International Journal of Undergraduate Research and Creative Activities

Volume 12 | Issue 1 Article 11

October 2023

Presence and abundance of antibiotic resistance genes at an organic goat dairy farm

Janice Marie Parks
park8495@pacificu.edu

Noah Askew
aske9121@pacificu.edu

Jack Woodrow Creagh-Grave
crea0737@pacificu.edu

Gyorgyi Nyerges
nyerges@pacificu.edu

Recommended Citation

Parks, Janice Marie; Askew, Noah; Creagh-Grave, Jack Woodrow; and Nyerges, Gyorgyi (2023) "Presence and abundance of antibiotic resistance genes at an organic goat dairy farm," *International Journal of Undergraduate Research and Creative Activities*: Vol. 12: Iss. 1, Article 11.
DOI: https://doi.org/10.7710/2168-0620.0309
Available at: https://digitalcommons.cwu.edu/ijurca/vol12/iss1/11

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Abstract

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IJURCA: International Journal of Undergraduate Research & Creative Activities

Volume 12 Article 8

August 16, 2020

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Janice M. Parks
Pacific University, park8495@pacificu.edu

Noah Askew
Pacific University, aske9121@pacificu.edu

Jack W. Creagh-Grave
Pacific University, crea0737@pacificu.edu

Gyorgyi Nyerges, PhD
Pacific University, nyerges@pacificu.edu

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How does the use of antibiotics in animal husbandry impact antibiotic resistance genes (ARGs) and integron prevalence? This article examines the prevalence of nine ARGs (*OXA*-2, *tet*B, *tet*G, bla_{CMY-2}, *ere*B, *NDM*, *erm*B, *sul*1, and *sul*2) and two class one integron sequences (*intI*1 and *CS*) at an organic goat dairy farm. To provide insight on how antibiotic use is contributing to the abundance of ARGs and integrons outside of where they are used, we made a comparison between our findings and the prevalence of ARGs and integrons at conventional farms from previously published studies. Samples were collected from an organic goat dairy farm in the Willamette Valley from the goat bedding and goat pellets, as well as non-agriculture soil from the Pacific University campus. DNA extraction, followed by PCR and agarose gel electrophoresis, was used to determine the ARG and integron presence in samples. Quantitative PCR was used to determine the ARG and integron abundance in the samples. Out of nine ARGs and two class 1 integron sequences, we found that four ARGs (*tet*G, *erm*B, *sul*1, and *sul*2) and both class 1 integron sequences (*intI*1 and *CS*) were present in the bacterial populations in our samples. *tet*G, *intI*1, and *sul*1 were chosen for qPCR analysis, and revealed equal or higher concentrations when compared to conventional farms. This study highlights the need to compare various practices to determine their impact on ARG prevalence.

Keywords
Antibiotic resistance, manure, organic goat farm, qPCR

Peer Review

This work has undergone a double-blind review by a minimum of two faculty members from institutions of higher learning from around the world. The faculty reviewers have expertise in disciplines closely related to those represented by this work. If possible, the work was also reviewed by undergraduates in collaboration with the faculty reviewers.

Acknowledgements

We would like to thank the goat dairy farm owners for their generosity for opening up their farm to us for this project. In addition, we would like to thank them for sharing their day to day experience and their wonderful freshly made cheese and freshly baked bread with us. We would also like to thank the Pacific University Dean's Office and Biology department for providing the funds to complete this project.

https://digitalcommons.cwu.edu/ijurca/vol12/iss1/11
DOI: 10.7710/2168-0620.0309

Although antibiotic resistance genes (ARGs) have been harbored by bacteria since as early as the Cambrian period, as antibiotics were produced naturally by fungi and bacteria, their prevalence was relatively low compared to today (Allen et al., 2010). As humans began to use antibiotics to treat bacterial infections that were at one-point life threatening, the prevalence of ARGs subsequently increased (Allen et al., 2010; Cantas et al., 2013). This is due to the high selective pressure that human antibiotic use puts on bacterial populations (Allen et al., 2010). In response to the ongoing increase in ARGs, many of the practices regarding antibiotic use have come into question. Although it has been recognized that many anthropogenically promoted ARGs are introduced into the environment through our waste water, antibiotic usage in animal agriculture has also come under scrutiny (Pallares-Vega et al., 2019).

ARGs are prevalent in the manure of livestock animals and this poses numerous human health risks. It is estimated that up to 70% of the antibiotics used in animal agriculture are used for purposes other than treating infection, and this broad and imprecise use of antibiotics is generally regarded as a driver of the trend of increasing antibiotic resistance (Cantas et al., 2013; Sharma et al., 2018). Due to this, the use of animal manures as a fertilizer for crops is assisting in the spread of ARGs. Based on current studies, there is evidence that the application of commercial organic fertilizers increases the levels of ARGs present in the soil, especially with repeated application (Zhou et al., 2017; Zhang et al., 2019). In addition, studies have found significantly higher abundance of ARGs in the manure and manure-based fertilizer treated soil in which antibiotics are used on animals than in non-agricultural soil (Hurst et al., 2019; Zhou et al., 2017). This is a concern since these genes could easily lead to an increase in antibiotic resistant bacteria (ARB), like MRSA, in the soils crops are being grown in. Since the soil containing these bacteria could contaminate crops, this is a human health risk as this could lead to an outbreak in human populations. ARB could be spread to the surrounding environment of the animal houses not only through manure, but in the form of aerosols serving as a risk for the individuals that work at these facilities (Chen et al., 2019a). Studies have also found that the integron gene *intI*1 is common in manure from conventionally farmed livestock, suggesting some of the bacteria could have multidrug resistance (Zhou et al., 2017; Dungan et al., 2019; Gillings et al., 2014).

Integrons are genetic structures that harbor attachment sites for genes that potentially encode for ARGs (Deng et al., 2015). *IntI*1 is a vital component of integrons responsible for incorporating new ARGs as gene cassettes and thus might serve as a useful proxy for estimating the abundance of ARGs in a bacterial population (Gillings et al., 2014). Determination of the composition of ARGs between the conserved regions of integrons has shown that unique combinations of ARGs can be found, which may lead to the development and transfer of unique patterns of antibiotic resistance via integrons (Levesque et al., 1995). These novel combinations of ARGs could be highly prevalent in soil microbial communities, with the soil being an environment that facilitates horizontal gene transfer (Cantas et al., 2013). Due to horizontal gene transfer, there is not an environment on Earth that we can definitively say has not been impacted by human antibiotic use (Allen et al., 2010).

The use of antibiotics in animal agriculture has spread to rural areas of the world, such as farming communities in Ecuador (Salinas et al., 2019). Unlike the majority of animal agriculture in the United States and China, some of the people who are raising livestock in Ecuador live in close proximity to their animals. These operations are also smaller than those that are commonly seen in the United States and China. As a result, settings such as these are important to help us understand how horizontal gene transfer could contribute to the spread of ARGs between

bacteria isolated from humans and animals. Based on one study that has been done on this system, it was found that ARGs are spread between bacteria isolated from humans and animals as they found shared ARGs (Salinas et al., 2019). Results such as these present a clear picture that the impacts antibiotic use have on the microbiomes of livestock animals are not isolated from the human microbiome.

Although the number of small-scale farms is few in the United States compared to places like Ecuador, we had the unique opportunity of working with one non-conventional organic goat dairy farm. Non-conventional organic farms, like the one we conducted our study at, cannot use antibiotics (USDA, 2019). In cases where they have to use antibiotics for the animal's health, that animal's products cannot be sold as organic based on the USDA's guidelines (USDA, 2019). Interestingly, one study has found that ARG presence and abundance is very similar between conventional and antibiotic free farms, with the exception of a select few ARGs (Hurst et al., 2019). Despite this information, there is a lack of knowledge on the prevalence of ARGs and integrons in organic farm settings. Closing this knowledge gap is important given that we know, from the studies described earlier, that ARGs can be transferred between bacteria in humans and animals, and from animal manure to the soil crops are grown in. In addition, if there are high levels of ARGs in the animal houses like has been seen in conventional farms, as described earlier, then the farm workers in these organic settings could be putting their health at risk. Without these individuals knowing the risks they are taking within these environments, proper safety protocols could not be taking place to protect human and animal health.

Aside from the human and animal health risks, making a comparison between the prevalence of ARGs and integrons at conventional and organic farm settings could give us insight on how antibiotic use is contributing to the abundance of ARGs and integrons outside of where they are used. Therefore, the aim of our study is to determine the prevalence of ARGs and integrons in an organic farm setting, and to make a comparison to published concentrations of ARGs and integrons in conventional farm settings.

Methods

Sample Collection. Samples were collected once at an organic goat dairy farm in the Willamette Valley in September 2019. We collected several goat pellets from within the goat barn and goat bedding (goat feces, biochar, and straw) from the goat barn. Soil was also collected on the same day from outside the Tran Library on the Pacific University campus to serve as a control. All samples were collected using sterile tongue depressors. Samples were put into sterile sampling bags and then stored at 4 °C on the same day as they were collected until DNA extraction.

DNA Extraction. Manufacturer's protocols were followed for the Qiagen DNeasy PowerSoil Kit to extract DNA from the goat pellet, goat bedding, and control soil samples. Optional additional incubation at 4 °C for 5 minutes was performed in steps 7 and 10 of the kit's protocol. A single variation from the kit's protocol was an extension of the vortexing time of the PowerBead tube for the goat pellet and goat bedding DNA extractions from 10 minutes to 25 minutes due to a large amount of plant matter in the samples. We then measured DNA concentration of each DNA sample on a BioTek Take3 Micro-volume plate in a BioTek Synergy HT microplate reader using BioTek Gen 5 software.

PCR and Agarose Gel Electrophoresis. PCR was performed using 12.5 µL Bioline MyTaq™ Red Mix, 0.5 µL of 10 µM forward primer, 0.5 µL of 10 µM reverse primer, 10.5 µL PCR grade water, and 1 µL DNA template ranging from 37.25 ng/µL to 55.75 ng/µL in each reaction. For all the reactions, we had an initial denaturation step at 95 °C. We then ran

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DOI: 10.7710/2168-0620.0309

our denaturation at 95 °C for 30 additional seconds, annealing for 30 seconds, and elongation at 72 °C for 30 seconds. These steps were repeated for 30 cycles when targeting *ere*B, *erm*B, *intI*1, *sul*1, *sul*2, and *bla*$_{CMY-2}$ genes and 35 cycles for the *NDM*, *OXA*-2, *tet*G, and *tet*B genes and the *CS* region. The final elongation for all of our reactions was at 72 °C for 7 minutes. Information on the annealing temperatures and primers used are shown in Table 1S.

Agarose gel electrophoresis was performed in order to visualize the result of the PCR. One percent agarose gels were used with Biotium GelRed and 1xTAE buffer. 8 µL of PCR product were used and 5 µL NEB 100 bp DNA ladder. Gels were run at 100 Volts for about an hour. Images of the gels were taken with the BioRad Gel Doc™ EZ Imager.

PCR Product Purification. PCR products with positive amplification of the expected size for the target gene and no nonspecific amplification underwent PCR purification using the Qiagen QIAquick PCR Purification Kit following the manufacturer's protocols.

For the *CS* amplicons, which have variable expected sizes, and the PCR products with amplification of the expected size for the target genes and nonspecific amplification, the PCR product was run on a 1.5% agarose gel at 100 Volts for 1.5 hours to clearly separate the nonspecific amplification from the band of the correct size. Afterward, selected individual bands were excised from the agarose gel and purified using the Qiagen QIAquick Gel Extraction Kit following the manufacturer's protocols.

Sequencing and Sequence Analysis. Purified PCR product with the corresponding primer were sent to Eurofins for Sanger sequencing following their specifications. Sequences were analyzed using the Qiagen CLC Main Workbench version 8. Portions of our sequences where the chromatogram showed a non-ambiguous sequence were imported into NCBI blastx for identification.

Quantitative PCR. Purified PCR product from the 16S rRNA, *intI*1, *tet*G, *sul*1 genes were diluted (10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2) to specific gene copies/µL for use as standards in the qPCR reaction. DNA templates were diluted to equal concentrations for use in the reaction. We followed the manufacturer's protocols for Applied Biosystems PowerUp SYBR Green to make the reaction. Reactions were run in the Applied Biosystems StepOnePlus Real-Time PCR System. For the 16S rRNA gene, we followed the Applied Biosystems PowerUp SYBR Green standard cycling mode for primers with melting temperatures less than 60 °C and used an annealing temperature of 58 °C. For the *tet*G gene, we followed the standard cycling mode instructions for primers with melting temperatures greater than 60 °C. For the *sul*1 gene, we followed the same protocol that we used for *tet*G except for during stage two where we denatured at 95 °C for 20 seconds, annealed at 58 °C for 20 seconds, and elongated at 72 °C for 20 seconds. To ensure there were not multiple products, we ran a melt curve following the Applied Biosystems PowerUp SYBR Green protocol. For *intI*1 we used the qPCR protocol described in Zhang et al., 2016, but with an annealing temperature of 60 °C. We generated our graphs and performed statistical tests using R version 3.6.1 in RStudio. We performed a one-way ANOVA on our data before performing the Tukey Test.

Results

Out of the nine ARGs and two class 1 integron sequences that we focused on, we found that three of the ARGs (*tet*G, *erm*B, *sul*1, and *sul*2) and both class 1 integron sequences (*intI*1 and *CS*) were present in the bacterial populations in our samples (Table 1). We confirmed these results by comparing the sequences to the NCBI database using blastx (Table 2S). Despite the presence of a band of the correct size, the identity of *tet*B in the control soil was not confirmed with sequencing therefore, we concluded that *tet*B was not present in this

sample (Table 1). Although there were bands that were potentially the correct size among the nonspecific amplification for *OXA*-2 and *NDM,* we did not generate sequences for these samples as the DNA concentration in our purified PCR product were too low. Additionally, it was unclear in our gel images if the bands we were evaluating were the correct size due to the high level of nonspecific amplification. We did not generate a sequence for *erm*B in the goat pellet sample as we did not initially count this sample as being positive due to how faint the band appeared in our gel image. As a result, we do not have conclusive evidence for the presence of *erm*B in the goat pellet.

Table 1. Presence/absence information for the tested ARGs for all three DNA samples.

ARG	Goat Pellet	Goat Bedding	Control Soil
*intI*1	+[¢]	+	-
OXA-2	NS[δ]	NS	-
*tet*B	-	-	+[*]
*tet*G	NS[†]	+	-
bla_{CMY-2}	-	-	-
*ere*B	-	-	-
CS	+	+	-
NDM	NS	-	-
*erm*B	+	+[¤]	-
*sul*1	+	+	-
*sul*2	-	+	-

[¢] (+) indicates that a PCR product of the appropriate size was visualized using agarose gel electrophoresis and (-) indicates that no PCR products were visualized using agarose gel electrophoresis

[δ] NS indicates that nonspecific amplification was visualized using agarose gel electrophoresis regardless if there was a PCR product of the appropriate size.

[*] Identity was not confirmed by sequencing.

[†] Identity was confirmed by sequencing.

[¤] A sequence was not produced for this sample leaving it unconfirmed.

The genes *tet*G, *intI*1, and *sul*1 were chosen for qPCR analysis because the primer pairs we were using produced shorter product lengths which work well with this technique. Furthermore, class 1 integrons such as *intI*1 can be an indicator for the presence of multidrug resistant bacteria, and antibiotics related to *sul*1 and *tet*G are commonly used in veterinary medicine (Qiang and Adams, 2004; Gillings et al., 2014). Although we intended to perform qPCR on *sul*2 as well, we were unable to complete this analysis due to the SARS-CoV-2 pandemic. The values we received for our control sample were below our detection limits for all three genes.

We calculated that the absolute abundance of *tet*G in 1 g of sample for the goat pellet to be $2.60 * 10^6$ copies/g and $3.30 * 10^7$ copies/g sample for the goat bedding (Figure 1). We also found that 0.1% of bacteria in the population from the goat pellet and 3.07% of bacteria in the population from the goat bedding had the *tet*G gene (Figure 2).

We calculated that the absolute abundance of *intI*1 in 1 g of sample for the goat pellet to be $7.16 * 10^6$ copies/g and $1.90 * 10^7$ copies/g sample for the goat bedding (Figure 1). We also found that 0.233% of bacteria in the population from the goat pellet had the *intI*1 integron and 1.75% of bacteria in the population from the goat bedding had the *intI*1 integron (Figure 2). For *sul*1, we calculated an absolute abundance in 1 g of sample for the goat pellet to be $1.42 * 10^8$ copies/g and $8.06 * 10^8$ copies/g for the goat bedding (Figure 1). When we calculated the relative abundance of the *sul*1 gene we found that 3.28% of the bacteria in the population from the goat pellet and 81.3% of the bacteria from the goat bedding had this gene.

We determined that the absolute abundance in the goat bedding is significantly higher for all three genes: *tet*G (Welch two sample t-test, n = 3, $P = 0.001$), *intI*1 (Welch two sample t-test, n = 3, $P = 0.005$), and *sul*1 (Welch two sample t-test, n = 3, $P = 0.0003$), than in the goat pellet, with n representing each replicate used per sample in qPCR (Figure 1).

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DOI: 10.7710/2168-0620.0309

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Figure 1. Absolute abundance of *tet*G, *intI*1, and *sul*1 in goat pellet and goat bedding samples. Quantification of each gene was performed using qPCR, then we calculated absolute gene copies per gram of sample. Abundance of these genes in the control soil were not calculated because the absolute abundance was below our detection limit. The vertical line at the top of each bar represents the standard error within our data. Different letters indicate significant differences ($P < 0.05$). Bars that are assigned the same letter are not significantly different ($P \geq 0.05$).

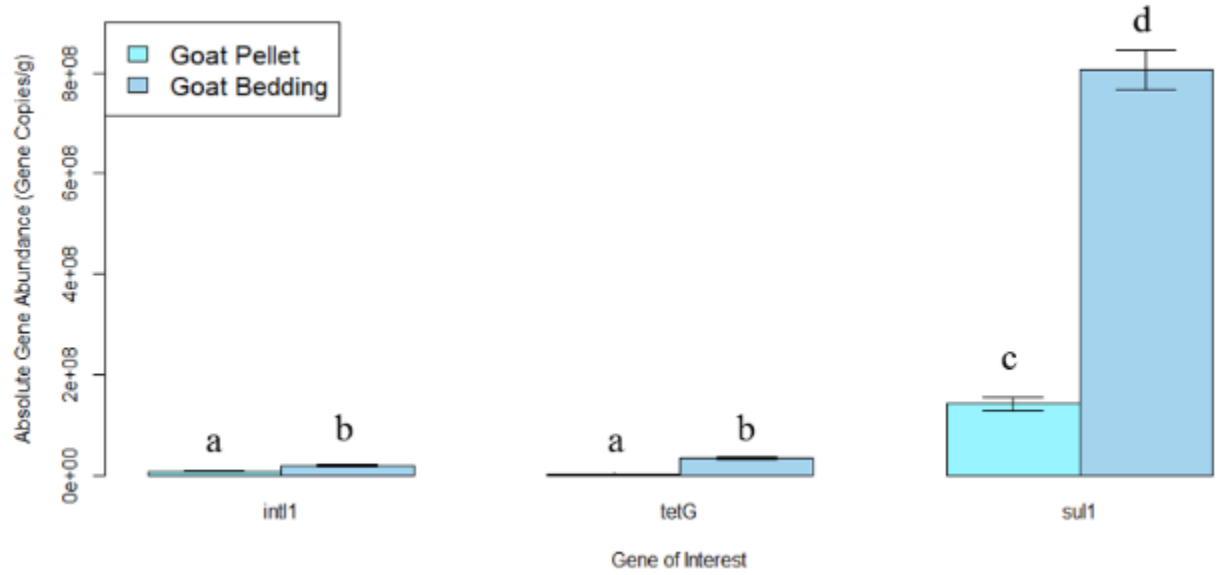
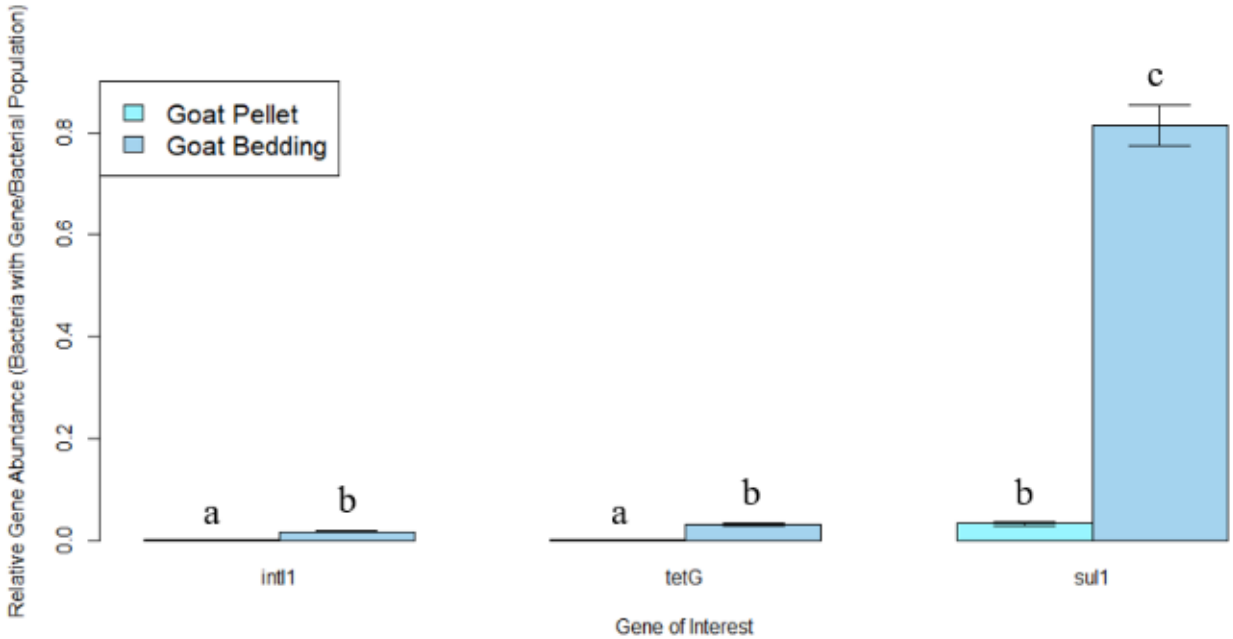


Figure 2. Relative abundance of *intI*1, *tet*G, and *sul*1 in goat pellet and goat bedding samples. Quantification of each gene was performed using qPCR, then we calculated the mean gene copies in each sample over the mean copies of 16S rRNA gene divided by four, to account for each bacterium containing on average four copies of the 16S gene, to determine the ratio of bacteria carrying the ARG to the total bacterial population of each sample. The relative abundance values for the control soil samples were not calculated because the absolute copy numbers were below the detection limit. The vertical line at the top of each bar represents the standard error in our data. Different letters indicate significant differences ($P < 0.05$). Bars that are assigned the same letter are not significantly different ($P \geq 0.05$).



We also found that the relative abundance in the goat bedding is significantly higher for all three genes: *tet*G (Welch two sample t-test, n = 3, $P = 0.001$), *intI*1 (Welch two sample t-test, n = 3, $P = 0.004$), and *sul*1 (Welch two sample t-test, n = 3, $P = 0.0008$), than in the goat pellet, with n representing each replicate used per sample in qPCR (Figure 2). There was significantly more *sul*1 than *tet*G and *intI*1 in both the goat bedding and the goat pellet for both the absolute and relative gene abundance (Tukey Test, $P \leq 0.0005$). No significant difference was detected between *tet*G and *intI*1 in both samples for both the absolute and relative gene abundance (Tukey Test, $P \geq 0.05$) (Figures 1 and 2). Significantly more ARGs were detected in the goat bedding for the relative gene abundance than in the goat pellet (Mann Whitney U Test, n1 = 9, n2 = 9, $P = 0.02$). No significant difference was found in the absolute abundance of ARGs between the goat pellet and the goat bedding (Mann Whitney U Test, n1 = 9, n2 = 9, $P = 0.09$).

Discussion

In our study, we measured the presence and abundance of multiple ARGs in the pellets and bedding at an organic goat dairy farm. Compared to the non-agricultural soil control, there was a greater occurrence of ARGs in the organic goat dairy farm samples (Table 1). This was expected based on results from a study in Ecuador (Salinas et al., 2019). ARG prevalence can occur in non-agricultural soils such as pastureland, residential, recreational, and forest soils though at lower levels than cropland (Dungan et al., 2019). Soils with manure applied from cattle not treated with antibiotics were shown to have significantly higher levels of ARGs than untreated soils (Hu et al., 2016; McKinney et al., 2018). Livestock gut microbiota can be easily transferred to the soil through excrement, and although animals in our study were not treated with antibiotics, it is still possible that ARG's exist within their gut microbiota. qPCR analysis found that the absolute and relative abundance of *intI*1, *sul*1, and *tet*G was significantly higher in the goat bedding than the goat pellet (Figures 1, 2). The difference in relative abundance between the goat pellet and bedding was 7.51 fold for *intI*1, 24.8 fold for *sul*1, and 30.7 fold for *tet*G. While the extent of the difference was surprisingly large, it has been shown that repeated application of organic manure to soil over time leads to accumulation of ARGs (Zhou et al., 2017). Bacteria likely can flourish in goat bedding, which is also an environment ripe for horizontal gene transfer (Cantas et al., 2013). Since bedding is comprised mostly of pellets and straw, it makes sense that ARGs would accumulate over time despite the fact that the bedding is removed and refreshed regularly. We found that the *sul*1 gene was present in a much higher number in both the goat pellet and the bedding compared to the other two genes tested. We seriously considered the possibility of contamination in our qPCR reaction, however, after reexamining all our results, we concluded that our finding is most likely correct and there must be another explanation. From discussion with the farm, we learned that they did need to resort to using antibiotics to treat one of their goats that had an infected wound. The goat was retired from the farm shortly after treatment and was moved off the farm. This occurrence could have had an impact on the abundance of *sul*1 despite the short time frame. Unfortunately, we were not provided with information on the type of antibiotic the goat was treated with, so we cannot conclude anything further. Another possibility is that there is some form of co-selection occurring in the bacterial population that is causing a high prevalence of *sul*1. For instance, the *sul*1 ARG could be linked to another gene in the bacterial genome that is being selected for, thus resulting in a higher prevalence of *sul*1 as well.

We compared the abundance of *intI*1, *sul*1 and *tet*G in the goat bedding and goat pellet samples with the literature, expecting our results would have a lower prevalence of ARGs compared to reported quantities of ARGs in conventional farms, also known as

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DOI: 10.7710/2168-0620.0309

concentrated animal feeding operations (CAFOs). Instead, we found that our results were on the same order of magnitude of 10^{-3} gene copies per 16S rRNA gene for *intI*1 and *tetG* in pellets, and a greater order of magnitude for *sul*1 (Lopatto et al., 2019; Cheng et al., 2013). Since our samples were not actively influenced by antibiotics, but have comparable values to many studies that did use soil influenced by antibiotics, this raises the question of whether antibiotic usage promotes ARGs in microbial populations. Numerous studies point to a correlation between antibiotic use in animals and ARGs in their manure (Bibbal et al., 2007; Heuer and Smalla, 2007; Peak et al., 2007; Hölzel et al., 2010). Evidence also exists that shows manure from animals not treated with antibiotics increased ARG prevalence in soil, although this effect is transient without antibiotics (Heuer and Smalla, 2007; Dungan et al., 2019). Alternatively, ARG presence in the soil may be due to small residual amounts of antibiotic in the soil. For instance, sulfonamide concentrations as low as 0.1 mg/kg of soil can impact soil bacteria populations (Heuer et al., 2008). ARGs can persist in the environment despite a lack of promotional selection (Martinez, 2009). Thus, it is possible that previous owners of the land used antibiotics, and the resulting ARGs have persisted. Therefore, the persisting ARGs could be impacting our results, however, the lands history is unclear. Alternatively, ARGs can be highly mobile in manure used as fertilizer, in wastewater, and even aerosols making it a possibility that the ARGs we detected are due to outside influences (Chen et al., 2019a; Hamscher et al., 2003: Martinez, 2009).

Future studies on the abundance of ARGs in organic farms would benefit from a direct comparison with CAFO farms. Understanding the role of subtherapeutic antibiotic usage on livestock compared to zero antibiotic usage is essential for evaluating the threat posed by antibiotics. In addition, although the bacteria found in livestock may not be pathogenic, they provide a reservoir of ARGs that could be horizontally transferred to pathogenic bacteria posing a human and livestock health risk. Finally, it may be important to determine the extent to which ARGs are present on produce grown from manure-treated soils, in which the manure comes from animals not treated with antibiotics. This is because one study showed that ARGs are more abundant in various parts of plants grown in manure-treated soil than those grown in soil where manure has not been applied (Zhang et al., 2019). If the ARG containing bacteria found on these plants are pathogenic, it is important to discern the difference in the risk between using antibiotic influenced manure and non-antibiotic influenced manure on crops.

The presence, accumulation, and spread of ARGs in agricultural soils continues to present a worldwide health issue, especially as more light is shed on how antibiotic use in food animals can lead to increases in the occurrence and abundance of these ARGs in the soil through the application of manure. There has been much less research to date, however, on the occurrence and abundance of ARGs in manure and manure-treated soils. Increased understanding of this topic with comparison to CAFOs and non-agricultural soils will help us to better understand what risks ARGs may present even in antimicrobial-free settings, and how they may be mitigated. Additionally, there is increasing concern about the mechanisms of transfer of ARGs within bacterial communities, the development of multidrug resistance, and the possible implications of these factors for human health. Further study of class 1 integron presence in antimicrobial-free settings will help determine how ARGs may be disseminated in areas where antibiotics are not used.

In conclusion, there are ARGs conferring resistance to common dairy farming antibiotics in the bedding of an organic goat dairy farm where antibiotics are not used on working livestock. In addition, the presence of *intI*1 in the manure and bedding at an organic goat dairy farm indicates that the development of novel patterns of antibiotic resistance is

possible even in agricultural settings where antibiotics are not used. Overall, these data suggest that further research on the occurrence and abundance of ARGs in antimicrobial free agricultural settings will be beneficial to the wider understanding of the dissemination of antibiotic resistance and risks to human health.

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Appendix: Supplemental Tables

Table 1S. Primers used to amplify gene products for ARGs and the 16S rRNA gene with associated sequences, annealing temperatures, and expected sizes of target gene products. When we used annealing temperatures different from the reference for a particular primer, the difference is noted.

ARG	5' - 3' primers	Annealing Temperature Used	Expected Size	Reference
*intI*1	HS463a: CTGGATTTCGATCACGGCACG HS464: ACATGCGTGTAAATCATCGTCG	60 ℃	473 bp	Zhang et al., 2019
OXA-2	*blaOXA-2* F: GCCAAAGGCACGATAGTTGT *blaOXA-2* R: GCGTCCGAGTTGACTGCCGG	57 ℃	701 bp	Anand et al., 2016
*tet*B	*tetB* F: GAGACGCAATCG AATTCGG *tetB* R: TTTAGTGGCTAT TCTTCCTGCC	48 ℃	224 bp	Anand et al., 2016
*tet*G	TetG-FW: GCAGAGCAGGTCGCTGG TetG-RV: CCYGCAAGAGAAGCCAGAAG	64 ℃	134 bp	Zhang et al., 2019
bla_{CMY-2}	CMY-2-A: ATGATGAAAAAATCGTTATGC CMY-2-B: TTATTGCAGCTTTTCAAGAATGC	55 ℃	Under 1145 bp	Mammeri et al., 2010
*ere*B	F: AGAAATGGAGGTTCATACTTACCA R: CATATAATCATCACCAATGGCA	52 ℃	546 bp	Sutcliffe et al., 1996
CS	3-CS: AAGCAGACTTGACCTGA 5-CS: GGCATCCAAGCAGCAAG	57 ℃	Variable length	Levesque et al., 1995
NDM	NDM-Fm: GGTTTGGCGATCTGGTTTTC NDM-Rm: CGGAATGGCTCATCACGATC	57 ℃	264 bp	Fomda et al., 2014
ermB	ermBFw: TGGAACAGGTAAAGGGCATT ermBRev: GCGTGTTTCATTGCTTGATG	52 ℃	Greater than 364 bp	Chen et al., 2019b
16S rDNA	1369 Fw: CGG TGA ATA CGT TCY CGG 1492 Rev: GGT TAC CTT GTT ACG ACT T	55 ℃	123 bp	Suzuki et al., 2000

Table 2S. Identity of purified PCR products from Sanger sequencing.

Sample Identity	Amplicon Size	Chromatogram Size	Quality	Identity
*intI*1-1	473 bp	355 bp	+*	Integrase [Klebsiella sp. A5] (100%)
*intI*1-2	473 bp	325 bp	+	Integron integrase [Pseudomonas aeruginosa] (100%)
*erm*B	~550 bp	436 bp	+	23S ribosomal RNA methyltransferase Erm, partial [Enterococcus faecalis] (100%)
*tet*B†	224 bp	149 bp	+	Hypothetical protein (45.65%)
*tet*G-1	134 bp	91 bp	+	MFS transporter, partial [Pseudomonas aeruginosa] (82.61%)
*tet*G-2	134 bp	80 bp	+/-	MFS transporter [Pseudomonas aeruginosa] (81.82%)
*CS*1-S	~150 bp	117 bp	+	Streptomycin 3"-O-adenlylyltransferase [Klebsiella oxytoca] (100%)
*CS*2-S	~150 bp	100 bp	+	Aminoglycoside resistance protein, partial [Klebsiella pneumoniae subsp. Pneumoniae KPNIH2] (100%)
*CS*1-SM	~290 bp	239 bp	+	Glycerol kinase [Bacteroides sp. CAG:545] (90.54%)
*CS*1-M	~500 bp	104 bp	+/-	ANT(3")-Ia family aminoglycoside nucleotidyltransferase AadA9 [uncultured bacterium] (94.12%)
*CS*2-M	~500 bp	106 bp	+/-	Aminoglycoside resistance protein, partial [Klebsiella pneumoniae subsp. Pneumoniae KPNIH2] (79.41%)
*CS*1-ML	~650 bp	541 bp	+	MULTISPECIES: class 1 SAM-dependent DNA methyltransferase [Ruminococcus] (73.45%)
*CS*1-B	~1000 bp	595 bp	+/-	ANT(3")-Ia family aminoglycoside nucleotidyltransferase AadA9 [Glutamicibacter arilaitensis] (89.77%)
*CS*2-B	~1000 bp	620 bp	+	Aminoglycoside 3'-adenlylyl transferase, partial [Escherichia coli] (95.26%)
*sul*1-P	137 bp	104 bp	+	Dihydropteroate synthase [Klebsiella pneumoniae] (100.00%)
*sul*1-B	132 bp	99 bp	+	Dihydropteroate synthase [Klebsiella pneumoniae] (100.00%)
*sul*2-B	161 bp	108 bp	+	Sulfonamide-resistant dihydropteroate synthase Sul2 [Shigella flexneri] (100.00%)

* + indicates that a high-quality sequence with little to no noise in the chromatogram was used to generate our data and +/- indicates some noise was present in the chromatogram.

† All sequences with the exception of *tet*B were identified as the expected product using blastx.

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DOI: 10.7710/2168-0620.0309