.** *EFSA J* 2019, **17**:e05709.

56. Su M, Satola SW, Read TD: **Genome-based prediction of bacterial antibiotic resistance.** *J Clin Microbiol* 2019, **57**:e01405-18.

57. Lal Gupta C, Kumar Tiwari R, Cytryn E: **Platforms for elucidating antibiotic resistance in single genomes and complex metagenomes.** *Environ Int* 2020, **138**:105667.

58. Stalder T, Press MO, Sullivan S, Liachko I, Top EM: **Linking the resistome and plasmidome to the microbiome.** *ISME J* 2019, **13**:2437-2446 (2019 13:10).

59. Bickhart DM, Watson M, Koren S, Panke-Buisse K, Cersosimo LM, Press MO, van Tassel CP, van Kessel JAS, Haley BJ, Kim SW, *et al.*: **Assignment of virus and antimicrobial resistance genes to microbial hosts in a complex microbial community by combined long-read assembly and proximity ligation.** *Genome Biol* 2019, **20**:1-18.

60. Agga GE, Cook KL, Netthisinghe AMP, Gilfillen RA, Woosley PB, Sistani KR: **Persistence of antibiotic resistance genes in beef cattle backgrounding environment over two years after cessation of operation.** *PLoS One* 2019, **14**:e0212510.

61. Santinello M, Diana A, de Marchi M, Scali F, Bertocchi L, Lorenzi V, Alborali GL, Penasa M: **Promoting judicious antimicrobial use in beef production: the role of quarantine.** *Animals* 2022, **12**:116.

62. Turcotte C, Thibodeau A, Quessy S, Topp E, Beauchamp G, Fravallo P, Archambault M, Gaucher M lou: **Impacts of short-term antibiotic withdrawal and long-term judicious antibiotic use on resistance gene abundance and cecal microbiota composition on commercial broiler chicken farms in Québec.** *Front Vet Sci* 2020, **7**:1067.

The authors demonstrated that short-term antibiotic withdrawal (15 month period) in commercial broiler chickens was ineffective in reducing antimicrobial gene abundance, but the judicious use of antibiotics for over a period of 6 years (long-term) was capable of reducing the antimicrobial gene abundance.

63. Kudo H, Usui M, Nagafuji W, Oka K, Takahashi M, Yamaguchi H, Tamura Y: **Inhibition effect of flavophospholipol on conjugative transfer of the extended-spectrum  $\beta$ -lactamase and *vanA* genes.** *J Antibiot* 2019, **72**:79-85.

64. Palencia-Gándara C, Getino M, Moyano G, Redondo S, Fernández-López R, González-Zorn B, de la Cruz F: **Conjugation inhibitors effectively prevent plasmid transmission in natural environments.** *mBio* 2021, **12**:e0127721.

65. Vrancianu CO, Popa LI, Bleotu C, Chifiriuc MC: **Targeting plasmids to limit acquisition and transmission of antimicrobial resistance.** *Front Microbiol* 2020, **11**:761.

66. Leite AAM, Nardi RMD, Nicoli JR, Chartone-Souza E, Nascimento AMA: **Carica papaya seed macerate as inhibitor of conjugative R plasmid transfer from *Salmonella typhimurium* to *Escherichia coli* in vitro and in the digestive tract of gnotobiotic mice.** *J Gen Appl Microbiol* 2005, **51**:21-26.