

# **Detection of Methicillin Resistance Among Staphylococci Species**

*Thesis*

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## ***List of Abbreviation***

<b>MRSA</b>	methicillin-resistant <i>S. aureus</i>
<b>CoNS</b>	coagulase-negative staphylococci
<b>PBP</b>	penicillin binding protein
<b>PSPs</b>	penicillinase-stable penicillins
<b>MIC</b>	Minimal Inhibitory Concentration
<b>ORSAB</b>	Oxacillin Resistant Screening Agar Base
<b>CRF</b>	coagulase reacting factor
<b>IgG</b>	immunoglobulin G
<b>TSST</b>	Toxic shock syndrome toxin
<b>SQS</b>	squalene synthetase
<b>ROS</b>	reactive oxygen species
<b>SCV</b>	Small colony variants
<b>DNA</b>	Deoxyribonucleic acid
<b>SE</b>	staphylococcal Enterotoxin
<b>EFT</b>	exfoliative toxins
<b>MSCRAMMS</b>	microbial surface components recognizing adhesive matrix molecules
<b>PIA</b>	polysaccharide intercellular adhesion
<b>AtlE</b>	autolysin E.
<b>Aap</b>	accumulation associated protein.
<b>PSMs</b>	phenol-soluble modulins
<b>Sar</b>	<i>staphylococcal</i> accessory regulator
<b>Embp</b>	Extracellular matrix binding protein
<b>sigB</b>	sigma factor
<b>PSM</b>	Phenol-soluble modulin
<b>AAP</b>	Accumulation Associated Protein
<b>FAME</b>	Fatty acid modifying enzyme
<b>Agr</b>	accessory gene regulator
<b>CSF</b>	cerebro-spinal fluid
<b>UDP</b>	Uridine diphosphate
<b>PEP</b>	phosphoenolpyruvic acid
<b>UDP-GlcNAc</b>	UDP-N-acetylglucosamine
<b>UDP-MurNAc</b>	UDP-Nacetylmuramic acid
<b>L-Ala</b>	L-alanine
<b>D-GluNH<sub>2</sub> or D-GluCOOH</b>	D-glutamic acid
<b>L-Lys</b>	L-lysine
<b>D-Ala-D-Ala</b>	D-alanyl-D-alanine
<b>DAP</b>	L-diaminopimelic acid
<b>CA</b>	clavulanic acid
<b>TZB</b>	tazobactam

NI	not included
EDTA	Ethylenediaminetetraacetic acid
MBL	Metallo-B-lactamases
ACT	AmpC type
CMY	Cephameycins
FOX	Cefoxitin
TEM	(Temoneira) name of patient
SHV	Sulphydryl variable
PC1	Penicillinase
CTX	Cefotaxime
PER	<i>Pseudomonas</i> extended resistant
VEB	Vietnam extended-spectrum $\beta$ -lactamase <i>pseudomonas</i> -specific enzyme
OXA	Oxacillin
CepA	Chromosomal cephalosporinase Ambler class A
KPC	<i>Klebsiella pneumoniae</i> carbapenemase
SME	<i>Serratia marcescens</i> enzyme
IMP	Imipenem
VIM	Verona integron-encoded metallo- $\beta$ -lactamase
L1	Labile enzyme
Sfh	<i>Serratia fonticola</i> carbapenem hydrolase
AmpC	Ambler class C
IND	<i>Chryseobacterium indologenes</i>
CphA	Gene encoding carbapenem-hydrolyzing metallo- beta-lactamase of <i>Aeromonas hydrophila</i>
cfiA	Gene encoding Cephalosporinase of <b><i>Bacteroides fragilis</i></b>
CAU	Gene encoding metallo-beta-lactamase of <i>Caulobacter crescentus</i>
UK	United Kingdom
NIDR	National Infectious Diseases Register
<b>BlaZ</b>	gene encodes $\beta$ -lactamase
<b>blaR1 -mecR1</b>	gene encoding a putative transmembrane signal transducer
<b>blaI -mecI</b>	gene encoding the repressor
<b>mecA</b>	gene encodes methicillin resistance in staphylococci
<b>BORSA</b>	borderline methicillin resistance in <i>S. aureus</i>
<b>Fem</b>	factors essential for methicillin resistance
<b>Aux</b>	auxiliary genes
<b>SCCmec</b>	Staphylococcal cassette chromosome mec
<b>OrfX</b>	open reading frame with unknown function
<b>AttBsc</b>	<i>bacterial chromosomal attachment site of scc</i>
<b>IS</b>	insertion sequence
<b>Ccr</b>	<i>Cassette chromosome recombinases</i>



<i>J</i>	<i>Joining regions</i>
<b>IWG-SCC</b>	International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements
<b>HA-MRSA</b>	Hospital-associated MRSA
<b>CA-MRSA</b>	Community acquired MRSA
<b>PVL</b>	Panton-Valentine leukocidin
<b>ESRD</b>	End-stage renal disease
<b>MSSA</b>	methicillin sensitive <i>S. aureus</i>
<b>DNase</b>	Deoxyribonuclease
<b>TBO</b>	Toluidine blue O
<b>PCR</b>	Polymerase Chain Reaction
<i>Nuc</i>	Nuclease
<i>Coa</i>	Coagulase
<i>Spa</i>	protein A
<b>OMSA</b>	<i>Oxacillin Mannitol salt agar</i>
<b>MSA</b>	Mannitol Salt Agar
<b>TAT</b>	Turnaround time
<b>CLSI</b>	Clinical and laboratory standard institute
<b>DD</b>	disk-diffusion
<b>EUCAST</b>	European Committee on Antimicrobial Susceptibility Testing
<b>BSAC</b>	The British Society for Antimicrobial Chemotherapy
<b>E-test</b>	Epsilometer test
<b>VA</b>	Vancomycin
<b>DM</b>	Daptomycin
<b>LZ</b>	Linezolid
<b>MRS</b>	methicillin resistant staphylococci
<b>CAMHB</b>	cation-adjusted Mueller-Hinton broth
<b>S</b>	Susceptible
<b>I</b>	Intermediate
<b>R</b>	Resistant
<b>BD</b>	Becton Dickinson
<b>mPCR</b>	Multiplex polymerase chain reaction
<b>dsDNA</b>	Double stranded DNA
<b>FDA</b>	Food and Drug Administration
<b>EMRSA</b>	Epidemic MRSA strains
<b>SSTI</b>	Skin and soft tissue infections
<b>SA</b>	<i>Staphylococcus aureus</i>
<b>REA</b>	Restriction Endonuclease Analysis
<b>RFLP</b>	restriction fragment length polymorphism
<b>PFGE</b>	Pulsed field gel electrophoresis
<b>AP-PCR</b>	Arbitrarily primed polymerase chain reaction
<b>RAPD</b>	random amplified polymorphic DNA
<b>MLST</b>	Multilocus sequence typing

<b>SLST</b>	Single-locus sequence typing
<b><i>Sma</i> I</b>	name in reference to <i>Serratia marcescens</i> from which it was derived
<b><i>TGP</i></b>	<i>Toxin gene profile typing</i>
<b>PPE</b>	Personal Protective Equipments
<b>CDC</b>	Centers for Disease Control and Prevention
<b>ATCC</b>	American Type Culture Collection
<b>FOX DD</b>	Cefoxitin disk diffusion test
<b>H<sub>2</sub>O<sub>2</sub></b>	<b>hydrogen peroxide</b>
<b>RNase</b>	ribonuclease
<b>dNTP</b>	<b>Deoxyribonucleotide triphosphate</b>
<b>Taq</b>	enzyme from <i>Thermus aquaticus</i>
<b>C<sub>t</sub></b>	Cycle threshold
<b>T<sub>m</sub></b>	Melting temperature
<b>OSA</b>	oxacillin screen agar
<b><i>p-value</i></b>	probability value
<b>PPV</b>	positive predictive value
<b>NPV</b>	negative predictive value
<b>SS</b>	statistically significant
<b>PSE</b>	pseudomonas-specific enzyme
<b>16S rRNA</b>	16 subunit ribosomal ribonucleic acid
<b>Fc region</b>	fragment crystallizable region

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## **Introduction**

The *Staphylococcus* species are divided into two large groups. The first group known as coagulase positive staphylococci which is mainly represented by *Staphylococcus aureus*, a pathogen that can cause a variety of infections in immunocompetent patients ranging from cutaneous to systematic infections. The second group, known as coagulase negative staphylococci comprises diverse species that are members of the normal flora of humans, mammals and birds, and they are involved in infectious processes in immunocompromised patients or patients using catheters (*Martins and Cunha, 2007; CDC, 2007*).

Methicillin was the drug of choice for treatment of staphylococcal infections before resistance had developed against it. The first case of methicillin-resistant *S. aureus* (MRSA) was reported in 1961 (*Louie et al., 2000*).

It is assumed that methicillin-resistance genes had evolved in coagulase-negative staphylococci (CoNS) and were then horizontally transferred among staphylococci. Staphylococci naturally have a protein in its cell wall penicillin binding protein (PBP), with trans-peptidase activity, play a key role in cell wall synthesis and are the target for B-lactam antibiotics. The

methicillin-resistant strains produce modified PBP called PBP2a with low affinity for B-lactam antibiotics. Resistance to methicillin mediated by *mecA* gene, responsible for production of PBP2a. *mecA* located on a region of chromosome called SCCmec (*Vaez et al., 2011*).

Accurate detection of methicillin resistance in Staphylococcal species by routine methods is difficult due to the presence of two subpopulation of *S. aureus* (one susceptible and another resistant) which may coexist within a culture. All cells in culture may carry the genetic information for resistance but a small number can express this kind of resistance in routine susceptibility testing performed in the laboratory. This phenomenon is termed heterogeneous resistance & occurs in Staphylococci resistant to penicillinase-stable penicillin such as oxacillin (*Brown, 2001*).

Accurate detection of *mecA*-mediated resistance to oxacillin and other penicillinase-stable penicillins (PSPs), i.e., methicillin, nafcillin, cloxacillin, dicloxacillin, and flucloxacillin, is necessary to ensure appropriate antimicrobial chemotherapy of staphylococcal infections (*Sasirekha et al., 2012*)

There are many methods for detection of methicillin resistance in Staphylococcal species. Most laboratories use disk

diffusion method for routine tests. The gold standard for antimicrobial susceptibility testing has been the Minimal Inhibitory Concentration (MIC) determined by a dilution or E-test method. In recent years MIC methods has been replaced by molecular methods that detect *mecA* gene. However the use of these assays are largely restricted to reference centers & not available in most routine diagnostic laboratories (*Madigan & Martinko, 2006; CLSI, 2007*).

The aim of this study is to determine the reliability of different routine methods for detection of MRSA&MRCoNS.