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Antibiotic resistance genes traced from manure to soil and water on Finnish farms

A new study has investigated the movement of antibiotic resistance genes between farm animals, soil and water in Finland. The results show that many of these genes are spread from animals to the soil through manure application; however, these genes do not appear to persist in soil. The study suggests that practices that minimise the use of antibiotics, as used in Finland, may lead to lower levels of clinically relevant resistance genes in agricultural soils.

It is suspected that the use of antibiotics in agriculture is contributing to the emergence of antimicrobial resistance (infection-causing bacteria which are resistant to antibiotics). Genes that cause antibiotic resistance are carried by microorganisms that live in the environment. These microorganisms can also be hosted by farm animals. Antibiotic-resistance genes (ARGs) can be transferred to bacteria associated with animals or humans, through 'horizontal gene transfer' (where genes are passed between organisms, rather than from parents to offspring). This process — a common mechanism in the evolution of bacteria — often takes place in animal intestines, and may also occur in agricultural soils.

When antibiotics are used in farm animals, bacteria in their gut which are resistant to antibiotics are promoted through forces of evolutionary selection. If manure is used as fertiliser, then these ARGs may increase in the environment and, from the environment, the ARGs can be cycled back to animals via fodder, suggest the researchers. This study looked for ARGs on four Finnish farms, to determine how they move and survive in manure, soil and water.

The farms studied were two dairy farms and two swine (pig) farms, which had previously been the focus of a narrower study looking for a small number of ARGs[1]. Animals on each farm had received antibiotic treatment — chiefly penicillin — for bacterial infections, but, as is generally the case in Finland, were not given antibiotics for any other reason. (In the EU, use of antimicrobials for growth promotion is not allowed in food-producing animals.) All sampled soils had been in regular agricultural use for several years and were fertilised annually with manure.

The researchers sampled fresh manure, stored manure, unfertilised soil and fertilised soil on the day the manure was applied; fertilised soil two and six weeks after manure application; and water from a drainage ditch, before and after manure application. During analysis, DNA was extracted from the samples to determine levels of ARGs present. The researchers also looked for mobile genetic elements (MGEs), which are parts of genes involved in gene transfer.

The researchers found 161 different ARGs in the samples and 21 MGEs. Amongst the ARGs, the researchers identified genes that confer resistance to groups of antibiotics, including sulphonamides, tetracyclines and aminoglycosides, as well as to disinfectants. The greatest diversity (130 types) of ARGs and MGEs was found in fresh and stored manure, followed by manured soils (97 types).

Manure also had the highest relative abundance of ARGs and MGEs, determined in relation to the abundance of 16S ribosomal RNA (the most established genetic marker used for bacterial identification and classification), and the relative abundance of more than 100 types of ARG was higher in stored manure compared to fresh. The researchers offer several possible explanations for this, including that horizontal dissemination of ARGs may be promoted in the presence of trace amounts of antibiotic or in cold storage conditions, or that there may be a shift in the bacterial community during storage.

Continued on next page.

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Source: Muurinen, J., Stedtfeld, R., Karkman, A., Pärnänen, K., Tiedje, J. & Virta, M. (2017). Influence of Manure Application on the Environmental Resistome under Finnish Agricultural Practice with Restricted Antibiotic Use. *Environmental Science & Technology*, 51(11), 5989-5999. DOI: 10.1021/acs.est.7b00551

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1. Ruuskanen, M., *et al.* (2016). Fertilizing with Animal Manure Disseminates Antibiotic Resistance Genes to the Farm Environment. *J. Environ. Qual.*, 45(2): 488–493

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Antibiotic resistance genes traced from manure to soil and water on Finnish farms *(continued)*

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Higher levels of ARGs were found in fertilised soil than in unfertilised, but the abundance and diversity of ARGs was lower six weeks following application compared to two weeks after. Only 29 types of ARG were found in unfertilised soil.

The lowest diversity of ARGs and MGEs was found in the ditch water. ARGs associated with manure were not detected in soil or ditch water prior to fertilisation, suggesting that when they were found in soil and water, it was due to manure application. However, the majority of ARGs found in manure were not found in water, indicating that large loads of ARGs are probably not being spread widely to water from the farms in the study. Genes conferring resistance to β-lactam antibiotics and genes conferring multidrug resistance were the most common ARGs in unfertilised soil and in ditch water, but not abundant in manure. Unlike in some other studies their abundance did not increase in fertilised soil.

The researchers state that their findings are consistent with the idea that farms are 'reservoirs' of ARGs. However, samples taken before the annual spreading of manure in spring did not show high concentrations of ARGs, even though they have been treated with manure for decades. In fact, concentrations were similar to those in soils uncontaminated by fecal residues that were analysed to demonstrate 'background levels' of ARGs[2], according to the researchers. In addition, the decreased levels found in fertilised soil six weeks after manure application, compared to the more recently manured soil, suggests that the ARGs originating in manure do not survive well in soil.

The researchers also note that there are other factors that might lead to low levels of ARGs in Finnish farm soils before fertilisation, such as the annual freeze–thaw cycle; manure application being limited to April–October; and the enforcement of prudent antibiotic use including the use of mostly narrow-spectrum antibiotics. Other studies have shown that higher levels are found in farm soils where antibiotics are used frequently and/or non-therapeutically (e.g. to increase animal growth), or prophylactically (i.e. to prevent disease)[3]. The researchers, therefore, propose that adopting agricultural practices similar to those in Finland could lead to low prevalence of clinically relevant ARGs in farm soils.

In contrast, the ARGs most prevalent in the unfertilised soil and ditch water are probably widely dispersed in the environment — but this is not due to human impact, say the researchers. However, the use of antibiotics on farm animals and the use of their manure as a fertiliser could increase the chances of these genes transferring from environmental bacteria to those that cause infections. They recommend, therefore, not underestimating the risk of ARG transfer from manure and the environment, and reducing antibiotic use on farms to reduce the load of ARGs in the environment.

2. See Wang, F., Stedtfeld, R.D., Kim, O.-S., Chai, B., Yang, L., Stedtfeld, T.M., Hong, S.G., Kim, D., Lim, H.S., Hashsham, S.A., Tiedje, J.M. and Sul, W.J. (2016). Influence of Soil Characteristics and Proximity to Antarctic Research Stations on Abundance of Antibiotic Resistance Genes in Soils. *Environ. Sci. Technol.*, 50 (23) 12621– 12629

3. See Schmitt, H., Stoob, K., Hamscher, G., Smit, E. and Seinen, W. (2006). Tetracyclines and Tetracycline Resistance in Agricultural Soils: Microcosm and Field Studies. *Microb. Ecol.*, 51(3) 267– 276; and Zhu, Y.-G., Johnson, T.A., Su, J.-Q., Qiao, M., Guo, G.-X., Stedtfeld, R.D., Hashsham, S.A. and Tiedje, J.M. (2013). Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc. Natl. Acad. Sci. U.S.A.,* 110(9): 3435–3440