Evaluation of the role of *bla* genes in beta lactam and methicillin resistant *Staphylococcus aureus*

By

Sarah Hussein Ahmed Hussein

B.Sc. Microbiology and Chemistry,
Faculty of Women for arts, science and education,
Ain-Shams University (2009)

Thesis

Submitted for partial fulfillment of Master Degree in Microbiology

Supervisors:

Prof. Yehia Ahmed El-Zawahry
Prof. of Microbiology,
Botany Department,
Faculty of Science,
Zagazig University

Dr. Sahar Tolba Mohamed
Associate Prof. of Microbiology,
Microbiology Department,
Faculty of Science,
Ain Shams University

Dr. Fifi Mohamed Reda
Assistant Prof. of Microbiology,
Botany Department,
Faculty of Science,
Zagazig University

Department of Microbiology
Faculty of Science
Ain Shams University

2018

Acknowledgement

Firstly, all praise to Allah for giving me the ability to achieve this work. I would like to express my sincere thanks and gratitude to Prof. **Yehia Ahmed El Zawahry** , Prof. of Microbiology, Botany Department, Faculty of Science, Zagazig University for supervising this work , encouragement, continuous support and valuable advice during the preparation of this thesis. Your supervision lend this work a panache.

I would be honored to express my deep appreciation and gratitude to Dr. **Sahar Tolba Mohamed,** Associate Prof. of Microbiology, Faculty of Science, Ain-Shams University for continuous assistance, useful discussions and sincere advice. Indeed, this work could not be accomplished without your potential

I wish to express my deep thanks to Dr. **Fifi Mohamed Reda,** Associate Prof. of Microbiology, Botany Department, Faculty of Science, Zagazig University for her help , constructive criticism and continuous encouragement .

I am sincerely grateful to all staff members , colleagues at the Botany department , Faculty of Science, Zagazig University for their support and continuous cooperation.

List of Tables

List of figures and photos

List of Abbreviation

Abbreviation	Full term
AMP	Ampicillin
AZM	Azithromycin
CA-MRSA	Community-acquired Staphylococcus aureus
CAZ	ceftazidime
CDC	Center for Disease and prevention
CFU	Colony Forming Unit
CIP	ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CPS	Coagulase positive staphylococci
CRO	ceftriaxone
CTX	cefotaxime
DAD	Disc agar diffusion
DNA	Deoxyribonuclic acid
DO	Doxycycline
E	Erythromycin
EDTA	Ethylenediamine tetra acetic acid
g.	Gram
h.	hour
HA	Hospital- acquired methicillin resistant *Staphylococcus aureus*
IPM	Imipenem
µg	Microgram
MDR	Multi-drug resistance
MEM	Meropenem
mL	millilitre
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
Ox	Oxacillin
PBP	Penicillin binding protein
PFGE	Pulse field gel electrophoresis
PCR	Polymerase Chain reaction
SAB	*Staphylococcus aureus* bacteremia
SCCmec	Staphylococcal cassette chromosome
VA	vancomycin

WHO	World Health Organization

Evaluation of the role of *bla* genes in beta lactam and methicillin resistant *Staphylococcus aureus*

Sarah H. Ahmed[1], Sahar T.M. Tolba[1], Yehia A. El-Zawahry[2]

[1] Department of Microbiology, Faculty of science -Ain-Shams University ,Cairo, Egypt

[2] Department of botany, Zagazig University, Zagazig, Egypt

Abstract

One hundred and nineteen clinical samples were isolated from patients admitted in different hospitals in El-Sharkia governorate. Sixty six isolates were confirmed to be *S. aureus*. Susceptibility to different antimicrobial agents and Minimum inhibitory concentration tests showed that all the isolates were resistant to β-lactam antibiotics, 77.2% (n=51) isolates were methicillin resistant *S. aureus* MRSA, while almost all the isolates were sensitive to vancomycin and tigecycline. Polymerase Chain Reaction (PCR) of *mec*A, encoding methicillin resistance, and *bla*Z, β-lactamase biosynthetic gene, revealed the coexistence of both genes in 56.8% (n= 29/51) of the isolates. Meanwhile, 11.7% (n=6/51) of MRSA isolates phenotypically resistant to oxacillin were found to be *mec*A^{-} .This data support the fact that the expression of *bla* genes enhanced the phenotypic expression of oxacillin resistance as a result of β-lactamase hyperproduction. On the other hand, 33% of MRSA (n=17/51) were *bla*Z^{-} suggesting a mutation event in *blaZ* or the existence of an alternative mechanisms for β-lactam resistance that may compete with *mec*A gene .

Key words: *Staphylococcus aureus* - *mec*A- *bla*Z- MRSA- MIC- β-lactamase resistant MRSA-

Introduction

Staphylococcus aureus is an extraordinarily versatile pathogen that can survive under hostile external environmental conditions, colonize mucous membranes and skin, and cause severe toxin-mediated disease or severe invasive purulent infections in humans **(Archer, 1998; Lowy, 1998).**

It represents an increasing problem, in hospitals for decades as well as in community-acquired infections.

The severity of staphylococcal infections combined with feeble response to antibiotic treatment is due to the specific suite of virulence and antibiotic resistance-associated genes **(Peacock et al., 2002).**

Most Methicillin-resistant *Staphylococcus aureus* (MRSA) infections occur in people who have been in hospitals or other health care settings, such as nursing homes and dialysis centers. Staphylococcal resistance to beta-lactam antibiotics is mediated by either of two mechanisms: (i) production of beta-lactamase and (ii) production of an altered target penicillin-binding protein (PBP), PBP2a.

Methicillin resistant gene mecA is embedded in a large heterologous chromosomal cassette, the SCCmec element **(Ito et al., 1999)**. Some MRSA strains carry upstream to the mecA gene

the regulatory genes mecI-mecR1 encoding for a repressor and a sensor/inducer of the *mec*A expression, respectively **(Hiramatsu et al., 1992)**.

This genetic organization is similar to the beta-lactamase locus that encodes for penicillin-resistance only, and contains the structural gene (*bla*Z), a repressor (*bla*I) and a sensor/inducer (*bla*R1). There is a cross-talk between both regulatory systems, as each one alone is able to control the transcription of mecA and blaZ **(Hackbarth and Chambers, 1993).**

Based on those observations, it has been postulated that full resistance to beta-lactamase of many contemporary MRSA clinical strains, implies a non-functional mecI-mecR1 regulatory system **(Hiramatsu et al., 1992).** The cross link between the mec genes and bla genes is not yet resolved. At present, there are many clonal complex disseminated in different healthcare settings.

In the current study, microbiological and molecular studies were used to study the prevalence of some resistance genes among MRSA isolates from inpatients at different hospitals in El-Sharkia Governerate.

Aim of the work

This study aims to assess the role of *blaZ* in β-lactam resistant methicillin resistant *S. aureus* isolates. The use of molecular techniques in the detection of MRSA is essential for rapid diagnosis.

Objectives:

To achieve the aim of the study, the following objectives have been carried out

1. Isolation and characterization of *Staphylococcus aureus* (*S. aureus*) isolated from nosocomial cases in different hospitals in El- Sharkia Governerate.
2. Investigating the sensitivity and the resistance of *S. aureus* isolates to different antibiotics commonly used in infectious diseases.
3. Determination of minimum inhibitory concentration (MIC).
4. Detection of *mec*A and *bla*Z genes in methicillin-resistant *S. aureus.*
5. Assessing the correlation between the *mec*A and *bla*Z genes in the isolates.

Review of Literature

Stapyhylococcus aureus has become a major health problem that causes many prevalent and fatal conditions in humans **(kluytmans *et al.*,2010)**. Although it is a commensal organism colonizing the skin and mucosal surfaces of its carriers including the anterior nasal nares, nasopharynx, intestine, upper respiratory tract, the widespread of the resistant form of *Staph.aureus* (MRSA) accompanied by the remarkable morbidity and mortality made it notoriously regarded as a pervasive pathogenic microorganism**(Chen *et al.*,2012; Melzer *et al.* 2013; Brown *et al.*2014).**

MRSA was first discovered in the UK nearly in 1961, 2 years after the introduction of methicillin and has now a worldwide spread , particularly in the hospitals and other healthcare settings where it is ordinarily termed a superbug.It could also be known as oxacillin-resistant *S.aureus* (ORSA) **(Ippolito *et al*.,2010).**

As rapidly as new antibiotics were introduced, *S.aureus* has developed many virulence factors and efficient mechanisms to neutralize them. Outbreaks have frequently been reported in neonatal and surgical intensive care units (ICU), inpatient wards, and operating rooms. In addition, the patient-to-patient nosocomial

transmission responsible for such outbreaks predominantly occurs through the hands of healthcare workers. Nosocomial infections caused by MRSA are related to long hospitals stays of patients , poor infection control practices, and this eventually add more financial burden on the society **(Mims *et al*.,2004)**

Unastonishingly , it is a main reason for a broad clinical spectrum of skin infections. Staphylococcal infections can turn deadly if the bacteria invade deeper into the body, entering the bloodstream, joints, bones, lungs or heart .It may lead, in some cases, to life-threatening systemic diseases, especially in patients accommodated in hospital wards. Among these diseases are pneumonia , sepsis, meningitis that result in nearly more deaths than HIV , viral Hepatitis, and tuberculosis combined **(Sebastian *et al*.,2012*).***

Reduced susceptibility faced by most of these *S.aureus* strains to antimicrobial agents nowadays has caused a global health concerns **(Hiramtsu *et al*.,2014, Spagnolo *et al*.,2014*)*.**

Infections caused by MRSA is not restricted to a certain geographic area ; it is a worldwide problem. Europe has a strong presence of MRSA, accounting for approximately 44% of nosocomial infections in the year 2008. Fortunately, this is improving thanks to surveillance programs and stringent outbreak