



BeeNet



Monitoraggio
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PHENOTYPIC AND GENOTYPIC RESISTANCE TO COLISTIN IN ENVIRONMENTAL BACTERIA ISOLATED IN EMILIA-ROMAGNA BY BIOMONITORING WITH *APIS MELLIFERA* COLONIES

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INTRODUCTION

Colistin, also known as polymyxin E, is a cationic polypeptide antibiotic belonging to the class of polymyxins. It was firstly isolated from *Paenibacillus polymyxa* subsp. *colistinus* in 1947. Colistin has been used for animal treatment for decades, especially in Italy, and it is still widely employed. However, in Europe, the use of colistin in veterinary medicine drastically decreased in recent years. The recent identification of the first transferable plasmid-located colistin resistance determinant, called *mobile colistin resistance 1 gene* (*mcr1*), led the studies on the antimicrobial-resistance. Nowadays, nine genes coding for colistin resistance mechanisms were found, generally are transmitted horizontally via mobile plasmids. To date, the spread of resistant bacteria and the presence of these genes in the environment is poorly investigated. The behavioral and morphological peculiarities of *Apis mellifera* have already favored the use of their colonies as environmental bioindicators of both resistance genes and antibiotic-resistant bacteria. Within the BeeNet project, the study aimed to evaluate the phenotypic and genotypic resistance to colistin of Gram-negative bacteria spread in the environment and, isolated from the body surface and gastrointestinal tract of foraging bees.

MATERIALS & METHODS

Thirty-three apiaries distributed in all provinces of the Emilia-Romagna region were investigated (Figure 1). Each apiary was composed of three colonies that were sampled in four different periods of the year, namely November 2021, March 2022, June 2022, and September 2022. For each investigated colony, approximately twenty-five worker bees were sampled from the external combs.

Ten foragers from the external combs of each hive and stored at 4°C until analysis. The bacterial isolation was done in a non-selective medium. For this study, only Gram-negative strains were selected. Phenotypic resistances of bacteria were determined through a microdilution assay by evaluating the minimum inhibitory concentration (MIC) through dilutions of the antimicrobial from 0.5 µg/ml to 256 µg/ml. Strains with MIC values > 2 µg/ml were considered resistant.

The presence of the nine *mcr* genes was performed by two different multiplex PCRs (one for *mcr1*-*mcr5* genes, the other for *mcr6*-*mcr9*).

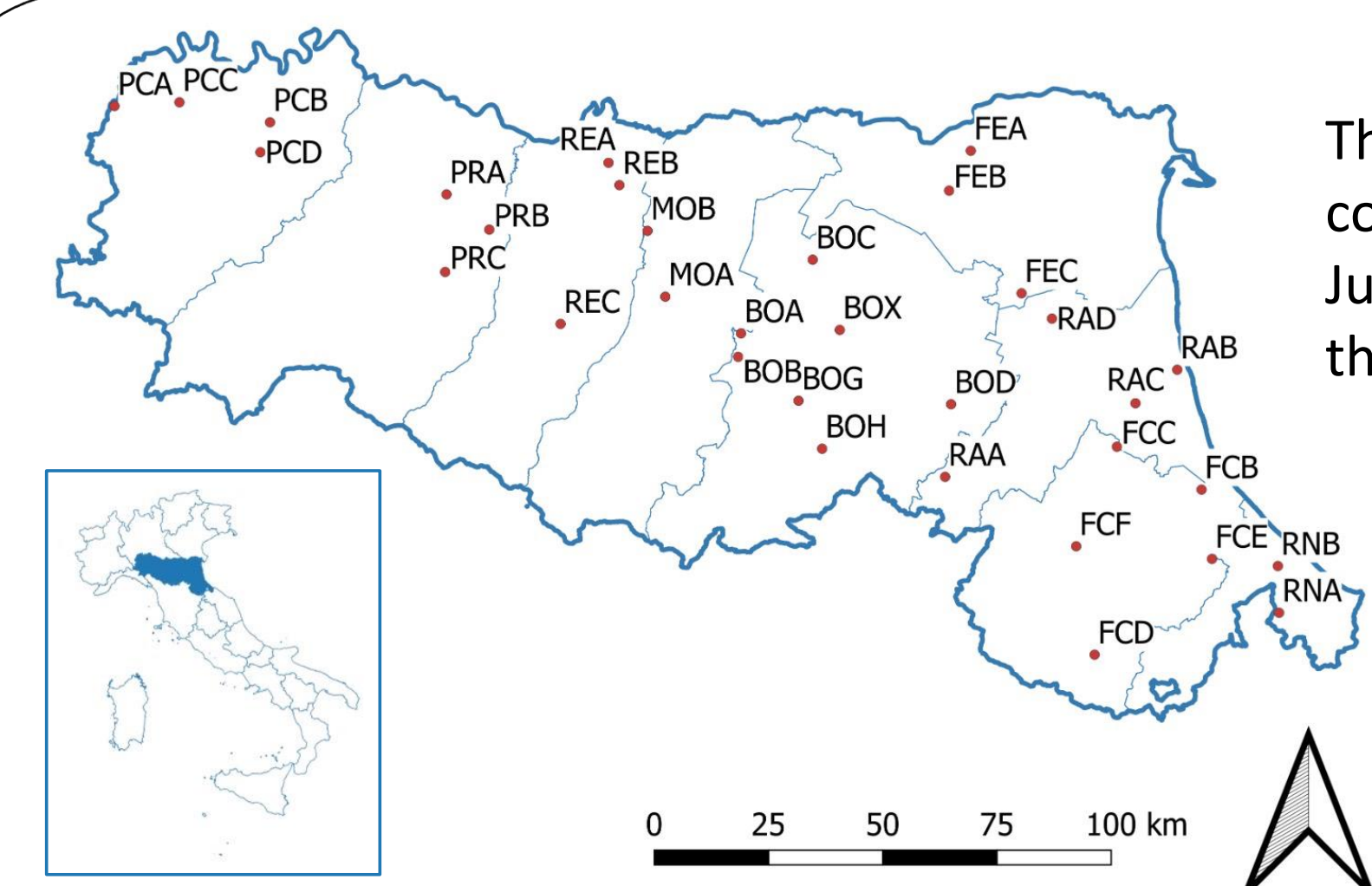


Figure 1. Geographical localization of the investigated apiaries in the Emilia-Romagna region.

RESULTS

In total, 257 bacteria strains were investigated for colistin resistance. At the MIC analysis, 176 were resistant with a value > 2 µg/ml. Higher resistance (256 µg/ml) was found in *Enterobacter*, *Klebsiella*, *Pantoea* and *Escherichia* (Figure 2).

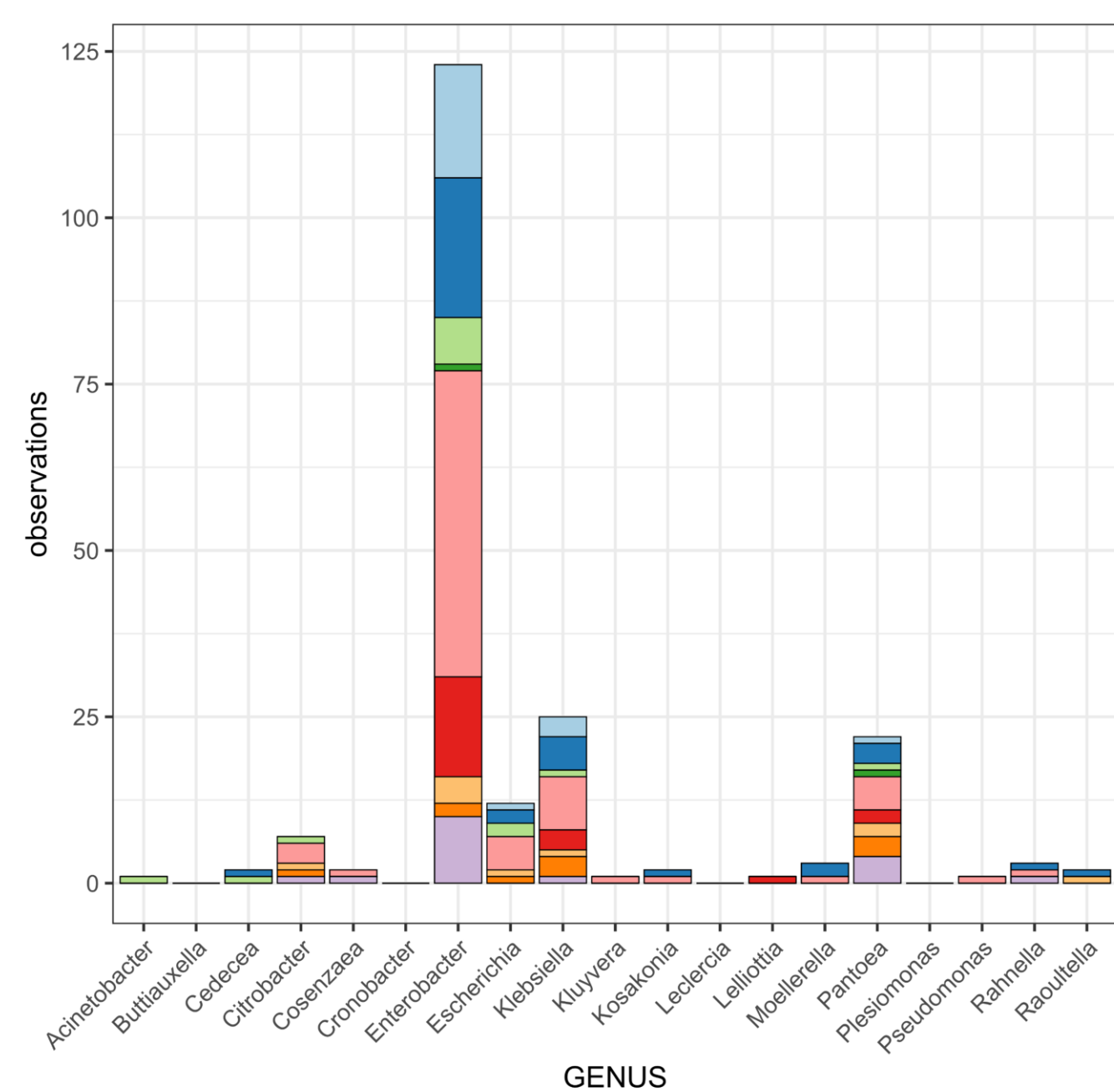


Figure 3. The number of observations for each *mcr* gene in relation to each isolated strain.

At least one *mcr* gene was found in 137 strains. The most frequent was *mcr5*, followed by *mcr2* and *mcr6*. The high frequency of resistances was found in *Enterobacter*, *Klebsiella*, *Pantoea* and *Escherichia* (Figure 3).

All nine *mcr* genes were distributed in all Emilia-Romagna provinces (Figure 4).

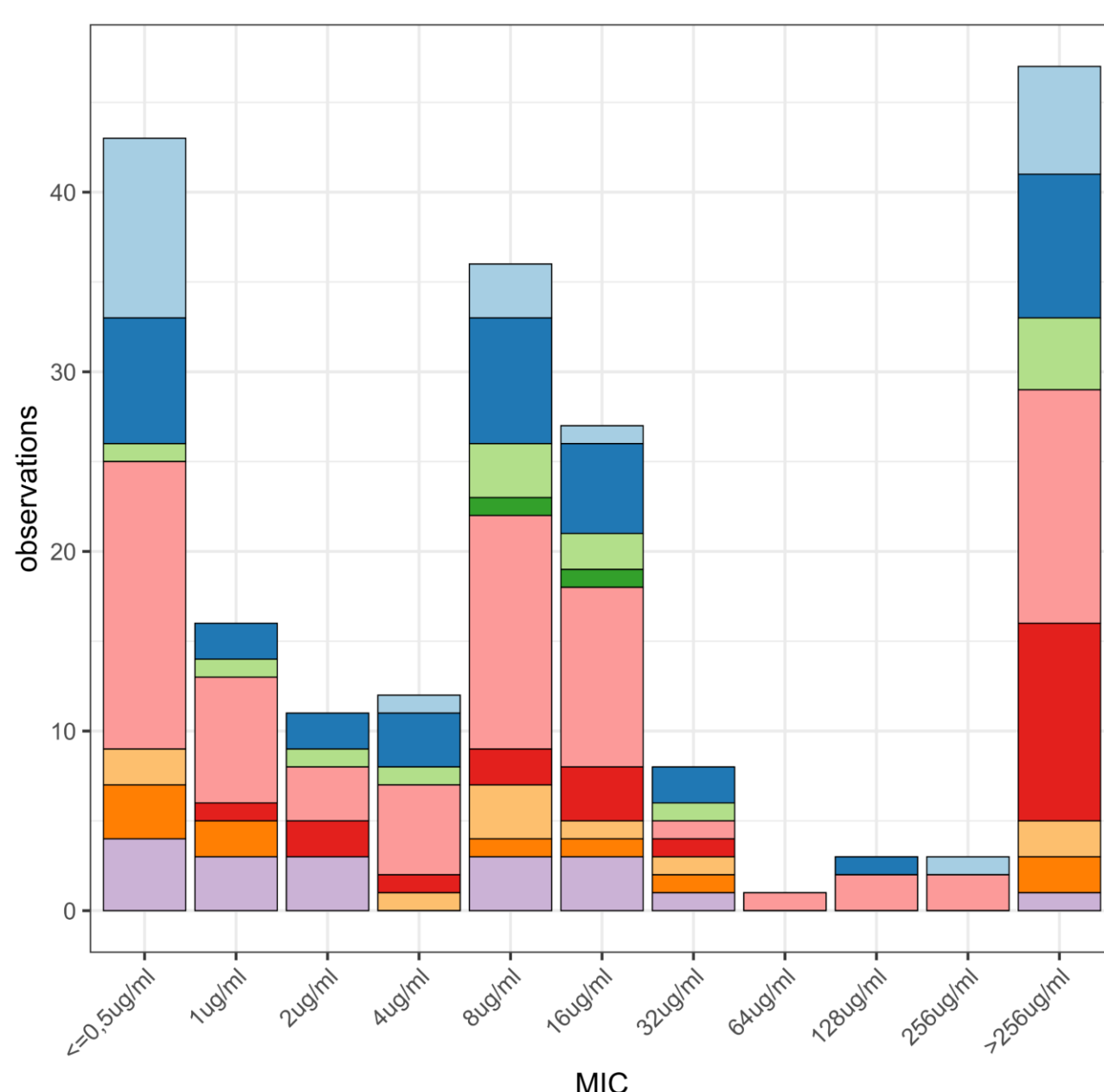


Figure 5. Relationship between the presence of *mcr* genes and MIC values

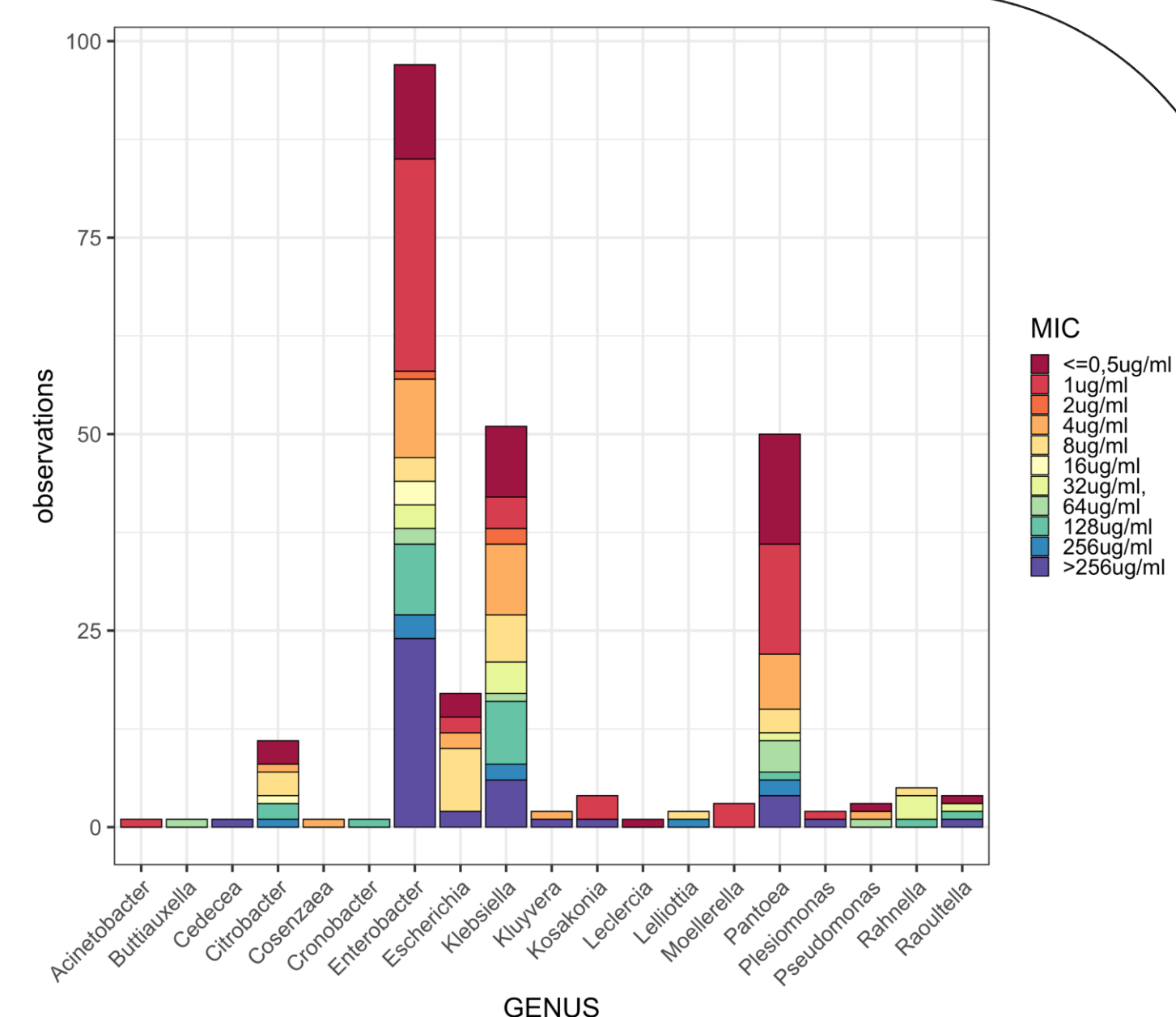


Figure 2. The number of observations for each MIC value in relation to each isolated strain.

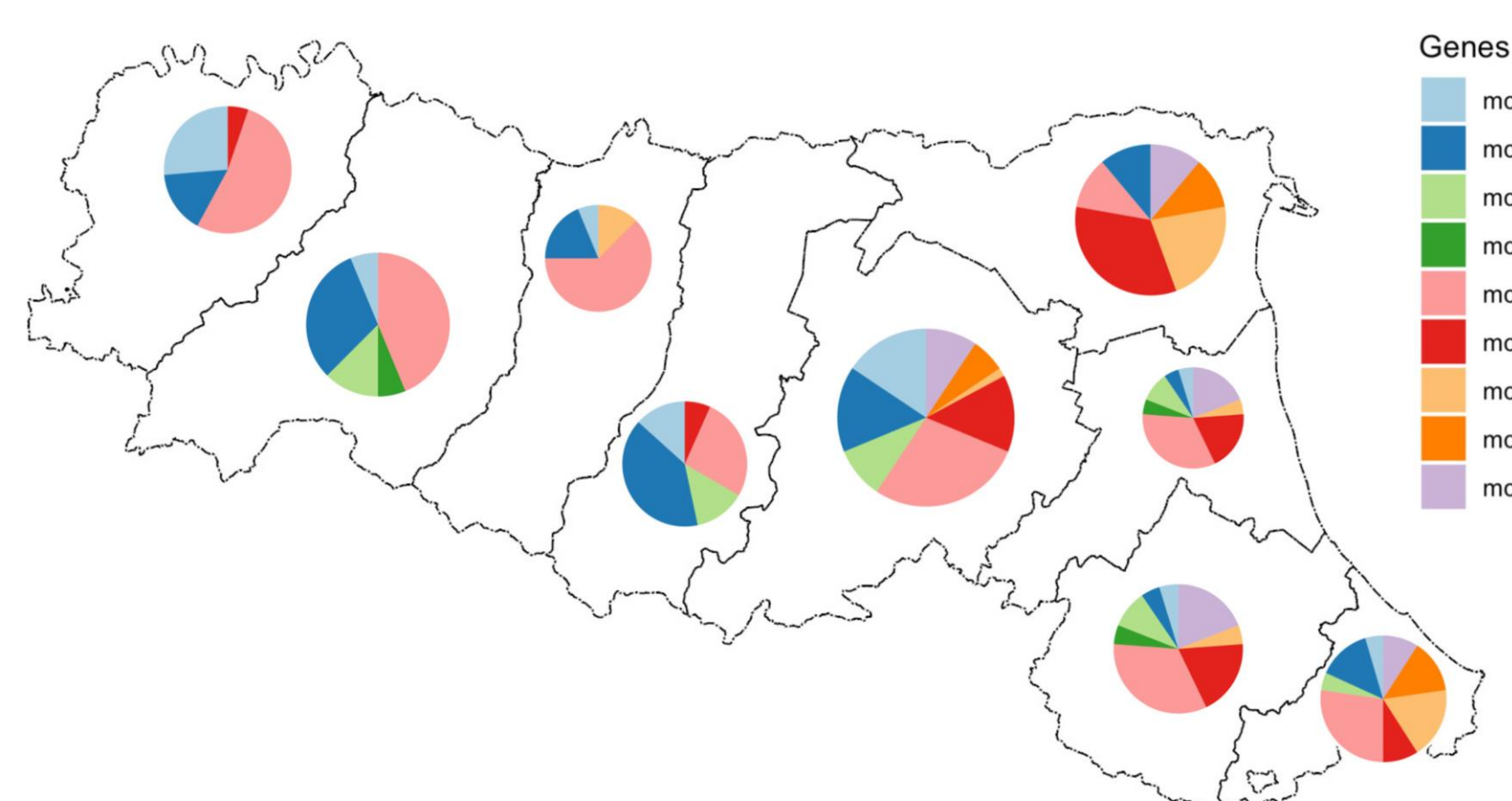


Figure 4. Geographical distribution of the investigated *mcr* genes in the Emilia-Romagna region.

DISCUSSION AND CONCLUSION

This investigation focused on the epidemiological surveillance of colistin, confirming the important role of honey bee colonies in monitoring the environmental spread of antimicrobials. The results obtained indicate the possibility of identifying both the presence and environmental distribution of specific colistin resistance genes.

The application of honey bees for environmental monitoring is of paramount relevance, particularly within the One-Health framework. This significance is underscored by the essential role played by colistin and other antimicrobials, widely applied to control diseases in humans, animals, and plants alike

