Summary
6. SUMMARY

- Totally 126 clinical wound isolates of *S. aureus* were collected from four medical centres in Erode, Salem, and Coimbatore districts. These were categorized by different age groups and different types of wound.

- Pre-confirmed *S. aureus* isolates were further conformed by lipolytic activity using LSM agar, Baird Parker Agar (BPA), Vogal Jonson Agar and coagulase test using tube and slide method.

- 23 antibiotic discs were used for antimicrobial susceptibility tests against *S. aureus*. Of 126 isolates, 82 Multi Drug Resistant (MDR) strains and 90 different antibiotic patterns were identified.

- CHROM agar *S. aureus*, Oxacillin Resistant Screening Agar Base (ORSAB), Baird Park Agar (BPA), Mannitol Salt Agar (MSA), Blood Agar (BA) and Mueller Hinton Agar (MHA) media with appropriate antibiotics were used for isolation of MRSA. 100% sensitivity was observed in CHROM agar in 48 hrs. Low sensitivity (68.5%) was observed in Mueller Hinton agar.

- Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were carried out for 54 MRSA isolates against ciprofloxacin, oxacillin and vancomycin. None of the MRSA susceptible to ciprofloxacin, 41 and 36 MRSA isolates were resistant to the oxacillin and vancomycin respectively.

- Genotypic expression of 126 *S. aureus* were examined using PCR, among that 54 isolates (53 MDR and 1 NMDR) were positive for *mecA* gene (533 bp) and remaining were negative. Low level of NMDR-MRSA also detected by using *mecA* gene but not in conventional methods.
The correlation study was carried out between major three antibiotics with Methicillin resistant and the presence and absents of mecA gene. In MRSA Ox, Cf, V resistant and the presents of mecA gene were 34, 40 and 39 isolates respectively. Ox, Cf, V sensitive and the presence of mec A gene were 14, 8, and 9 isolates respectively. Methicillin sensitive with presence of mecA gene isolates were observed very low percentage (<3.2%).

12 MDR- MRSA isolates were selected for plasmid profile determination. No molecular variation (21.22 kb) was observed in 12 strains, compared with a standard marker of λ DNA (Eco RI digestion).

Nine plant seeds of Elettaria cardamomum, Mangifera indica, Moringa oleifera, Phoenix dactylifera, Tamarindus indica, Annona squamosa, Artocarpus heterophyllus, Cucurbita maxima and Momordica charantia were selected for antimicrobial screening. Ethanol, methanol, acetone, petroleum ether, and chloroform extracts were assayed against selected 12 MDR-MRSA by agar disc diffusion method. Out of 9, 5 seeds were showed promising results especially in high polar ethanol, methanol and acetone extract of Elettaria cardamomum, Mangifera indica, Moringa oleifera, Phoenix dactylifera, and Tamarindus indica. Minimum zone was observed in chloroform and petroleum ether extracts.

MIC in checkerboard assay, the ethanol, methanol and acetone extract of M. oleifera, M. indica, P. dactylifera, E. cardamomum and T. indica were showed between 0.11 mg/ml to 1.87 mg/ml against 12 MDR-MRSA. M. oleifera and M. indica showed minimum MIC of 0.11 mg/ml.

In time killing assay, ethanol, methanol and acetone extracts of five seeds were showed 99.9% bactericidal for 12 of 12 isolates after 24 hrs at 1xMIC concentration. The MDR-MRSA strain did not respond in 0.5 hrs even at 4xMIC concentration in all the three extracts. These results were concluded that the ki1 by all five seed extracts depends on both time and concentration.
• No mutagenic effect was observed with *S. typhimurium* TA 98 and TA 100 in the presence and absence of the S9 metabolic activation in five seed extracts. No significant colony variation was observed in different concentration (10 and 30 mg/ml) of seed extracts. 2-aminofluorene (AF2) and 2-aminoanthracene (2-AA) mutagens were used as a positive control.

• *Mangifera indica* seed extract was fractionated by column chromatography on silica gel eluted with isopropyl alcohol and ethyl acetate concentration. Fractions were pooled according to their physical similarity and Rf value and anti MDR-MRSA activity was tested by checkerboard assay. Fraction 14-16 showed most effective activity against MDR-MRSA, no activity was observed in fraction 38-45.

• The antibacterial properties of *Mangifera indica* seed suggested its potential usefulness in traditional medicine for the treatment of chronic wound contaminated with MDR-MRSA. The two compounds were purified by HPLC, then structural elucidation of these compounds by Nuclear Magnetic Resonance (NMR), Mass Spectroscopy (MS) and Infrared (IR). The isolated compound -1 which showed antibacterial activity was identified as complex tannin and compound -2 was sucrose.

• *Moringa oleifera* seed also showed good antibacterial activity against MDR-MRSA, but not studied in compound level. So further studies are needed in order to elucidate the mechanisms of action of particular compound and their derivatives.

This research has triggered the subsequent research work aims at evaluating the anti MRSA potential of the plant seeds.