Biology | John Maurer

Exploring the transfer of antibiotic resistance genes within poultry litter

Antibiotic resistance is a major threat to human and animal health. Bacteria containing antibiotic resistance genes are found in poultry litter, usually from commercial chicken production. Since poultry litter is often used as a soil fertiliser, there is a risk that this antibiotic resistance could be transferred into the soil. Professor John Maurer at Virginia Tech University, USA, has shown that antibiotic resistance is transmitted through carriers called plasmids in the laboratory. However, plasmid transfer was not seen when the same experiment was carried out in poultry litter, suggesting other mechanisms may be at play.

Agriculture, including animal husbandry, requires environmental input, such as water provision and feed production. However, the by-products of animals can also give back to the environment. In the USA alone, it is estimated that poultry farms, including chicken and turkey, produce around 20 million tonnes of manure each year. This mostly consists of poultry litter, the mix of materials, spilled feed, manure, and feathers that result from poultry production, which has then been broken down by microbes.

Animal manure, especially poultry manure, can be a beneficial addition to soil as it increases nitrogen and carbon content, compounds that are both important for soil fertility and crop growth. Poultry manure contains almost all 13 essential plant nutrients, including nitrogen, potassium, and phosphorus. Nitrogen, in particular, encourages green leafy growth. Indeed, poultry manure is often sold in a dry form by garden centres for domestic use. This is because fresh poultry litter can contain bacteria that are harmful to humans. Dried products are often sterilised during manufacture, meaning they pose lower risk if being used within a home environment.

PUBLIC HEALTH CONSIDERATIONS

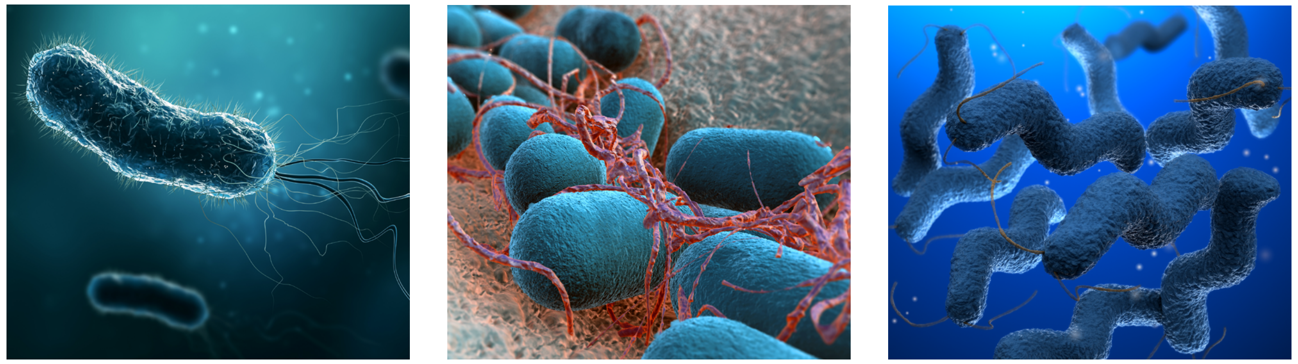
Professor John Maurer works within the School of Animal Sciences at Virginia Tech University, USA. His research concentrates on the ecology of microbial resistance in poultry production, the interaction between animals and microbes (bacteria, viruses, fungi), and its implications for animal health. Maurer's research group also aims to learn more about the spread of disease from animals to humans, especially within poultry production systems.

Maurer explains that poultry litter contains 100 million bacterial cells per gram. While many of these bacteria have benefits for soil and plant health, around 1–10% of the bacteria may contaminate the soil with antibiotic resistance microorganisms.

Poultry litter also contains bacteria capable of causing disease, such as *Salmonella*, *E. coli* or *Campylobacter*, that can be transferred into soil. Perhaps the most well-known pathogenic (disease-causing) bacteria within poultry is *Salmonella*, which has decreased in recent years due to national control programmes, strict biosecurity measures, and regular testing of chicken flocks. Each microorganism will require its own specific control measures, due to the differences between bacterial species.

ANTIBIOTIC RESISTANCE

Antibiotic resistance is a rising challenge in human and animal health and poses a major threat to global health and food security. Antibiotic resistance occurs when bacteria are no longer killed by existing medications, making infections harder to treat as the antibiotics used to treat them become less effective. The Centers for Disease Control and Prevention estimates that antimicrobial-resistant bacteria and fungi cause almost 3 million infections every year in the United States.



Poultry litter contains bacteria capable of causing disease, such as *Salmonella* (left), *E. coli* (middle), or *Campylobacter* (right).

While some level of antibiotic resistance happens naturally, as bacteria replicate and random genetic mutations occur, antibiotic usage selects for the dominance of these mutations. Previous studies done by Maurer's research group explored whether agricultural use contributes to antibiotic resistance in *Salmonella* found in chickens. They found that antibiotic treatment did not increase the level of *Salmonella* resistance in their trial; neither did transfer of a reservoir of antibiotic resistance genes into poultry. Maurer concludes that other factors are responsible for driving antibiotic resistance in *Salmonella*.

Scientists are able to characterise the number of resistance genes found in bacteria in poultry litter (the 'resistome'). Maurer reports that specific antibiotic resistance genes are relatively prevalent in poultry litter and can be found in harmless as well as disease-causing bacteria. This suggests that antibiotic resistance genes can be passed between bacteria within poultry litter. One way this may occur is through plasmids, genetic elements that package up and ferry antimicrobial resistance genes between bacteria. Generally, transfer of resistance requires physical cell-to-cell contact. Within plasmids, antibiotic resistance genes are often associated with elements, called integrons, that can exchange specific genes and thereby build upon existing resistances.

TRANSMISSION THROUGH PLASMIDS

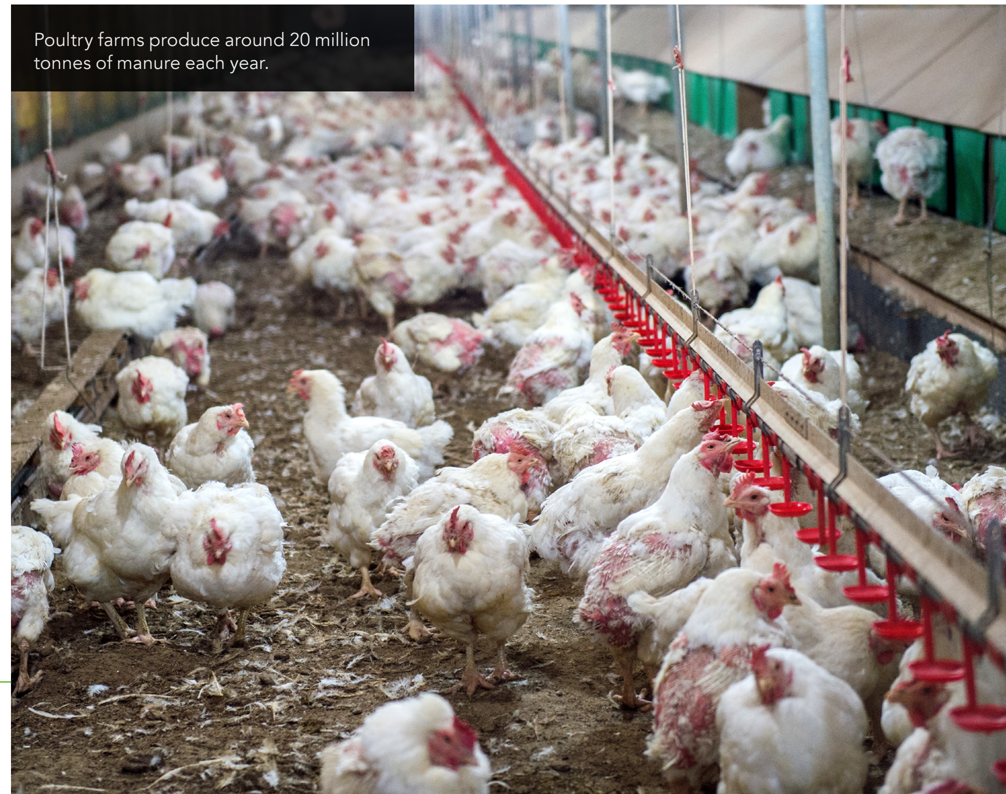
Maurer and colleagues investigated whether a plasmid carrying antimicrobial resistance could move from one type of bacteria to another, notably from an antibiotic-resistant *E. coli* to an antibiotic-sensitive *Salmonella*.

Firstly, the researchers extracted bacteria from the poultry litter and calculated the levels of antimicrobial resistance against four different antibiotics. The poultry litter was from two sources, chicken flocks kept for experimental purposes at Virginia Tech and commercial broiler chicken houses. The results showed that the highest level of resistance among the bacterial ecosystem was to the antibiotics streptomycin and tetracycline.

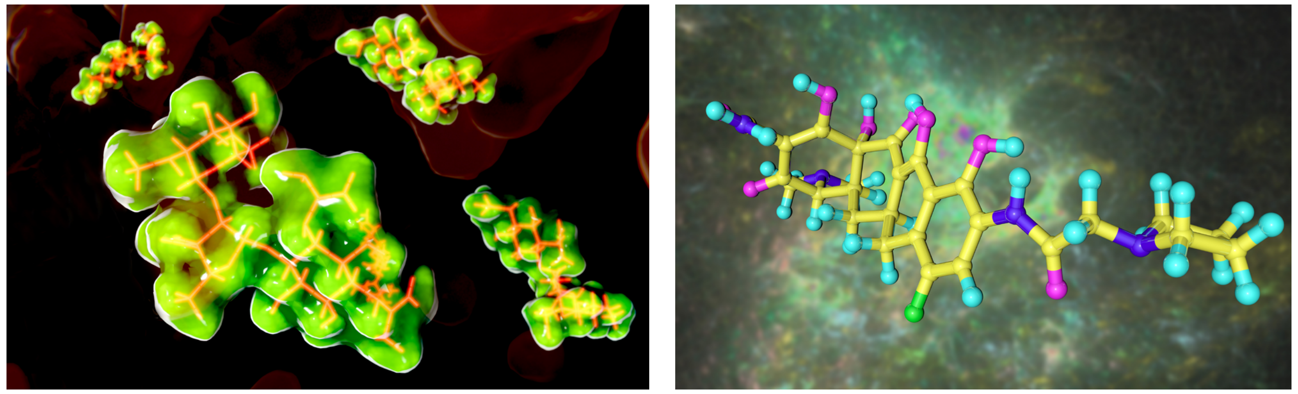
The researchers hypothesised that specific conditions may be needed for antimicrobial resistance to be transferred between bacteria; they tested multiple conditions that varied the strength of the bacterial growth media, temperature, and length of contact time between bacterial cells to try and identify optimal conditions leading to antibiotic resistance transfer between bacteria in poultry litter.

Next, plasmids were studied in a laboratory setting, and the results showed that it was possible for plasmids to move between different types of bacteria. These small DNA molecules are important for bacterial survival especially when antibiotics are present as they allow genetic information to be passed between bacterial cells.

Poultry litter contains bacteria capable of causing disease that can be transferred into soil.



Poultry farms produce around 20 million tonnes of manure each year.



Antibiotics streptomycin (left) and tetracycline (right).

Plasmids varied in their ability to transfer antibiotic resistance depending on whether conditions were set to 98°F (body temperature) or 75°F (temperature of a poultry house).

The researchers also tested whether this exchange of antibiotic resistance still happened when there were other types of bacteria being added into the mix. The results showed that even if other bacteria from poultry litter were present, antibacterial resistance still moved from an antibiotic-resistant *E. coli* to an antibiotic-sensitive *Salmonella*, ferried by plasmids.

After identifying conditions that led to the highest level of plasmid transfer in the presence of poultry litter bacteria, the group also explored whether the type of plasmid or strains of resistant bacteria influenced their previous results when antibiotic-resistant *E. coli* and antibiotic-sensitive *Salmonella* were added to poultry litter.

However, when the same approach was used to study transfer of antibiotic resistance within a poultry litter microcosm (a miniature version of what commercial poultry litter would look like), no plasmid exchange of antibiotic resistance was observed, despite the same types of bacteria being used.

The research group wondered whether low levels of some types of bacteria containing resistance genes may be the reason that plasmid transfer was not detected in poultry litter. They also tried using a different type of plasmid that was more suited to the temperature of a poultry house. The findings showed that although there were high levels of resistance genes being expressed by bacteria in litter, they do not seem to be transferred between bacteria by plasmids.

OTHER FACTORS AT PLAY

There may be several reasons why plasmid transfer of antibiotic resistance was not observed, possibly because the poultry litter did not support growth of the selected experimental bacterial strains or may produce factors that interfere with plasmid abundance or transfer. The physical nature of the litter, such as the pH or type of bedding material, may also play a role, as studies by other research groups have shown differing levels of plasmid transfer. Maurer suggests that future studies could investigate whether certain types of litter or poultry management practices are more likely to promote the spread of antibiotic resistance genes between bacteria in poultry litter.

Maurer concludes that bacteria from poultry litter do contain plasmids for transmitting antibiotic resistance. However, their potential to transmit this information between them appears limited under standard experimental conditions, despite a wide variety of conditions examined in this study. Even though there is transfer of antibiotic resistance via plasmids in nature, this does not appear to be likely in poultry litter, or between litter and soil.

Even though there is transfer of antibiotic resistance via plasmids in nature, this does not appear to be likely in poultry litter, or between litter and soil.

Behind the Research

Dr John J Maurer

E: jjmaurer@vt.edu **T:** +1 540-231-9134 **W:** www.apsc.vt.edu/OurPeople/Faculty/John-J-Maurer.html

Research Objectives

Professor Maurer studies antibiotic resistance in poultry litter.

Detail

Bio

John J Maurer, PhD, is professor in the Department of Animal and Poultry Sciences at Virginia Tech. He is a member of the American Association of Avian Pathologists and American Society for Microbiology. His research interests include the development, validation, and implementation of molecular tools for on-farm surveillance of foodborne pathogens and the ecology of antibiotic resistance and foodborne pathogens in the food production environment. His current USDA-funded research is focused on remediating antimicrobial resistance in poultry litter. Dr Maurer has 79 peer-reviewed publications and 19 book chapters and reviews.

Funding

USDA National Institute of Food and Agriculture

Collaborators

Margie D Lee, DVM, PhD (Virginia-Maryland College of Veterinary Medicine, Virginia Tech)

References

Oxendine, A, et al, (2023) Conditions necessary for the transfer of antimicrobial resistance in poultry litter. *Antibiotics (Basel, Switzerland)*, 12(6), 1006. doi.org/10.3390/antibiotics12061006

Bythwood, TN, et al, (2019) Antimicrobial resistant *salmonella enterica* typhimurium colonizing chickens: the impact of plasmids, genotype, bacterial communities and antibiotic administration on resistance. *Front Sustain Food Syst*, 3. doi.org/10.3389/fsufs.2019.00020

Nandi, S, et al, (2004) Gram-positive bacteria are a major reservoir of class 1 antibiotic resistance integrons in poultry litter. *PNAS*, 101(18), 7118–7122. doi.org/10.1073/pnas.0306466101

Personal Response

What inspired you to conduct this research?

" The ultimate goal of our research project is to identify poultry litter incubation conditions, like composting, that can reduce antibiotic resistance in this animal manure and prevent transfer of this resistance to harmful bacteria like *Salmonella*. "

If plasmids are not responsible for transfer of antimicrobial resistance within poultry litter, and between litter and soil, what do you think the alternative mechanism may be?

" We have actually documented transfer of antibiotic resistance to *Salmonella* in chickens. We believe that this transfer occurs in the chicken's intestine, where *Salmonella* naturally resides. "

VIRGINIA TECH