Antibiotic resistance levels in soils from urban and rural land uses in Great Britain

Kieran Osbiston, Anne Oxbrough and Lorena Teresa Fernández-Martínez*

Abstract

Although soil is one of the largest microbial diversity reservoirs, the processes that define its microbial community dynamics are not fully understood. Improving our understanding of the levels of antibiotic resistance in soils with different land uses in Great Britain is not only important for the protection of animal health (including humans), but also for gaining an insight into gene transfer levels in microbial communities. This study looked at the levels of antibiotic-resistant bacteria (ARB) able to survive inhibitory concentrations of chloramphenicol, erythromycin and vancomycin, as well as subinhibitory (10 µg ml⁻¹) erythromycin concentrations. Soils from nine different sites across Great Britain with three distinct land uses (agricultural, urban and semi-natural) were sampled and the percentage of ARB was calculated for each site. Statistical analyses confirmed a significant difference in the level of ARB found in agricultural land compared to urban or semi-natural sites. The results also showed that resistance levels to vancomycin and chloramphenicol in the agricultural and urban sites sampled were significantly higher than those for erythromycin, whilst in semi-natural sites all three antibiotics show similar resistance levels. Finally, although the levels of resistance to a subinhibitory (10 µg ml⁻¹) erythromycin concentration were significantly higher across land use types when compared to the levels of resistance to an inhibitory (20 µg ml⁻¹) concentration, these were much less marked in soil from agricultural land compared to that from urban or semi-natural land use soil.

INTRODUCTION

Antimicrobial resistance (AMR) is one of the most serious problems affecting global animal health (including humans). Although the increase in AMR is mostly driven by antibiotic and antimicrobial use, research suggests that the natural environment plays a key role in the wider spread of AMR [1–3]. While the use of antibiotics as growth promoters was phased out in the European Union between 1999 and 2006, their use in farming is still a major contributing factor to their release in large quantities to local ecosystems [4]. In addition, other anthropogenic activities in agriculture, including municipal wastewater irrigation [5] and the use of biosolids [6] and manure [7] in agricultural soils, can contribute to the spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in these soils.

Agricultural intensification, like many anthropogenic activities, impacts negatively on the environment [8]. Examples of these impacts include deforestation, soil degradation and pollution of water and soil. The preventative and therapeutic use of antibiotics in animal farming has been shown to contribute to an increase in AMR, via manure storage and the use of manure solids or wastewater as soil fertilizer [9–11]. The constant use of vast amounts of antibiotics in animal farming, leading to their release into the local environment, together with the increasing prevalence of ARB and ARGs, is a concern. While the use of antibiotics can increase the number of ARB, the relationship between ARB/ARGs, land use practices and antibiotic use in agriculture is poorly understood [12].

AMR is a natural phenomenon that predates the clinical use of antibiotics [13–15]. Although antibiotic resistance genes occur naturally in soils independently of anthropogenic activities [16], research suggests that their abundance in agricultural soils has been increasing since antibiotics were introduced for growth promotion purposes in animal farming [17], making their way into agricultural fields via manure application [16]. Manure application can transfer ARB and
ARGs to soils, causing the expansion of antibiotic resistance reservoirs in comparison with non-manured soils [18–21]. The ARGs that confer resistance to antibiotics are currently considered to be environmental contaminants [22, 23]. Major sources of ARG pollution include animal-derived faeces and manure entering the environment via direct soil application [18, 24–30]. Antibiotics used in farm animals can accumulate in the soil and consequently spread as fertilizer on the farmland at low concentrations [31], but the effects of these low antibiotic concentrations on selection for resistant bacteria in the environment or specific bacterial community responses remain uncertain [29, 32, 33].

Although AMR levels in land used for agricultural purposes have been relatively well studied, not much is known about urban or more natural soil environments. It is important to consider that the increase in AMR in clinics could also be linked to horizontal gene transfer from natural ARG reservoirs from these understudied environments [34]. In order to define the level of anthropogenic impact in different land uses, AMR levels in semi-natural soil environments must be established [35].

Antibiotics commonly used in medicine include chloramphenicol and erythromycin. Although both antibiotics impede bacterial growth by inhibiting protein synthesis, the resistance mechanisms for them differ [36, 37]. Erythromycin is a macrolide, one of the most common antibiotic classes routinely used in both human and veterinary medicine. Erythromycin was the first macrolide antibiotic used clinically to treat human infections [38] and it is still commonly prescribed due to its wide-spectrum activity against Gram-positive and some Gram-negative bacteria [39]. Vancomycin is a last-resort clinical antibiotic that blocks cell wall biosynthesis [40]. Resistance to vancomycin can arise through the modification of cell wall precursors [41, 42]. These three antibiotics are naturally produced by soil bacteria [43] and therefore natural levels of resistance to all three drugs would be expected to be present in soil environments independently of anthropogenic activities.

The objective of this study was to evaluate the levels of bacteria resistant to chloramphenicol, erythromycin and vancomycin present in soils across Great Britain with distinct land uses and to establish whether there is a relationship between resistance level and land use type, particularly regarding human activity. This study also aimed to determine the effect of differing concentrations on one particular antibiotic, erythromycin, on the number of ARB. Erythromycin at subinhibitory and inhibitory concentrations was chosen due to its historical use in veterinary practice and its stability in soil environments.

**METHODS**

**Sampling sites**

Samples were collected from nine sites across Great Britain (Fig. 1). The selected sites represent three distinct land uses: ‘urban’, comprising improved grasslands in public parks embedded within urban areas that have a high daily volume of visitors; ‘agricultural’, comprising improved grasslands in public parks with intensive cattle grazing, recently limed and spread with cow manure, but less human access; and, ‘semi-natural’, comprising mature Scots pine plantation forests in remote areas with little human or captive animal interaction.

**Soil sampling**

Six 10 g soil samples were taken from each of the 9 sites in this study, giving a total of 54 samples. The six sampling points within each site were selected in homogenous habitat areas and away from the edges. Soil was collected from the top 10 cm soil layer by using a small trowel, which was washed with sterile water and 70% ethanol in-between sampling points. Soil samples were immediately placed in 15 ml sterile Falcon tubes labelled with the site name and the GPS location. Samples were kept refrigerated in a cooler bag during sampling and stored at 4°C as soon as possible.

**Testing of soil samples for antibiotic resistance strains**

The antibiotics we selected for study – chloramphenicol, erythromycin and vancomycin – are critically important in human medicine [44], belong to different antimicrobial classes and therefore possess different resistance mechanisms, and cover a wide range of bacterial hosts [45, 46].

One gram from each soil sample collected was used to generate 10^0 to 10^-3 dilutions. Twenty microlitres of each dilution was plated in triplicate onto R2A plates containing nystatin (50 µg ml^-1^) as a control or nystatin (50 µg ml^-1^) plus one of the following antibiotics: chloramphenicol (20 µg ml^-1^), erythromycin (10 µg ml^-1^), erythromycin (20 µg ml^-1^) and...
vancomycin (20 µg ml⁻¹). This process was repeated five times with 1 g of soil from each sampling point.

R2A plates were incubated for 7 days at 30 °C. Nystatin (50 µg ml⁻¹) was added to all plates to prevent fungal growth. The number of ARB c.f.u. ml⁻¹ present was calculated for both control and antibiotic-containing plates (Table S1, available in the online version of this article).

**Statistical analysis**

The percentage of ARB c.f.u. ml⁻¹ on plates containing antibiotics with respect to the total number of c.f.u. ml⁻¹ present in their control plates was measured for each antibiotic and/or concentration of antibiotic. Then, the mean percentage of ARB was calculated using the percentage of ARB c.f.u. ml⁻¹ values obtained for each dilution on each of the five replicates taken per sample. To determine whether land use and antibiotic type influenced the number of culturable antibiotic strains, percentage ARB was tested using generalized linear mixed models (GLMMs) with land use and antibiotic (at 20 µg ml⁻¹ concentration) as fixed factors and site as a random factor. ARB was centred around zero to improve model fit and a Gamma distribution was used for zero bounded data. Where fixed factors in the final model were significant these were explored graphically. Final models were checked to ensure that they met the assumptions of the GLMMs following procedures outlined elsewhere [47].

To determine whether antibiotic concentration influences the number of culturable antibiotic strains, percentage ARB was tested using GLMMs with concentration of erythromycin and land use type as fixed factors, and site as a random factor. Although land use type was not of primary interest here, its inclusion allows us to test the interaction between concentration and land use. ARB was centred around zero, a Gamma distribution was used and models were checked and tested for significance as described above for the GLMMs. All analyses were carried out in the R statistical programme (version 4.0.0) [48]. GLMM used the ‘lmer’ function in the lme4 package [49]. Significance was tested using the ‘Anova’ function in the car package [50].

**RESULTS**

**Levels of ARB in soil depend on land use and resistance differs between tested antibiotics**

Overall, there was a significant difference in the mean percentage of ARB among the land use types (χ² =9.91, df=2, P =<0.007); where there was a higher percentage of ARB in agricultural land compared with semi-natural or urban land uses, which showed similar levels of resistance to each other (Fig. 2a). There was a similar mean percentage of ARB for each antibiotic of ~20% (Fig. 2b), but, when accounting for differences in site location, there was a highly significant difference in the mean percentage of ARB for the antibiotic types (χ² =25.5, df=2, P =<0.00001) (Fig. 2c). Overall, the mean percentage of ARB was lower for erythromycin than for both vancomycin and chloramphenicol, which did not differ from each other, for both agricultural and urban land use. However, the interaction between land use and antibiotic was marginally significant (χ² =9.6, df=4, P =0.05).

In the semi-natural land use the mean percentage of ARB among all three antibiotics did not differ from each other (Fig. 2c). Trends by individual site can be seen in Fig. S1.

**Agricultural land presents high levels of resistance to high erythromycin concentrations**

There was a highly significant difference in the mean percentage of ARB between the concentration levels of erythromycin (χ² =11.2, df=1, P =0.0008), where there were significantly more ARB at 10 µg ml⁻¹ than at 20 µg ml⁻¹ (Fig. 3). Further, there was a highly significant interaction between erythromycin concentration and land use type (χ² =19.4, df=2, P =<0.00001), where the difference in ARB between the concentrations was much smaller for agricultural land use soil, compared to semi-natural and urban land use soil (see Fig. S2).
**DISCUSSION**

In order to gain an insight into the levels of ARB to three antibiotics in different land use soils in Great Britain, we investigated soils with distinct human activities, i.e. agricultural and urban, and compared these to semi-natural soils. The results from this study show that levels of ARB in agricultural land are significantly higher than those found in either urban or semi-natural land use soils across Great Britain. Similar results have been obtained by previous studies comparing levels of ARB and ARGs between forest and agricultural land in different locations worldwide [51–54]. Our study also showed that there is no significant difference in the levels of ARB present in semi-natural and urban areas across the sites sampled. The level of ARB found in semi-natural and urban areas is likely to reflect the level of antibiotic-producing bacteria present in those soil environments. Naturally occurring antibiotic producers will also carry the genes that confer resistance mechanisms to said molecules. The constant presence of low levels of antibiotics and ARGs in these environments is enough to maintain antibiotic resistance levels within those particular soil populations [55–58]. The levels of ARB found in non-agricultural background soils can provide a good baseline of antibiotic resistance levels in a particular location [59, 60] and therefore can help to determine the impact of management practices on antibiotic resistance within an agricultural system more accurately.

---

**Fig. 2.** Mean percentage of antibiotic-resistant bacteria (ARB) by (a) land use type, (b) antibiotic type and (c) the interaction between these main terms for the three antibiotics chloramphenicol (Chl), erythromycin (Ery) and vancomycin (Van) at 20 µg ml⁻¹. Outcomes of generalized liner mixed models are shown in the top left. Significant differences arising from Tukey post-hoc tests are indicated above each treatment by different letters.

**Fig. 3.** Mean percentage of antibiotic-resistant bacteria (ARB) to different erythromycin concentrations (10 µg ml⁻¹ and 20 µg ml⁻¹) among the three land use types, agricultural, semi-natural and urban. Outcomes of generalized linear mixed models are shown in the top left. Significant differences between concentrations within each land use, arising from Tukey post-hoc tests, are indicated above each treatment by different letters.
The significantly higher levels of ARB found across agricultural land in this study are likely to be linked to the use of animal slurry in all farms visited. Several studies to date have shown that the use of animal manure in agricultural soil increases the number of ARGs and therefore ARB [61–64], at least for a transient period after their application [65, 66]. The accumulation of ARB in manured agricultural soil depends on the contribution of ARB originated from the gut of the animals, their horizontal gene transfer to soil bacteria, and the selective pressure of antibiotic residues and other chemical stressors present in the soil [9, 64, 67, 68]. Further, although ARGs responsible for macrolides (erythromycin), amphenicols (chloramphenicol) and glycopeptides (vancomycin) have been extensively reported in other soil studies [52, 61, 69–71], to our knowledge, this is the first study that compares the levels of resistance to these three antibiotics within different land use soils in Great Britain.

Interestingly, our results show that resistance levels to vancomycin and chloramphenicol in both agricultural and urban locations are significantly higher than the levels of erythromycin resistance, whilst in semi-natural locations all three antibiotics show similar resistance levels. Vancomycin is considered to be a last-resort antibiotic, and therefore the presence of high vancomycin resistance levels in both agricultural and urban soils in Great Britain not only demonstrates the highly extensive scope of the soil resistome in those locations, but should also be a cause of concern. Similarly high levels of antibiotic resistance to erythromycin, chloramphenicol and vancomycin have previously been reported for both agricultural and urban soils in different countries [69, 72, 73], whilst other studies report negligible levels of resistance to vancomycin compared to those for erythromycin [74]. It is therefore essential to conduct localized antibiotic resistance analyses with respect to specific land uses in order to determine the levels of resistance for different antibiotics in that particular location. In order to determine why the resistance levels to vancomycin and chloramphenicol are significantly higher in the soils with higher levels of human activity sampled in Great Britain in this particular study, further work should aim to identify whether the antibiotic resistance mechanisms of bacteria isolated from different land use soils are due to the acquisition of specific ARGs or multidrug efflux pumps and to establish correlations between resistance mechanisms, soil composition (particularly regarding the levels of metals present) and human activity.

This study also looked at the effect that using different concentrations of erythromycin (10 µg ml⁻¹ and 20 µg ml⁻¹) had on the number of ARB across the different land use soils. These concentrations were selected based on the lowest minimum inhibitory concentration (MIC) value for erythromycin observed for most bacterial species recorded in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) database [75], ~16 µg ml⁻¹. Therefore 10 µg ml⁻¹ represents a subinhibitory concentration of erythromycin, whilst 20 µg ml⁻¹ represents a concentration above the MIC. The results show that, across the different land use soils, and as we would expect, there are significantly higher levels of bacteria able to grow in the presence of 10 µg ml⁻¹ compared to 20 µg ml⁻¹ of erythromycin. As with other antibiotics, resistance to erythromycin is not confined to a single mechanism. Several mechanisms of erythromycin resistance have been observed, including reduced penetrability of the cell membrane or active efflux of the molecule to decrease its intracellular concentration, ribosome modification or protection, and drug modification via macrolide phosphotransferases and macrolide esterases [76]. The efficient efflux-mediated mechanisms abundant in soil bacteria can be a source of non-specific multidrug resistance [77, 78], which could be responsible for the increased levels of ARB at subinhibitory concentrations of erythromycin.

It is not surprising that we found that the levels of resistance to different erythromycin concentrations were much less marked in agricultural land soil with respect to the other land use soils. Many findings to date have demonstrated that the use of animal manure in soil increases the reservoir of clinically relevant ARGs [61, 62], and in particular, levels of erythromycin-specific ARGs such as ermB are present in high abundance in agricultural soils [52, 69]. While linking an ARG to a specific phenotypic resistance is difficult, especially in the mixed microbial communities common in soil environments, metagenomic analyses to identify the ARGs found in ARB communities able to grow at concentrations of 10 and 20 µg ml⁻¹ of erythromycin, respectively, would allow us to detect whether there are differences in the resistance mechanisms present in each ARB population.

In summary, the present study highlights that agricultural land can act as a main reservoir for resistance to clinically relevant antibiotics in Great Britain, compared to urban or semi-natural locations. Understanding the development and spread of antibiotic resistance levels in different land use soils is important in protecting human, animal and ecological health. In this study, we also found that levels of resistance to different antibiotics are dependent on land use. The geographical location and management practices of different countries are likely to have an effect on these differences. Therefore, it is important to conduct localized studies on levels of resistance to specific antibiotics in order to perform appropriate antibiotic resistance risk assessments. Metagenomic analyses to identify the mechanisms driving levels of resistance to different antibiotics in these soil microbial communities would also increase our limited knowledge on how antimicrobial resistance is spread amongst different land use soils.

Funding information
This work was supported by Biotechnology and Biological Sciences Research Council (BBSRC) grant no. BB/S016651/1.

Acknowledgements
The authors would like to thank Ben Jennings for the semi-natural field samples he collected for this study. We would also like to thank private landowners and the Forestry Commission for allowing us to collect soil samples for this study.
References
1. Collignon P, Beggs JJ, Walsh TR, Gandra S, Laxminarayan R. Anthropological and socioeconomic factors contributing to global antimicrobial use: a univariate and multivariable analysis. Lancet Planet Health 2018;2:e398–e405.
2. Aga D, Davies J, Gandra S, Kasprzyk-Hordern B, Larsson J et al. Initiatives for addressing antimicrobial resistance in the environment: current situation and challenges 2018.
3. Graham D, Giesen M, Bunce J. Strategic approach for prioritising local and regional sanitation interventions for reducing global antibiotic resistance. Water 2018;11:27.
4. Prescott JF. History of antimicrobial usage in agriculture: an overview. Antimicrob Resist Bact Anim Orig American Society of Microbiology 2019:19–28
5. Volkman H, Schwartz T, Bischoff P, Kirchen S, Obst U. Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (TaqMan). J Microbiol Methods 2004;56:277–286.
6. Topp E, Monteiro SC, Beck A, Coelho BB, Boxall ABA et al. Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field. Sci Total Environ 2008;396:52–59.
7. Zhao L, Dong YH, Wang H. Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. Sci Total Environ 2010;408:1069–1075.
8. Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. Nature 2002;418:671–677.
9. Binh CTT, Heuer H, Kaupenjohann M, Smalla K. Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids. FEMS Microbiol Ecol 2008;65:25–37.
10. Negreanu Y, Pasternak Z, Jurkевич E, Cytryn E. Impact of treated wastewater irrigation on antibiotic resistance in agricultural soils. Environ Sci Technol 2012;46:4800–4808.
11. Chantziaras I, Boyen F, Callens B, Dewulf J. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. J Antimicrob Chemother 2014;69:827–834.
12. Williams-Nyugen J, Sallach JB, Bartelt-Hunt S, Boxall AB, Durso LM et al. Antibiotics and antibiotic resistance in Agroecosystems: state of the science. J Environ Qual 2016;45:394–406.
13. Miteva VI, Sheridan PP, Brenchley JE. Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. Appl Environ Microbiol 2004;70:202–213.
14. D’Costa VM, King CE, Kalan L, Morar M, Sung WWL et al. Antibiotic resistance is ancient. Nature 2011;477:457–461.
15. Bhullar K, Waglechner N, Pawlowski A, Koteva K, Banks ED et al. Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One 2012;7:e34953.
16. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74:417–433.
17. Knapp CW, Dolfing J, Ehlers PAI, Graham DW. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environ Sci Technol 2010;44:580–587.
18. Heuer H, Smalla K. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. Environ Microbiol 2007;9:657–666.
19. Cytryn E. The soil resistome: the anthropogenic, the native, and the unknown. Soil Biology and Biochemistry 2013;63:118–23.
20. Amarakoon ID, Surac S, Zvomuya F, Cessna AJ, Larney FJ et al. Dissipation of antimicrobials in a seasonally frozen soil after beef cattle manure application. J Environ Qual 2016;45:1644–1651.
21. McKinney CW, Dungan RS, Moore A, Leytem AB. Occurrence and abundance of antibiotic resistance genes in agricultural soil receiving dairy manure. FEMS Microbiol Ecol 2018;94 [Epub ahead of print 01 03 2018].
22. Sanderson H, Fricker C, Brown RS, Majury A, Liss SN. Antibiotic resistance genes as an emerging environmental contaminant. Environ Rev 2016;24:205–218.
23. Iwu CD, Korsten L, Okoh AI. The incidence of antibiotic resistance within and beyond the agricultural ecosystem: a concern for public health. Microbiol Biotechnol. 2020;9:e10135.
24. Czekalski N, Berthold T, Caucci S, Egli A, Bürgmann H. Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into lake Geneva, Switzerland. Front Microbiol 2012;3:106.
25. Graham DW, Olivesas-Riemont S, Knapp CW, Lima L, Werner D et al. Antibiotic resistance gene abundances associated with waste discharges to the Almendares river near Havana, Cuba. Environ Sci Technol 2011;45:418–424.
26. Heuer H, Focks A, Lamshöft M, Smalla K, Matthies M et al. Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. Soil Biol Biochem 2008;40:1892–1900.
27. Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C et al. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci Total Environ 2013;447:345–360.
28. Zhang Y, Marrs CF, Simon C, Xi C. Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter spp. Sci Total Environ 2009;407:3702–3706.
29. Martínez JL. Effect of antibiotics on bacterial populations: a multi-hierarchical selection process. F1000Res 2017;6:51.
30. Dolliver H, Gupta S. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J Environ Qual 2008;37:1227–1237.
31. Larsson DGG. Antibiotics in the environment. Ups J Med Sci 2014;119:108–112.
32. Bengtsson-Palme J, Larsson DGG. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environ Int 2016;86:140–149.
33. Yim G, de la Cruz F, Spiegelman GB, Davies J. Transcription modulation of Salmonella enterica serovar typhimurium promoters by sub-MIC levels of rifampin. J Bacterial 2006;188:7898–7991.
34. Nesme J, Simonet P. The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. Environ Microbiol 2015;17:913–930.
35. Singer RS, Ward MP, Maldonado G. Can landscape ecology untangle the complexity of antibiotic resistance? Nat Rev Microbiol 2006;4:943–952.
36. Schwarz S, Keihenberger C, Doublot B, Cloeckaert A. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev 2004;28:519–542.
37. Varaldo PE, Montanari MP, Giovanetti E. Genetic elements responsible for erythromycin resistance in streptococci. Antimicrob Agents Chemother 2009;53:353–353.
38. Gaynor M, Mankin A. Macrolide antibiotics: binding site, mechanism of action, resistance. Curr Top Med Chem 2003;3:949–960.
39. Kanfer I, Skinner MF, Walker RB. Analysis of macrolide antibiotics. J Chromatogr A 1998;812:255–286.
40. Ruzin A, Singh G, Severin A, Yang Y, Dushin RG et al. Mechanism of action of the mannopeptimycins, a novel class of glycopeptide antibiotics active against vancomycin-resistant gram-positive bacteria. Antimicrob Agents Chemother 2004;48:728–738.
41. Gold HS. Vancomycin-Resistant enterococci: mechanisms and clinical observations. Clin Infect Dis 2001;33:210–219.
42. Arthur M. Depardieu F, Snaith HA, Reynolds PE, Courvalin P. Contribution of VanY D,D-carboxypeptidase to glycopeptide resistance in Enterococcus faecalis by hydrolysis of peptidoglycan precursors. Antimicrob Agents Chemother 1994;38:1899–1903.
43. Mahajan GB. Antibacterial Agents from Actinomycetes - A Review. Front Biosci 2012;16:240–253.
44. Organization WH. WHO | Critically important antimicrobials for human medicine, 6th revision. World Health Organization; 2019.
45. van Hoek AHAM, Mevius D, Guerra B, Mullany P, Roberts AP et al. Acquired antibiotic resistance genes: an overview. Front Microbiol 2011;2:203.
46. Roberts MC, Schwarz S. Tetracycline and Phenicol resistance genes and mechanisms: importance for agriculture, the environment, and humans. J Environ Qual 2016;45:576–592.
47. Thomas R, Lello J, Medeiros R, Pollard A, Robinson P et al. Data Analysis with R Statistical Software: A Guidebook for Scientists. UK: Eco-explore; 2017.
48. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2020.
49. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw 2015;67.
50. Fox J, Weisberg S. An R companion to applied regression. third. 2019.
51. Chaer G, Fernandes M, Myrold D, Bottomley P. Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. Microb Ecol 2009;58:414–424.
52. Dungan RS, Strausbaugh CA, Leytem AB. Survey of selected antibiotic resistance genes in agricultural and non-agricultural soils in south-central Idaho. FEMS Microbiol Ecol 2019;95 [Epub ahead of print 01 08 2019].
53. Malik A, Khan IF, Aleeem A. Plasmid incidence in bacteria from agricultural and industrial soils. World J Microbiol Biotechnol 2002;18:827–833.
54. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MOA et al. The shared antibiotic resistome of soil bacteria and human pathogens. Science 2012;337:1107–1111.
55. Gulberg E, Cao S, Berg OG, Ilbäck C, Sandgren L et al. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog 2011;7:e1002158.
56. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J et al. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 2010;8:251–259.
57. Alonso A, Sanchez P, Martinez JL. Environmental selection of antibiotic resistance genes. minireview. Environ Microbiol 2001;3:1–9.
58. Martinez JL. Antibiotics and antibiotic resistance genes in natural environments. Science 2008;321:365–367.
59. Durso LM, Wedin DA, Gilley JE, Miller DN, Marx DB. Assessment of selected antibiotic resistance in Ungrazed native Nebraska prairie soils. J Environ Qual 2016;45:454–462.
60. Rothrock MJ, Keen PL, Cook KL, Durso LM, Franklin AM et al. How should we be determining background and baseline antibiotic resistance levels in Agroecosystem research? J Environ Qual 2016;45:420–431.
61. Heuer H, Schmitt H, Smalla K. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr Opin Microbiol 2011;14:236–243.
62. Ruuskanen M, Muurinen J, Meierjohan A, Pärnänen K, Tamminen M et al. Fertilizing with animal manure Disseminates antibiotic resistance genes to the farm environment. J Environ Qual 2016;45:488–493.
63. Sengelov G, Agero Y, Halling-Sørensen B, Baloda SB, Andersen JS et al. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environ Int 2003;28:587–595.
64. Udićov-Kolić 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.
65. Martí R, Tien Y-C, Murray R, Scott A, Sabourin L et al. Safely coupling livestock and crop production systems: how rapidly do antibiotic resistance genes dissipate in soil following a commercial application of swine or dairy manure? Appl Environ Microbiol 2014;80:3258–3265.
66. Liu P, Jia S, He X, Zhang X, Ye L. Different impacts of manure and chemical fertilizers on bacterial community structure and antibiotic resistance genes in arable soils. Chemosphere 2017;188:455–464.
67. Martí R, Scott A, Tien Y-C, Murray R, Sabourin L et al. 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.
68. Xie W-Y, Shen Q, Zhao FJ. Antibiotics and antibiotic resistance from animal manures to soil: a review. Eur J Soil Sci 2018;69:181–195.
69. Yi X, Wang M, Zhou Z. The potential impact of naturally produced antibiotics, environmental factors, and anthropogenic pressure on the occurrence of ERM genes in urban soils. Environ Pollut 2019;245:282–289.
70. Armalytė J, Skerniaškytė J, Bakiene E, Krasauskas R, Šiugždinienė R et al. Microbial diversity and antimicrobial resistance profile in microbiota from soils of conventional and organic farming systems. Front Microbiol 2019;10:892.
71. Zhu Y-G, Johnson TA, Su J-Q, Qiao M, Guo G-X et al. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc Natl Acad Sci U S A 2013;110:3435–3440.
72. Zhao Y, Cocerva T, Cox S, Tardif S, Su J-Q et al. Evidence for co-selection of antibiotic resistance genes and mobile genetic elements in metal polluted urban soils. Sci Total Environ 2019;656:512–520.
73. Baral D, Dvorak BL, Admiraal D, Jia S, Zhang C et al. Tracking the sources of antibiotic resistance genes in an urban stream during wet weather using shotgun metagenomic analyses. Environ Sci Technology 2018;52:9033–9044.
74. Sen S, Sarkar K. Screening for ESBL producing bacterial isolates of agricultural soil and profiling for multidrug resistance. Ann Agrar Sci 2018;16:272–280.
75. European Committee on Antimicrobial Susceptibility Testing. EUCAST: EUCAST 2016. https://www.eucast.org/ (accessed May 4, 2020).
76. Golkar R, Zielinski M, Berghuis AM. Look and outlook on enzyme-mediated macrolide resistance. Front Microbiol 2018;9:9.
77. Dolejska M, Villa L, Poirel L, Nordmann P, Carattoli A. Complete sequencing of an IncHI1 plasmid encoding the carbapenemase NDM-1, the armA 16S RNA methylase and a resistance-nodulation-cell division/multidrug efflux pump. J Antimicrob Chemother 2013;68:34–39.
78. Walsh F, Duffy B. The culturable soil antibiotic resistome: a community of multi-drug resistant bacteria. PLoS One 2013;8:e65567.