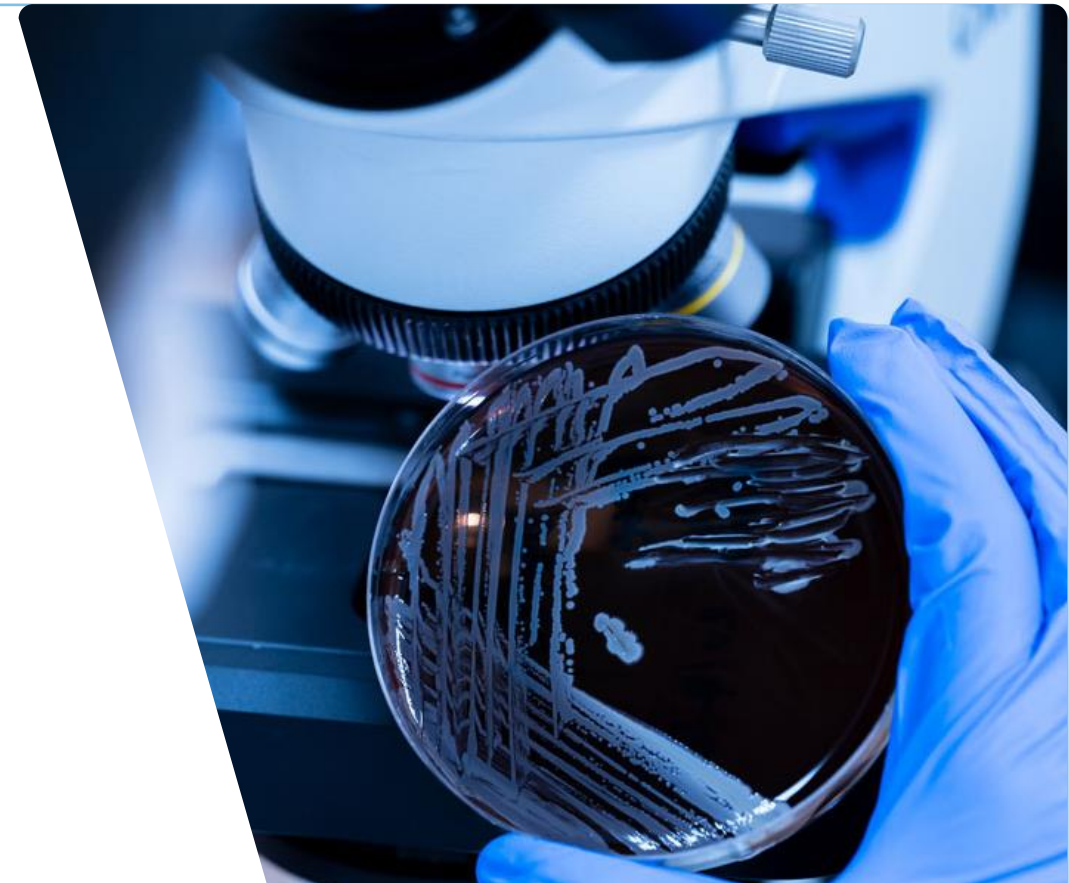


Promising Research on Non-Vaccine Alternatives To Antimicrobials in Africa.

Case Study: Development of COMMI Nanoformulation from Commiphora Plant Resins for Innovative Mastitis Treatment

Gaymary George Bakari

Christian Nkanga & Xin Zhao





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Introduction

Antimicrobial Resistance is a Global One Health threat, with Africa heavily affected.

Introduction

Livestock sector contributes via

- **Overuse** and **misuse of antibiotics.**
- Inadequate **dosing** or **duration.**
- Poor infection control practices.
- Lack of regulation or enforcement.
- Use of antibiotics as growth promoters.

Introduction

Developing a **nanotechnology**-based Herbal alternative such as **Commi nano Teat Infusion** with antibacterial properties to reduce dependence on traditional antibiotics.



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Advantages of encapsulating the resin with nano particles

A

Improves
solubility and
bioavailability
of resin
compounds.

B

Enables
controlled
and targeted
delivery to
infected
udder tissue.

C

Potentially
stable at room
temperature,
easing
logistics in
rural Africa.

D

Enables
controlled
and targeted
delivery to
infected
udder tissue.



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Why Commiphora Resins?



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2009–2022

Awardee of AFNET (PhD) and AESA RISE postdoc fellowship (AAS) to Formulate, validate and promote products from *Commiphora swynnertonii* resin for veterinary and medical uses

Commiphora swynnertonii, a family Burseraceae

- Distributed in Africa and Asia and is among the plant **Treatment of wounds, gastric ulcers, arthritis, Antimicrobial, anticoccidial and anti-inflammatory conditions** (Bakari et al 2011–2022.,)



C. swynnertonii plant



Stem part showing resin
oozing out of the tree



Raw resin





CommiCare





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Development of Commi nano Fractions for treat mastitis (IDRC through Inno Vet 2-AMR project).



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Prevalence of mastitis and AMR in Tanzania



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Preliminary Survey on KAP of Mastitis and AMR.

- The study was conducted in five regions of Tanzania:
 - **Arusha, Tanga, Njombe Iringa and Mbeya**
- Involved 665 respondents (**105 from each regions**).
- **3,858** milk sample collected from different farms
- Training to **665 farmers** on mastitis prevention, **AMR**, good manufacturing practice of milk and milk products and records keeping.



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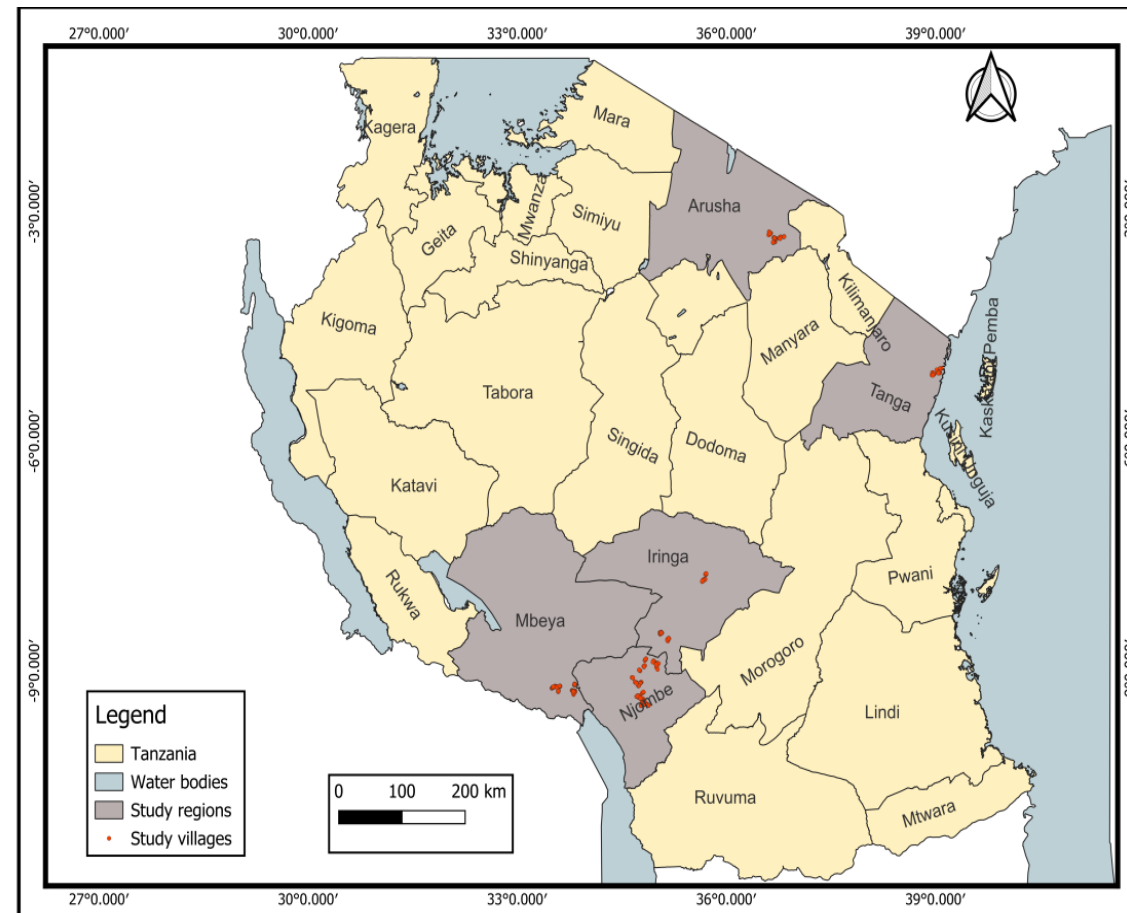
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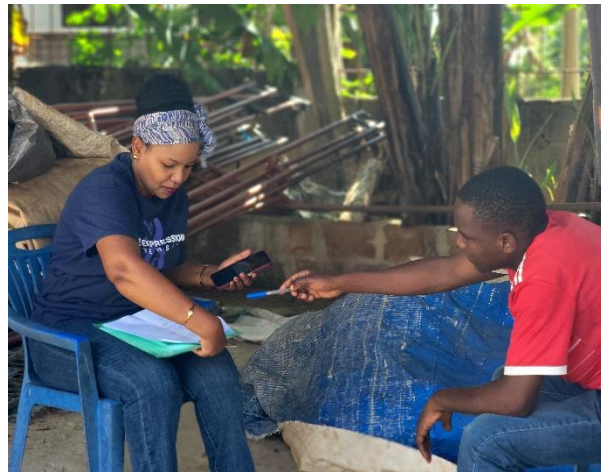


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Tanzania map showing study regions





**Face to face
Interview
To farmers.**



**Focus
Group
Discussion.**



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Sample Collection And CMT Testing



**Provision of
Result
feedback &
training to
Farmers**



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Analysis of sample Collected for Mastitis & AMR.

1. Prevalence of subclinical mastitis (CMT Test)

- **Total animals sampled:** 975
- **Milk samples collected:** 3,858 (from active quarters)
- Samples were collected **aseptically** using **CMT paddles**
- **CMT Results:**
 - **Positive for mastitis:** 1,425 quarters (**36.94%**) and
Negative: 2,433 quarters (**63.06%**)
- **Mastitis prevalence** (based on CMT screening): **36.9%**



**Testing of milk
Using **CMT**
For subclinical
Mastitis.**

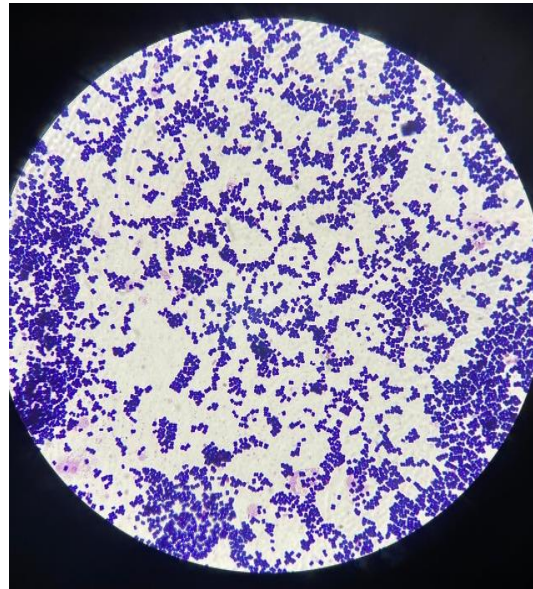
2. Bacteria culture and Isolation.

- **Total animals sampled:** 975
- **Milk samples collected:** 3,858 (from active quarters)
- A total of 400 Staphylococcus spp and 100 Coliform isolated from by using culture method **(12.9%)**
- Out of 400 Staphylococcus spp 182 were confirmed Staphylococcus aureus
- Out of **100** Coliforms, **8** were Escherichia coli.

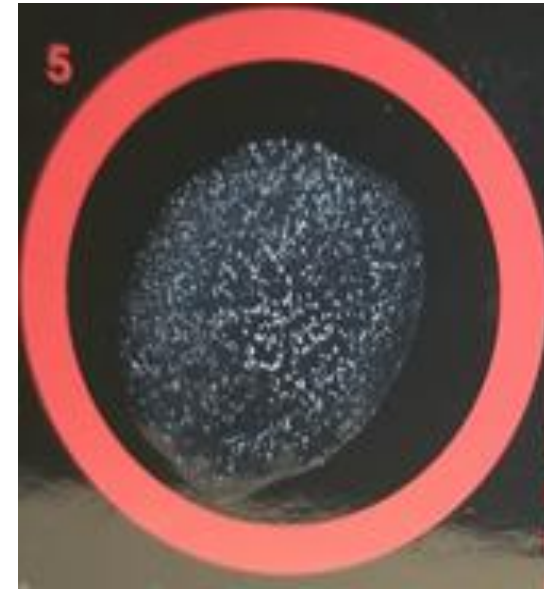
Isolation of *Staphylococcus aureus*



Isolation of
Staphylococcus spp
on Mannitol salt agar



Confirmation by Gram
Stain slide showing
Staphylococcus spp



Coagulase test differentiate
Staphylococcus aureus
using Staphrex reagent

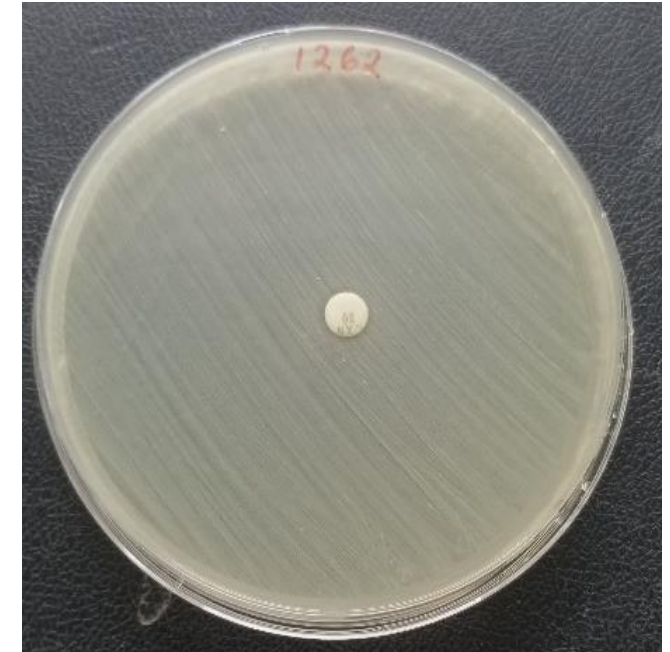
3. Antimicrobial susceptibility test



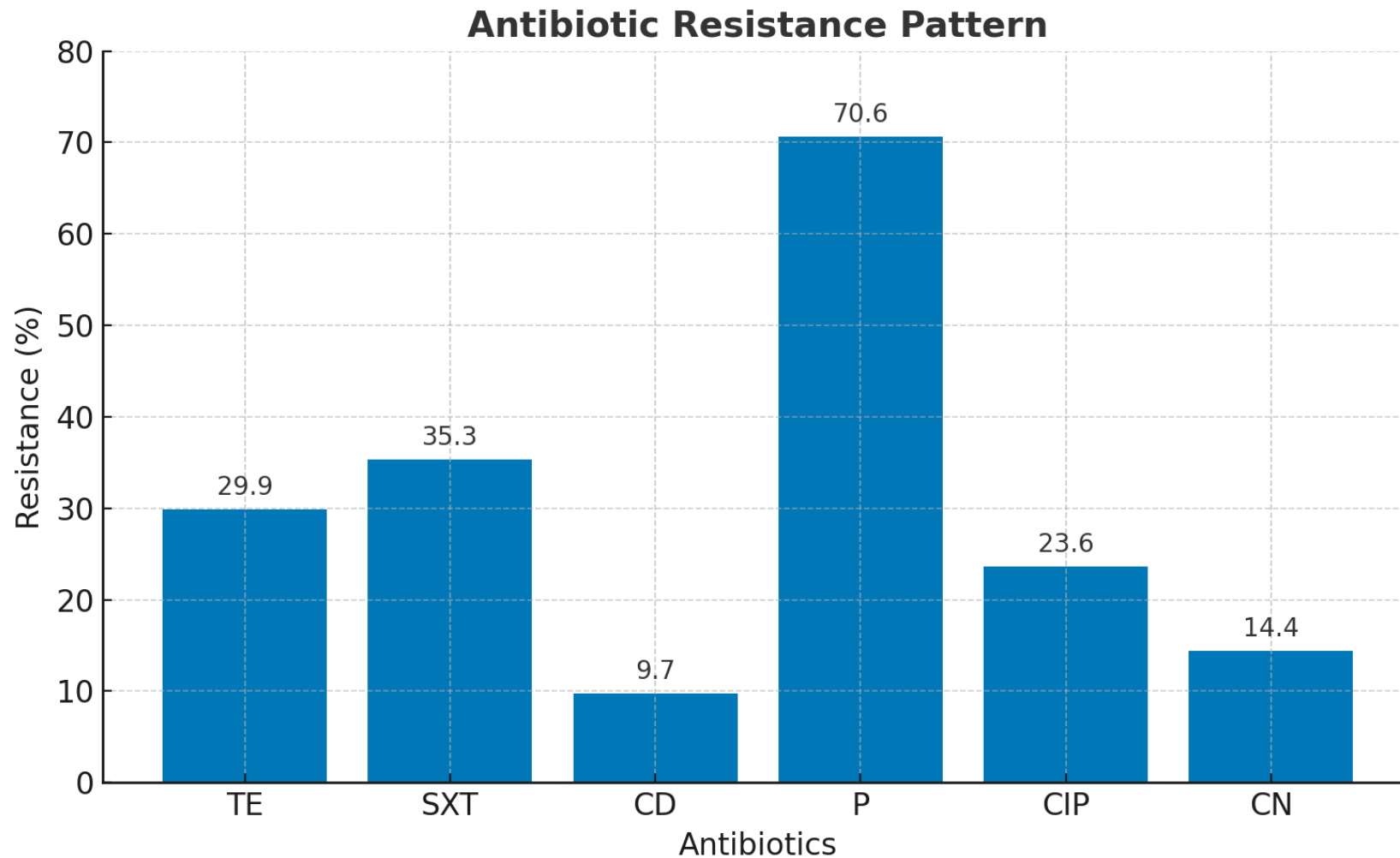
MDRS showing Inhibition Zone.



AST showing Inhibition Zone.



MRSA using Cefoxitin.



Penicillin (P) with the highest resistance (70.6%) and Clindamycin (CD) with the lowest (9.7%).

Figure 2: Percentage of antimicrobial resistance among bacterial isolates obtained from milk samples.



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4. PCR amplification of Staphylococcus spp 16S rRNA gene



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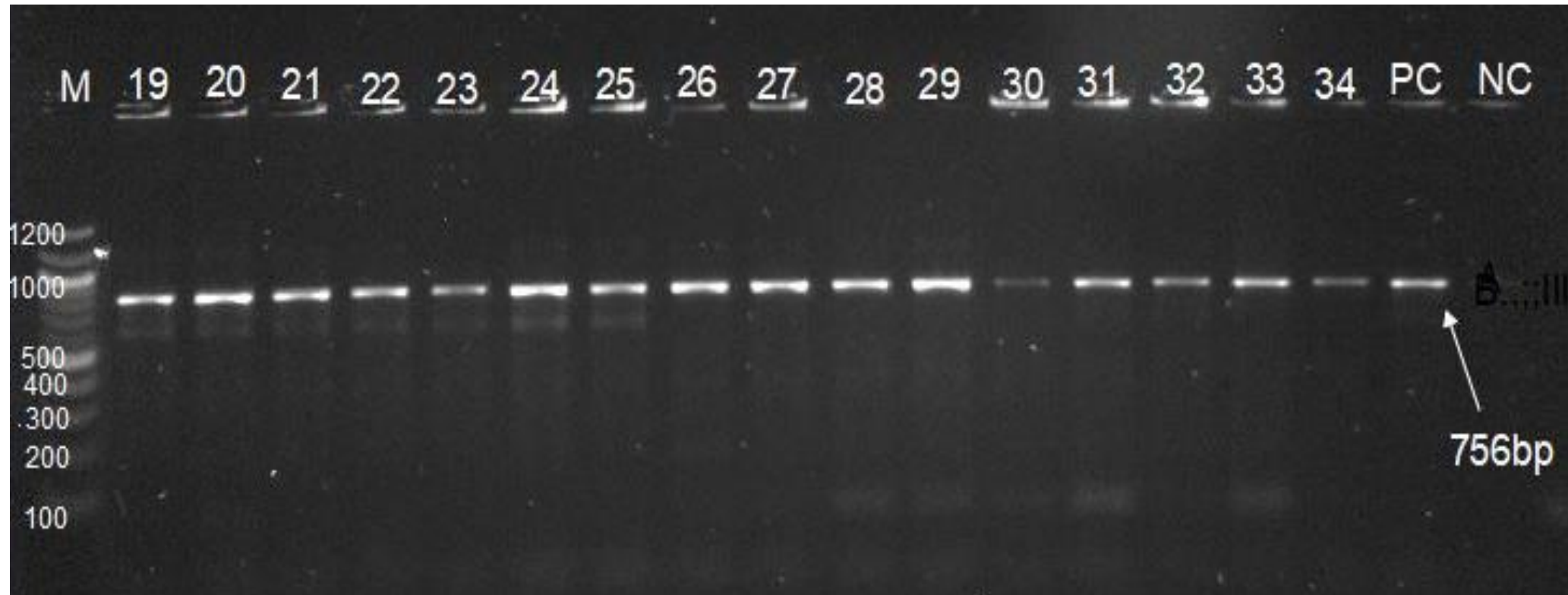
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Figure: PCR amplification of Staphylococcus species 16S rRNA gene. M is a 100bp marker and lanes 19 to 34 are representatives of positive samples, NC is negative control and PC is Positive Control.





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Validation of Commi nano Fractions in vitro particles Tested on bacterial culture.



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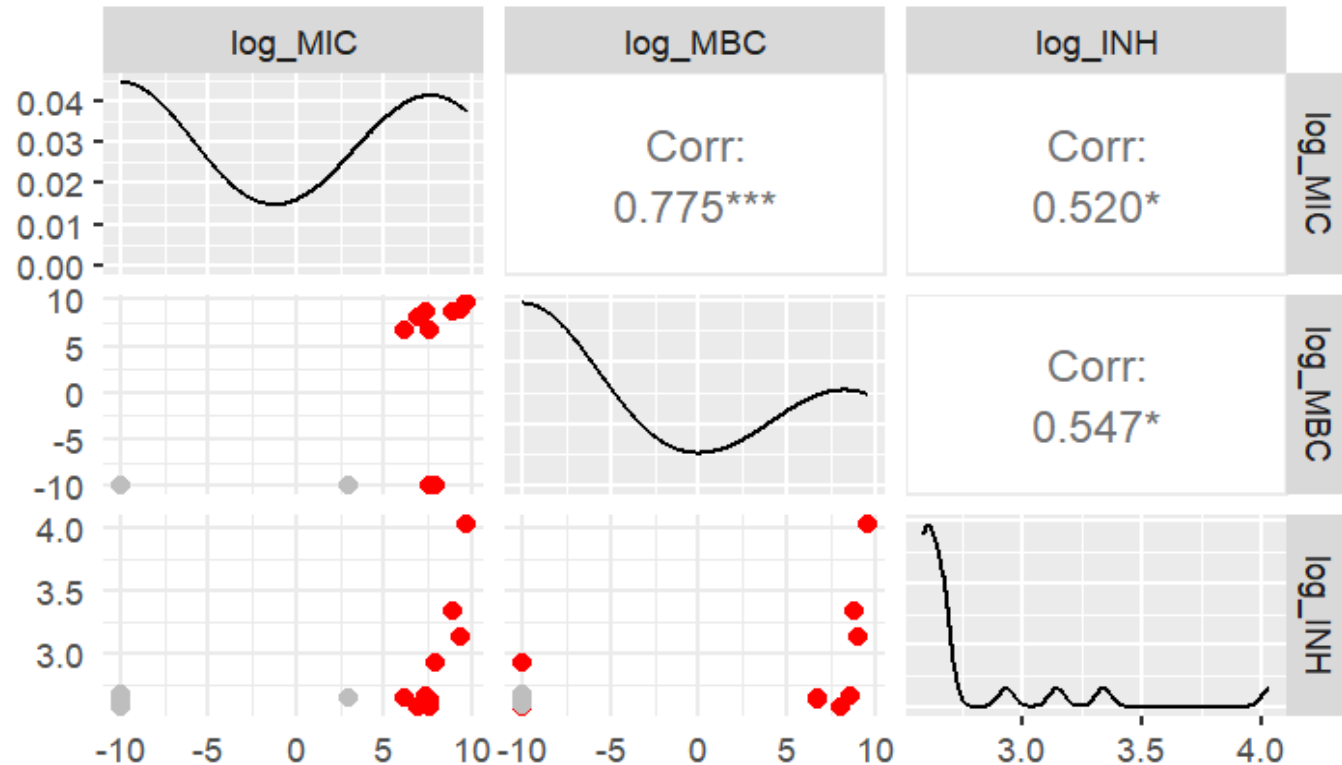
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Antimicrobial Sensitivity and Potency Activity of 20 Drugs Against *S. aureus*



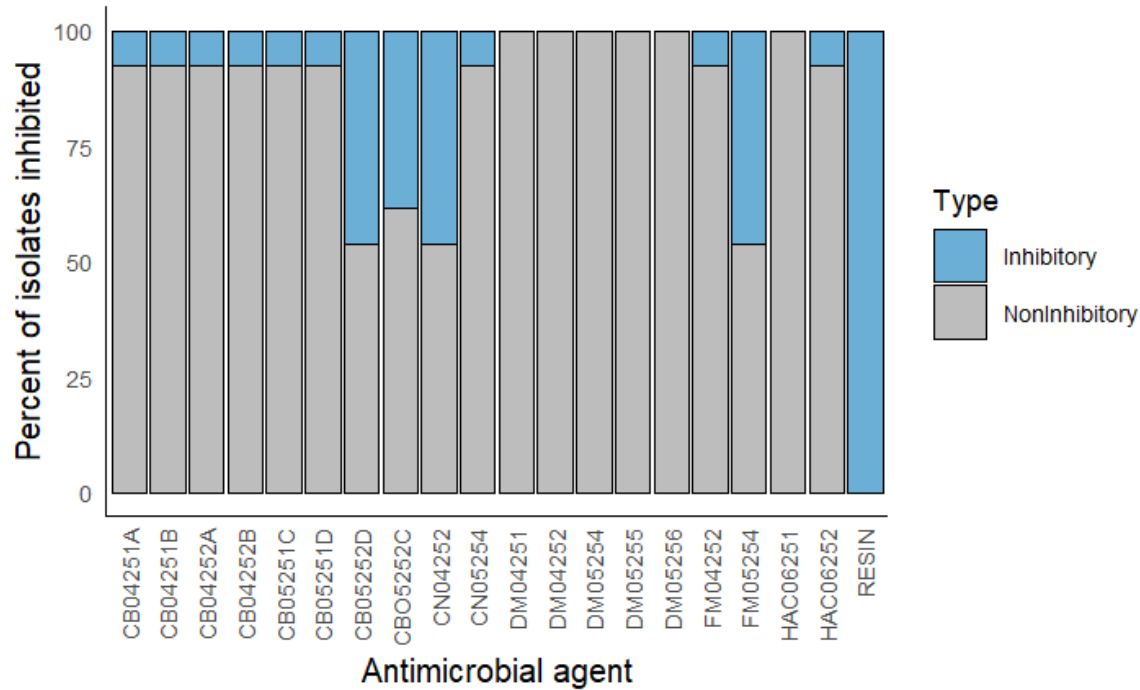
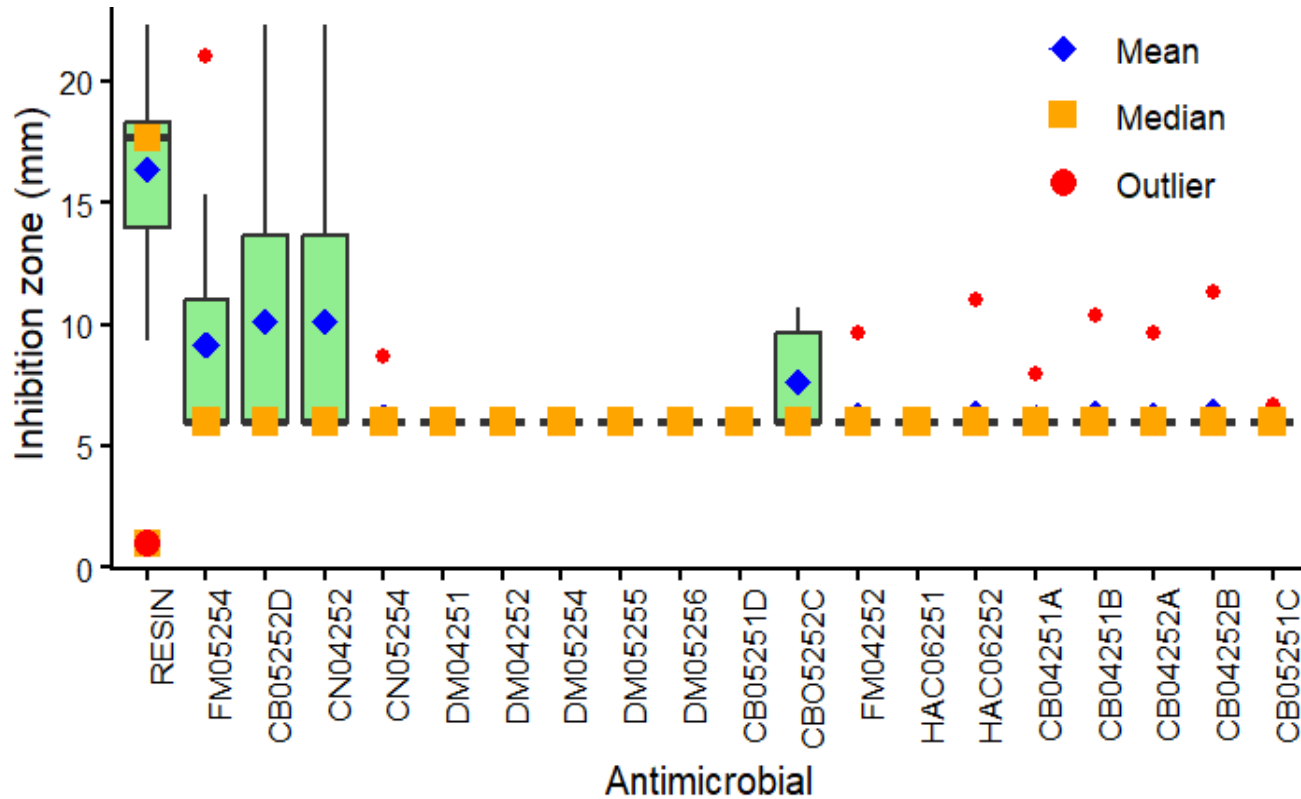


Fig 1: Bar graph showing the proportion of IHZ values >6 across antimicrobials, with Resin exhibiting the highest frequency against *S.aureus*

Key Findings – Frequency of Inhibition results

- FM05254, CB05252D, CN04252 each inhibited 46.2% of isolates.
- CBO5252C inhibited 38.5%, while several others showed only 7.7% inhibition.
- DM04251, DM04252, DM05254–DM05256, HAC06251 showed no inhibition.



Key Findings – Descriptive/ Distribution of INH zone values

**[CB05252D=CN04252,] ›
FM05254 › CB05252C showed
inhibition**

Fig 2: Box plots showing distribution of IHZ values across antimicrobials, with Resin exhibiting the highest IHZ against *S. aureus*

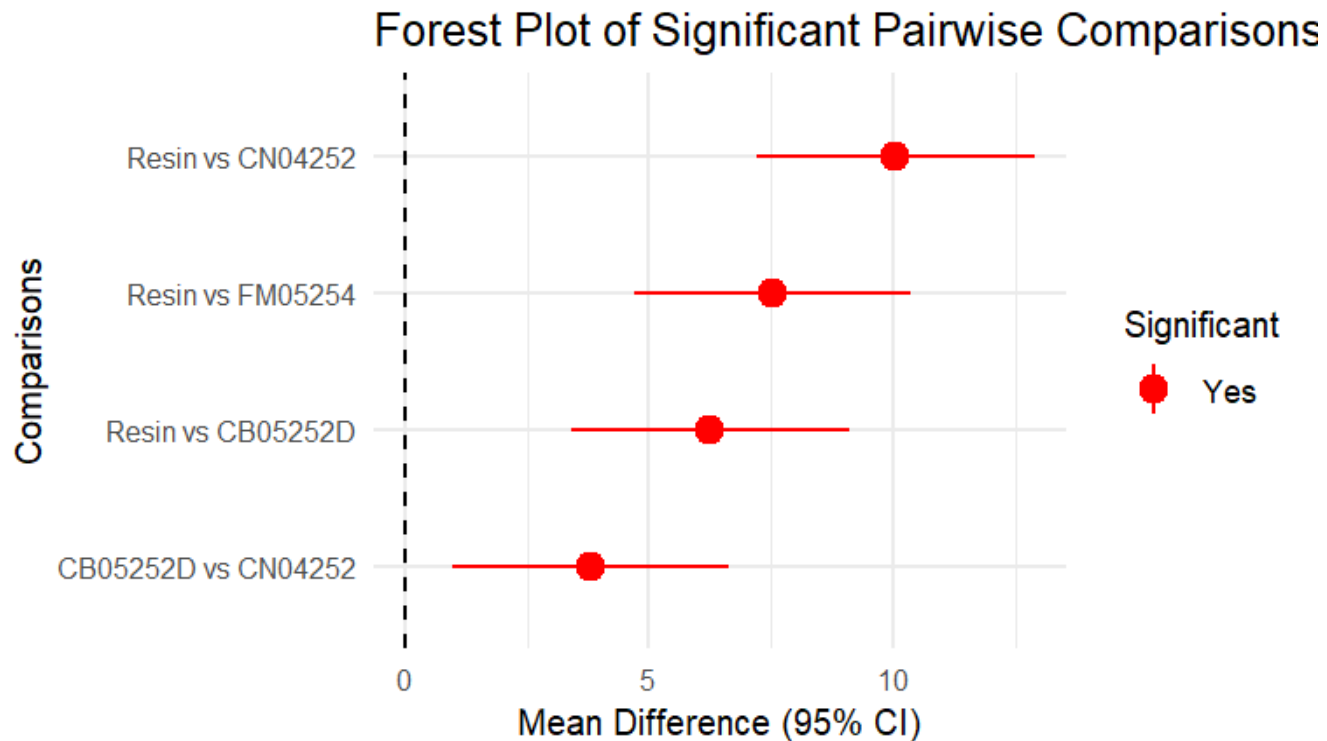


Fig 3: Analysis of Variance (ANOVA) and multiple comparison test results showing significant differences in IHZ values across antimicrobials, with Resin exhibiting the highest and most consistent inhibitory effect against *S. aureus*.

Key Findings – Comparative inhibition results

ANOVA showed significant variation among antimicrobial agents ($P < 0.0001$).

Resin differed **most significantly** from all others (mean diff. **6.26–10.36**, $P < 0.001$).

Moderate differences observed among **FM05254, CB05252D, CN04252, CN05254** (mean diff. **1.2–4.1**).

Minimal or non-significant differences found within **DM, CB, and HAC** groups, indicating **similar effects**.

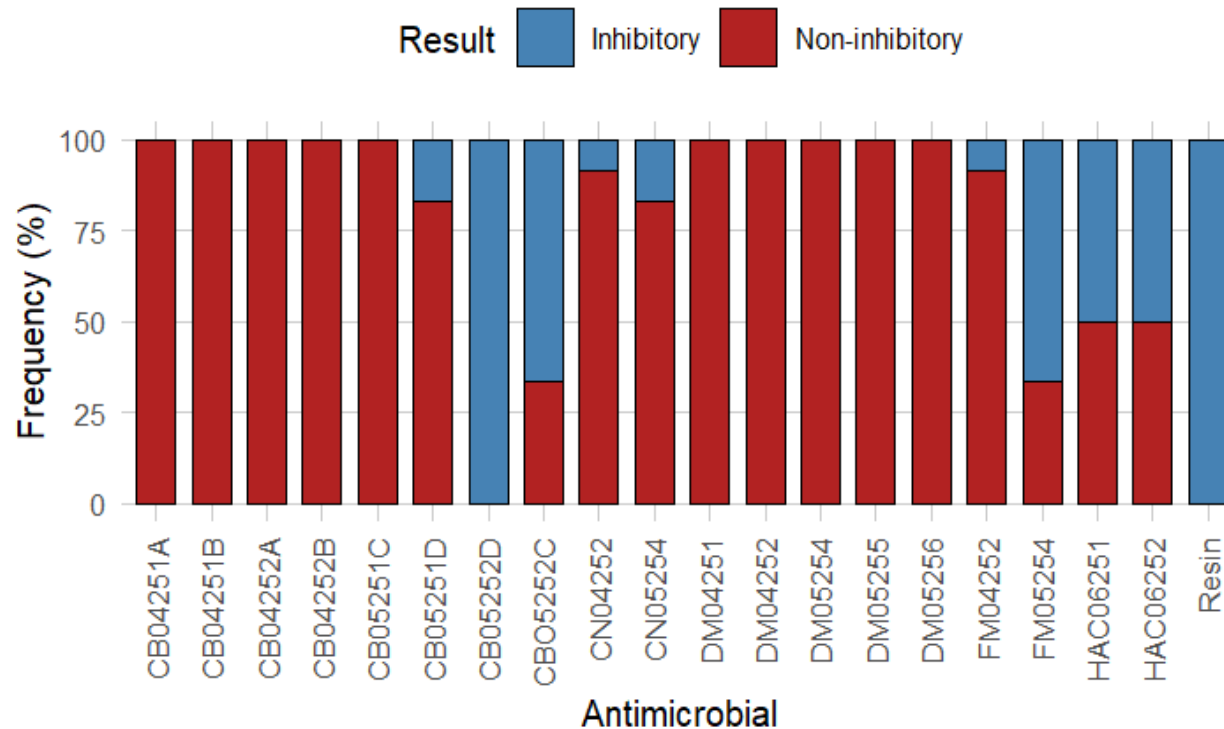


Fig 4: Bar graph showing the proportion of MIC values >0 across antimicrobials, with Resin & CB05252D exhibiting the highest frequencies against *S.aureus*

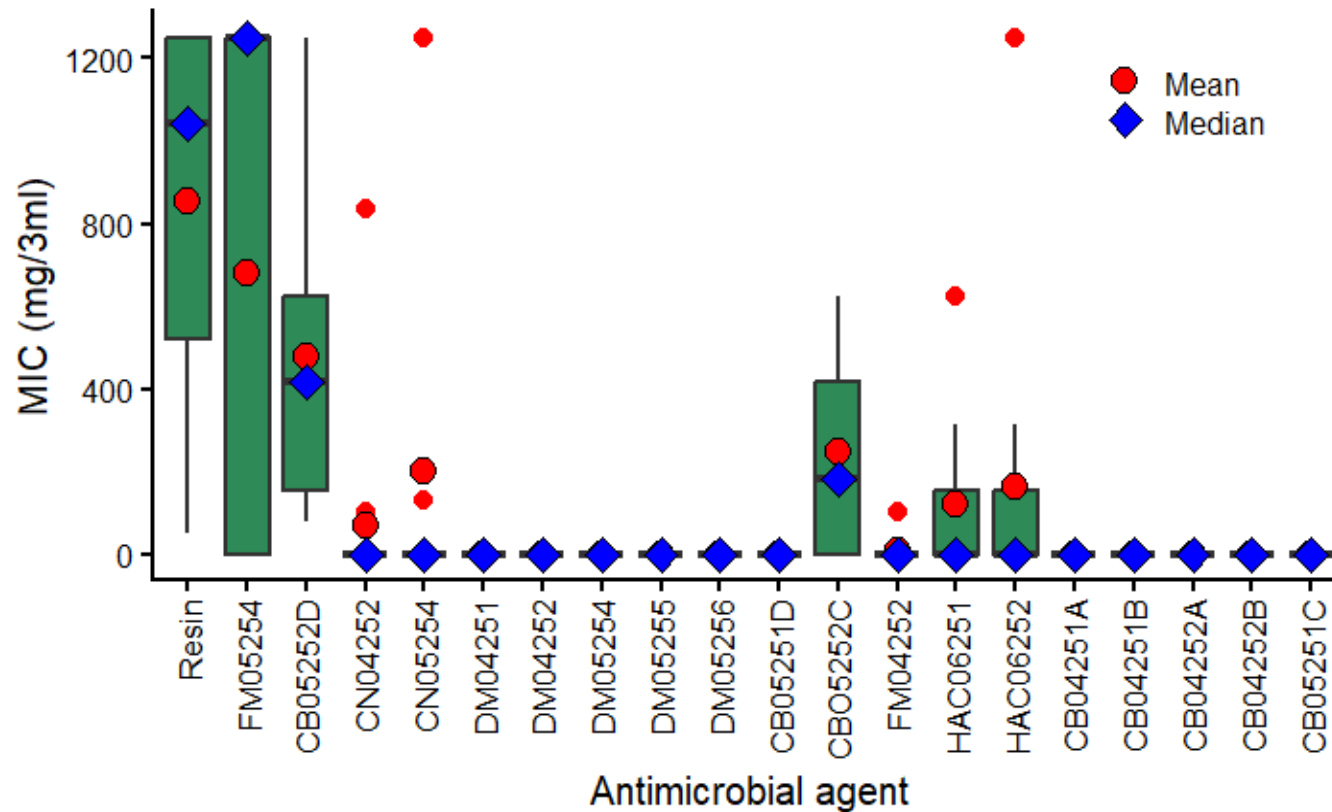
Key Findings – Frequency of MIC results

7 Nano resin fractions showed inhibitory effects

**CB05252D > CBO5252C >
FM05254, > HAC06251 > HAC06252**

**CB05251D, CN04252, CN05254 &
FM04254 : inhibited $\leq 23\%$**

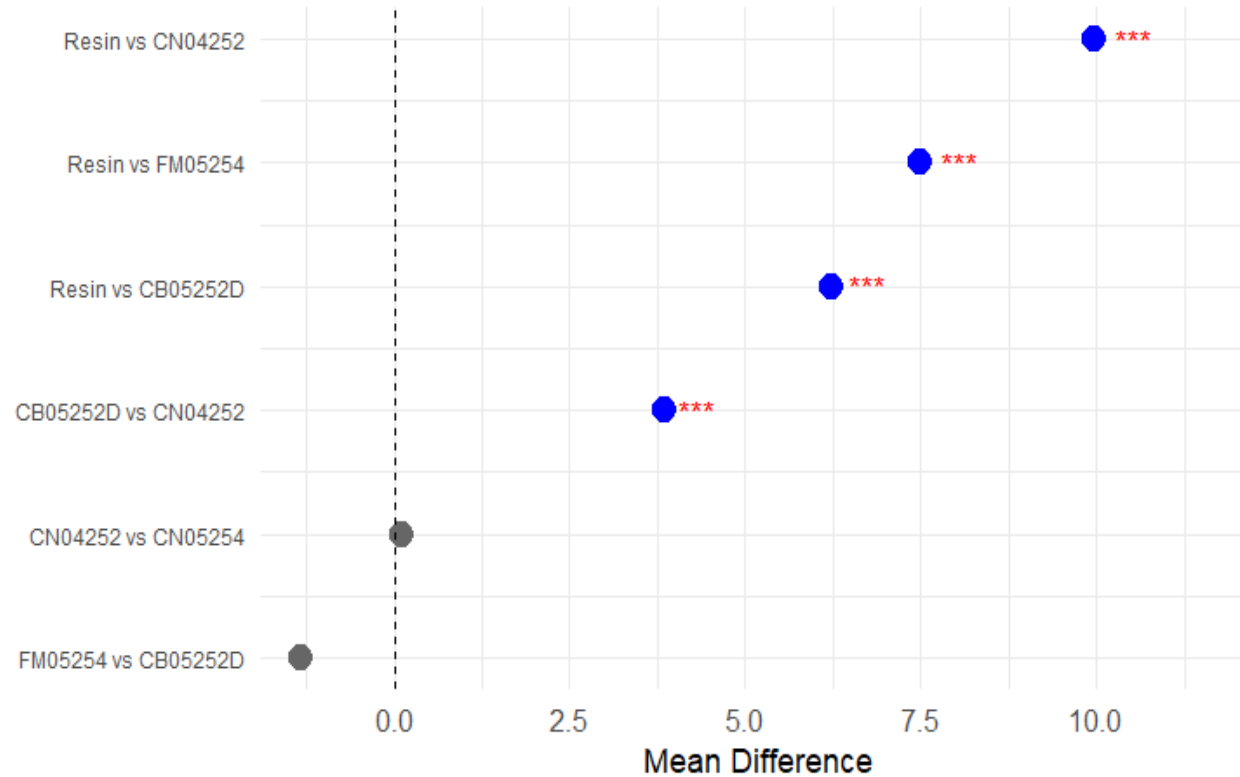
Others = None



Key Findings – MIC Distribution and Potency Against *S. aureus*

Most potent antimicrobials: HAC06251, & HAC06252 – strong inhibition at **very low MICs**.

Fig 5: Box plots showing distribution of MIC>0 values across antimicrobials



Key Findings–ANOVA and Pairwise Comparison of MIC Values Across Antimicrobials

ANOVA: Significant variation among tested drugs (**P < 0.0001**)

Overall: CB05252D & CN04252 were different.

Fig 6: Analysis of Variance (ANOVA) and multiple comparison test results showing significant differences in MIC values across antimicrobials, with Resin driving the highest effect (and less potent)

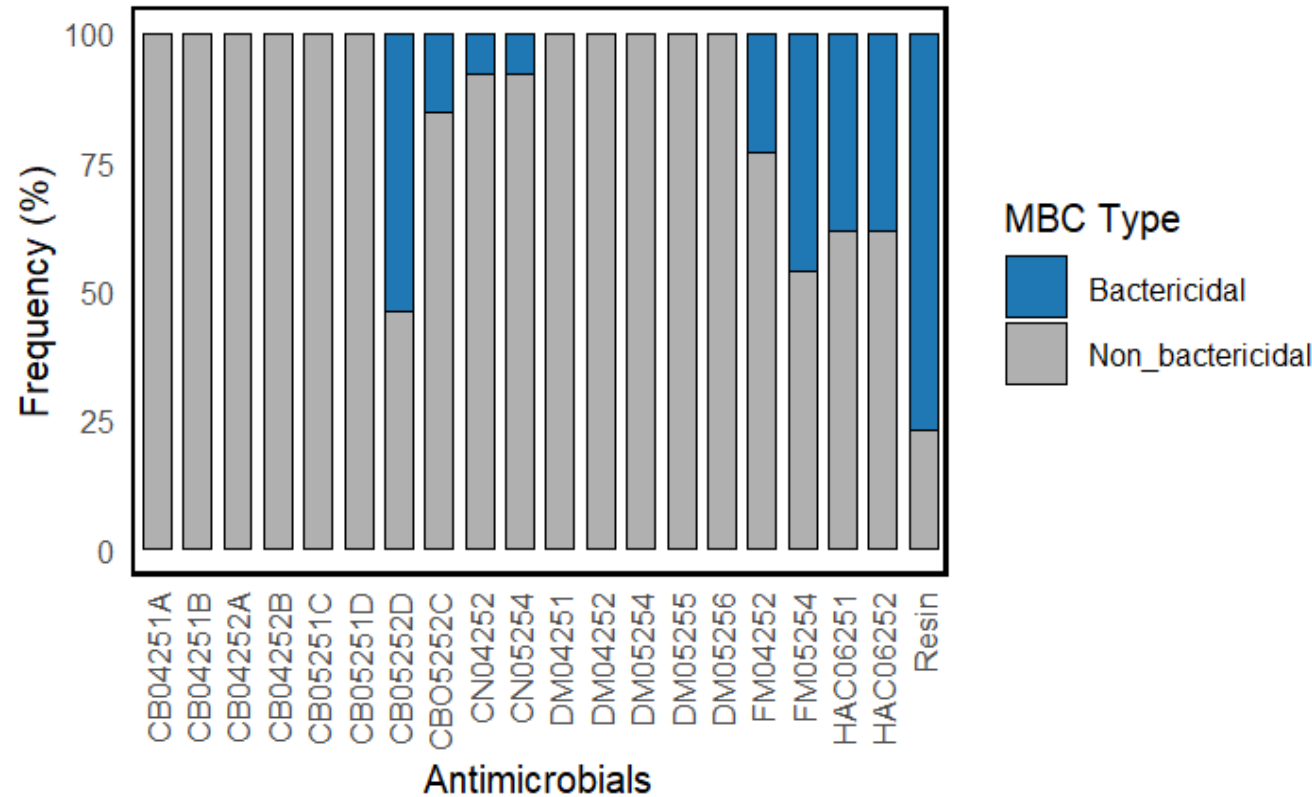


Fig 7: Bar graph showing the proportion of MBC values >0 across antimicrobials, with Resin & CB05252D exhibiting the highest frequencies against *S.aureus*

Key Findings–Bactericidal Activity of Antimicrobials Against *S. aureus* (MBC)

CB05252D and FM05254: – 54% and 46% respectively.

CB05252C, CN04252, CN05254:
minimal activity – 8% inhibition.

**CB04251B, CB04252A/B,
CB05251C/D, DM04251–DM05256:
no bactericidal effect**

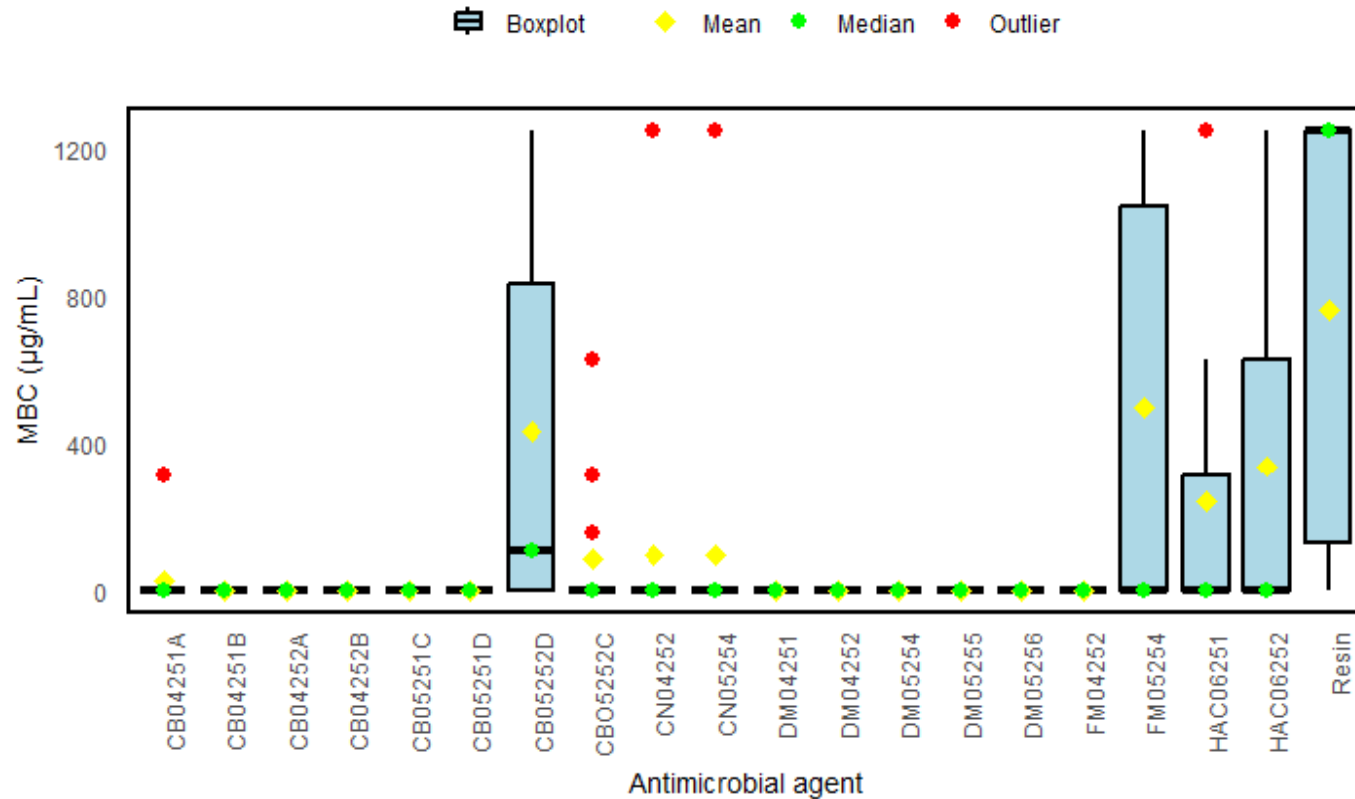


Fig 8: Box plots showing distribution of MBC values against *S. aureus* across antimicrobials,.

Key Findings – MIC Distribution and Potency Against *S. aureus*

Most potent antimicrobials: HAC06251, & HAC06252 – strong inhibition at **least low MBCs**.

Least potent: Resin – highest mean MIC (763.2 mg/mL), indicating **lowest inhibitory effect**.

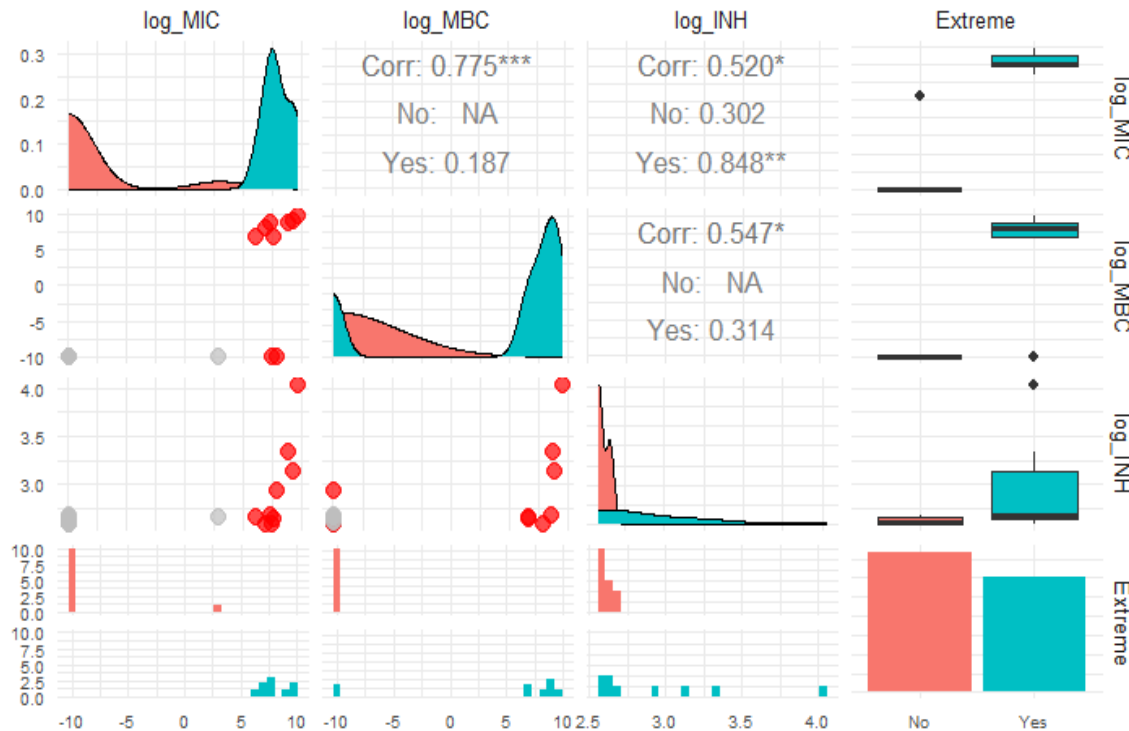


Fig 9: “Pairwise correlation of log2-transformed MIC, MBC, and INH values for 20 antimicrobials against 13 *S. aureus* isolates. Points in red indicate ‘extreme’ antimicrobials with the top 25% of values, representing drugs requiring higher concentrations to inhibit/kill and thus lower potency. MIC and MBC show strong positive correlation, while INH correlates moderately with both,

Key Findings – MIC Distribution and Potency Against *S. aureus*

Most antimicrobials clustered at low concentrations (**gray points**).

A few high-concentration drugs (**red points**) as extremes: Resin, FM05254, CB05252D, CN04252, CN05254, CB05251D, CBO5252C, HAC06251, HAC06252

Strong correlation between **MIC** and **MBC**.

Moderate correlation of **INH** with the other two.

Key Findings–Antimicrobial Assay Performance

ROC Curve: MIC & MBC vs INH

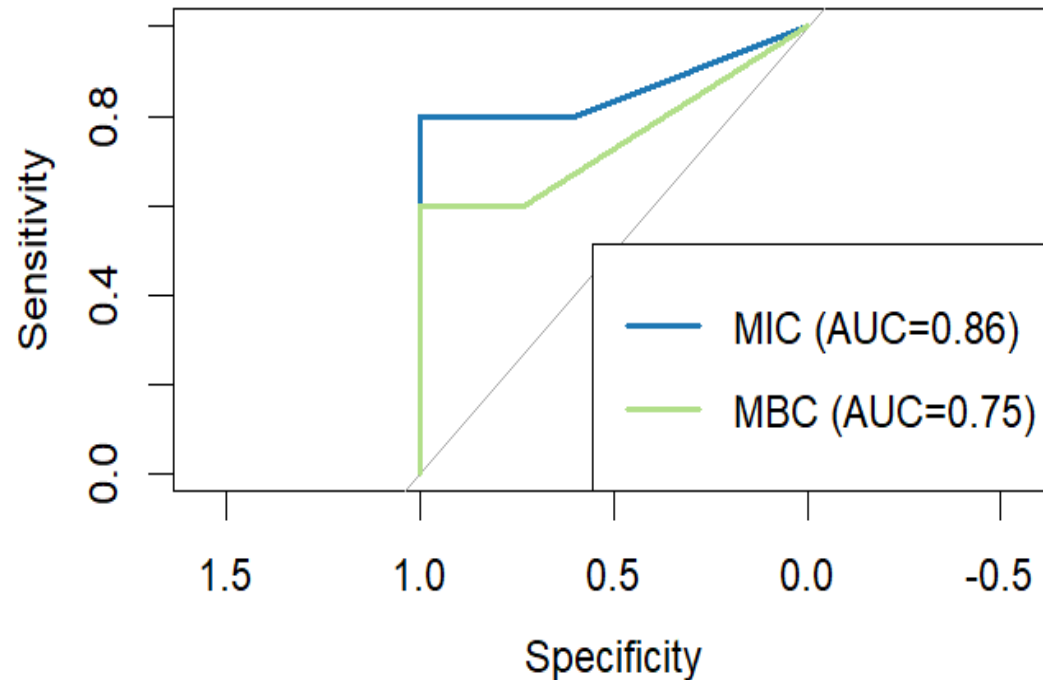


Fig 10: Receiver operating characteristic (ROC) curves for MIC and MBC compared with INH as the reference standard. The MIC curve (blue) demonstrates higher discriminative ability (AUC = 0.86) than MBC (green, AUC = 0.75).

Proportion inhibited: INH = 60%, MIC = 50%, MBC = 35%.

ROC performance: MIC AUC = 0.86, MBC AUC = 0.75 – MIC better discriminates inhibitory isolates.

Kappa agreement with INH: MIC = 0.21 (fair), MBC = 0.17 (slight).

Takeaway: MIC shows **better overall performance and alignment** with the INH reference than MBC.



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In vivo trials for validation of nano fractions using **rat model.**



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Female pregnant and new born rats



Lactating rat showing teats





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**Prof. Christian Nkanga, UNIKIN
(christian.nkanga@unikin.ac.cd)**

**Prof Xin Zhao, McGill University Canada
(xin.zhao@mcgill.ca)**



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Any Question ?



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THANK YOU