Detection of Methicillin Resistance Among Staphylococci Species

Thesis

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Mai Mabrouk Salem

List of Tables

List of Figures

List of Abbreviation

MRSA	methicillin-resistant *S. aureus*
CoNS	coagulase-negative staphylococci
PBP	penicillin binding protein
PSPs	penicillinase-stable penicillins
MIC	Minimal Inhibitory Concentration
ORSAB	Oxacillin Resistant Screening Agar Base
CRF	coagulase reacting factor
IgG	immunoglobulin G
TSST	Toxic shock syndrome toxin
SQS	squalene synthetase
ROS	reactive oxygen species
SCV	Small colony variants
DNA	Deoxyribonucleic acid
SE	staphylococcal Enterotoxin
EFT	exfoliative toxins
MSCRAMMS	microbial surface components recognizing adhesive matrix molecules
PIA	polysaccharide intercellular adhesion
AtlE	autolysin E.
Aap	accumulatiom associated protein.
PSMs	phenol-soluble modulins
Sar	*staphylococcal* accessory regulator
Embp	Extracellular matrix binding protein
sigB	sigma factor
PSM	Phenol-soluble modulin
AAP	Accumulation Associated Protein
FAME	Fatty acid modifying enzyme
Agr	accessory gene regulator
CSF	cerebro-spinal fluid
UDP	Uridine diphosphate
PEP	phosphoenolpyruvic acid
UDP-GlcNAc	UDP-N-acetylglucosamine
UDP-MurNAc	UDP-Nacetylmuramic acid
L-Ala	L-alanine
D-GluNH_2 or D-GluCOOH	D-glutamc acid
L-Lys	L-lysine
D-Ala-D-Ala	D-alanyl-D-alanine
DAP	L-diaminopimelic acid
CA	clavulanic acid
TZB	tazobactam

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

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

SLST	Single-locus sequence typing
***Sma* I**	name in reference to *Serratia marcescens* from which it was drived
TGP	*Toxin gene profile typing*
PPE	Personal Protective Equipments
CDC	Centers for Disease Control and Prevention
ATCC	American Type Culture Collection
FOX DD	Cefoxitin disk diffusion test
H_2O_2	**hydrogen peroxide**
RNase	ribonuclease
dNTP	**Deoxyribonucleotide triphosphate**
Taq	enzyme from Themnus aquaticus
C_t	Cycle threshold
T_m	Melting temperature
OSA	oxacillin screen agar
p-value	probability value
PPV	positive predictive value
NPV	negative predictive value
SS	statistically significant
PSE	pseudomonas-specific enzyme
16S rRNA	16 subunit ribosomal ribonucleic acid
Fc region	fragment crystallizable region

Contents

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.