

Development of gold nanoparticle-based platform for rapid detection of Gram-negative pathogens and their antibiotic resistance genes

Yashaswini V¹, Nupura P¹, Jnana A¹, Satyamoorthy K², and Murali T.S^{1*}

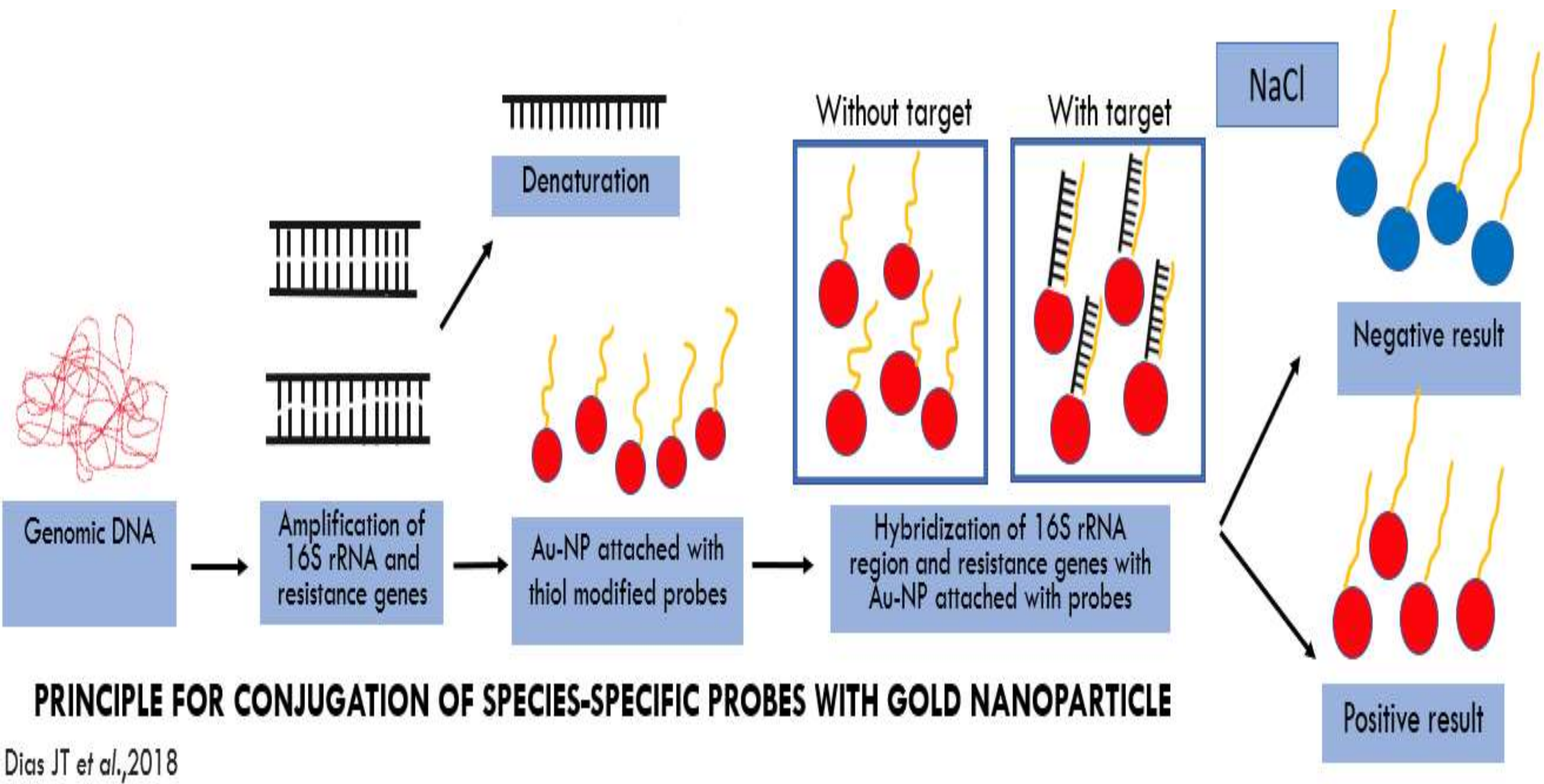
¹Department of Biotechnology, School of Life Sciences, Manipal Academy of Higher Education, Manipal - 576104, India

²Department of Cell and Molecular Biology, School of Life Sciences, Manipal Academy of Higher Education, Manipal - 576104, India

*Corresponding author, e-mail: murali.ts@manipal.edu

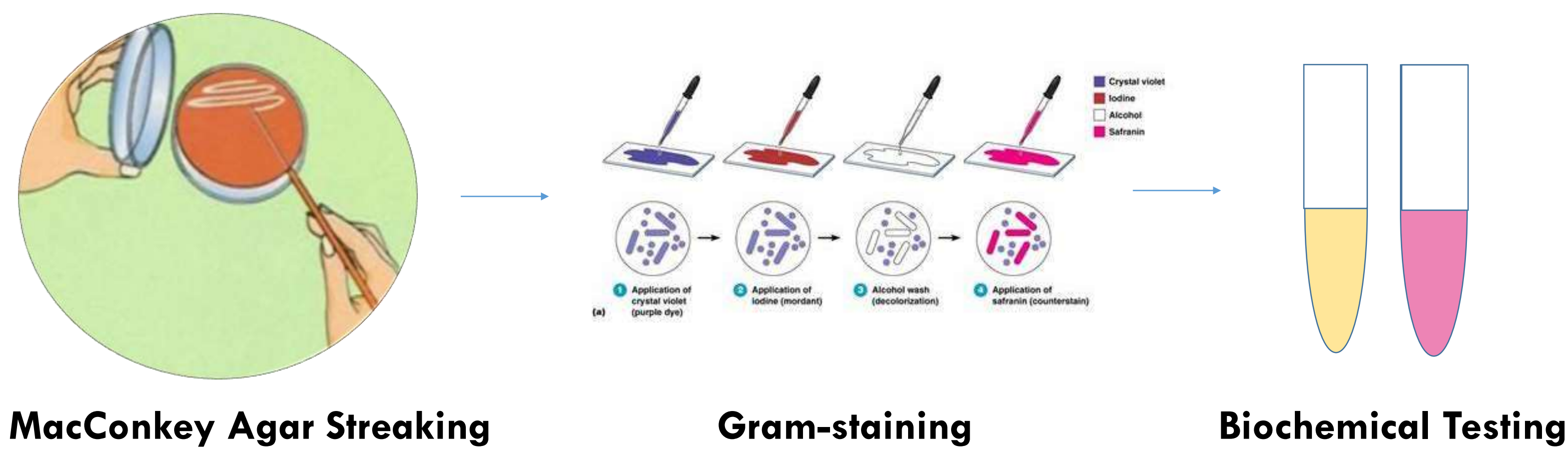
INTRODUCTION

- Diabetic foot ulcers (DFU) : condition associated with diabetes mellitus, results in exacerbation of wounds in the foot due to poor blood circulation, damage of the nerve, and high blood sugar.
- STUDY FOCUS: Developing a gold nanoparticle-based diagnostic platform that can be used for identification and detection of antibiotic resistance in Gram-negative bacterial pathogens commonly associated with DFU pathogenesis (*Acinetobacter baumannii*, *Enterobacter* spp., *Pseudomonas aeruginosa* and *Proteus mirabilis*)
- The principle of the platform is based on nucleic acid detection, combined with the colorimetric property of gold nanoparticles.
- This diagnostic platform intends to provide a clinical solution for DFU therapy that is cost-effective, rapid and accurate, necessary to combat the emerging public health threat of multidrug resistant.

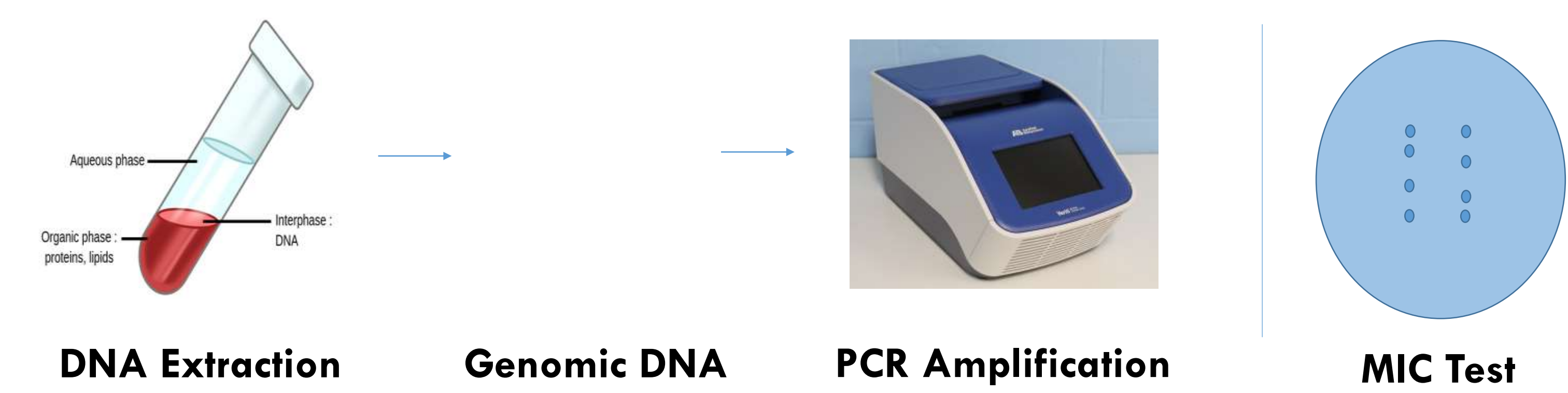


METHODOLOGY

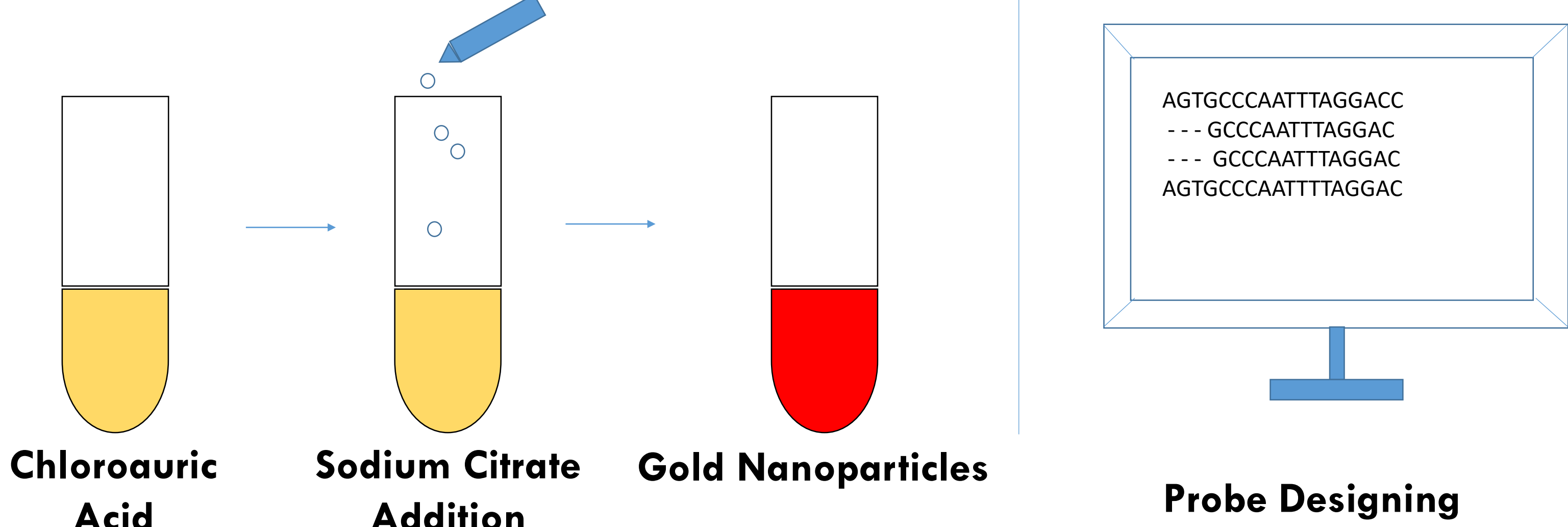
1. Revival and Characterization of Bacterial Isolates



2. DNA isolation and PCR of 16S rRNA and resistance genes (chosen based on the Minimum Inhibitory Concentration Test)



3. Gold Nanoparticle Synthesis and Probe Designing

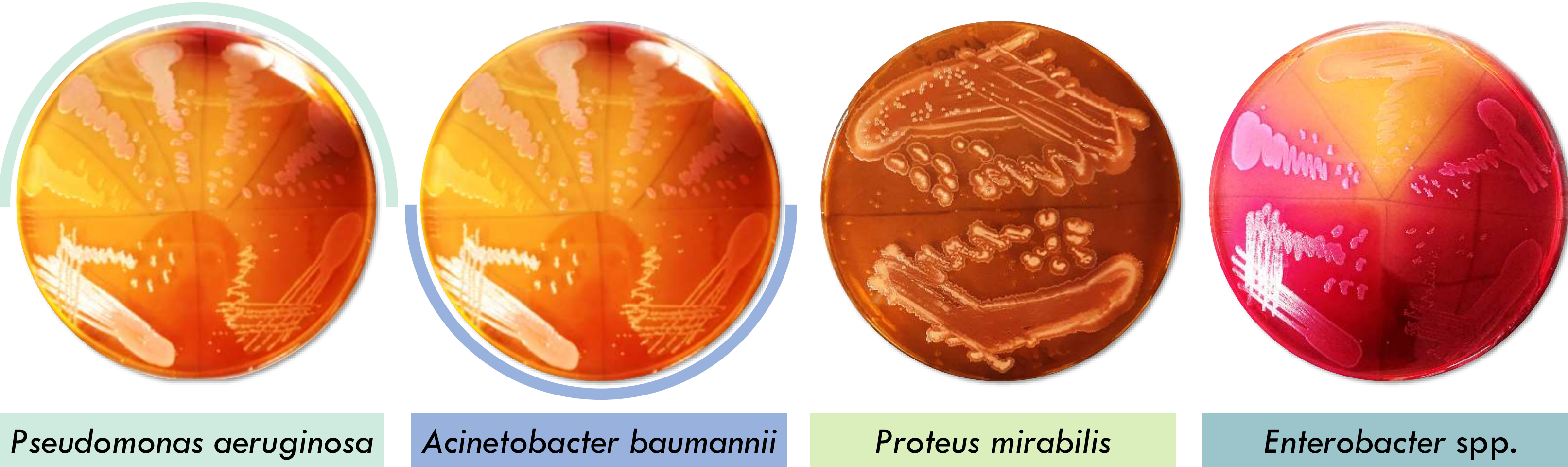


CONCLUSION

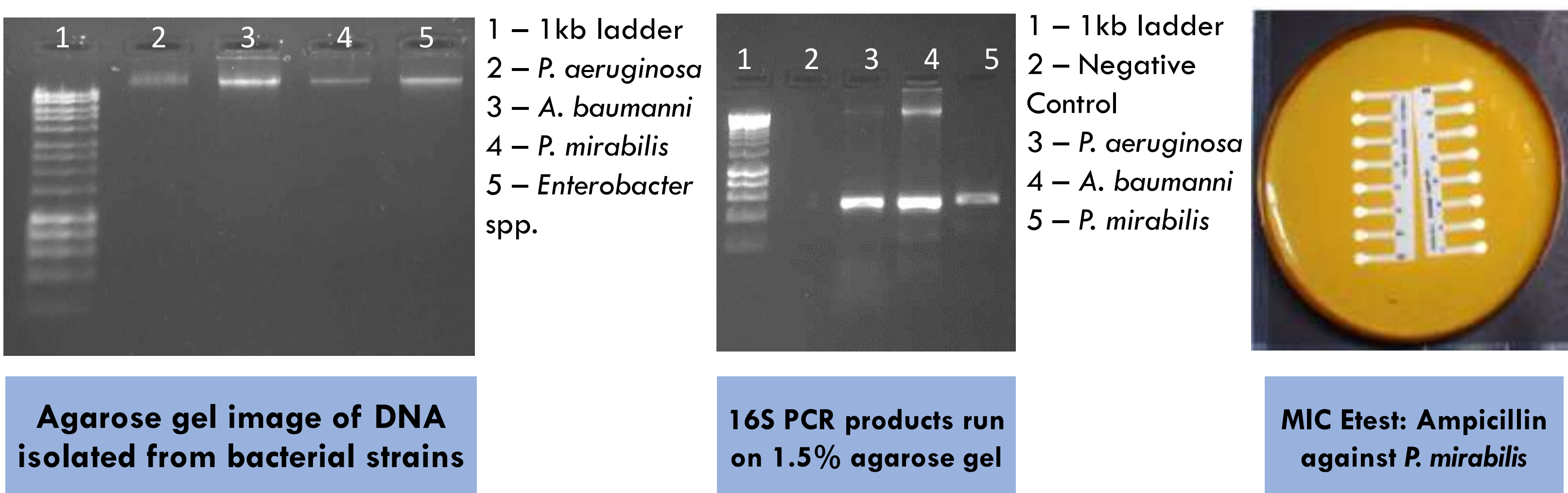
- Development of this assay will provide us with a rapid, sensitive, and specific culture-independent method that will provide information on the drug-resistance of the bacterial pathogens
- The creation of a solid phase system will be better suited for the purpose of clinical application.
- The system will facilitate rapid testing for DFU, thereby possibly preventing amputations.

RESULTS

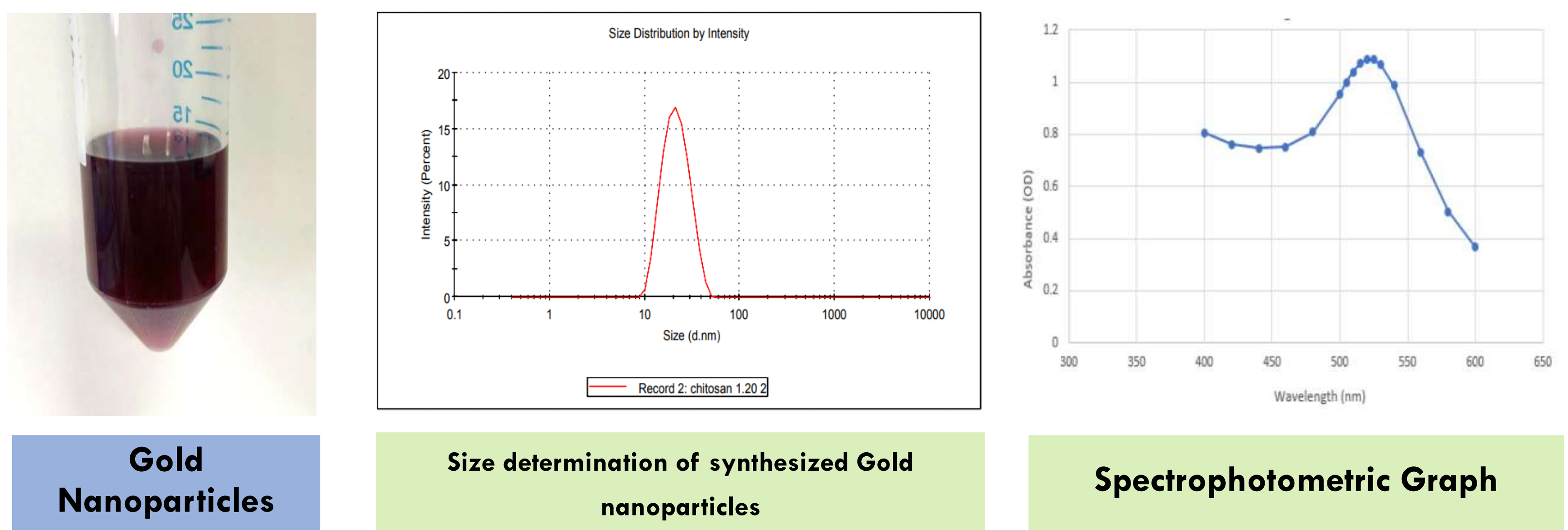
1. Chosen Bacterial isolates



2. Gel Electrophoresis of Isolated DNA, 16S rRNA amplification, and MIC



3. Gold Nanoparticle Characterization



REFERENCES

- Dias JT, Svedberg G, Nystrand M, Andersson-Svahn H, Gantelius J. Rapid nanoprobe signal enhancement by *in situ* gold nanoparticle synthesis. *JoVE*. 2018;57297.
- Ferri M, Ranucci E, Romagnoli P, Giaccone V. Antimicrobial resistance: A global emerging threat to public health systems. *Crit. Rev. Food. Sci. Nutr.* 2017;57:2857-2876.
- Jnana A, Muthuraman V, Varghese VK, Chakrabarty S, Murali TS, Ramachandra L et al., Microbial community distribution and core microbiome in successive wound grades of individuals with diabetic foot ulcers. *Appl. Environ. Microbiol.* 2020;86:1-14.
- Murali TS, Kavitha S, Spoorthi J, Bhat DV, Prasad AS, Upton Z, et al. Characteristics of microbial drug resistance and its correlates in chronic diabetic foot ulcer infections. *J. Med. Microbiol.* 2014;63:1377-85.

ACKNOWLEDGEMENT

Manipal School of Life Sciences, MAHE, for providing the opportunity, infrastructure and research support required to carry out this project.