

PROFILE OF ANTIMICROBIAL RESISTANCE GENES ACROSS SWINE WASTE MANAGEMENT

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BACKGROUND

In Brazil, manure management and treatment are performed using two types of system: waste stabilization ponds (WSPs) and covered lagoon biodigesters (CLBs). The digested manure from both systems is used as organic fertilizer. Soil fertilization processes are widely used; however, the maintenance and dissemination of antimicrobial resistance genes (ARGs) in this sustainable and important system have not been investigated. Therefore, this study analyzed the persistence of ARGs in soils fertilized with digested manure and after swine manure management.

MATERIALS AND METHODS

Manure and soil samples were collected from 43 Brazilian swine farms, being 20 farms that use WSP system located in South Brazil, and 23 farms that use CLB system located in Midwest and Southeast Brazil. For each farm, manure samples were collected from swine waste treatment entry, that is the raw swine manure and outlet point, that is the digested manure. Also, soil samples from non-organic fertilized (NFS) and organic fertilized (FS) areas were sampled in each farm.

From all the samples metagenomic DNA was isolated and sequenced by shotgun sequencing in an Illumina NovaSeq 6000 platform (Illumina), 2 × 150 bp, with 30 GB/sample depth. Resistome analysis was performed by the Resistance Gene Identifier on the Comprehensive Antibiotic Resistance Database followed by statistical analyses (Kwatra, 2021; Alcock et al., 2023)).

The ARGs' diversity rates were assessed by Shannon index ($p < 0.05$), PERMANOVA ($p < 0.05$), and PCoA using Bray Curtis metrics. Statistical comparisons were performed between management systems and comparing waste and soil collection points. In addition, the relative abundance of ARGs' antimicrobial classes across swine waste treatments was determined. Differential abundance of antimicrobial classes was assessed by analysis of compositions of microbiomes and statistical comparisons among manure and soil samples in each treatment, were assessed by microeco (Liu et al., 2021).

RESULTS

In total, 19,798 ARGs were found among the samples, which include 492 different genes from 37 different antimicrobial drug classes.

From all the sampled farms, the identified ARGs encoded antimicrobial resistance to many antimicrobial classes. The most common classes were aminoglycosides, multidrug resistance (MDR), tetracyclines and macrolides. As consequence, the most part of resistance mechanisms were related to antibiotic target alteration and antibiotic inactivation.

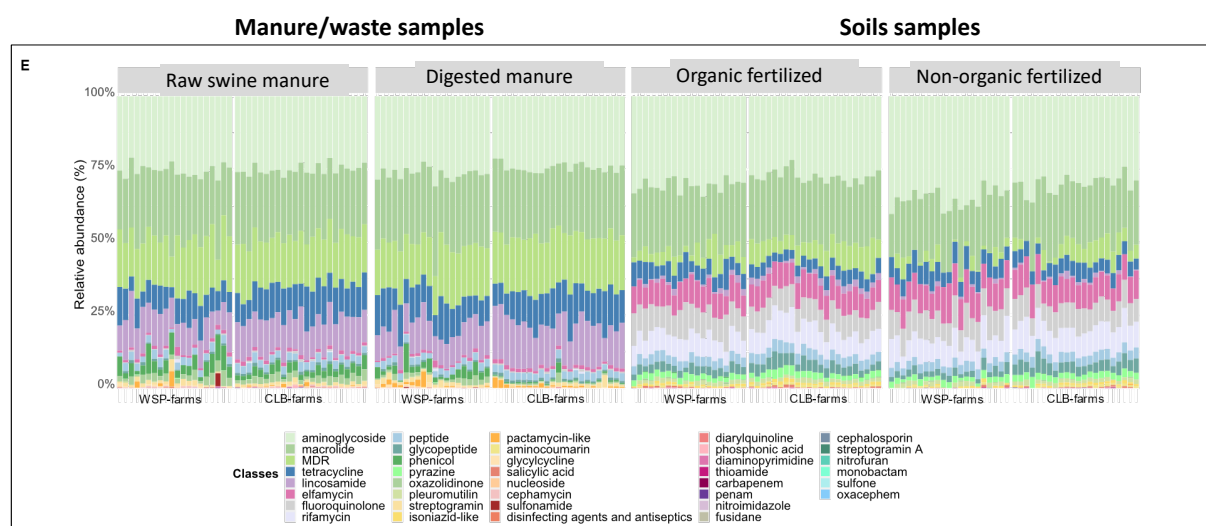
When we analyzed ARGs diversity rates, we can see that ARG diversity is related to the kind of sample. Manure samples diversity were very similar among them, similarly to soils samples, which diversity rates were also very similar. This observation was independently of the management system (CLB or WSP).

Additionally, the statical results indicate that the use of either CLB or WSP did not influence ARGs composition in both manure and soils sample.

The relative abundance analysis showed little abundance variation between the kind of samples (Figure 1). The manure ARGs abundance was maintained during the digestion process, with high similar abundance when raw manure and digested samples were compared. Also, this similarity was founded in both management systems.

Furthermore, as can see in Figure 1, relative abundance of the ARGs found in soils were very similar across all samples, independently of both the manure application as fertilizer and the farm-system.

Figure 1. Relative abundance for the classes from the identified antimicrobial resistance genes



To determine the role of ARGs in the antimicrobial spread differential abundance analysis was performed. The results showed some ARGs that were significantly more abundant in digested manure, from both systems, such as the coding resistance to, aminoglycosides, macrolides and tetracyclines. Interestingly, these drug classes are extensively used; therefore, molecules accumulation in the lagoons can be a selective source for resistant bacteria multiplication. On the Other hand, there were classes that were reduced after manure management. Finally, in the soil's samples, also, there were classes that were differentially present in either fertilized soils or non-fertilized ones.

The probable implication of ARGs persistence in soils because manure land application was investigated. We identified 36 ARGs in fertilized soils that were shared exclusively with digested manure (Figure 2). Interestingly, six of them encoded to MDR, while other ones encoded to high-priority antimicrobial resistance classes, such as carbapenems and vancomycin. Noteworthy, these resistance classes genes were observed only in fertilized soils from WSP farms.

Figure 2. Antimicrobial resistance genes in soils originating from digested swine manure

Gene	Antimicrobial class	CLB-farms	WSP-farms
tet family	tetracycline	X	X
Erm family	macrolide; lincosamide; streptogramin; streptogramin A; streptogramin B		X
mel	macrolide; streptogramin		X
OXA-347	carbapenem; cephalosporin; penam		X
aadA9	aminoglycoside	X	X
ANT(6) family	aminoglycoside	X	X
cmx	phenicol	X	X
vanG	glycopeptide		X
vanW_in_vanG_cl	glycopeptide		X
Efac_EFTu_GE2A	elfamycin		X
mphG	macrolide	X	X
mef(C)	macrolide; tetracycline	X	
Bhyo_23S_TYL	macrolide	X	
Ecol_23S_CHL	phenicol		X
Ecol_23S_CLR	macrolide	X	
Mhom_23S_MAC	macrolide		X
Mfer_23S_MAC	macrolide		X
Ccol_ACT_CHL	phenicol		X
LnuH	lincosamide	X	
LnuP	lincosamide	X	
aadA27	aminoglycoside		X
qacEdelta1	disinfecting agents and antiseptics	X	X
cfrE	lincosamide; streptogramin; oxazolidinone; phenicol; pleuromutilin	X	X
cmlA9	phenicol	X	X
qacG	disinfecting agents and antiseptics		X
EstT	macrolide; glycyclcycline; tetracycline		X

DISCUSSION and CONCLUSION

The results from this study highlight that ARGs can persist in the environment after manure treatment. Moreover, neither of the analyzed swine manure management systems were completely effective in removing ARGs. However, our findings indicate that CLB system provides a more robust ARGs removal in comparison to the WSPs. Previous research determined that although livestock manure application on agricultural grassland alters its microbiome and resistome by increasing bacterial diversity and ARGs abundance, the overall impacts were short term (Tyrrell et al., 2023).

Swine manure is a source of antimicrobial residues, which can enhance the selective pressure for antimicrobial resistance by the dissemination of ARGs. Understand the dynamic of ARGs throughout manure treatment processes is essential to determine practical actions to minimize antimicrobial resistance spread. Nevertheless, our results indicate that ARGs composition in soils are not influenced by the use of digested manure as organic fertilization source.

Altogether, the persistence of ARGs in environment implies the need of further improvements in swine manure management systems focusing on genetic material.

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