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HAL Authorization

Antibiotic resistance genes in sewage sludge submitted to anaerobic digestion

Céline Roose-Amsaleg¹, Romain Causse-Védrines², Cédric Wolf¹, Garance Demarquest¹, Thomas Lehembre¹, Sabrina Guérin-Rechdaoui³, Vincent Rocher³, Ariane Bize⁴, Annet Laverman¹

¹ CNRS, ECOBIO-UMR 6553, Université Rennes 1, 35000 Rennes, France

² Biogenouest Genomics, ANAEE-Fr and the EcogenO core facility of Rennes (OSUR)

³ SIAAP, Innovation department, 82 avenue Kléber, 92700 Colombes, France

⁴ Université Paris-Saclay, INRAE, PROSE, 92160, Antony, France

Introduction

Antibiotic resistance is considered as a major threat to public health by the WHO due to its wide spread. As a consequence, antibiotic resistance genes (ARG) represent emerging pollutants, not only present in hospitals but also in all environments such as soil or water and notably wastewater.

Wastewater treatment plants could concentrate fecal biomass and also ARG. This questions about bio-safety of their final product: sewage sludges and how they could be sanitized.

Two processes, composting and drying, significantly reduced the relative abundance of ARG as well as mobile genetic element genes (MGEG) in organic waste (1). However, anaerobic digestion (AD) could be preferred because of the energy it allows to recover or its integration in circular bioeconomy at local scale. AD has been demonstrated affecting the number/fate of ARG: either reducing or increasing but in diverse lab-scale digesters (2). We hypothesize that reduction of ARG may occur during dry co-digestion of sewage sludge with manure.

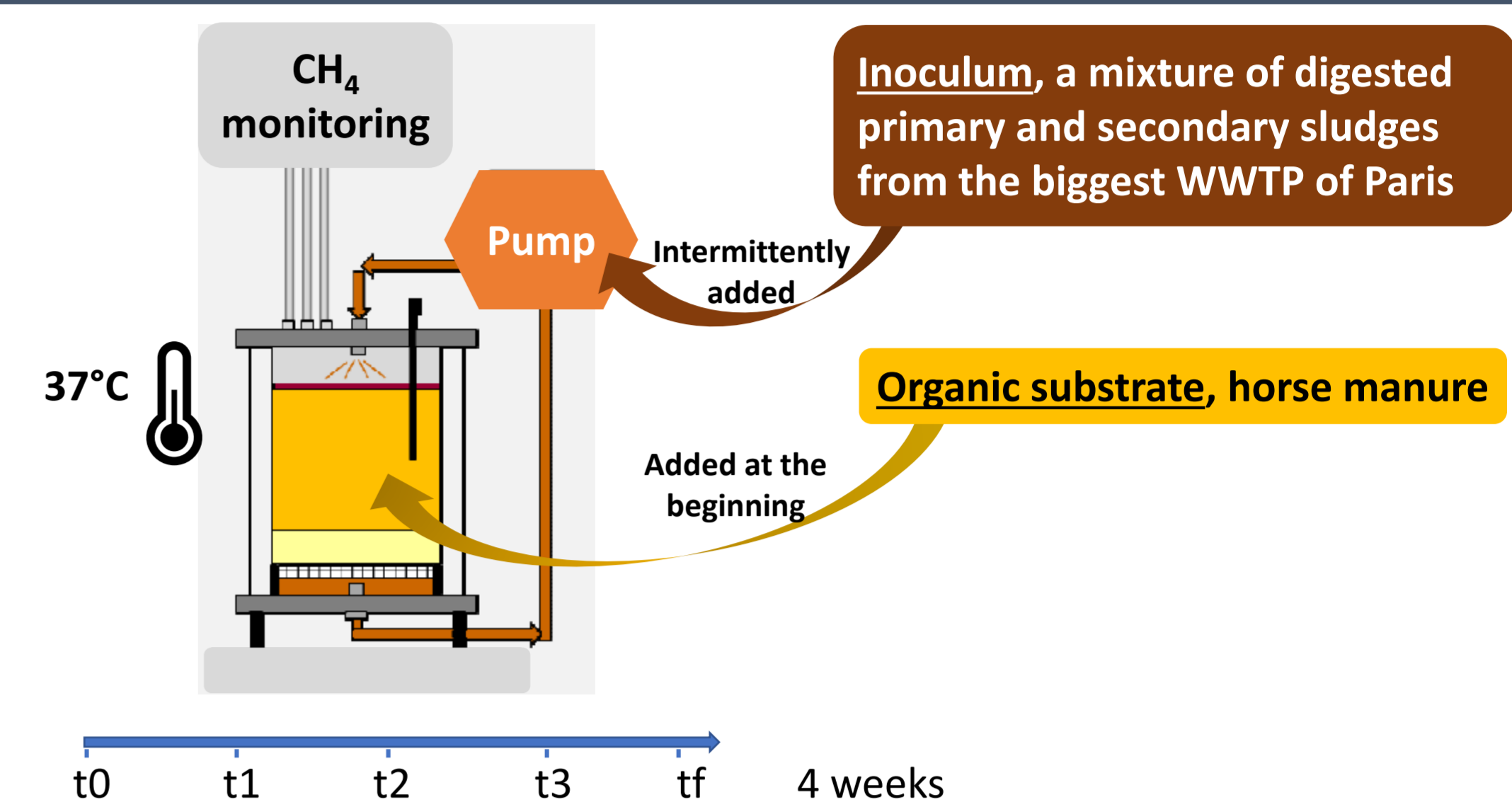
One of the main methods developed to detect ARG in such complex environmental matrices as sewage sludges is the high-throughput quantitative PCR (HT-qPCR). Several suppliers proposed thermocyclers able at the same time, to minimize the size of the well and to increase the number of samples (and of replicates)

The aim was to survey the amounts of ARG and MGEG in sewage sludge during dry anaerobic digestion.

Material and Methods

1. Experimental design:

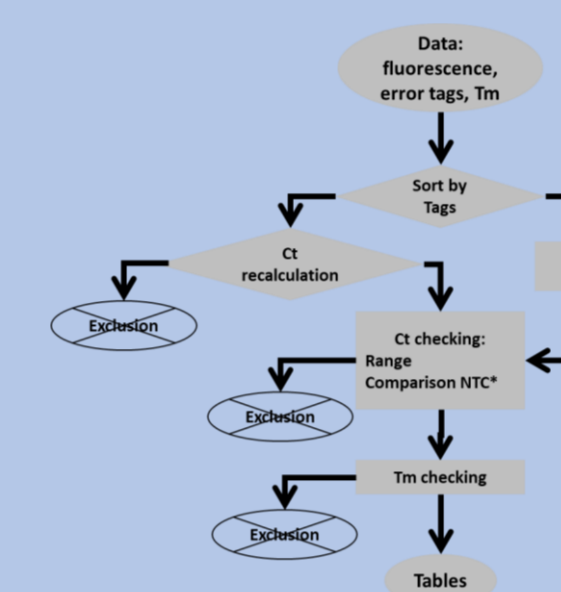
Dry mesophilic anaerobic digestion
60 L-pilot reactor



2. ARG detection

DNA extracted from digestate (Dneasy Powersoil Kit, Qiagen)

- qPCR: • WaferGen™ SmartChip, Takara
• LightCycler® 480 SYBR Green I Master mix
• 382 primer pairs (see 3): ARG, MGEG and two *16S*rDNA
• $T_H = 60^\circ\text{C}$, 45 cycles
• Duplicate reactions (5 x 2) and two NTC*



Data analysis automatization: R script

*NTC: no template control

**Ct: Cycle threshold

Results and Discussion

Detection in the digested sewage sludge: 58/380 genes

DETECTED OR NOT?

- No variation of the number of genes during anaerobic digestion
→ All time points considered altogether in the following results (10 reactions per gene)
- Relevant detection was defined as detection of 6/10 reactions per gene (Figure 1)

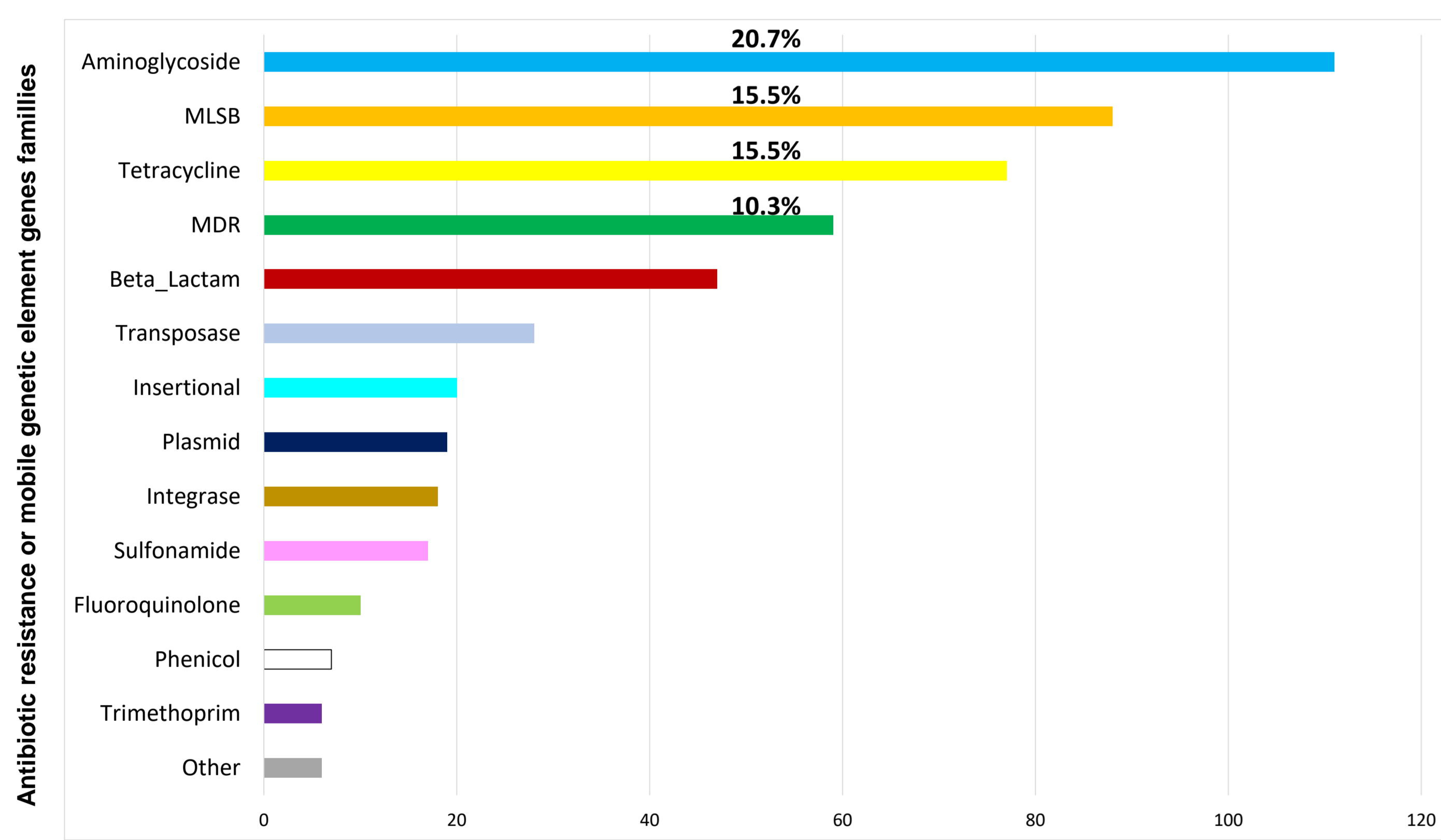


Figure 1: Repartition (sum of positive reactions) by antibiotic resistance or MGE family of the relevant detected genes in the studied digested sewage sludge (% of detected genes)

- Aminoglycosides and MLS (macrolides-lincosamides, streptogramins) genes were also the most detected genes in other anaerobic digested sewage sludge (2).
- No genes encoding for a resistance to Amphenicol (e. g. *mdtL*, *rarD*, *catA*) or Vancomycin (e. g. *vanA*, *vanB*, *vanC*...) or encoding for insertional (e. g. *TN5*, *IS1111*...) or MGE (e. g. *IS 26*, *IS200*, *mobA*...).

WHICH LEVEL OF DETECTION?

Ct checking and recalculation

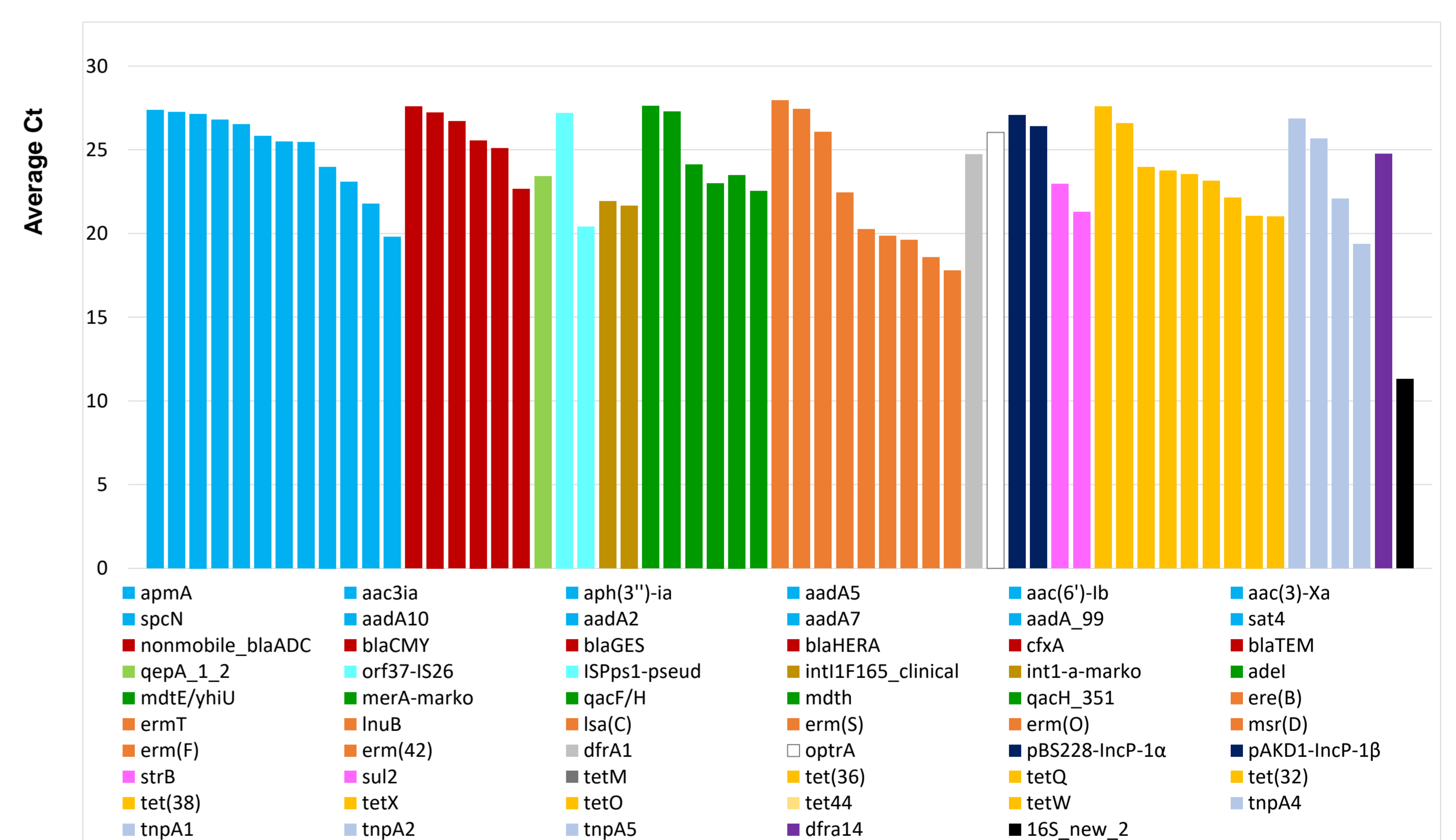


Figure 2: Level of detection (represented by Ct) by genes (and sorted by families, same colors as in Figure 1) in the studied digested sewage sludge

Ct range: 17.7 (*erm(42)*) to 27.9 (*ere(B)*) << 11.3 (*16S RNA gene*)

Conclusions

Anaerobic digestion does not eliminate all the ARG present in sewage sludges (15.2% remained).

Generalization of the script to other experimental designs.

1- Goulas *et al.*, 2020, Environmental evidence, 9
2- Syafiuddin and Boopathy, 2021, Bioresource Technology, 330
3- Stedtfeld *et al.*, 2018, FEMS Microbiology Ecology, 94