

**Comparison between a Chromogenic MRSA
Selective Medium and Mannitol-Salt Medium
with Cefoxitin for the detection of Methicillin-
Resistant *Staphylococcus aureus***

Thesis

Submitted for Partial Fulfillment of Master Degree in Medical
Microbiology and Immunology

Submitted By

Yasmin Mohamed Ahmed Mahmoud

M.B.B.Ch, Ain Shams University

Under the supervision of

Dr. Nehal Mohamed Anwar Fahim

*Professor of Medical Microbiology and Immunology
Faculty of Medicine, Ain Shams University*

Dr. Lamia Fouad Fathi

*Lecturer of Medical Microbiology and Immunology
Faculty of Medicine, Ain Shams University*

Dr. Hesham Ali Helal

*Lecturer of plastic and reconstructive surgery
Faculty of Medicine, Ain Shams University*

**Faculty of Medicine
Ain Shams University
2012**

مقارنة بين المستنبت اللوني المنتقى للمكورات العنقودية
المقاومة للمثيسلين و مستنبت المنيتول الملحي المزود
بالسفكسيدين للكشف عن المكورات العنقودية الذهبية المقاومة
للمثيسلين

رسالة

توطئة للحصول على درجة الماجستير
في الميكروبيولوجيا الطبية والمناعة

مقدمة من

الطبيبة/ ياسمين محمد أحمد محمود

بكالوريوس الطب و الجراحة

كلية الطب - جامعة عين شمس

تحت اشراف

ا.د / نهال محمد أنور فهميم

استاذ الميكروبيولوجيا الطبية والمناعة

كلية الطب - جامعة عين شمس

د./ لمياء فؤاد فتحى

مدرس الميكروبيولوجيا الطبية والمناعة

كلية الطب - جامعة عين شمس

د./ هشام على هلال

مدرس جراحة التجميل والإصلاح

كلية الطب - جامعة عين شمس

كلية الطب

جامعة عين شمس

٢٠١٢

6. SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem. Early recognition of patients colonized or infected with MRSA can have a direct impact on the selection of antibiotic therapy and the decision to initiate isolation procedures. Most laboratories struggle to determine the optimal use of resources, considering options to balance cost, speed, and diagnostic accuracy. An ideal method for MRSA detection should have a high sensitivity and a short time to the reporting of the results

This study was conducted during period from February 2010 to November 2011 at the Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University.

The aim of the work was to compare MRSA-Select, a commercially available chromogenic medium, as a rapid method for detection of MRSA in clinical isolates to mannitol salt agar with cefoxitin (MSA-Cefoxitin). The results were evaluated using PCR as a gold standard.

ACKNOWLEDGMENT

Praise be to ALLAH, the Lord of the Worlds, by whose grace this work has been completed.

The candidate wishes to express her faithful thanks to **Dr. Nehal Mohamed Anwar Fahim** *Professor of Medical Microbiology and Immunology Faculty of Medicine, Ain Shams Universtiy* and **Dr. Lamia Fouad Fathi** *Lecturer of Medical Microbiology and Immunology Faculty of Medicine, Ain Shams University* for excellent supervision during the progress of this work and efforts in revising the manuscript and valuable advices in this work. Also, The candidate is grateful to **Dr. Hesham Ali Helal** *Lecturer of plastic and reconstructive surgery Faculty of Medicine, Ain Shams University* for his valuable guidance and supervision this thesis.

The candidate is also grateful to the members of the *Medical Microbiology and Immunology Dep., Faculty of Medicine, Ain Shams University* for their sincere and fruitful help throughout my work as well as to all who present any kind of help to me particularly my college **Shimaa Ahmed Abd El-Salam .**

I am particularly grateful to my family members specially my father **Dr. Mohamed Ahmed Mahmoud** *Professor of soil chemistry ,Fac. of Soils Dept. fac. of Agric., Ain Shams Univercity.* for their help and continuous encouragement during the period of executing this work,

Contents

Title	Page No.
1.Introduction	۱
2. Review of Literature	4
2.1. Genus staphylococcus	4
2.1.1 Structure of Staphylococcus aureus	5
2.1.2 Pathogenesis and Immunity	11
2.1.2.1. Adhesion proteins	15
2.1.2.2 Staphylococcal Toxins	15
2.1.2. 3. Staphylococcal enzymes	۲۶
2.1.3 Clinical Diseases of Staphylococcus aureus	۳۰
2.1.3.1.Skin infections	۳۱
2.1.3.2. Diseases due to toxin production	۳۳
2.1.3.3. Other diseases	۳۵
2.1.4.Laboratory Diagnosis of Staphylococcus aureus	۳۷
2.1.4.1.Phynotepic methods	۳۸
2.1.4.2.Molecular methods	۴۱
2.2. Methicillin Resistant Staphlococcus aureus	۴۳
2.2.1. History of MRSA	۴۳
2.2.2. Evolution and Epidemiology of MRSA	45
2.2.2.1 Mode of transmission	54
2.2.2.2.Reservoir of MRSA	55
2.2.2.3.Risk factors of MRSA and colonization	56
2.2.3.Resistance to Antimicrobials	58
2.2.3.1.Penicillin resistance	59
2.2.3.2.Methicillin resistance	61
2.2.3.3.Vancomycin resistance	66
2.2.4.Small-colony variants ⁱⁱⁱ	70
2.2.5. Identification of MRSA	72

2.2.5.1. Susceptibility testing for methicillin resistance	73
2.2.5.2. Latex agglutination	77
2.2.5.3. Automated methods	77
2.2.5.4 Chromogenic media	78
2.2.5.5. Molecular methods	80
2.2.6. Typing of <i>Staphylococcus aureus</i>	81
2.2.6.1. Pulsed-Field Gel Electrophoresis (PFGE)	82
2.2.6.2. Multilocus Sequence Typing (MLST)	85
2.2.6.3. Staphylococcal Cassette Chromosome mec (SCCmec) typing	87
2.2.6.4. Spa typing	88
2.3. Treatment, Prevention and Control of MRSA Infections	91
2.3.1. Treatment of MRSA infections	91
2.3.1.1. Vancomycin	93
2.3.1.2. Linezolid	96
2.3.1.3. Daptomycin	98
2.3.1.4. Tigecycline	100
2.3. 2. Staphylococcal Vaccines and Immunotherapies	101
2.3.3. New Therapeutic Approaches	104
2.3.3.1. Iclaprim	105
2.3.3.2. Ceftobiprole	106
2.3.3.3. Ceftaroline	107
2.3.3.4. Deformylase inhibitors	108
2.3.3.5. Pleuromutilins	108
2.3.4. Strategies to Control MRSA	109
2.3.4.1. Appropriate antimicrobial use	111
2.3.4.2. Treatment of colonized patients	113
2.3.4.3. Tests for clearance	115
3. Materials and Methods	117

3.1. Materials	117
3.1.1.Culture Media	117
3.1.2.Mannitol salt agar (MSA)	119
3.1.3. Chemicals	120
3.1.4.Cefoxitin Discs	120
3.2. Methods	120
3.2.1. Collection and Processing of Pathological Material	120
3.2.2. Bacteriological examination of the growth	120
3.2.3. Identification of staphylococcus aureus isolates	121
3.2.4. Determination of methicillin resistance in Staphylococcus aureus isolates	123
3.2.4.1. MRSA-Select	123
3.2.4.2. Cefoxitin disc diffusion method	123
3.2.4.3. Detection of mecA gene using PCR technique	125
3.2.5. Statistical methods	131
4. Results	132
4.1. Identification of different isolates	132
4.2. Cefoxitin Disc Diffusion Methods	134
4.3. MRSA-Select plats	136
4.4. Detection of mecA gene using Polymerase Chain Reaction (PCR) technique	139
4.5. Comparison between results of MSA-Cefoxitin, MRSA-Select and PCR	141
5. Discussion	147
6. Summary	
7. References	
Arabic Summary	

List of Tables

No. of Table	<i>Title</i>	No of page
Table (1)	<i>S. aureus Surface proteins</i>	8
Table (2)	<i>S. aureus toxins</i>	24
Table (3)	<i>S. aureus Enzymes</i>	28
Table (4)	<i>Comparison of SCCmec allotypes</i>	65
Table (5)	<i>Zone of inhibitions of cefoxitin disc diffusion method</i>	73
Table (6)	<i>Concentrations of oxacillin and cefoxitin to detect resistance of S. aureus</i>	75
Table (7)	<i>Number and identification of pink and white colonies growing on MRSASelect after 20 and 48 h incubation</i>	139
Table (8)	<i>Comparison between PCR and MRSA-Select after incubation period of 48 h</i>	138
Table (9)	<i>Comparison between PCR and MSA-cefoxitin after incubation of 18-20 h.</i>	142
Table (10)	<i>Number and identification of pink and white colonies growing on MRSASelect after 20 and 48 h incubation</i>	145

List of Figures

No. of Figures	Title	Page
Fig. (1)	Type II <i>mec</i> (SCC <i>mec</i> II and Type IV <i>mec</i> (SCC <i>mec</i> IV)	48
Fig.(2)	A timeline of the four waves of antibiotic resistance in <i>Staphylococcus aureus</i> .	59
Fig.(3)	a) Induction of staphylococcal β -lactamase synthesis in the presence of the β -lactam antibiotic penicillin. b) Mechanism of <i>S. aureus</i> resistance methicillin.	61
Fig.(4)	Mechanisms of <i>S. aureus</i> resistance to vancomycin	69
Fig.(5)	Mechanisms of <i>S. aureus</i> resistance to vancomycin	70
Fig.(6)	Schematic representation of how antibiotic resistance evolves via natural selection.	72
Fig.(7)	Colonies of MRSA growing on chromID TM MRSA agar (A) and MRSA Select agar (B)	80
Fig.(8)	number of isolates obtained from specimens	133
Fig. (9)	Culture of <i>S. aureus</i> isolates on Mannitol-salt agar media. <i>S. aureus</i> fermented mannitol resulting in conversion of colure of media from red to yellow	134
Fig.(10)	mannitol-salt agar plate equally streaked by <i>S. aureus</i> broth with equal	135

	density to a standard 0.5 McFarland tube and supplied with 30 µg cefoxitine disc. The plat shows zone of inhibition of 10 mm.	
Fig.(11)	mannitol-salt agar plate equally streaked by S. aureus broth with equal density to a standard 0.5 McFarland tube and supplied with 30 µg cefoxitine disc. The plat shows zone of inhibition of 29 mm.	136
Fig.(12)	Rose pink colonies on MRSA-Select (Bio-Rad laboratories).	137
Fig.(13)	White colonies on MRSA-Select	138
Fig.(14a)	gel-electrophoresis of PCR.	140
Fig.(14b)	<i>gel-electrophoresis of PCR</i>	141
Fig. (15)	<i>sensitivity, specificity, ppv and npv of MSA-Cefoxitin</i>	143
Fig. (16)	sensitivity, specificity, ppv and npv of MRSA-Select after incubation of 48 h.	146

List of abbreviations

abbreviations	Full name
CA-MRSA	community-associated methicillin resistant staphylococcus aureus
CA-MSSA	community-associated methicillin susceptible staphylococcus aureus
CCs	Clonal complexes
CDC	Centers for Disease Control and Prevention
ClfA and ClfB	clumping factor A and B
Cna	collagen-binding protein
CoNS	Coagulase-negative staphylococci
DHFR	Dihydrofolate reductase
DNase	deoxyribonuclease
ET-A	epidermolytic toxin A
ET-B	epidermolytic toxin B
ETs	Exfoliative toxins
FAME	fatty acid modifying enzyme
FnBpA and FnBpB	fibronectin-binding proteins A and B
GISA	Glycopeptide-intermediate <i>S. aureus</i>
HA-MRSA	healthcare-associated infections
J regions	junkyard regions
MLST	multilocus sequence typing
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MSA	mannitol-salt agar
MSCRAMMs	microbial surface components

	recognizing adhesive matrix molecules
PBP2a	penicillin binding protein 2a
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PL	Plasmin
PLG	plasminogen
PMN	polymorph neuclear cell
PVL	Panton-Valentine leukocidin
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SAK	Staphylokinase
SCC <i>mec</i>	Staphylococcal Chromosome Cassette <i>mec</i>
SCVs	Small-colony variants
SEA	staphylococcal enterotoxins A
SEIs	staphylococcal enterotoxin-like proteins
SEs	staphylococcal enterotoxins
SSSS	staphylococcus scalded skin syndrome
SSTIs	skin and soft-tissue infections
ST	sequence type
TMP	Trimethoprim
TNFR1	tumor necrosis factor receptor 1
TNF- α	tumor-necrosis factor- α
TSS	toxic shock syndrome
TSST-1	Toxic shock syndrome toxin 1
VISA	vancomycin intermediate-resistant <i>S. aureus</i>
VRSA	vancomycin resistant <i>S. aureus</i>

1. Introduction

Staphylococci are the main causative agents of nosocomial diseases. Over the last few years, the increase in the number of methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major clinical problem. *MRSA* infections are associated with considerable morbidity, mortality, and excess cost (*Engemann et al., 2003*).

It has been shown that, in most cases, the source of *Staphylococcus aureus* causing bacteremia is the patient's nose, and colonization with MRSA leads to autoinfection at a higher rate than colonization with methicillin-susceptible isolates (*Stoakes et al., 2006*).

An increase in the number of *MRSA* has become a serious clinical and epidemiological problem, as resistance to this antibiotic implies resistance to all β -lactam antibiotics. (*Velasco et al., 2005*). Methicillin-resistance is attributable to the *mecA* gene, encoding penicillin-binding protein (PBP) 2a, which presents low affinity for β -lactam antimicrobials (*Fuda et al., 2004*).

Introduction

The detection of the methicillin resistance represents a real challenge for the routine clinical microbiology laboratories since molecular methods, the gold standard method, are not available for most medical institutions. A wide range of techniques has been used to detect and identify *MRSA* from clinical specimens. Selective and differential culture media, especially mannitol salt agar (MSA), are most widely employed. However, all MSA media are not the same: they differ in their salt, and their performances may not be comparable. In general, MSA media are not sensitive enough to be used for detection of *Staphylococcus aureus* including MRSA in clinical specimens (***Stoakes et al, 2006***).

Rapid detection of MRSA is essential to optimize therapy, to minimize patients' discomfort and to reduce costs. Chromogenic media were thus developed to detect MRSA strains in one single step. Growth of non-staphylococcal strains is repressed by high salt concentration and enzymic substrate is used to achieve a specific colour reaction to detect *S. aureus*. Growth of methicillin-susceptible *Staphylococcus aureus* (MSSA)