COMPARATIVE STUDY BETWEEN A CHROMOGENIC MEDIUM (ORSAB) & ROUTINE IDENTIFICATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

Thesis

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ABSTRACT

Staphylococcus aureus is one of the most commonly identified pathogens in human medicine and is a major cause of nosocomial and community acquired infections. Resistance of *S. aureus* to methicillin was reported for the first time in 1961 Methicillin resistance in *S. aureus* is mediated by the production of an altered Penicillin-Binding Protein; PBP2a, the production of which is regulated by *mec A* gene complex Often, applicable antibiotics for treatment of MRSA are only glycopeptides like vancomycin and teicoplanin. However, during the last few years, a great problem has emerged; as some strains of MRSA have displayed intermediate (VISA) or full resistance (VRSA) to vancomycin. Accordingly, new treatment options for MRSA infections include daptomycin, linezolid, tigecycline and quinupristin/dalfopristin. Moreover, more recent anti-MRSA drugs are under development; including dalbavancin, telavancin and oritavancin

Key word:

Staphylococcus spectra, Methicillin resistant staphylococcus aurous , Laboratory Diagnosis of staphylococcal infections, treatment of staphylococcal infections

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Contents

List of Abbreviations

A-MRSA	Community Acquired Methicillin Resistant *Staphylococcus aureus*
cr	Cassette chromosome recobminases
DC	Center for Disease Control and prevention
HIP	Chemotaxis Inhibitory Protein
LSI	Clinical and Laboratory Standards Institute
oNS	*Coagulase Negative Staphylococci*
SA	Chromagar selective medium
SF	Cerebrospinal fluid
-Ala-D-Ala	D-Alanyl-D-Alanine
-Ala-D-Lac	D-Alanyl-D-Lactate
HFR	Dihydrofolate reductase enzyme
NA	Deoxyribonucleic acid
Nase	Deoxyribonuclease test
MRSA	Epidemic Methicillin Resistant *Staphylococcus aureus*
m	factors essential for methicillin resistance
ISA	Glycopeptide Intermediate *Staphylococcus aureus*
A-MRSA	Hospital acquired MRSA

HCW	Health Care Workers
ICU	Intensive care unit
Ig	Immunoglobulin
MHC	Major Histocomptability
MIC	Minimal Inhibitory Concentration
mRNA	Messenger RNA
MRSA	Methicillin Resistant *Staphylococcal aureus*
MRSE	Methicillin Resistant *Staphylococcal Epidermidis*
MSSA	Methicillin Sensitive *Staphylococcal aureus*
NaCl	Sodium chloride
NAMRU-3	Naval Medical Research Unit No.3
NCCLS	National Committee for Clinical Laboratory Standards
ORSAB	Oxacillin Resistance Screening Agar Base
PBP	Penicillin Binding Protein
PCR	Polymerase-Chain-Reaction
PFGE	Pulsed Field Gel Electrophoresis
PVL	Panton Valentine Leukocidin
Q-P	Quinupristin-Dalfopristin
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
S. aureus	*Staphylococcus aureus*
SCCmec	Staphylococcal Cassette Chromosome mec
TMP-SMZ	Trimethoprim-Sulphamethoxazole

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INTRODUCTION

Staphylococcus aureus is one of the most commonly identified pathogens in human medicine and is a major cause of nosocomial and community acquired infections. Resistance of *S. aureus* to methicillin was reported for the first time in 1961 ***(Cherkaoui et al., 2007)***.

Methicillin resistance in *S. aureus* is mediated by the production of an altered Penicillin-Binding Protein; PBP2a, the production of which is regulated by *mec A* gene complex ***(Francois et al., 2003).***

Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for a large number of nosocomial infections worldwide, and has become of greater public health concern since the emergence of community-acquired MRSA ***(Nahimana et al., 2006)***.

Many hospitals struggle with increasing amounts of MRSA which are "multiresistant" against all beta-lactam antibiotics. Commonly, affecting patients with a longer length of stay in the intensive care unit (ICU) especially a surgical ICU, dialysis unit, burn unit, and patients with permanent indwelling catheters or percutaneous medical devices ***(Domann, 2000)***.

Community acquired MRSA infections have a more aggressive clinical course and involve mostly skin and lungs. These infections appear as outbreaks among prisoners, sportsmen, homosexual men and military personnel ***(Noriega et al., 2008)***.

To efficiently prevent dissemination of MRSA, rapid and reliable identification and a close collaboration between clinicians and microbiologists are required ***(Badawi et al., 2001).***

Detection of *mec A* gene is the most accurate method for identification of methicillin resistance in *S. aureus*, but it cannot be routinely done ***(Wichelhaus et al., 2009).*** Accordingly, alternative reliable tests are needed for rapid detection of MRSA in the laboratories for the control of MRSA in hospitals and for the initiation of appropriate antimicrobial treatment in critically ill patients ***(Farr and Jarvis, 2002)***. New chromogenic media for screening of MRSA have recently become available as MRSA-ID, MRSA-Select, chromagar MRSA & ORSAB (Oxacillin Resistance Screening Agar Base) ***(Cetincol, 2008).***

Often, applicable antibiotics for treatment of MRSA are only glycopeptides like vancomycin and teicoplanin. However, during the last few years, a great problem has emerged; as some strains of MRSA have displayed intermediate (VISA) or full resistance (VRSA) to vancomycin. Accordingly, new treatment options for MRSA infections include daptomycin, linezolid, tigecycline and quinupristin/dalfopristin. Moreover, more recent anti-MRSA drugs are under development; including dalbavancin, telavancin and oritavancin ***(Naqao, 2009)*.**

Aim of Work

The aim of the present study was to compare a chromogenic medium known as Oxacillin Resistance Screening Agar Base (ORSAB) to the ordinary methods applied in our laboratories for identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA).

Staphylococcus Species

Morphology and Identification

Staphylococci are Gram-positive spherical cocci of about 1 um in diameter. They divide incompletely in three perpendicular planes to form pairs, tetrads, short chains and irregular grape like clusters (Greek staphyle; a bunch of grapes) ***(Ryan, 2004).***

They are non-motile, non-spore forming, and occasionally capsulated. Most are catalase positive and oxidase negative cocci. With the exception of one species, *Staphylococcus saccharolyticus*; which is a true anaerobe, staphylococci ferment glucose and are facultatively anaerobes (grow better in presence of air). Staphylococci require a medium containing amino acids and growth factors for anaerobic growth. They also require uracil and carbon source ***(Forbes et al., 2002).***

Staphylococci grow well on most routine laboratory media, such as nutrient agar or trypticase soy agar. For primary isolation from clinical material, sheep blood agar is recommended. Most species are able to grow in the presence of 10% sodium chloride. Their optimum temperature ranges from 18-42 ^{o}C; i.e. they are typically mesophiles while their optimum pH ranges from 7.0-7.5. Colonies are smooth, low convex, glistening, opaque and sometimes surrounded by a narrow zone of haemolysis on blood agar depending on the strain ***(James et al., 2004)***.

Taxonomy

Staphylococci belong to the family Micrococcaceae which includes four genera; Planococcus, Micrococcus, Somatococcus and Staphylococcus ***(Forbes et al., 2002).***

Habitat

Staphylococci are widespread in nature. They are generally found on skin and mucous membrane of mammals and birds. They may be found in the mouth, pharynx, conjunctiva, blood, mammary glands, intestinal, genitourinary, and upper respiratory tracts. Nasal carriage of *Staphylococcus aureus (S. aureus)* occurs in 20-50% of the population. Staphylococci are also found regularly on clothing, bed linen and other fomites in human environment ***(Von et al., 2001).***

Staphylococci may become pathogenic if they gain entry into the host tissue through trauma of the cutaneous barrier, inoculation by needles, or direct implantation of medical devices (foreign bodies). Infected tissue of the host may support large subpopulations of Staphylococci and in some situations, they may persist for long periods ***(Cole et al., 2001).***

Classification of *Staphylococcus* species

There are 33 species recognized in the genus Staphylococcus. *Staphylococcus* species are classified on the basis of their DNA relatedness using DNA-DNA hybridization ***(Sheagren and Schaberg, 2004).***

Practically, staphylococci are mainly classified according to the presence or absence of coagulase production into; Coagulase-positive (the most important is *S. aureus*) and coagulase-negative staphylococci ***(Corrigan et al., 2009)***.

Staphylococcus aureus

In medical microbiology the term coagulase-positive Staphylococcus refers to *S. aureus.* It is the most important human pathogen among *Staphylococcus* species and is characterized by producing coagulase enzyme. Coagulase enzyme is an extracellular enzyme which binds to prothrombin in the host to form a complex called staphylothrombin.The protease activity; characteristic of thrombin, is activated in the complex, resulting in the conversion of fibrinogen to fibrin. Thus, the bacteria could protect themselves from phagocytic and immune defenses by causing localized clotting ***(Deresinski et al., 2005).***

Epidemiology:

S. aureus is a common pathogenic commensal bacterium found in warm, moist areas of the body, particularly the nose, axilla and perineum. The basic human habitat of *S. aureus* is the anterior nares with a nasal carriage of about 20-50% of the population, but it does not cause harm and does not require treatment. However, some of *S. aureus* carriers constitute a source of infection by disseminating the organism to others ***(Haddadin et al., 2002).***

Most community acquired *S. aureus* infections are autoinfections with strains carried in anterior nares, on skin, or both. Community acquired

outbreaks are usually associated with poor hygiene and fomite transmission from person to person ***(Lowy, 2003)***.

Hospital outbreaks may occur by transmission of the organism from patient to another on the hands of hospital personnel. Nasal or perineal carriage among medical, nursing or other hospital personnel, may also be a source of an outbreak, especially if carriage is heavy and numerous organisms are disseminated. The most hazardous source is a hospital personnel who works despite having staphylococcal lesion as a boil. Bacteriophage typing and antibiograms are used as epidemiologic tools to detect carriers who may be the source of the outbreak ***(James et al., 2004)***.

Pathogenesis

I. Mode of transmission:-

A. Endogenous transmission

Staphylococci are colonizers of various skin and mucosal surfaces. Infections are frequently acquired when *S. aureus* spreads to normally sterile sites by traumatic introduction (e.g., surgical wound or microabrasions) ***(Humphreys, 2004).***

B. Person to person transmission

S. aureus may be transmitted from person to person by fomites, hands or from the infected skin of health-care workers to patients. Person to person spread in hospitals can lead to patients becoming colonized and potentially infected with antibiotic resistant strains ***(Forbes et al., 2002).***

II. Virulence factors:-

S. aureus possesses a large number of cell associated and extracellular factors, some of which contribute to the ability of the organism to overcome the body's defenses, invade, survive in and colonize the tissues. Though the role of each individual factor is not fully understood, it is likely that they are responsible for the establishment of infection, enabling the organism to bind to connective tissue, opposing destruction by bactericidal activities of humoral factors such as complement and overcoming uptake and intracellular killing by phagocytes ***(Humphreys, 2004)***. The different virulence factors are summarized in **Table (1)**.

Table (1): Virulence factors of *S. aureus*

Virulence factor	Activity
▪ **Cell wall polymers**	
1.Peptidogiycan	It is a polysaccharide polymer. It elicits the production of interleukin-I and opsonic antibodies by monocytes. It can be a chemoattractant for polymorphonuclear leucocytes, has endotoxin like activity and activates complement and coagulation cascades.
2.Teichoic acids	They are polymers of ribitol phosphate. They mediate adherence of staphylococci to mucosal cells.
▪ **Cell surface proteins**	
1.Protein A	It binds to the Fc region of IgG, thereby preventing the activation of complement. As a consequence, no C3 is produced, resulting in great reduction of the opsonization and phagocytosis of the organisms.
2. Clumping factor	Binds to fibrinogen.
3. Fibronectin-binding protein	Binds to fibronectin.