Biochemically and Molecularly Characterization of Multi-Resistant Bacteria to Antibiotics of the ESKAPE Group from Hospital Wastewater and the WWTP of Panama.

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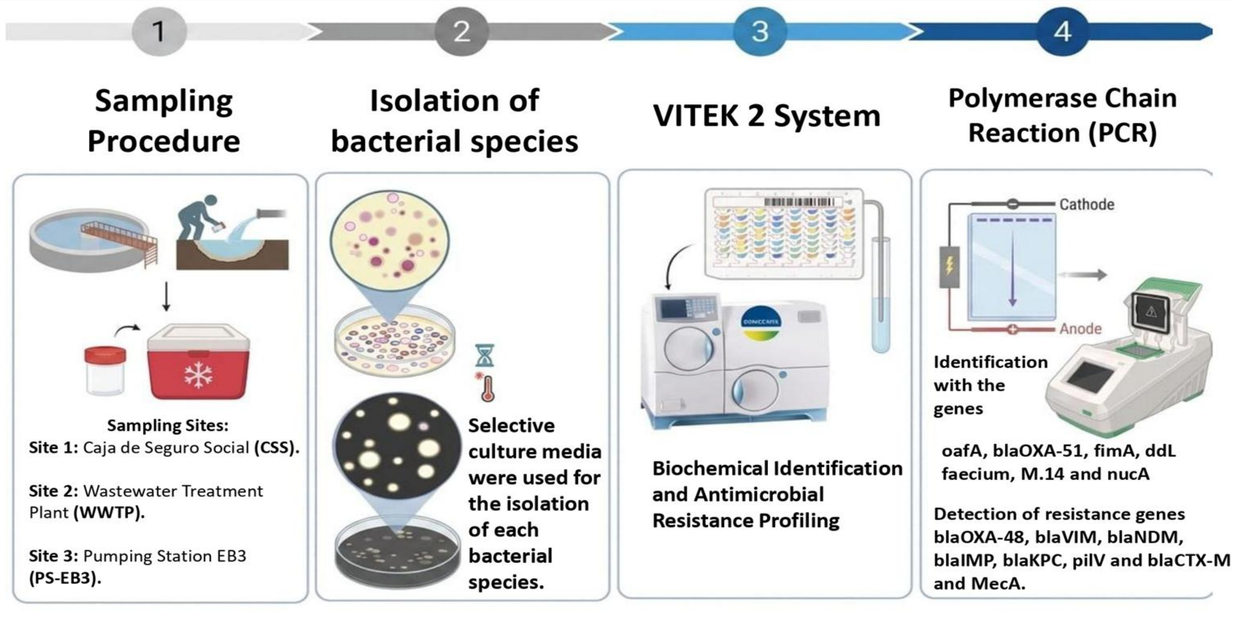
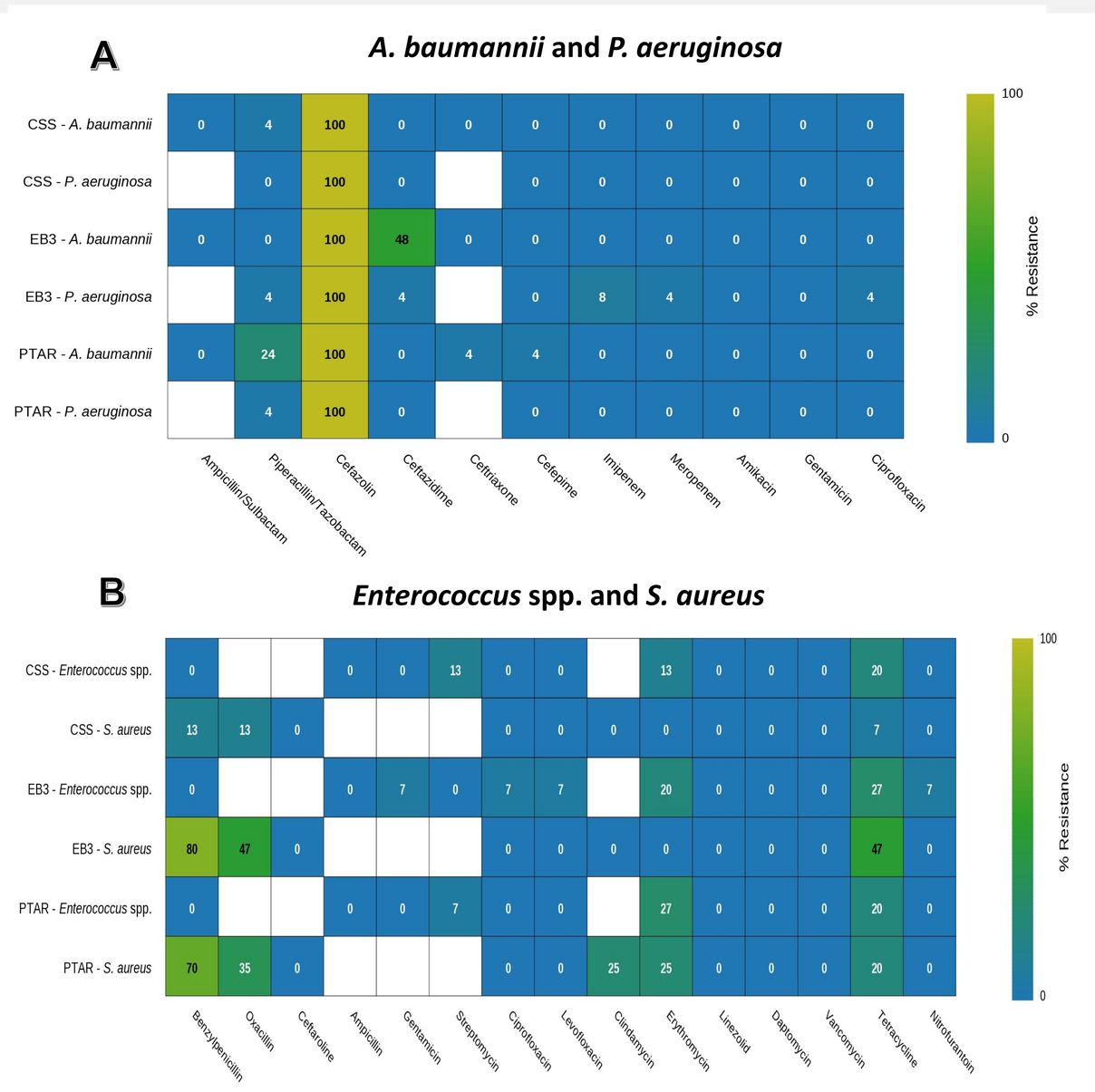
Introduction

Antimicrobial resistance (AMR) is a critical threat to global public health(2). Consequently, the WHO has prioritized the study of the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.), pathogens renowned for their ability to evade multiple drugs. However, this issue transcends the clinical setting; untreated wastewater has been identified as a critical reservoir and a strategic point for the environmental dissemination of these microorganisms and their genetic determinants(1).

Objective: The aim of this study was to characterize biochemically and molecularly the ESKAPE group and *Serratia marcescens*, as well as to determine the antimicrobial resistance profiles of strains isolated from hospital effluents and the Wastewater Treatment Plant (WWTP) of Panama City.

Materials and Methods





Results

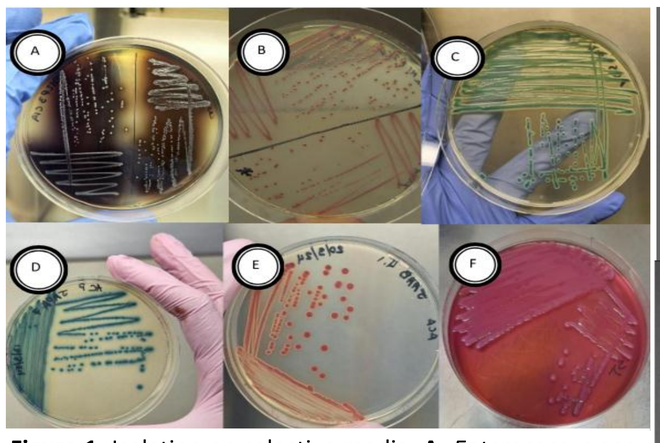


Figure 1. Isolation on selective media: **A.** *Enterococcus spp*. on Bile Esculin Agar**. B.** *S. aureus* on CHROMagar Staph. **C.** *Serratia marcescens* on CHROMagar Serratia. **D.** *Pseudomonas aeruginosa* on CHROMagar Pseudomonas. **E.** *Acinetobacter baumannii* on CHROMagar Acinetobacter. **F.** *Klebsiella spp.* on EMB agar.

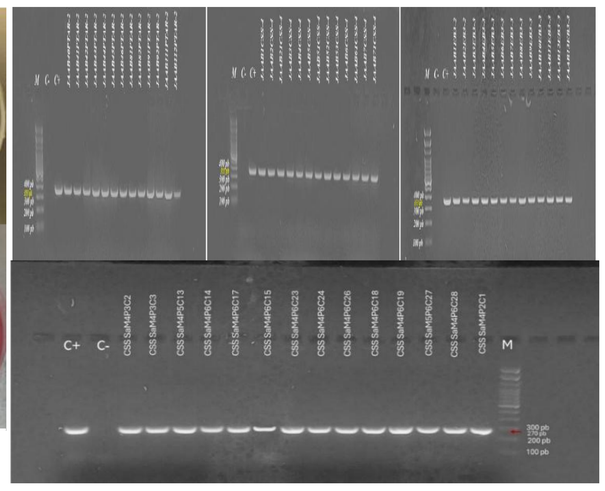


Figure 2. Detection of blaOXA-51 (353 bp) and nucA (270 bp) genes for the identification of *A. baumannii* and *S. aureus,* respectively.

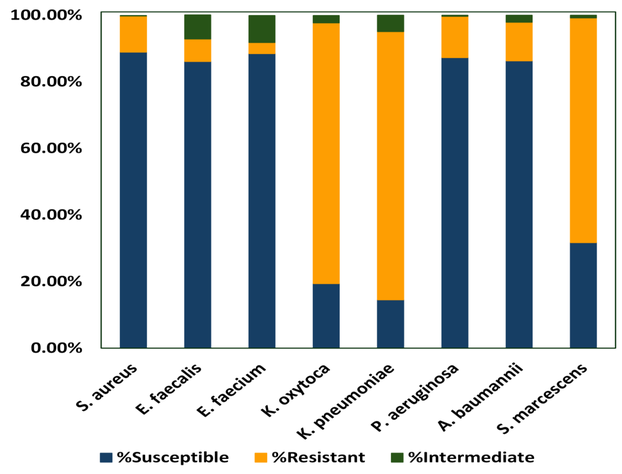


Figure 3. Antimicrobial resistance distribution by species (Vitek 2 System)

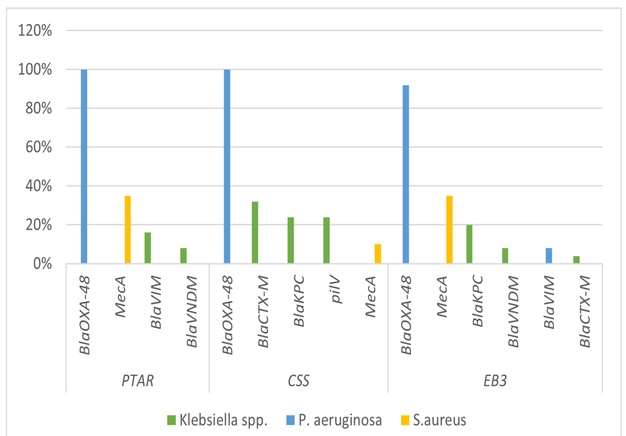


Figure 4. Distribution of antimicrobial resistance genes by pathogen (ESKAPE Grupe)

Figure 5. Antimicrobial resistance percentage by species and sampling site

Antimicrobial resistance profile by species and sampling site. Distribution per site: 25 strains of *Acinetobacter spp., P. aeruginosa, and Klebsiella spp*. Specific counts include *S. marcescens* (WWTP: 5, EB3: 4), *S. aureus* (WWTP: 20, EB3/CSS: 15), and 15 *Enterococcus spp*. strains across all locations.

Conclusions

- ESKAPE group isolates were obtained from the three sampling sites. Identification was confirmed via biochemical testing and validated through molecular techniques.
- *Klebsiella* spp. and *Serratia marcescens* presented the highest multidrug resistance (MDR) profiles to beta-lactams, ciprofloxacin, and fosfomycin, while resistance to ceftazidime was identified in *A. baumannii*.
- Genotypic analysis validated the MRSA phenotype (*mecA*+) in *S. aureus* and detected *bla*VIM and *bla*OXA-48 carbapenemases in *P. aeruginosa*. *Klebsiella* spp. harbored the highest burden of resistance markers (*bla*KPC, *bla*NDM, *bla*CTX, *bla*VIM, *pilV*), evidencing the dissemination of these resistance mechanisms in hospital effluents and the WWTP.

References

1. Savin, M., Bierbaum, G., Hammerl, J. A., Heinemann, C., Parcina, M., Sib, E., . . . Kreyenschmidt, J. (2020). Antibiotic-resistant bacteria and antimicrobial residues in wastewater and process water from German pig slaughterhouses and their receiving municipal wastewater treatment plants. Science of The Total Environment, 727, https://doi.org/10.1016/j.scitotenv.2020.138788.
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