

# The swine waste Resistome: occurrence of ARGs in the associated microbial communities

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## Background and Aim

Due to the huge intensive livestock farming, **veterinary antimicrobials** have been used routinely to preserve animal health and productivity. However, this **overuse** resulted in the **development and subsequent transmission of antibiotic resistant bacteria and antibiotic resistance genes (ARGs)** between animals and from animals to humans. The concentration of antibiotics in agricultural-related environments exerts **selective pressure** on bacteria, playing a role in the acquisition of ARGs, which can spread in the environment through **Horizontal Gene Transfer (HGT) mechanisms**. HGT exploits **mobile genetic elements**, such as transposons, integrons and plasmids. In particular, **integron class 1** is the most frequently identified as responsible for spreading antibiotic resistance determinants amongst commensals and pathogens of humans and domesticated animals. However, despite the large number of research covering this topic, little has been done to optimize the ARGs' detection and traceability on livestock-related matrices.

This study aims to evaluate **presence and abundance of the most diffuse ARGs and integron class 1 (*int1*)** in samples from 3 livestock farms, finding a possible association among ARGs, HGT and chemical characterization of farms' liquid manure.

## Highlights

- Chemical characterization of manure's samples along the supply chain of three swine' farms.

- Isolation of *Escherichia coli* strains from pigs' manure samples and characterization of antibiotic resistance phenotypes (Vitek, Biomereux) for the most common human and veterinary antibiotics.

- Presence evaluation of **10 most common ARGs and of both clinical and environmental *int1* gene** in the characterized *E. coli* strains.

- Quantification of the **6 detected ARGs and *int1*** in farms' manure samples.

## Material

Table 1. Pig manure sampling in farms, which have different management plant.

Farm A		
MW.A	Manure	Weaning stall
MG.A	Manure	Growing stall
MF.A	Manure	Fattening stall
M.A	Manure	Treatment plant inlet
LP.A	Liquid phase	Liquid phase tank
SP.A	Solid phase	Solid phase collecting area
STOCDep.A	after treatment	Treatment plant tank
Farm B		
MW.B	Manure	Weaning stall
MF.B	Manure	Fattening stall
DIG.B	M_F_B digested in biogas plant	Outlet biogas plant
LP.B	Liquid phase from R_DIG	Liquid phase tank
LS.B	Solid phase from R_DIG	Solid phase collecting area
STOC.B	Stock Manure	Storage tank
Farm C		
MW1.C	Manure	Weaning stall
MW2.C	Manure	Weaning stall
MF1.C	Manure	Fattening stall
MF2.C	Manure	Sow stall
STOC.C	Stock Manure	Storage tank

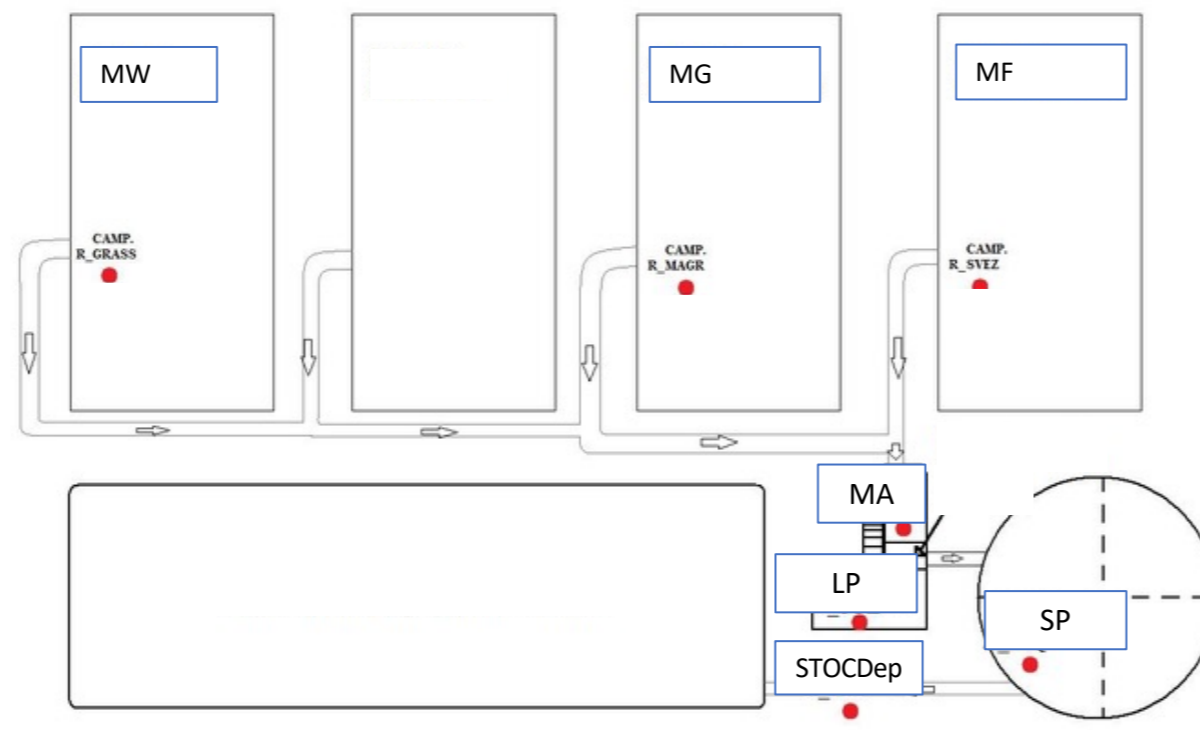


Figure 1. Example of pigs' waste management plant scheme (Farm A).

## Main Results

Table 2. Presence of the 10 most common ARGs and of *int1* gene in *E. coli* strains isolated from slurry samples of the three livestock farms.

Farm	strain	β-lactams		quinolones		macrolides		phenicols			tetracyclines	<i>int1</i>	Clinical <i>int1</i>
		<i>blaTEM</i>	<i>qnrS</i>	<i>ermB</i>	<i>ermA</i>	<i>floR</i>	<i>cmlA</i>	<i>fexB</i>	<i>fexA</i>	<i>tetA</i>			
A	STL 1/1	p										P	P
A	STL 1/2			p								P	P
A	STL 1/3	p										P	P
A	STL 1/4	p				p		p				P	P
A	STL 1/5							p				P	P
A	STL 1/7	p						p				P	
A	STL 1/8	p						p				P	
A	STL 1/9	p						p				P	
A	STL 2/1			p				p				P	
A	STL 2/2	p						p				P	P
A	STL 2/5	p						p				P	P
B	A1							p				P	P
C	AB/1	p	p	p		p		p				P	P
C	AB/2	p	p	p		p		p				P	P

p=present

In *E. coli* strains, *tetA*, *ermB*, *cmlA*, *floR*, *qnrS* and *blaTEM*, as well as *int1* gene, both clinical and environmental type, were detected



## Abundance of the same genes in 18 farms' manure samples

### Characterization of samples according to chemico-physical features and ARGs/*int1* gene distribution

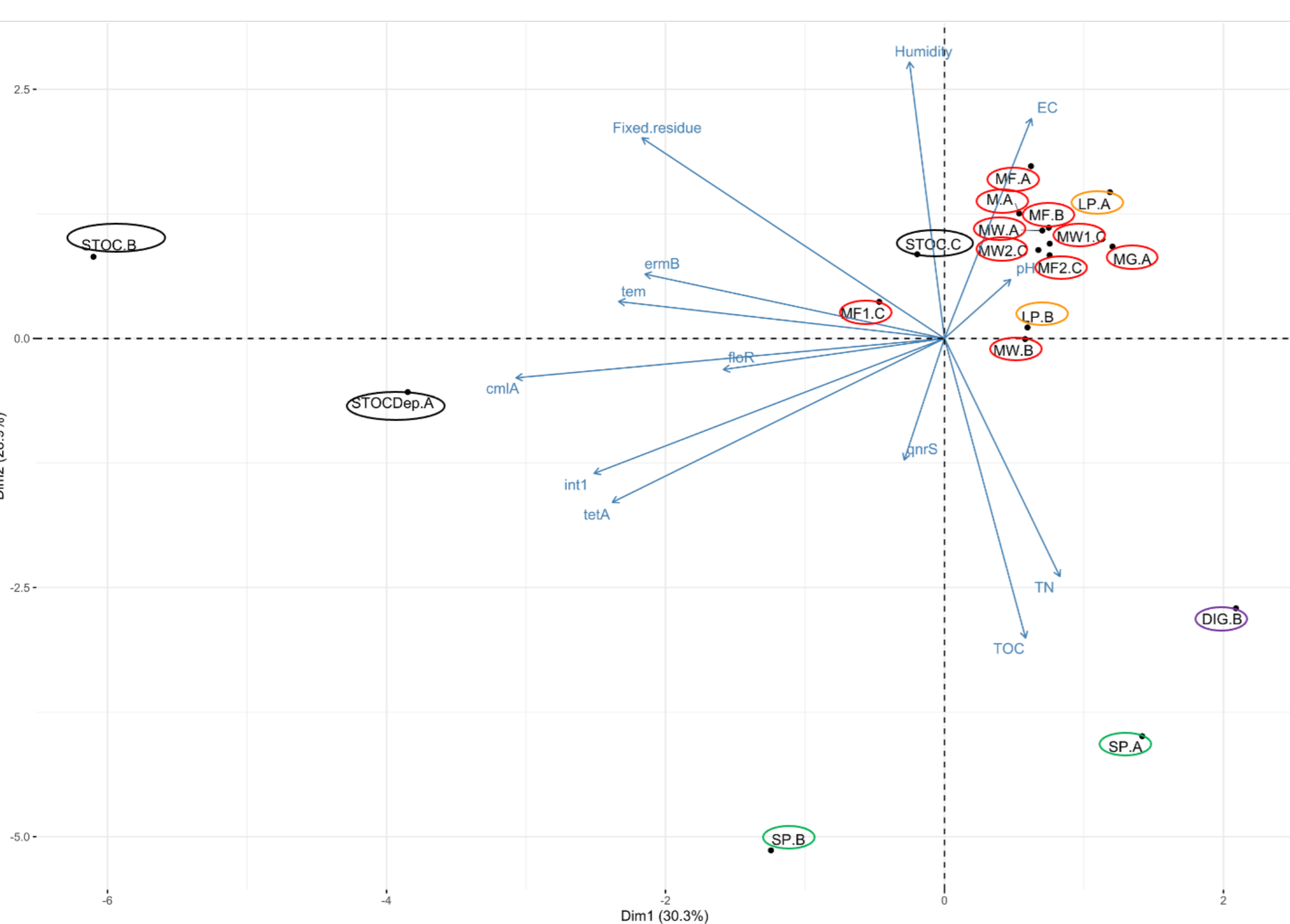


Figure 2. Biplot of manure samples in terms of ARGs/*int1* gene distribution and chemico-physical characterization.

Abbreviation: EC=Electrical conductivity, TOC=Total Organic Carbon; TN=Total Nitrogen.

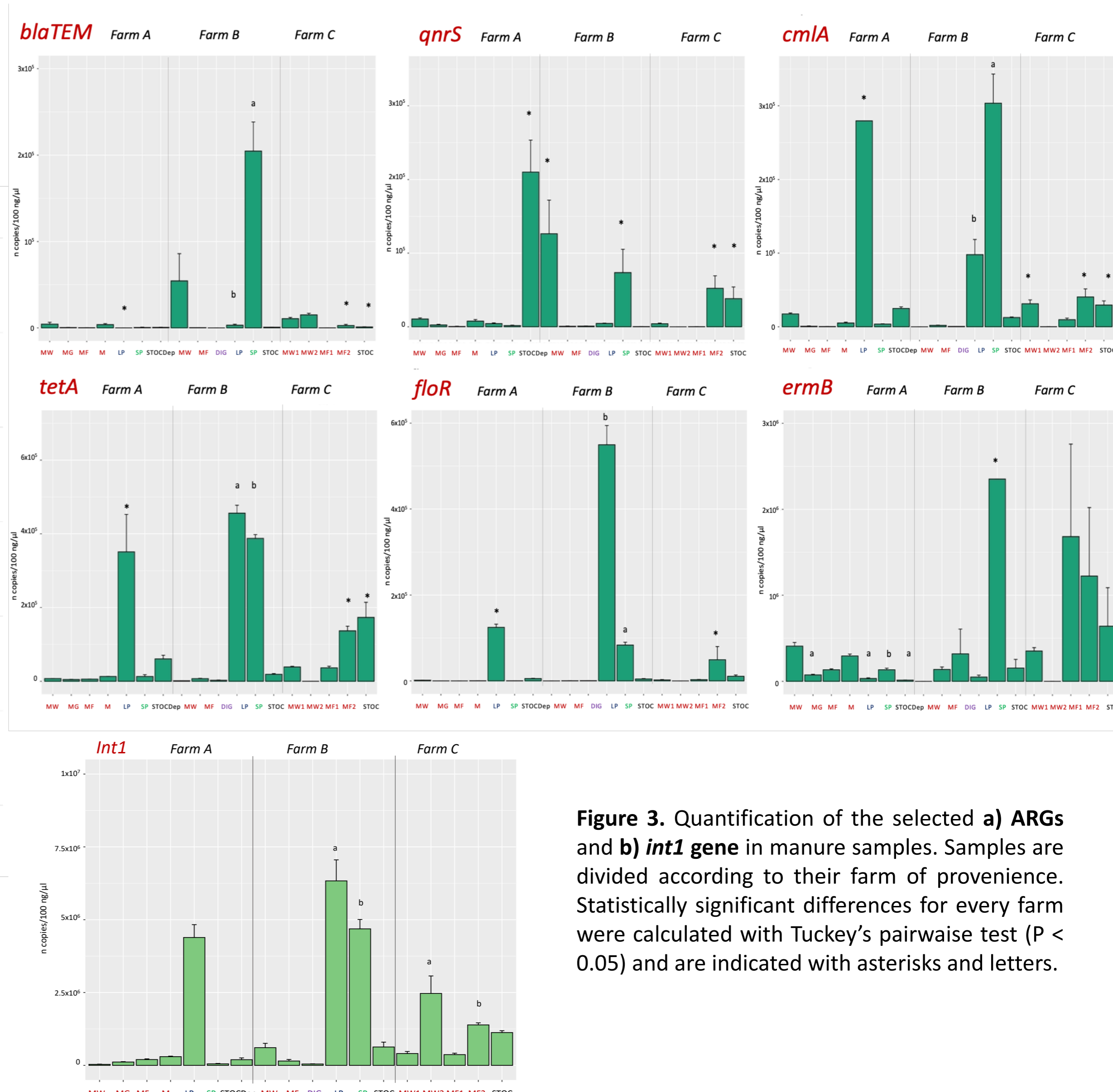


Figure 3. Quantification of the selected a) ARGs and b) *int1* gene in manure samples. Samples are divided according to their farm of provenience. Statistically significant differences for every farm were calculated with Tuckey's pairwise test ( $P < 0.05$ ) and are indicated with asterisks and letters.

## Conclusions

Results showed a high presence ( $5 \times 10^5$ ) of ARGs associated with high presence of *int1*, at the final collection points of liquid manure, in all three farms.

Indeed, *int1* cassette is always reported as one of the main mechanisms of HGT in different environments, such as fresh water, sediments and sludge, where it showed significantly positive correlation with the relative ARGs abundance (Zhao et al., 2019).

Interestingly, most parts of farms' final collection points (STOC and SOLID PHASE samples) are similar for ARGs distribution. Contrarily, MANURE and LIQUID PHASE samples are similar for the chemical characterization, in particular in terms of pH and electric conductivity.

Our findings confirm the risk of ARGs spread through HGT inside livestock microbial communities in particular environmental conditions, promoting the development of strategies for the studies of dynamics and for the control of ARGs' dissemination (Checcucci et al., 2020).