Prevalence of Panton Valentine Gene in both Community-acquired and Healthcare-acquired Methicillin Resistant *Staphylococcus aureus* Isolates

Thesis

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Dedication

This work is dedicated to . . .

My beloved father, to whom I owe everything I ever did in my life and will achieve.

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List of Abbreviations

Abb.	Full term
AST	*Active surveillance testing*
CA-MRSA	*Community acquired methicillin resistant Staphylococcus aureus*
CDC	*Centers for Disease Control*
CLSI	*Clinical Laboratory Standard Institute*
CoNS	*Coagulase negative Staphylococci*
CSF	*Cerebrospinal fluid*
DNA	*Deoxyribonucleic acid*
ELISA	*Enzyme-linked immunosorbent assay*
EIA	*Enzyme immunoassay*
EMRSA	*Epidemic methicillin resistant Staphylococcus aureus*
HA-MRSA	*Hospital acquired methicillin resistant Staphylococcus aureus*
H_2O_2	*Hydrogen peroxide*
Hrs	*Hours*
ICT	*Immunochromatographic test*
ICUs	*Intensive care units*
MIC	*Minimum inhibitory concentration*
Min	*Minute*
Ml	*Milliliter*
μL	*Microliter*
mm	*millimeter*
MRCNS	*Methicillin resistant coagulase negative Staphylococcus aureus*
MRSA	*Methicillin resistant Staphylococcus aureus*
MSSA	*Methicillin sensitive Staphylococcus aureus*
MSA	*Mannitol salt agar*
PBP2a	*penicillin-binding protein 2a*
PCR	*Polymerase chain reaction*

List of Abbreviations (Cont...)

Abb.	Full term
PVL	*Panton Valentine Leukocidin gene*
ROS	*Reactive oxygen species*
RTI	*Respiratory tract infection*
S. aureus	*Staph aureus*
SCC mec	*Staphylococcal cassette chromosome encoding methicillin resistance*
SD	*Standard deviation*
UK	*United Kingdom*
VRSA	*Vancomycin-resistant Staphylococcus aureus*
WHO	*World health organization*

ABSTRACT

Panton-Valentine leukocidin (PVL) toxin is mainly associated with necrotic lesions involving the skin or mucosa. PVL has been linked by epidemiological studies to community-associated methicillin resistant Staphylococci (CA-MRSA). There is a relatively few data about the incidence of this toxin in nosocomial infections.

The aim of this study was to compare the prevalence of PVL gene in healthcare acquired and community acquired *Staphylococcus aureus* isolates at Ain Shams University Hospitals. This study was a comparative cross-sectional study carried on eighty-nine (89) clinical samples obtained from patients attending outpatient clinics with community-acquired pyogenic infections and patients with healthcare acquired pyogenic infections in Intensive Care Units (ICUs) at Ain Shams University hospitals. From the eighty-nine (89) clinical samples, thirty-eight (38) clinical samples were from patients with nosocomial infections admitted to Postoperative ICU, Trauma ICU, Chest ICU and Internal Medicine ICU; and fifty-one (51) clinical samples were from patients with community acquired infections. These samples were conventionally processed in order to isolate fifteen (15) Hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) from patients with nosocomial infections and fifteen (15) Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) from patients with community acquired infections. Detection of the *mecA* gene and Panton Valentine Leukocidin gene (PVL) was performed by polymerase chain reaction (PCR). Results: *mecA* gene was positive in 40% (6/15) of HA-MRSA isolates compared to 47.6% (7/15) of CA-MRSA isolates, while *pvl* gene was positive 53.3% (8/15) in HA-MRSA isolates compared to 26.7% (4/15) of CA-MRSA isolates, and there was no significant correlation between *mecA* gene and *pvl* gene among the studied thirty (30) MRSA isolates (P value =1), as 50% (3/6) only of positive *mecA* were positive for *pvl*, and 44.4% (4/9) of negative *mecA* were negative *pvl*. The results of detection of *pvl* gene in both community and hospital isolates made this gene not a sole genetic marker for diagnosis of CA-MRSA.

Keywords:

Staphylococcus aureus (S. aureus), *mecA* gene, Panton valentine leucocidin gene (PVL), Hospital acquired methicillin resistant *Staphylococcus aureus* (HA- MRSA), Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA).

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a major human pathogen that causes a wide range of clinical infections. It is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections **(Coates *et al.,* 2014)**.

Methicillin resistant Staphylococcus aureus (MRSA)is a bacterium responsible for several difficult-to-treat infections in humans. MRSA strains have acquired a gene that makes them resistant to nearly all beta-lactam antibiotics. The resistance of MRSA to β-lactam antibiotics is associated with penicillin-binding protein 2a, encoded by the mecA gene **(Velasco *et al.,* 2005).**

MRSA has been considered a major pathogen in healthcare facilities, known as Healthcare associated MRSA (HA-MRSA). It has been observed emerging in the community as well, known as community associated MRSA (CA-MRSA) **(Tavares *et al.,* 2013).**

Panton-Valentine leukocidin (PVL) is one of many toxins associated with *S. aureus* infection. PVL acts through the synergistic activity of two non-associated secretory proteins, component S and component F. These two toxins are encoded by two genes, LukS-PV and LukF-PV **(Bradley, 2007).**

Panton Valentine leukocidin is present in majority of community associated MRSA isolates and rarely present in hospital isolates, therefore it is recognized as a marker of community acquired strains **(Li *et al.,* 2010)**.

PVL has a major cytotoxic effect, as the release of PVL by staphylococcal strains caused rapid and premature cell death, which is different from the physiological (and programmed) cell death of neutrophils following phagocytosis and degradation of virulent bacteria. These results have important implications especially for infections with CA-MRSA strains **(Labandeira-Rey *et al.,* 2007)**.

AIM OF THE WORK

The objective of this study was to compare the prevalence of PVL gene in healthcare acquired and community acquired Methicillin resistant *Staphylococcus aureus* isolates at Ain Shams University Hospitals.