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Antimicrobial susceptibility of *Staphylococcus aureus* strains against *Melipona bicolor* honey

Bárbara Kornin Gabardo[1], Luana Siqueira de Souza[1,2], Elisana Julek[1,2],Juliana Chiesse Da Silva Zatta[1], Julia Arantes Galvão[1,2]

Quality Control and Food Safety Laboratory - Federal University of Paraná , Curitiba/Paraná, Brazil[1]

Post-Graduation Program in Veterinary Sciences - Federal University of Paraná, Curitiba/Paraná, Brazil[2]

INTRODUCTION & AIM

The research of novel antimicrobial compounds with efficacy against ever evolving and resistance acquiring bacteria is an area of research with many outstretching arms, including the exploration of natural compounds such as honey; taking into consideration promising studies with *Apis mellifera* honey and their other byproducts, the questioning if other bee species manifest latent antimicrobial activity in their products arises [1-2]. *Melipona bicolor* is a species of stingless bee native and endemic to Brazil. Currently there is no other study that explores it's honey effectiveness against any bacterial strains. Bearing in mind the clinical relevance of *Staphylococcus aureus* infections in both prevalence and gravity of infections, the aim of this study was to appraise if this honey could be an effective inhibitor of *S. aureus* growth.

METHOD

Staphylococcus aureus strains used were isolated and later had the characterization of their profile of susceptibility and resistance to common antimicrobial agents. The antibacterial potential of *M. bicolor* honey (pH 3,07) against multidrug-resistant and sensitive *S. aureus* strains was assessed using the minimum inhibitory concentration (MIC) method in 96-well microdilution plates [3].

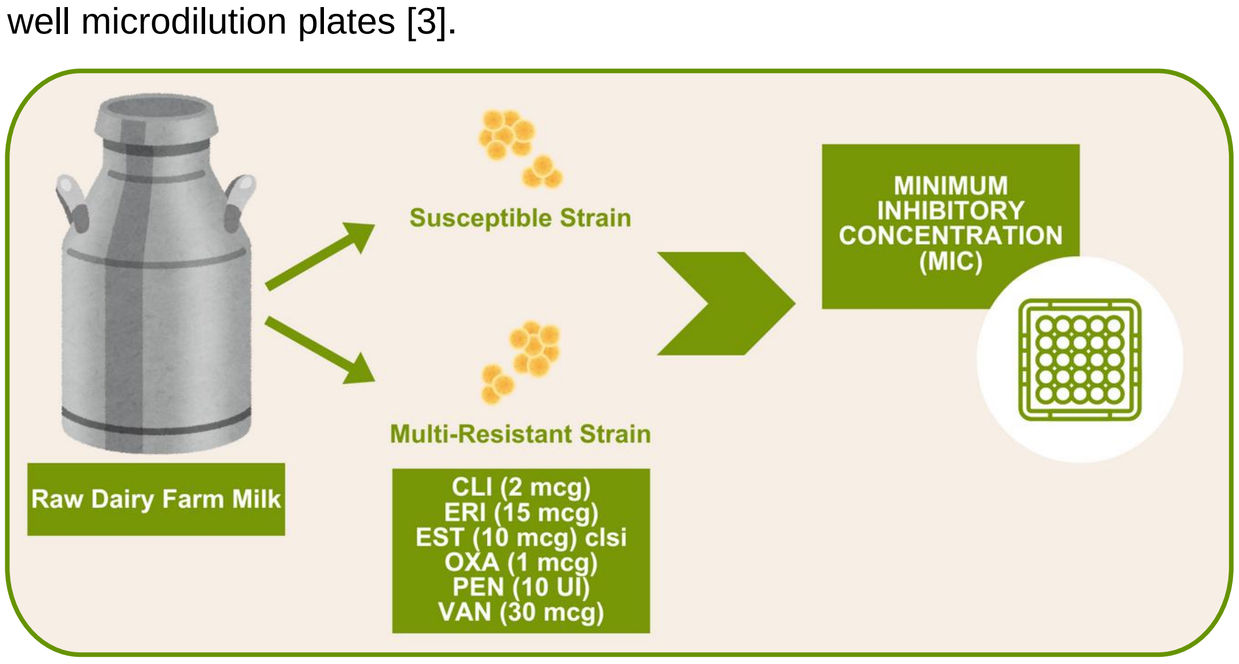

Figure 1. Diagram highlighting the origin and characteristics of *S. aureus* strains researched and approach taken to determine MIC.

From isolated colonies, the individual inoculum solutions were prepared with a standard concentration of colony forming units (CFUs). The dilutions of honey tested were made with sterile honey obtained through filtration with a 0.22µm 30mm PES membrane syringe filter and the dilution of the filtered aliquot in Muller–Hinton broth (MHB). 50 µl of each dilution tested was added to sequential wells of the 96-well plates, followed by 50 µl of the suspensions of the bacteria tested in isolation. The microplate was then incubated at 35°C for 24 hours to allow bacterial growth, if possible.

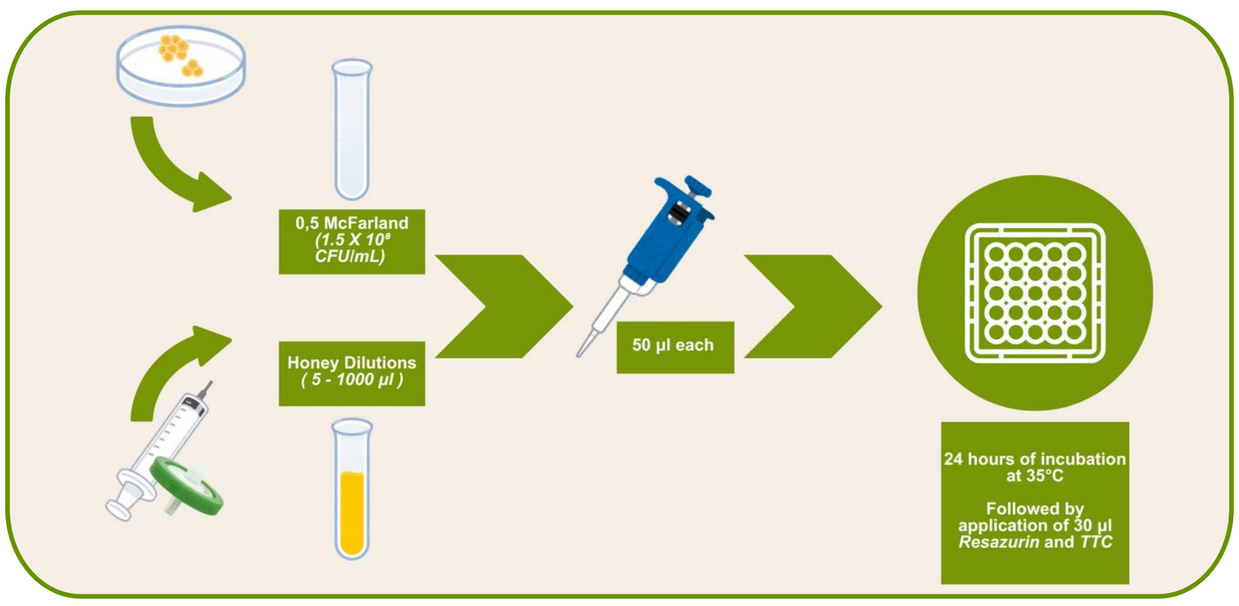

Figure 2. Scheme illustrating the workflow of preparation of one microplate for analysis.

MIC value was then investigated by applying 30 µl of the viability and aerobic cell metabolism detector dyes Resazurin and 2,3,5-triphenyltetrazolium chloride (TTC) to each dilution and inoculum containing well [4].

RESULTS & DISCUSSION

The growth of both strains was observed in all of the following honey concentrations tested: 5, 10, 20, 30, 50, 75, 100, 125, 150, 200, 225, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 e 1000 µl.

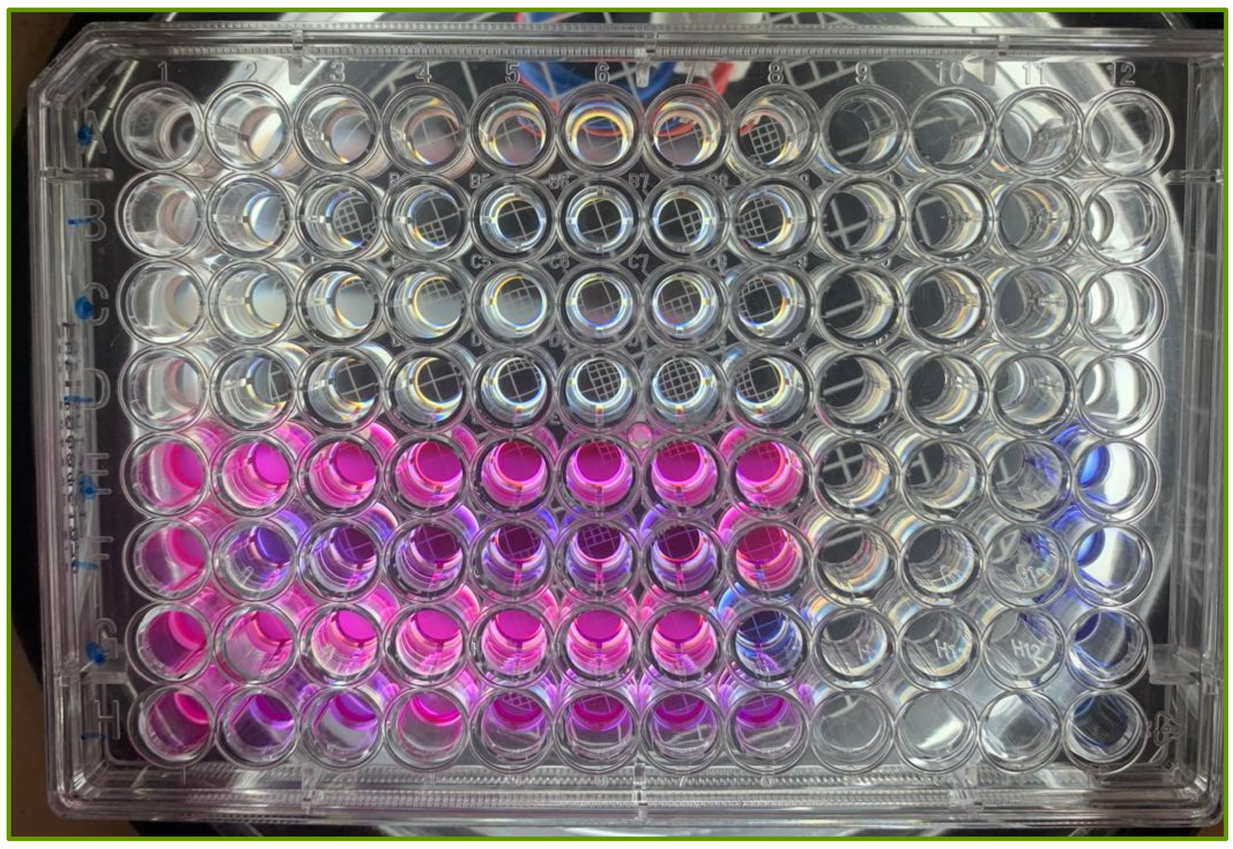

Figure 3. Microplate with reagent dyes applied; TCC applied in the first 4 columns immediately reacted, turning pink and then transparent before the picture was taken. Resazurin applied in the bottom 4 rows revealed purple to bright pink colors, again confirming the presence of bacteria with active metabolism present in all test wells. Dilutions tested in this microplate include 5, 10, 20, 30, 50, 100, 150, 200, 250 and 500 µl

CONCLUSION

The antimicrobial effect of *M. bicolor* honey against the *S. aureus* strains studied was not confirmed. Further trials involving different bacteria species against *M. bicolor* honey are to be considered.

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