Annexure

Annexure-I. List of abbreviations used

°C	Degree Celsius
h	Hour
µg	Microgram
µl	Microlitre
α	Alpha
β	Beta
γ	Gamma
µM	Micromolar
~	Approximately
A_{620nm}	Absorbance at 620nm
agr	*S*taphylococcal accessory gene regulator genes
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
bap	Biofilm associated protein
BHI	Brain heart infusion
BLAST	Basic local alignment search tool
BLS	Biofilm like Structures
bp	Base pair
CA	Community associated
CFU	Colony forming units
CLSI	Clinical and Laboratory Standards Institute
CLSM	Confocal Laser Scanning Microscopy
cm	Centimetre(s)
CRA	Congo Red Agar
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease

eDNA	Extracellular DNA
EDTA	Ethylene- diamine tetra acetic acid
EMRSA	Epidemic Methicillin Resistant *Stphylococcus aureus*
EPS	Extra Polymeric Substance
Fig.	Figure
g	Gram
i.e.	*id est*
ica	Intercellular adhesion genes
L	Litre
Mar	Multiple Antibiotic Resistance
MDR	Multi Drug Resistant
MEGA	Molecular Evolutionary Genetic Analysis
mg	Milligram
MHA	Mueller-Hinton agar
MIC	Minimum Inhibitory Concentrations
min	Minutes
mL	Milliliter
MLST	Multi-Locus Sequence Typing
MR	Methyl red
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
MSA	Mannitol salt agar
MSCRAMM	Microbial surface components recognizing adhesive matrix molecules
n	Number
nm	Nanometre
NA	Nutrient agar
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
NCCLS	National committee on clinical laboratory standards
OD	Optical Density

PBP	Penicillin-binding protein
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PIA	Polysaccharide Intercellular Adhesin
PVC	Polyvinyl Chloride
PW	Peptone water
rpm	Revolutions per minute
S	Second(s)
SCV	Small Colony Variants
SD	Standard deviation
SEM	Scanning Electron Microscopy
sigB	Sigma factor B
t test	Two sample test
TAE	Tris acetate EDTA
TBE	Tris Borate EDTA
TM	Tube Method
TMS	Tetra methyl Saline
TPH	Total Petroleum Hydrocarbons
UTI	Urinary Tract Infections
UV	ultraviolet
VP	Voges- Proskauer
WT	Wild Type
W/V	Weight/volume

Annexure- II. Reagents and Media used.

(A) Media:

1. Blood Agar (g/L)

Sheep blood agar base	40.5g
Distilled water	1000ml
pH 7.2±0.2 at 25 °C	

Blood agar base was dissolved and autoclaved for 15min at 121 °C and cooled to 45 °C. 10ml of the agar base was dispensed in a petri plate and 5ml agar containing 5% sheep blood was added. The mixture was tilted gently to spread out uniform layer.

2. Brain Heart Infusion agar (g/L)

Beef heart infusion (solids)	5.0g
Peptic digest of animal tissue	10.0g
Calf brain infusion (solids)	12.5g
Dextrose	2.0g
Sodium chloride	5.0g
Di-sodium phosphate	2.5g
Agar	20.0g
Distilled water	1000ml
pH 7.4±0.2 at 25°C	

Desired quantity of the media was dissolved in distilled water maintaining the total volume and autoclaved for 15min at 121 °C and cooled to 45 °C and dispensed in petri plate accordingly.

3. Brain Heart Infusion broth (g/L)

Beef heart infusion (solids)	5.0g
Peptic digest of animal tissue	10.0g
Calf brain infusion (solids)	12.5g

Dextrose	2.0g
Sodium chloride	5.0g
Di-sodium phosphate	2.5g
Distilled water	1000ml
pH 7.4±0.2 at 25°C	

Desired quantity of the broth was dissolved in distilled water maintaining the total volume and autoclaved for 15min at 121 °C.

4. Caesin Agar (g/L)

Caesin A:

Skimmed milk	100g
Demineralized water	1000ml

Caesin B:

Agar	20.0g
Demineralized water	1000ml

Caesin B, was autoclaved 15min at 121 °C and cooled to 45 °C and added to Caesin A, mixed well and dispensed in petri dish uniformly.

5. Congo Red Agar (g/L)

Brain Heart Infusion	3.7g
Sucrose	5.0g
Agar	20.0g
Congo red stain	0.08g

Desired quantity of the media was dissolved in distilled water maintaining the total volume and autoclaved for 15min at 121 °C and cooled to 45 °C and dispensed in petri plate accordingly.

6. Egg yolk agar (g/L)

Nutrient agar	900ml
Egg yolk saline	100ml

For preparing egg yolk saline 4 nos. of Hen egg yolk was mixed with 1000ml saline under sterile condition and filtered. Nutrient agar media was dissolved in water maintaining the total volume and autoclaved for 15min at 121 °C and cooled to 45 °C and mixed with egg yolk saline and dispensed in a petri plate accordingly

7. Mannitol Salt agar (g/L)

Meat extract	1.0g
Peptone	10.0g
D(-) mannitol	10.0g
Sodium chloride	75.0g
Phenol red	0.025g
Agar	20.0g
Distilled water	1000ml

pH: 7.1±0.2 at 25°C

Desired quantity of the media was dissolved in distilled water maintaining the total volume and autoclaved for 15min at 121 °C and cooled to 45 °C and dispensed in petri plate accordingly.

8. Mueller Hinton Agar (g/L)

Meat infusion powder	30.0g
Caesin acid hydrosylate	17.5g
Starch soluble	1.50g
Agar Agar	20.0g

pH 7.3± 2 at 25°C

Desired quantity of the media was dissolved in distilled water maintaining the total volume and autoclaved for 15min at 121 °C and cooled to 45 °C and dispensed in petri plate accordingly.

9. Nutrient Agar (g/L)

Beef extract	10.0g
Peptone	10.0g
Sodium chloride	5.0g
Agar	20.0g
Distilled water	1000ml

pH: 7.3±0.1 at 25°C

Desired quantity of the media was dissolved in distilled water maintaining the total volume and autoclaved for 15min at 121 °C and cooled to 45 °C and dispensed in petri plate accordingly.

10. Peptone water (g/L)

Peptic digest of animal tissue	10.0g
Sodium chloride	5.0g
Distilled water	1000ml

pH: 7.3±0.1 at 25°C

Desired quantity of the media was dissolved in water maintaining the total volume and autoclaved for 15min at 121 °C and cooled to 45 °C and dispensed in a petri plate accordingly.

11. Toludine blue agar (g/L)

Deoxyribonucleic acid (DNA)	0.30g
Calcium chloride	0.11g
Sodium chloride	10.0g
Toluidine blue	0.09g

Tris (hydroxymethyl) amino methane	6.06g
Agar	10.0g
Distilled water	1000ml
pH 9.0±0.2 at 25°C	

Desired quantity of the media was suspended in distilled water and heated to boil to boil for 1 to 2 minutes and dispensed in petri plates.

12. Tributyrin agar (g/L)

Peptic digest of animal tissue	5.0g
Yeast extract	3.0g
Agar	20.0g
Tributyrin	10ml
Distilled water	1000ml
pH: 7.5±0.2 at 25°C	

Desired quantity of the media was dissolved in water maintaining the total volume and autoclaved for 15min at 121 °C and cooled to 45 °C and dispensed in a petri plate accordingly.

(B) Reagents:

1. Tris-borate EDTA (TBE) buffer (10X stock solution; g/L)

Tris Base	108.0 g
Boric acid	55.0 g
EDTA (pH=8.0)	20ml
Distilled water	1000ml

2. Tris-acetate EDTA (TAE) buffer (10X stock solution; g/L)

Tris-HCl	48.4g
Glacial acetic acid	11.42ml

EDTA (pH=8.0)	20 ml

3. 6X loading dye/buffer (g/L)

Sucrose	40g
0.04% bromophenol blue solution	50ml

The final volume of the solution was adjusted to 100ml.

4. Phosphate buffer saline (g/L)

Sodium chloride	8g
Potassium chloride	0.20g
Di-sodium hydrogen phosphate	1.15g
Potassium di-hydrogen phosphate	0.20g
Distilled water	1000ml

pH: 7.2

All the ingredients were dissolved in distilled water, autoclaved at 121°C, cooled and stored at room temperature.

(C) Staining solutions:

1. Crystal Violet stain (g/L)

Solution A

Crystal Violet	2.5g
Ethanol (95%)	25ml

Solution B

Ammonium Oxalate	1.0g
Distilled Water	100ml

Solution A and solution B were mixed, filtered and stored at room temperature.

2. Gram's Iodine solution (g/L)

Stock solution:

Iodine	5.0g
Potassium iodide	10.0g
Distilled water	100ml

Working solution:

The above solution was diluted in 1:5 ratio with distilled water for preparing working solution.

3. Safranin stain (g/L)

Stock solution:

Safranin	2.5g
Ethanol (95%)	100ml

Working solution:

The above solution was diluted in 1:4 ratio with distilled water for preparing working solution.

4. Ethidium bromide solution (10mg/ml)

Ethidium bromide	0.1g
Distilled water	10ml

The above reagent was dissolved in distilled water and stored in room temperature.