

Can wastewater antibiotic resistant bacteria survive a healthy human gut microbiome?

Nazareno Scaccia*, Ivone Vaz-Moreira, Célia M. Manaia

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Arquiteto Lobão Vital, 172, 4200-374 Porto, Portugal



CATÓLICA
FACULTY
OF BIOTECHNOLOGY
PORTO

*nscaccia@porto.ucp.pt

Background and Objectives

The implications on the dissemination of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment due to water reuse are recognized. However, how this contamination may threaten human health is still a major question.

The entry via food chain is a likely possibility, although it is arguable that the complex human gut microbiome may hamper the survival of environmental ARB and ARG.

This was the major aim of this study in which was assessed the survival of known wastewater ARB in the presence of human faecal material.

Methodology



Faeces-based microcosm assays (FMAs)

Faecal material was collected from healthy donor, never subjected to antibiotherapy.

➤ FMAs spiked with wastewater ARB harboring known ARGs:

<i>Escherichia coli</i> A2FCC14	<i>bla</i> _{TEM} , <i>bla</i> _{CTX} , <i>bla</i> _{OXA-A}
<i>Enterococcus faecalis</i> H1EV10	<i>vanA</i>

These assays were also conducted under selective pressure.

FMA + ARB

FMA + free-DNA

Aerobic vs Anaerobic

➤ FMAs spiked with free-DNA extracts from the same bacteria

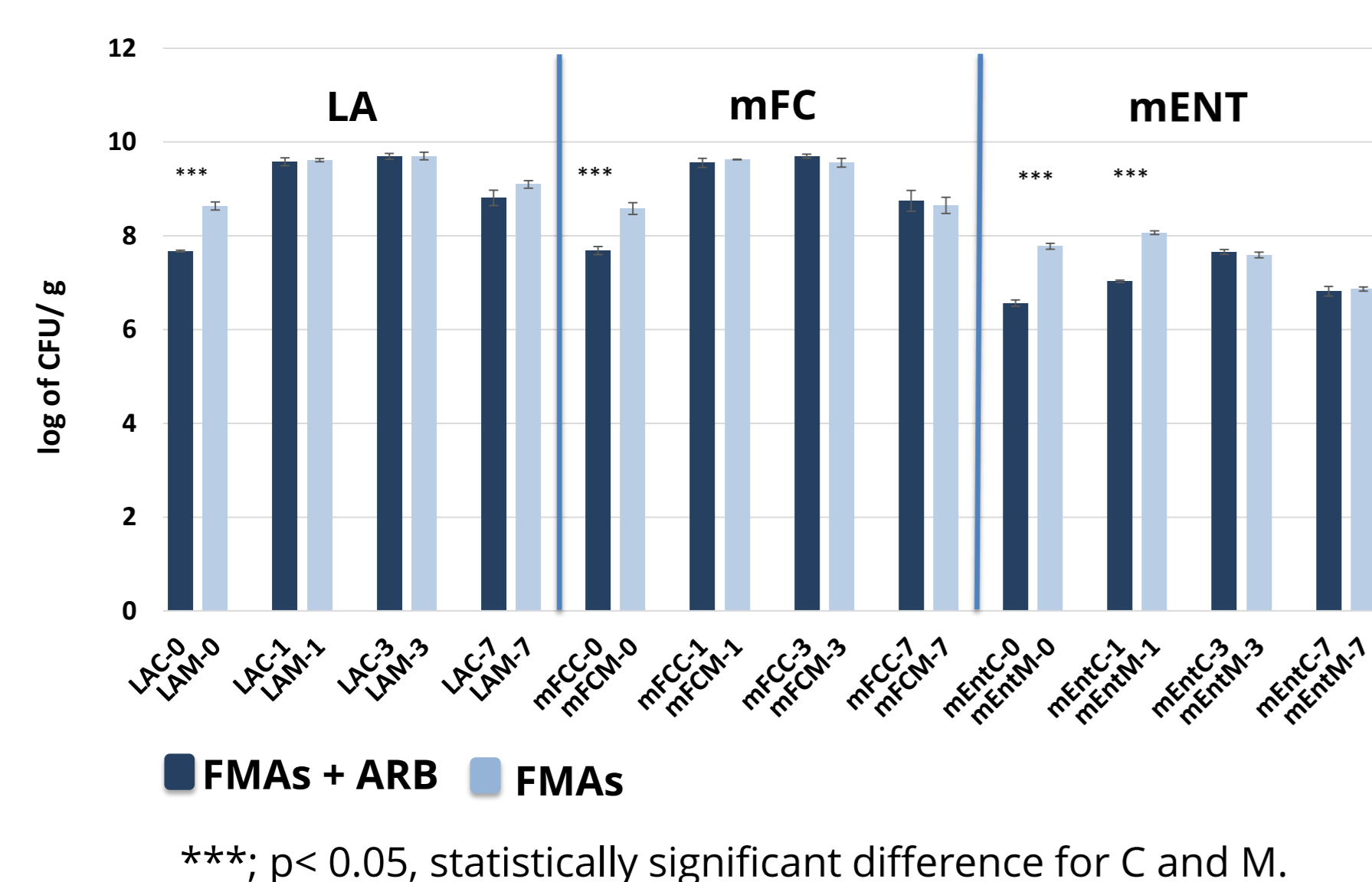
➤ FMAs spiked with same ARB were conducted under aerobic and anaerobic conditions

ARB were monitored based on cultivation methods (LA, m-FC and m-Enterococcus agar) and ARGs based on quantitative PCR (qPCR).

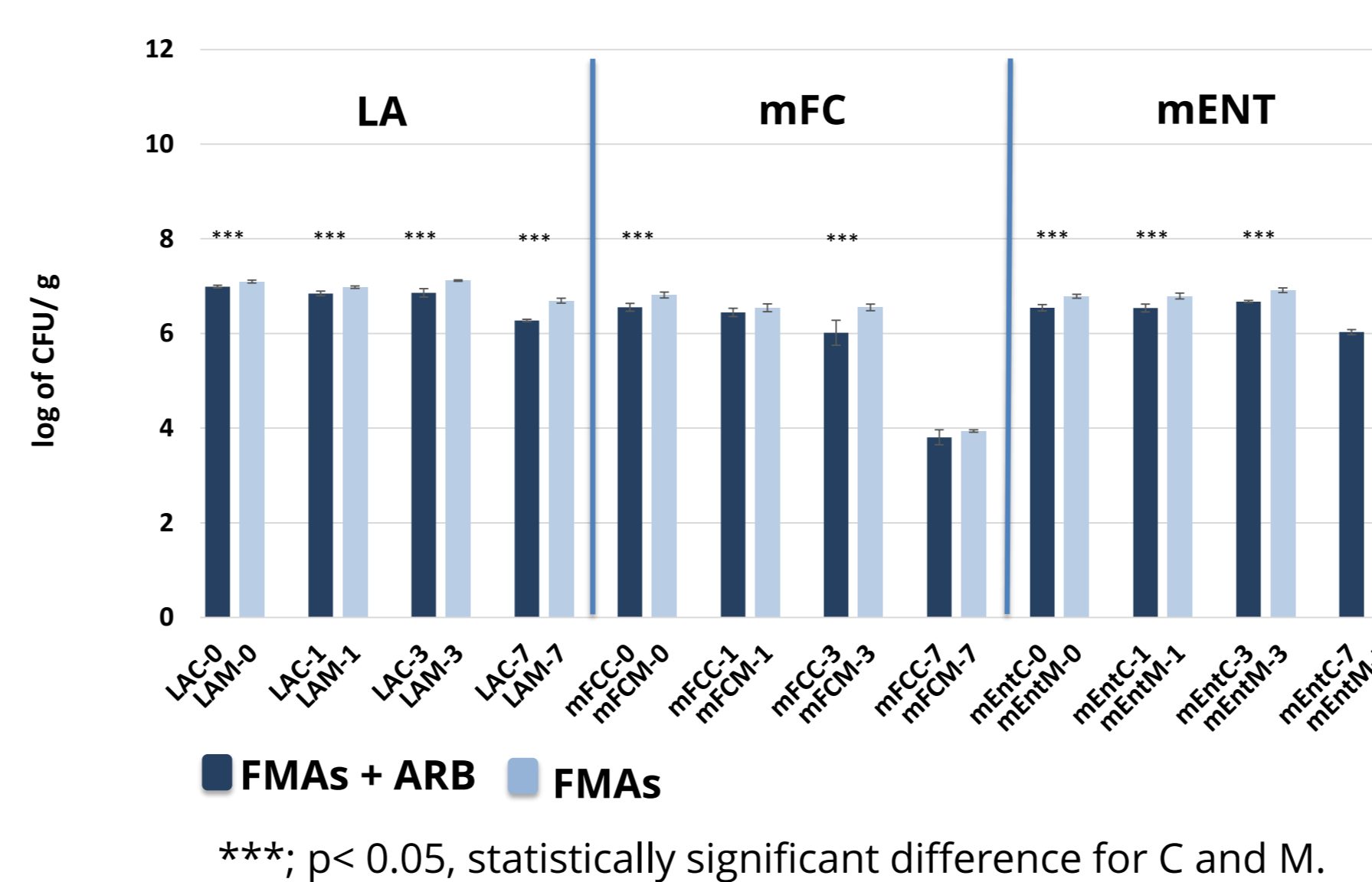
Results

ARB survival & ARGs abundance in FMAs

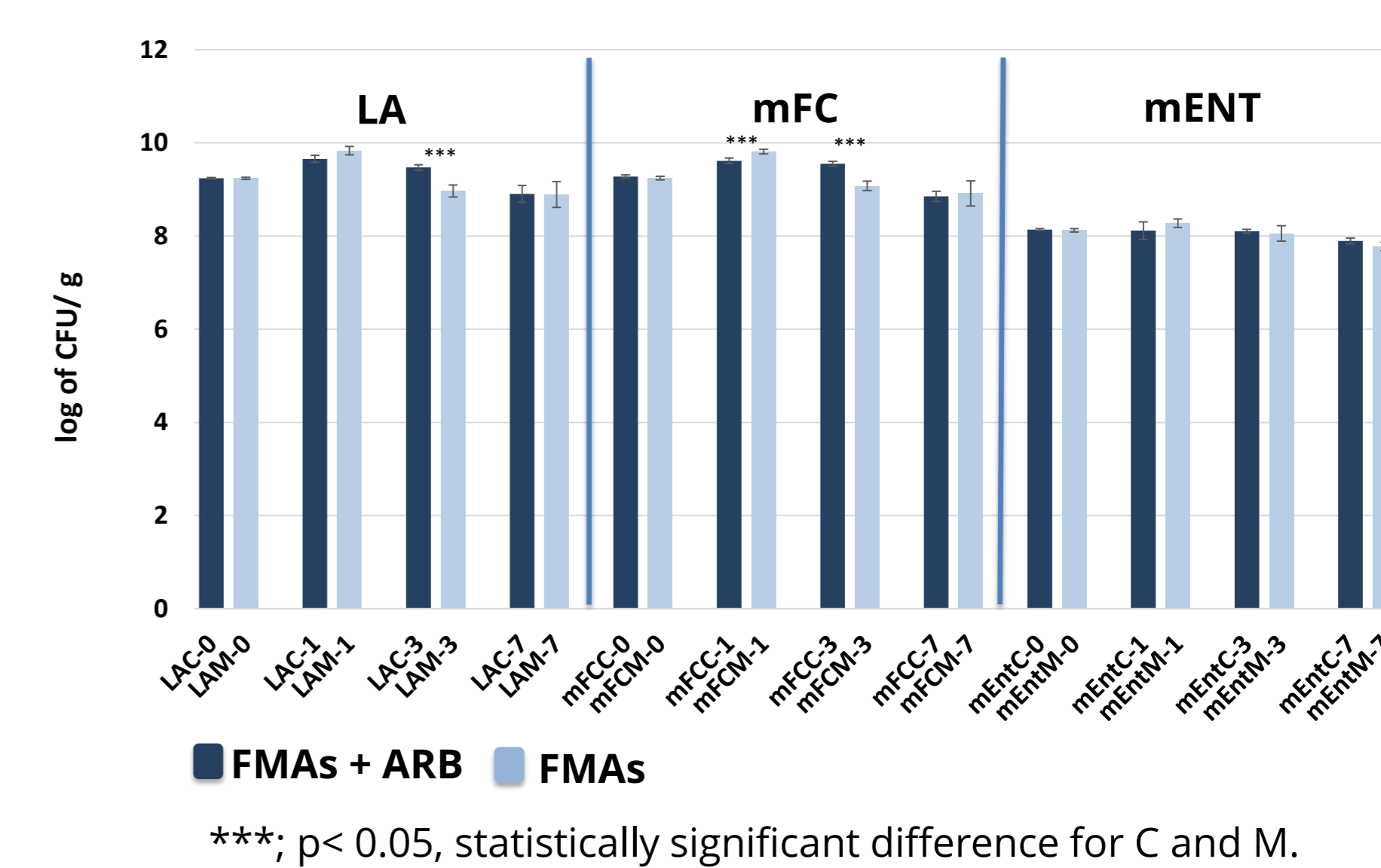
ARB SURVIVAL (AEROBIC CONDITIONS)



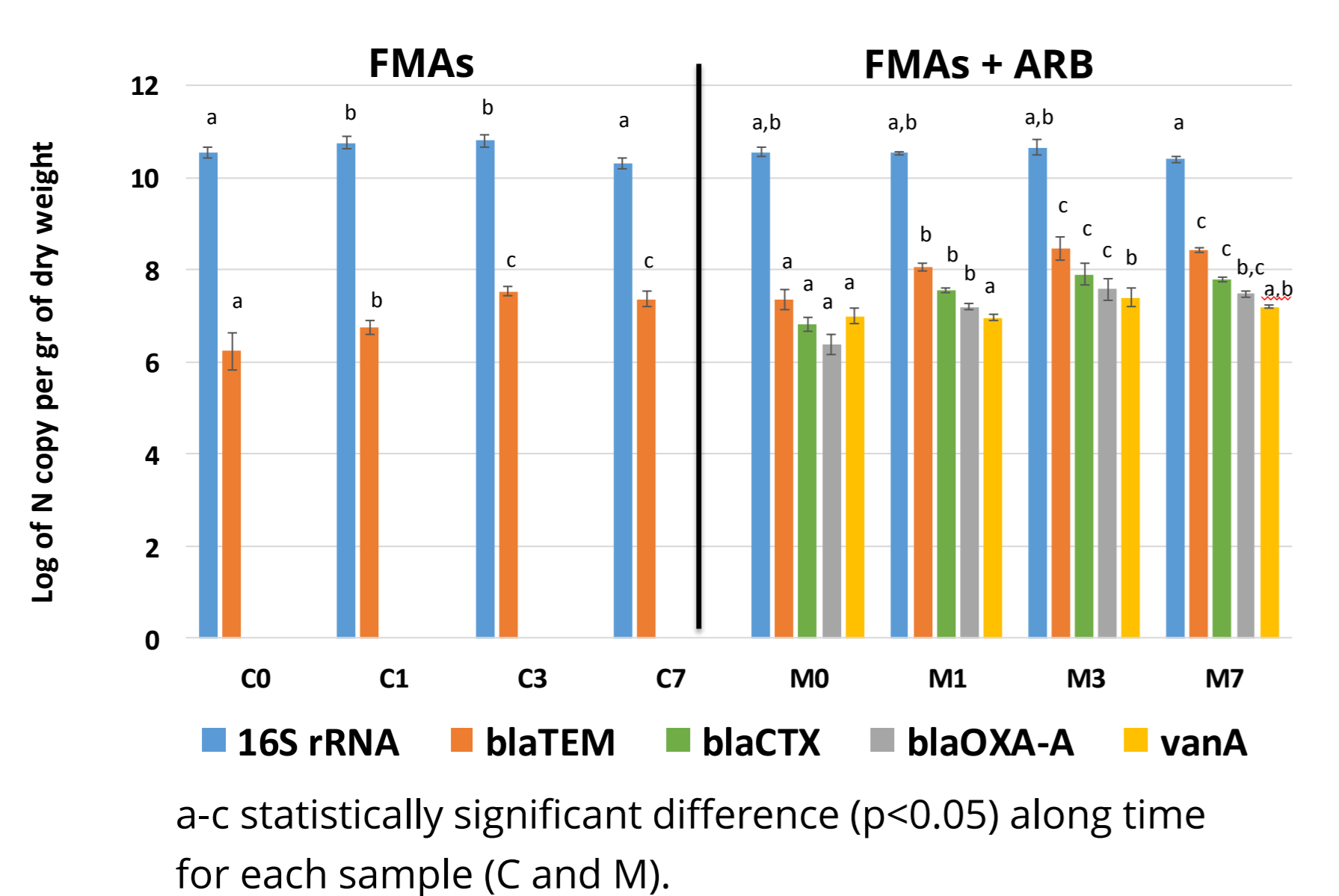
ARB SURVIVAL (ANAEROBIC CONDITIONS)



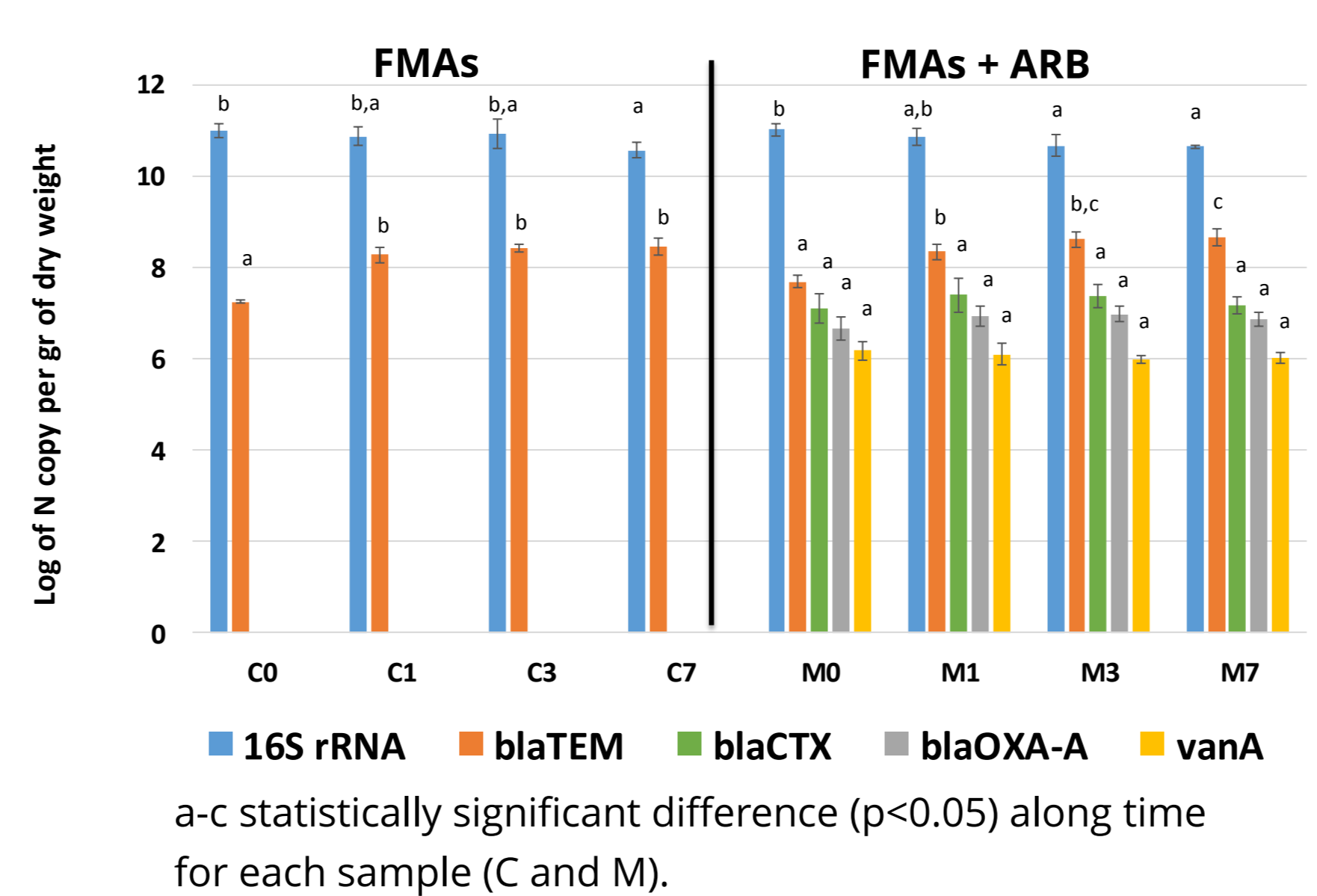
EFFECT OF FREE-DNA IN FMAs BACTERIA (AEROBIC CONDITIONS)



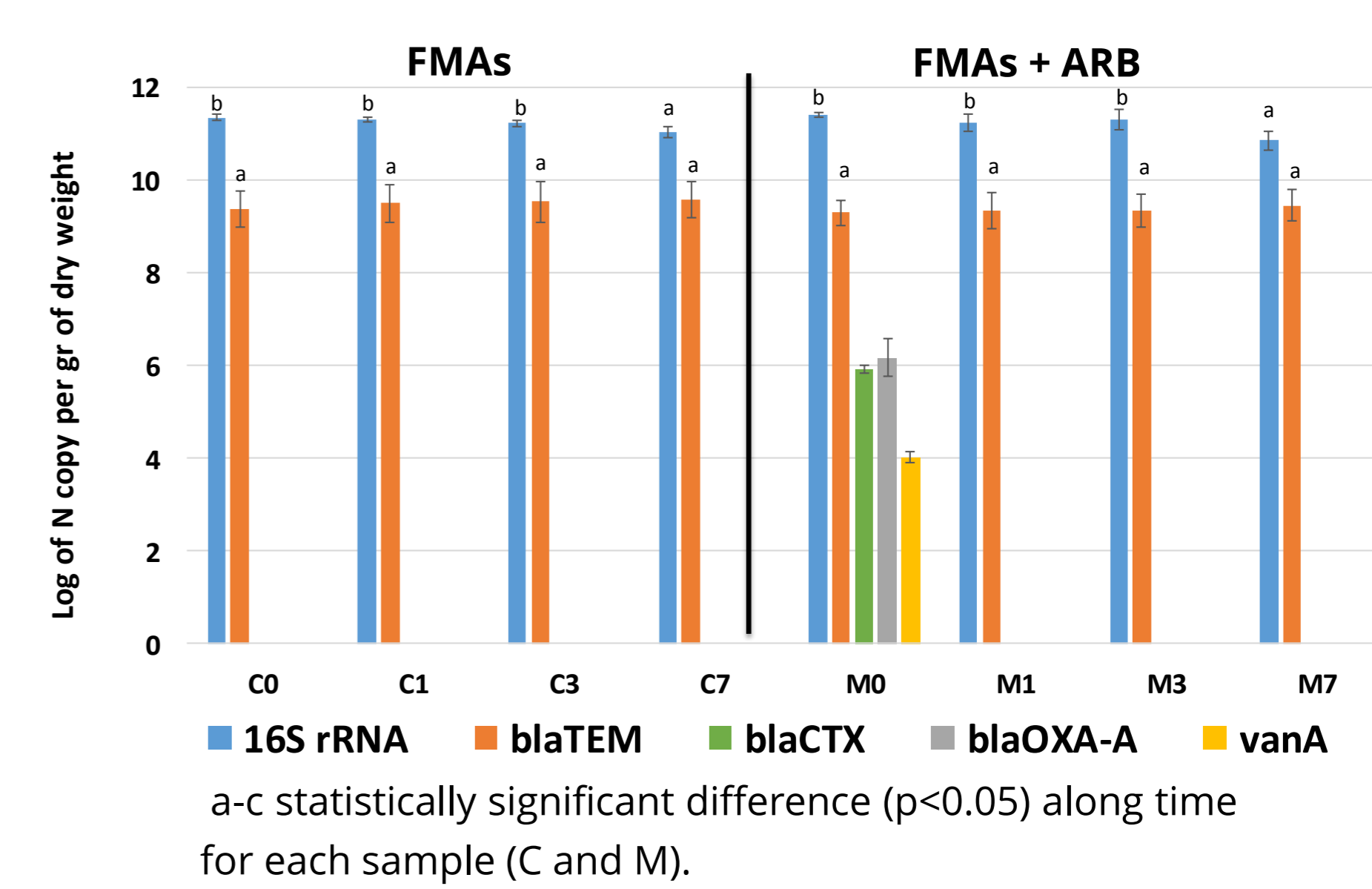
ARGs ABUNDANCE (AEROBIC CONDITIONS)



ARGs ABUNDANCE (ANAEROBIC CONDITIONS)



ARGs PERSISTENCE (AEROBIC CONDITIONS)



The survival of ARB under selective pressure (e.g. during antibiotherapy), did not show differences among FMAs inoculated with ARB in the presence of one dose of sub-inhibitory concentration of cefotaxime or vancomycin. However, another set of experiments of FMAs in presence of constant concentration of antibiotic is ongoing.

Conclusions

- ARB survived in the presence of the faecal microbiota at least for a week and their ARGs could be detected and quantified up to 7 days;
- After addition of free-DNA, the ARGs *bla*_{CTX}, *bla*_{OXA-A} and *vanA* decayed in 24 hours. Moreover, free-DNA did not imply an increase of antibiotic resistance;
- Comparing the microcosms conducted under aerobic or anaerobic conditions, the strain *Enterococcus faecalis* H1EV10 behaved similarly, while *E. coli* A2FCC14 has a sharper reduction anaerobically after 7 days. Nevertheless, in anaerobiosis the ARGs abundance did not change while in aerobiosis they had a slight increase (< 1 log).
- **The main conclusion of this research so far, is that wastewater ARB are able to survive in faecal microcosms and are not outcompeted by the autochthonous microflora until a week, and their ARGs can be quantified for longer periods.**

Acknowledgements

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