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Molecular characterization and Antibiogram of Methicillin Resistant *Staphylococcus aureus* in West Bank-Palestine

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Molecular characterization and Antibiogram of Methicillin Resistant *Staphylococcus aureus* in West Bank-Palestine

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Thesis Approval

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Dedication

To my God who always inspires me how to believe in myself and my dreams...

To those dearest to me, my great Mom & Dad, I will never finish thanking you for your continuous love...support...trust...patience and for everything you do every day for me...

To Dr. Kifaya Azmi, my supervisor and best friend, for her trust and the endless motivation...

To my brothers, my sisters, my unconditional special friends and all people who have always been there to support me...

To my finite disappointment moments, to all of those whom hope and courage are their unique habits...

I dedicate this work...

Thank You All.

Etaf Sliman Ibraheem Hadyeh

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed.....

Etaf Sliman Ibraheem Hadyeh

Date: 5.5.2018

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major cause of hospital-acquired and community-acquired infections. This study aimed to investigate the epidemiological and genetic diversity of MRSA isolates in healthcare settings from 2015 to 2017 and to characterize the major MRSA clones and antibiogram trends in Palestine.

Methodology: Samples and data were obtained from 112 individuals admitted to different hospitals of West Bank and Jerusalem. Antibiotic susceptibility patterns and staphylococcal chromosomal cassette *mec* (SCC*mec*) typing were determined. Further genes were typed by *Staphylococcus aureus* protein A (*spa*) typing and detection of panel of toxin genes, including: Panton-Valentine Leukocidin (PVL), ACME-*arc*A *(*a surrogate marker for arginine catabolic mobile element I), Toxic Shock Syndrome Toxin-1 (*TSST*-1) and Exfoliative Toxin A (ETA).

Results: All the 112 isolates of MRSA were susceptible to Vancomycin (100%). Also, almost all isolates were trimethoprim sulfamethoxazole (SXT) susceptible (81.3%). Resistance rates of the MRSA strains were as the following: all MRSA isolates were resistant to cefoxitin, penicillin G, amoxicillin/clavulanic acid, ceftriaxone and meropenem (100%), (63.4%) were resistant to erythromycin, (34.8%) were resistant to clindamycin, (38.3%) were resistant to ciprofloxacin, (23.2%) were resistant to gentamicin and (18.8%) were resistant to SXT. Of all the isolates, 32 isolates (28.6%) were multi-drug resistant (MDR), in which these isolates were resistant to at least three different non-β-lactam antibiotic groups. The majority of the isolates were identified as SCC*mec* type IV (86.6%), which is considered as a community associated "CA- MRSA". Among 29 different found *spa* types, 5*spa* types: t386, t008, t044, t223 and t037 were predominant and represented 14 (12.5%), 12 (10.7%), 12 (10.7%), 11 (9.8%), 10 (8.9%) isolates, respectively. Thirty three isolates (29.5%) were positive for the PVL toxin gene, while the ACME-*arcA* toxin gene was present in 18.8 % of the isolates and 23.2% had the *TSST*-1 gene. All *spa* type t991 isolates were ETA positive (5.4%). The four major

MRSA clones were: CC22 (15.2%), CC1 (13.4%), CC8 (13.4%) and CC80 (13.4%). In this study, USA-300 clone was found in 9 isolates (8.0%). This clone is considered as both resistant and highly virulent and can cause rapid tissue destructive infections in healthy young adults and children. It has caused great morbidity and mortality in the recent decades in USA.

Conclusions: Our results provide important insights into the epidemiology of MRSA strains in Palestine with high carriage of different toxins. We report a diversity of MRSA strains in hospitals in Palestine, with frequent community acquired SCC*mec* type IV carriage. Knowledge of the dominant MRSA clones in our region with the antimicrobial susceptibility profile is necessary for selection of the appropriate empirical antimicrobial treatment for MRSA infections.

Key words: MRSA, antibiogram, MDR, *spa*, SCC*mec*, PVL, ETA, *TSST*-1, *arc*A, USA-300 clone.

التوصيف الجزيئي واختبار أنماط الحساسية للمضادات الحيوية لبكتيريا العنقودية الذهبية المقاومة للميثيسيلين في فلسطين.

إعداد: عطاف سليمان ابراهيم هادية

إشراف: د. كفاية عزمي

الملخص:

خلفية الدراسة: أصبحت بكتيريا العنقودية الذهبية المقاومة للميثيسلين (MRSA) سبباً رئيسياً للعدوى المكتسبة من المستشفيات وبين أفراد المجتمع. هدفت هذه الدراسة إلى دراسة التنوع الوبائي والجيني لعينات بكتيريا المارسا (MRSA) من مراكز الرعاية الصحية في الفترة من 2015 إلى 2017 وتوصيف مستنسخات بكتيريا المارسا الرئيسية (major MRSA clones) ومدى استجابتها للمضادات الحيوية في فلسطين.

منهجية البحث: تم الحصول على عينات وبيانات من 112 فرداً مصابين بعدوى المارسا من مستشفيات مختلفة في الضفة الغربية والقدس. تم تحديد أنماط الحساسية للمضادات الحيوية (antibiotic susceptibility patterns) وأنواع الجين المسؤول عن مقاومة الميثيسلين (SCC*mec* typing). تم تحديد جينات أخرى متعلقة ببكتيريا المارسا، منها بروتين (*Staphylococcus aureus* protein A (*spa*) typing) بالإضافة إلى تحديد جينات مسؤولة عن تكوين في مواد سامة في هذه البكتيريا، بما في ذلك: الجين المسؤول عن سم Panton-Valentine Leukocidin toxin (PVL)، والجين المسؤول عن سم Arginine Catabolic mobile element I toxin (ACME-*arc*A)، والجين المسؤول عن متلازمة الصدمة السمية Toxic Shock Syndrome Toxin-1 (*TSST*-1) والجين المسؤول عن سم Exofoliative Toxin A (ETA).

النتائج: أظهرت نتائج البحث أن جميع عينات المارسا المعزولة (112) تستجيب لمضاد الـ vancomycin (100 ٪). أيضاً، تقريباً أغلب العينات معزولة كانت تستجيب لمضاد الـ SXT (81.3 ٪). كانت معدلات مقاومة سلالات MRSA للمضادات الحيوية كما يلي: جميع عينات مارسا المعزولة MRSA كانت مقاومة لكل من: cefoxitin و penicillin G و amoxicillin/clavulanic acid و ceftriaxone و meropenem (100)٪، في حين (63.4٪) من العينات المعزولة كانت مقاومة لمضاد الـ Erythmycin و(34.8٪) كانت مقاومة إلى مضاد الـ clindamycin و(38.3٪) كانت مقاومة لمضاد الـ ciprofloxacin و(23.2٪) كانت مقاومة لمضاد الـ gentamicin و (18.8٪) كانت مقاومة لـمضاد الـ SXT. من بين جميع عينات المارسا المعزولة، كانت هناك 32 عينة (28.6٪) متعددة المقاومة للأدوية (MDR)، حيث كانت هذه العينات مقاومة لما لا يقل عن ثلاث مجموعات مختلفة من المضادات الحيوية الغير اللاكتامية (non-β-lactam antibiotics). تم تحديد غالبية العينات المعزولة

على أنها من نوع SCC*mec* IV (86.6%)، والذي يعتبر نوعاً مرتبطًا بـالعدوى المكتسبة من المجتمع " CA-MRSA".

من بين 29 نوعًا مختلفًا وجدت من خلال الـ *spa* typing ، 5 أنواع من الـ *spa* types: t386 و t008 و t044 و t223 و t037 كانت سائدة وتمثل 14 (12.5٪) و 12 (10.7٪) و 12 (10.7٪) و 11 (9.8٪) و 10 (8.9 ٪) من العينات المعزولة، على التوالي. أيضاً، 33 عينة مارسا (29.5٪) عُزل منها PVL toxin gene، في حين كان جين ACME-*arc*A toxin gene موجود في 18.8٪ من العينات المعزولة و 23.2٪ من العينات كان يحمل الجين المسؤول عن متلازمة الصدمة السمية *TSST*-1 toxin gene. جميع العينات المعزولة من نوع t991كانت تحمل ETA gene (5.4٪). المستنسخات الأربعة الرئيسية (CCs) للمارسا كانت:(15.2%) CC22 ، (13.4%) CC1، CC8 (13.4) و (13.4%) CC80. في هذه الدراسة، تم العثور على استنساخ clone الـ USA-300 في 9 عينات (8.0٪). ويعتبر هذا الاستنساخ مقاوم وممرض للغاية حيث يمكن أن يسبب عدوى سريعة ومدمرة للأنسجة لدى الأصحاء البالغين والأطفال. هذا الاستنساخ تسبب في معدلات كبيرة للوفاة والوفيات في العقود الأخيرة في الولايات المتحدة الأمريكية.

الاستنتاجات والتوصيات: تظهر نتائج هذه الدراسة رؤية مهمة حول التنوع الوبائي لسلالات المارسا الموجودة في فلسطين مع وجود جينات مختلفة من السموم. كما تُقر هذه الدراسة وجود تنوع في سلالات بكتيريا المارسا في المستشفيات في فلسطين والتي تحمل النوع المكتسب من المجتمع SCC*mec* IV. أيضاً، إن معرفة مستنسخات المارسا المهيمنة في منطقتنا مع أنماط حساسيتها للمضادات الحيوية ضروري جداً من أجل الاختيار المناسب والفعال لعلاج عدوى بكتيريا العنقودية الذهبية المقاومة للميثيسلين.

الكلمات المفتاحية: مارسا، أنماط الحساسية للمضادات الحيوية، متعددة المقاومة للأدوية، *spa*، SCC*mec*، PVL، ACME-*arc*A، *TSST*-1، ETA، USA-300 clone.

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List of abbreviations

Abbreviation	Full Term
S. aureus	*Staphylococcus aureus*
SSTIs	Skin and soft tissue infections
MRSA	Methicillin Resistant *Staphylococcus aureus*
PBP	Penicillin-binding protein
CA-MRSA	Community acquired Methicillin Resistant *Staphylococcus aureus*
HA-MRSA	Hospitals acquired Methicillin Resistant *Staphylococcus aureus*
PVL	Panton-Valentine Leukocidin
SCC*mec*	Staphylococcal Cassette Chromosome *mec*
ccrA and *B*	Cassette chromosome recombinases A and B
SXT	Trimethoprim-sulfamethoxazole
VISA	Vancomycin Intermediate Staphylococcus aureus
VRSA	Vancomycin-resistant Staphylococcus aureus
PCR	Polymerase chain reaction
Spa	*Staphylococcus aureus* protein A
Ig	Immunoglobulin
bp	Base pair
SSRs	Short sequence repeats
TSST-1	Toxic shock syndrome toxin-1
ETs	Exfoliative Toxins
ACME	Arginine Catabolic Mobile Element
SSSS	Staphylococcal scalded skin syndrome
MLST	Multi -locus sequence typing
BURP	Based Upon Repeat Pattern
CCs	Clonal complexes
TAE	Tris Acetate EDTA
EDTA	Ethylenediaminetetraacetic acid
UV	Ultra-violet light

CSF	Cerebrospinal fluid
CVP	Central venous pressure
CMSA	Coagulase Mannitol Salt Agar
BHI	Brain heart infusion
FOX	Cefoxitin Antibiotic
CLSI	Clinical Laboratory Standards Institute
MOH	Ministry of Health
SPSS	Statistical Package for Social Sciences
MH	Mueller-Hinton
ICU	Intensive Care Unit
NT	Non-typeable
Pen G	Penicillin G
AMC	Amoxicillin/Clavulanic acid
CRO	Ceftriaxone
MEM	Meropenem
E	Erythromycin
C	Clindamycin
CIP	Ciprofloxacin
CN	Gentamicin
V	Vancomycin

Chapter One:

Introduction

1.1 Background:

Staphylococcus aureus (*S. aureus*) is an important bacterial pathogen in both community and healthcare-related settings in different parts of the world. It is one of the major human pathogens that can cause a broad variety of human diseases ranging from mild skin and soft tissue infections (SSTIs) to sever life-threatening invasive infections; such as: endocarditis, osteomyelitis, necrotizing pneumonia, bacteremia, septicemia, septic arthritis, meningitis, food poisoning and toxic shock syndrome (Bazzoun, Harastani, Shehabi, & Tokajian, 2014; Harastani & Tokajian, 2014; Kobayashi, Malachowa, & DeLeo, 2015; Serray et al., 2016; Tong, Davis, Eichenberger, Holland, & Fowler, 2015).

S. aureus is gram-positive cocci, arranged in clusters and described as "grape like". It is catalase positive, coagulase positive, mostly beta hemolytic on blood agar medium and a salt tolerant organism (tolerates up to 10% salt concentration). It can grow at a wide range of temperatures between 18 °C and 40 °C, (optimal 35 °C), aerobically or anaerobically (facultative). This bacteria have many potential toxins and virulence factors that can easily evade the immune system and disrupt phagocytosis, such as the capsule and protein A which is considered as an immunoglobulin binding protein (Foster, 1996; Taylor & Unakal, 2018).

S. aureus is a pervasive member of the human microbiological flora with up to 20-30% of humans that are persistently asymptomatically colonized with *S. aureus* on their skin or mucous membranes. However, 50-60% are occasionally colonized in the anterior nares (Frank et al., 2010; Gorwitz et al., 2008; J. A. Kluytmans & Wertheim, 2005; Otto, 2013; Wertheim et al., 2005; Williams, 1963). Moreover, a higher rate was reported for *S. aureus* as a human colonizer in approximately 30-50% of individuals, elsewhere (Noble, Valkenburg, & Wolters, 1967; Sowash & Uhlemann, 2014).

When *S. aureus* invades the skin or the mucosal surfaces, it can cause severe diseases and invasive infections. However, *S. aureus* carriage has been seen as a significant risk factor for *S. aureus* infections, in both healthy and chronically ill patients, especially the immunocompromized and hospitalized patients (Kooistra-Smid, Nieuwenhuis, van Belkum, & Verbrugh, 2009; Laupland, Ross, & Gregson, 2008). However, *S. aureus* pathogenesis and severity of the infections are usually related to different factors; such as: host colonization, immune system, infection site, acquisition of novel genetic elements and virulence factors and toxins carried by *S. aureus* (Krakauer & Stiles, 2013; Krishna & Miller, 2012; Liu, 2009). So, the carriage of *S. aureus* is a major risk factor and appears to play an important role in the epidemiology and pathogenesis of infections (J. Kluytmans, van Belkum, & Verbrugh, 1997; von Eiff, Becker, Machka, Stammer, & Peters, 2001).

Unfortunately, no effective licensed vaccine for *S. aureus* is approved till now. Thus, no complete protective measures are available in the community. Also, *S. aureus* infections depend on antibiotic therapy that are evolving resistance and treatment difficulties (Giersing, Dastgheyb, Modjarrad, & Moorthy, 2016; Spellberg & Daum, 2012).

S. aureus was susceptible to many different antibiotics that have been developed. However, the ability to acquire antibiotic resistance mechanisms has resulted in epidemics and pandemics waves of antibiotic resistant *S. aureus* strains in the past 60 years. Penicillin has become non-effective against *S. aureus* within 10 years after its introduction for use in people. To overcome this resistance, the semi-synthetic penicillin, methicillin, was developed. Yet, in 1961, only two years after methicillin was introduced, Methicillin Resistant *Staphylococcus aureus* (MRSA) was first reported in England (Jevons, 1961).

Worldwidely, MRSA is a dangerous resistant pathogen and is considered as a major public health threat in both community acquired (CA-MRSA) and hospital acquired infection (HA-MRSA) and has become a global epidemic (Carroll, 2008; Grundmann,

Aires-de-Sousa, Boyce, & Tiemersma, 2006; Vindel et al., 2009; Zhang, McClure, Elsayed, Louie, & Conly, 2005). It is accompanied by changes in the characteristics of MRSA strains emerging in different parts of the world. Methicillin resistance is caused by reformed penicillin-binding protein (PBP). PBP is an enzyme that mediates the transpeptide cross-linking of the peptidoglycan of the bacterial cell wall (Chatterjee & Otto, 2013; Green et al., 2012; Stapleton & Taylor, 2002). This alteration in the PBP lowers the affinity to all the beta-lactam antibiotics; including: all penicillins, cephalosporins (except the fifth generation ceftaroline) and carbapenems (CDC, 2017; Duplessis & Crum-Cianflone, 2011).

Moreover, several kinds of PBPs are involved in the resistance mechanism of MRSA which are encoded by the *mec* gene. For example, PBP2c are encoded by the *mec*C gene and PBP2a are encoded by the *mec*A gene which is the more common resistant gene among MRSA strains (Garcia-Alvarez et al., 2011).

In the last two decades, exclusively, MRSA isolates have been shifted from being hospital associated (HA-MRSA) to community associated (CA-MRSA), which is the most common cause of skin and soft tissue infections (Miller & Kaplan, 2009; Talan et al., 2011). The emergence and increase in the incidence of CA-MRSA as an opportunistic pathogen occurred over the past 15–20 years with many critical implications (Chambers, 2001; Conly & Johnston, 2003; Uhlemann, Otto, Lowy, & DeLeo, 2014). New MRSA clones were recognized as CA-MRSA isolates that do not only spread rapidly in community among healthy people, but also can cause different important and invasive infections in the health care related settings as well (David & Daum, 2010). Reduction in MRSA infections is considered as an important medical challenge, because these infections are associated with a serious economic burden on the healthcare resources and increased hospitalization costs due to the prolonged courses on more complex antibiotics and extended hospital days (Gould, Reilly, Bunyan, & Walker, 2010; Siddiqui & Whitten, 2018).

Accordingly, epidemiologic typing of MRSA using a combination of both phenotypic and genotypic typing methods has contributed to the understanding of the clonal changes

the epidemiological distribution of MRSA among different populations. Moreover, the molecular characterization of MRSA is crucial to monitor and limit the occurrence and development of new epidemic clones of MRSA (Grubb, 1988; Montesinos, Salido, Delgado, Cuervo, & Sierra, 2002; Sun et al., 2013). The genotyping is also important in MRSA identification and correlation studies. Several studies have shown that the molecular epidemiology of MRSA is changing and the antimicrobial resistance rates associated with MRSA are increasing in the last two decades (David et al., 2008; Enright et al., 2002).

1.2 Problem statement

MRSA has become a real international problem everywhere. It's a major public health concern in Palestine, responsible for both hospital and community associated infections.
The increase in the prevalence of MRSA in Palestine requires special attention and infection control programs for protecting patients and health care workers. This includes: patient education, awareness of presenting signs, proper antibiotic use and appropriate hand and clinic hygiene. In Palestine, data on MRSA are generally rare and with relatively little details regarding its molecular epidemiology and common epidemic clones. Until recently, MRSA molecular characteristics in our region are poorly studied and the scale of the problem is largely unknown.

It is important to study the molecular characteristics and the antimicrobial susceptibility testing (antibiograms) of MRSA in Palestine to understand the evolutionary dynamics and any probable presence of significant clones or clustering in our hospitals.

1.3 Study justification:

The frequency of both community acquired and hospital acquired MRSA in Palestine is increasing. The prevalence of MRSA and the epidemic clones in Palestine is not very clear and need more investigations. Also, the characterization of the molecular epidemiology of MRSA is essential to monitor and limit the occurrence and development of new resistant epidemic clones of MRSA in our region.

1.4 Study hypotheses:

1. Presence of unique MRSA clones in our hospitals.
2. There is an association between the molecular features and the antibiograms and resistance trends of MRSA in West Bank-Palestine.
3. MRSA isolates in our region have toxin genes which are related to MRSA virulence and invasion.

1.5 Study Goal:

To characterize the molecular epidemiology, virulence toxin genes and the antibiogram of MRSA in the West Bank-Palestine.

1.6 Study aim and objectives:

- **Aim**: This study aimed to investigate the epidemiological and genetic diversity of MRSA isolates in healthcare settings in the West Bank-Palestine, from 2015 to 2017.

- **Objectives**:

1. To study the molecular characteristics and the epidemic clones of MRSA strains in the West Bank-Palestine.
2. To compare and combine the molecular features with the antibiograms and resistance trends of MRSA.
3. To identify some toxin genes of MRSA which are related to MRSA virulence and invasion.

1.7 Summary of Thesis Chapters:

This thesis is divided into five main chapters. The first chapter reviews an introduction for the study subject and describes the problem we have analyzed, showing our main goals and objectives. In chapter two, we have focused on the history of MRSA and how these resistant bacteria have developed over the years, and we have studied the most common methodologies that are currently used for the molecular characterization of the resistant genes related to MRSA. Chapter three demonstrates the detailed methodologies used in this study, while chapter four focuses on the results we obtained, and correlates our results with the patient's antibiograms and the demographic data we could obtain from hospitals. In chapter five, our results and findings are discussed and compared with the findings of others and near countries. In the last part, we conclude our recommendations, strengths and limitations of this study and finally our future plans and goals. Chapters six and seven display the references used in this study and the appendices, respectively.

Chapter Two:

Literature review

2.1 History of MRSA, epidemiology and target populations:

Penicillin was introduced as a medical treatment in early 1940s for most S. *aureus* infections. Few years later, resistance to penicillin and beta-lactam antibiotics has started to develop due to the acquisition of a plasmid and coding for penicillinase enzyme which cleaves the beta-lactam ring of the penicillin antibiotics (Bondi & Dietz, 1945). Now, more than 90% *S. aureus* isolates can produce the penicillinase enzyme, regardless of the clinical setting and become no more sensitive to this antibiotic (Lowy, 2003).
In 1959, methicillin was introduced to overcome infections caused by *S. aureus* that produces the hydrolyzing enzyme beta-lactamase. Two years later, in 1961, first MRSA strain had developed quickly and spread worldwide. This development has reduced the therapeutic options for these bacteria (Jevons, 1961; Lowy, 2003; Parker & Hewitt, 1970; Schito, 2006). Initially, MRSA strains were developed only in hospitals, but in the late 1990s, first virulent CA-MRSA clones have been detected (David & Daum, 2010). CA-MRSA infections were related to the presence of the toxin Panton-Valentine Leukocidin (PVL) which had appeared rapidly and unexpectedly (DeLeo & Chambers, 2009; Enright et al., 2002; Green et al., 2012; Vandenesch et al., 2003).

MRSA-related skin and soft tissue hospital infections have increased to double over ten years since 2005 (Green et al., 2012; Moran, Abrahamian, Lovecchio, & Talan, 2013). MRSA infections had accounted for more than 60% of nosocomial *S. aureus* infections in the intensive care units with a significant increase and change in the epidemiology of MRSA in US hospitals between 1992-2003 (Klevens et al., 2006). Moreover, the incidence of *S. aureus* infections increased 50% from 1999 to 2005 and estimated to be about 13.8 per 1000 hospitalizations in US hospitals in 2005 (Klein, Smith, & Laxminarayan, 2007). Also, about 80,461 invasive MRSA infections and 11,285 related deaths occurred in the United States of America by 2011 with an estimated annual burden

of between $1.4 billion and 13.8 billion that was attributed to CA-MRSA (Abdulgader, Shittu, Nicol, & Kaba, 2015; CDC., 2013; Lee et al., 2013). In the European Union, it has been reported that MRSA infections annually affect more than 150,000 patients with high associated costs to the healthcare systems (Kock et al., 2010).

The *mec*A gene provides a general resistance for many beta-lactam antibiotics, such as the penicillins and almost all cephalosporins (Munita & Arias, 2016). Also, a novel mobile genetic element called Staphylococcal Cassette Chromosome *mec* "SCC*mec*" element may contain genes responsible for resistance to broad spectrum antimicrobial agents beside the beta-lactams (Chatterjee & Otto, 2013; Ito et al., 2001; Reygaert, 2013). A SCC*mec* element was identified in a Japanese *S. aureus* strain at a specific site of the N315 chromosome in 1981 and characterized by cloning the chromosomal region surrounding *mec*A region consisting of *mec*A, its regulatory genes (*mec*I and *mec*R1) and the insertion sequence IS431(Ito et al., 2001; Ito, Katayama, & Hiramatsu, 1999; Katayama, Ito, & Hiramatsu, 2000), (Figure 2.1).

The SCC*mec* is specified from other SCCs by the carriage of methicillin resistant-determinant. The determination of the entire nucleotide sequence and subsequent comparison with the corresponding region of MRSA strain revealed that the *mec* DNA has a specific structure for the genetic trait of methicillin resistance. Also, the island SCC*mec* carries two site-specific recombinase homologues and was named as a new family of staphylococcal genomic islands and its recombinases as cassette chromosome recombinases A and B (*ccrA* and *B*), located at the 3' end of *orfX* (Ito et al., 1999).

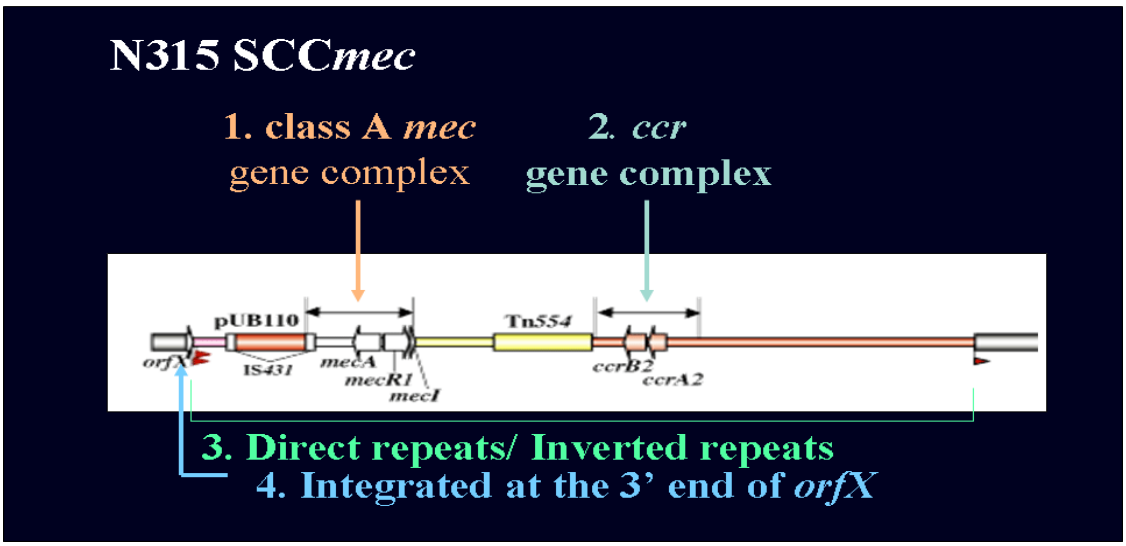


Figure 2.1: Genetic organization of the *mec*A gene complex in the SCC*mec* sequenced strains (N315) isolated in Japan in 1981. As shown, the SCC*mec* mobile element is characterized by *mec*A region consisting of *mec*A, its regulatory genes, *mec*I and *mec*R1, and the insertion sequence IS431. Also, the island SCC*mec* carries two site-specific recombinase homologues sites; the cassette chromosome recombinases A and B (*ccrA* and *B*), located at the 3' end of *orfX*. (Source: http://www.staphylococcus.net).

The biodiversity of novel strains and SCC*mec* elements has been described in recent years. The SCC*mec* types I, II and III are the most common types associated with HA-MRSA and are rarely found among the healthy population. However, the epidemiology of MRSA has been developed since the 1990s with the emergence of new SCC*mec* elements; such as SCC*mec* types IV and V. These types (IV and V) can spread easily outside the hospitals, infecting not only patients, but also colonizing healthy contact persons (Monecke et al., 2011; Okuma et al., 2002; Xiao et al., 2002). SCC*mec* types IV and V are CA-MRSA and can harbor genes encoding the toxin Panton-Valentine Leukocidin (PVL) which is a serious health problem risk due to its virulence and invasion toxic mechanisms (Kaneko & Kamio, 2004; Watkins, David, & Salata, 2012). These CA-MRSA types have increased mobility and the potential for horizontal spread of MRSA carriage or infections among populations (Albrich & Harbarth, 2008; File, 2007; Weber, 2005).

However, the CA-MRSA strains are more susceptible to the non beta-lactam antibiotic groups than the HA-MRSA strains, such as: aminoglycosides "mainly gentamicin",

tetracyclines and trimethoprim-sulfamethoxazole “SXT”, (Changchien, Chen, Chen, & Chu, 2016; Deresinski, 2005; Lina et al., 1999; Naimi et al., 2003).

In summary, since 1940 to 2000, four waves of antibiotic resistance in *S. aureus* have developed, as reviewed by Chambers and Deleo (Chambers & Deleo, 2009).
Wave one began in 1940, after the introduction of penicillin and continues up to day. Resistant strains of wave one had produced a plasmid that encoded the enzyme responsible for the hydrolysis of the beta-lactam ring (essential for penicillin activity) of penicillin, called penicillinase. It has been found that these strains can cause both community acquired infections as well as hospital acquired infections, mainly by a clone called: phage-type 80 (Blair & Carr, 1960; Bynoe, Elder, & Comtois, 1956; Rountree & Beard, 1958).

Wave two began in 1960, after two years upon the introduction of methicillin into clinical practice containing the SCC*mec* type I.

Later on, in 1970, wave three had developed with new SCC*mec* types (type II & III). Also, in wave three, Vancomycin Intermediate *Staphylococcus aureus* (VISA) has developed due to the upsurge in Vancomycin usage for treatment of complicated MRSA infections.

In the mid-to-late 1990s, wave four has begun with the emergence and shift to the CA-MRSA with a new SCC*mec* type (type IV). Also, in wave four, in 2002 Vancomycin-resistant *Staphylococcus aureus* (VRSA) strains has been first identified. Figure 2.2 shows a timeline of the four waves of antibiotic resistance in *Staphylococcus aureus* as reviewed by (Chambers & Deleo, 2009).

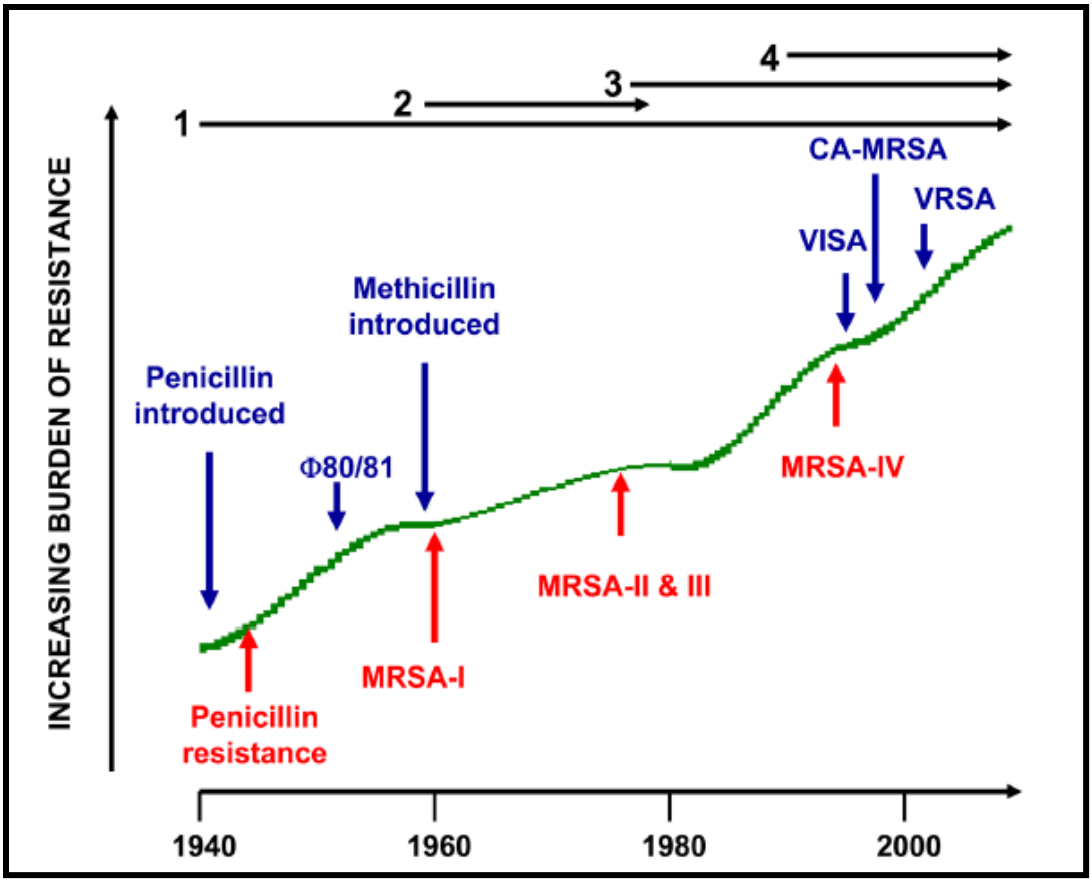


Figure 2.2: A timeline of the four waves of antibiotic resistance in *S. aureus* (Chambers & Deleo, 2009).

2.2 Common methodologies used for MRSA genotyping and toxin genes profiling

The application of several new molecular typing techniques depending on PCR for identification of MRSA strains and their gene encoding toxins gave noteworthy into the epidemiology of MRSA (Bukowski, Wladyka, & Dubin, 2010; Johnson et al., 1991).

2.2.1 SCC*mec* typing:

The SCC*mec* multiplex polymerase chain reaction (PCR) typing technique provides a strong evidence for the origin of MRSA, either HA-MRSA or CA-MRSA (Naimi et al., 2003).

The first multiplex PCR for SCC*mec* typing was reported by (Oliveira, Tomasz, & de Lencastre, 2001). However, three clonal types (Iberian, Brazilian, and Hungarian clones) were previously identified as the same as the European isolates of MRSA from England and Denmark in which all were closely related to a common background, named as background A. Also, there was a completely different genetic background in New York and Japan known as background B. Oliveira and his colleagues have shown that three recently characterized SCC*mec* types were associated with different pandemic clones in which both the SCC*mec* types I and III were related to the MRSA isolates of genetic background A with few structural variations, but the SCC*mec* type II was associated to the MRSA isolates of the genetic background B. Moreover, the SCC*mec* type IV has been identified to be related to background A as well as B in some MRSA strains (Oliveira, Tomasz, et al., 2001).

The multiplex SCC*mec* typing was improved in 2007 by Milheirico and his colleagues as a multiplex PCR involving a primer pair identifying the cassette chromosome recombinases (ccr) genes. They have updated a modified strategy for the method to identify the SCC*mec* types IV and V (Milheirico, Oliveira, & de Lencastre, 2007b).

The multiplex PCR developed by Zhang and his colleagues is used worldwide (Zhang, McClure, Elsayed, Louie, & Conly, 2005). This multiplex has designed new sets of SCC*mec* types with specific primers that can detect the SCC*mec* types I, II, III, IV and V with a concomitant detection of the *mecA* gene which is the methicillin resistant gene and can serve as an internal control for more validation of the multiplex PCR and better identification of MRSA with high sensitivity and specificity. This novel assay has shown a simple, rapid and useful typing technique for MRSA identification and epidemiological characterization (Zhang et al., 2005).

By 2011, eleven major SCC*mec* types (I to XI) have been identified with additional subtypes and variants (Shore et al., 2011; Turlej, Hryniewicz, & Empel, 2011).

2.2.2 *Staphylococcus aureus* protein A *(spa)* typing

The *spa* typing method was first applied in 1987 by Pickenhahn. Now, there are about 17641 identified *spa*-types with 390928 strains and 756 repeats registered in the Ridom *spa*-server, (http://www.spaserver.ridom.de) (Miao et al., 2017).

Protein A is a surface binding protein which can bind the Fcγ domain of immunoglobulin (Ig) molecules of the host and prevent phagocytosis by the host immune system, promoting host immune system suppression. This makes the *spa* one of the major virulence factors of *S. aureus* (Falugi, Kim, Missiakas, & Schneewind, 2013; Kobayashi & DeLeo, 2013; Votintseva et al., 2014). The *spa* contains three main regions: the Fc binding region, the X region and the C terminal. Its whole length is 2150 bp. However, the *spa* typing technique is based on DNA sequencing of short sequence repeats (SSRs) of the polymorphic X region of the staphylococcal protein A gene which consists of a variable number of 21-bp to 27-bp repeats, with commonly 24 bp (Koreen et al., 2004). This typing technique has many advantages in terms of accuracy, speed, interpretation, reproducibility, stability and inter-laboratory comparison (Faria, Carrico, Oliveira, Ramirez, & de Lencastre, 2008; Ruppitsch et al., 2006). Moreover, the *spa* typing of MRSA has been found to agree with the whole genome sequencing method in a great and reliable percentage of 97%, as described elsewhere (Bartels et al., 2014).

This method has been used for long term epidemiological and surveillance studies (Hallin et al., 2007). The exact function of the polymorphic X region of protein A is not well known, but the SSR variations are clearly related to bacterial pathogenesis, invasion to host tissues, evasion of the host immune system, attachment to the cell wall and virulence (Reischauer, Bernkopf, & Webersinke, 2010; Ruppitsch et al., 2006). However, the X region coding sequence in *spa* is a well conserved region and this allows the use of primers for PCR amplification and direct sequence typing (Shopsin et al., 1999). The *spa* typing has a discriminatory power for outbreak investigations and differentiation of different MRSA clones between different populations (Oliveira, Crisostomo, et al., 2001).

2.2.3 Toxin gene profiling (PVL, *TSST*-1,ETA & ACME-*arc*A)

Among multiple virulence factors, MRSA has the ability to produce several exotoxins which are directly associated with many diseases and symptoms.

2.2.3.1 Panton-Valentine Leukocidin (PVL):

PVL is one of the most critical toxins that can be produced by some MRSA strains. It was designated initially in 1894 by Van deVelde. In 1932, Panton and Valentine first associated PVL with skin and soft tissue infection, even before the introduction of MRSA (Panton & Valentine, 1932). This pore-forming cytotoxin can highly cause leukocyte destruction and tissue necrosis. The PVL-producing MRSA strains are emerging as a serious problem worldwide because this leukotoxin can cause lysis of the leukocytes (Leukocidine), inhibiting the host immune system (Boyle-Vavra & Daum, 2007; Melles et al., 2006). In addition, presence of PVL toxin is not only related to SSTIs, but also it has been reported to be related to necrotizing lung infections and pneumonia with high mortality rates associated with these infections (Al-Talib, Hasan, Yean, Al-Ashwal, & Ravichandran, 2011).

2.2.3.2 Toxic Shock Syndrome Toxin-1 (*TSST*-1):

This toxin is considered as a superantigen and was first reported in 1978 as an exotoxin encoded by the *tst* gene. It was associated mainly with strains isolated from patients with toxic shock syndrome in the USA. This toxin can stimulate the hallmark features of toxic shock syndrome mediating fever, hypotension, rash and multi-organ dysfunction. Also, it can lead to host adaptive immune suppression and dysfunction (Kulhankova, King, & Salgado-Pabón, 2014). Experimentally, it has been found that this exotoxin can produce fever in rabbits and enhance susceptibility to lethal shock and death (Chatterjee & Otto, 2013; Schlievert, Shands, Dan, Schmid, & Nishimura, 1981).

2.2.3.3 Exfoliative Toxins (ETs):

The ETs are superantigens and also known as “epidermolytic” toxins. ETs can lead to hydrolysis of the superficial skin layers and proteins leading to cutaneous infections (Bukowski et al., 2010).

There are two serologically and biologically active *S. aureus* exotoxins: exfoliative toxin A (ETA) and exofoliative toxin B (ETB) (Wiley & Rogolsky, 1977). These toxins are highly related to skin infections and dermatitis, especially in infants and children. However, it has been known that the exofoliative toxins of *S. aureus*, (mainly ETA), are responsible for the staphylococcal scalded skin syndrome (SSSS), which is also known as Ritters disease (Leung, Barankin, & Leong, 2018; Patel & Finlay, 2003). The SSSS is mainly characterized by blistering on the superficial skin layer, but does not affect the mucosa or deeper skin layers (Bukowski et al., 2010; Ladhani, Joannou, Lochrie, Evans, & Poston, 1999; Plano, Gutman, Woischnik, & Collins, 2000; Yamasaki et al., 2005).

2.2.3.4 Arginine Catabolic Mobile Element (ACME):

The ACME is associated with pathogenicity of the MRSA isolates which enhances both virulence and the ability of MRSA to colonize human skin. It is carried mainly by the USA-300 clone and related to the CA-MRSA of type IV (Diep et al., 2006; Mehrotra, Wang, & Johnson, 2000). This element is a mobile genetic element and has an important role in the growth and transmission of CA-MRSA strains (Watkins et al., 2012).
However, there are two main gene clusters identified in ACME: the *arc* genes (*arcA*, *arcB*, *arcC*, and *arcD*) and the oligopeptide permease operon (*opp*) genes (*opp-3A*, *opp-3B*, *opp-3C*, *opp-3D*, and *opp-3E*). Regarding the *arc* gene cluster, *arc*A is considered as a surrogate marker for ACME I (Diep et al., 2006; Miragaia et al., 2009).

The routine detection for all these toxins is usually by individual PCR for toxin-encoding genes or random amplified polymorphic DNA analysis, as it has been described elsewhere (Bukowski et al., 2010; Johnson et al., 1991; Lina et al., 1999).

2.3 Global epidemic status of MRSA:

One of the most dramatic epidemics of a single clone of CA-MRSA is that of the USA lineage: **USA-300**. This clone is a pandemic clonal lineage that is both resistant and highly virulent and can cause rapid tissue destructive infections in healthy young adults and children. It has caused great morbidity and mortality in the recent decades in USA (DeLeo, Otto, Kreiswirth, & Chambers, 2010).

It is a community acquired clone, found first in the USA around 17 years ago, and then it was found in South America, Europe and Asia-Pacific region. The USA-300 clone is the *spa* type t008 characterized by the carriage of both the PVL and ACME-*arc*A toxin genes (David et al., 2013; Strauss et al., 2017).

The recent emergence and spread of CA-MRSA has become a challenge even for countries that have so far maintained low rates of MRSA carriage (DeLeo et al., 2010). Continuous efforts to understand the changing epidemiology of MRSA infection in animals and human are necessary for appropriate antimicrobial treatment, effective infection control programs, and to monitor the evolution of the MRSA strains (Gould, 2005; Stefani et al., 2012).

Recently, Al Laham and his colleagues have studied MRSA clinical isolates from Gaza which gave a recent community widespread carriage of a the clonal complex (CC22) with *TSST-1* gene, known as the “Gaza strain”, (Al Laham et al., 2015)

Chapter Three

Methodology

3.1 Bacterial strains and data collection

In the present cross sectional descriptive study, a total of 112 MRSA isolates were collected between 16th of November 2015 and 13th of July 2017 from different hospitals/clinics. Major isolates were collected from "Al- Makassed Islamic Charitable Society Hospital" in Jerusalem. Other isolates were collected from the "Palestine Medical Complex" in Ramallah and "Beit-Jala Hospital" in Bethlehem. Few isolates were collected from the "Red Crescent Society Hospital", "Augusta Victoria Hospital", "Sehat Al-Quds" in Jerusalem , "Alia Hospital" in Hebron and from "Al-Watani Hospital, "Rafidia Hospital" and 'Al-Najah Hospital" in Nablus.

Samples were collected from different sources, including: wound, blood, nasal swabs, urine, pus, tissue, abscesses, ear swabs, sputum and other clinical sources such as: axillary swabs, cerebrospinal fluid (CSF) culture, central venous pressure (CVP) tip culture, skin swabs, synovial fluid culture and trap.

Demographic and clinical data including: age, sex, place of residence, date of administration and hospitalization, type of infection, isolate antimicrobial susceptibility testing, specimen origin and date of isolation were collected from medical records.

3.2 Bacterial Culture and identification of MRSA strains

MRSA isolates were identified phenotypically by colony morphology on blood agar, gram stain, and catalase and coagulase tests. Also, the isolates were sub-cultured on coagulase mannitol salt agar base (CMSA), which is recommended for the primary

isolation and differentiation of pathogenic *Staphylococci* from specimens or for classifying pure cultures (HIMEDIA, 2011). This medium is a selective medium for *Staphylococci* and differential for *S. aureus* from the other coagulase negative *Staphylococci* on the basis of coagulase production and mannitol fermentation by which the medium turns yellow and turbid as coagulase positive *S. aureus* grows on.

The strains of methicillin resistant *S. aureus* were detected by the disk agar diffusion method using a cefoxitin disk/30µg (FOX) on Mueller-Hinton agar plates (MH), incubated at 35^0C for 24h; a zone of inhibition ≤ 22 mm is considered as resistant according to the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). This was done mostly by the hospitals and confirmed for some isolates in our lab.

At the molecular level, the strains of MRSA were confirmed by the detection of *mec*A "methicillin resistant" gene as described elsewhere (Geha, Uhl, Gustaferro, & Persing, 1994), using the following primers: *mec*A1F (5'-GTAGAAATGACTGAACGTCCGATAA-3') and *mec*A2R (5'-CCAATTCCACATTGTTTCGGTCTAA-3'), yielding to 310-bp amplicons. Isolates that were confirmed to be methicillin sensitive by the disk diffusion method then by the absence of the *mec*A gene were excluded from this study, whereas, only MRSA isolates were included and characterized at the molecular level.

3.3 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was determined for few isolates by the hospitals using the Kirby-Bauer disk diffusion method according to CLSI recommendations (CLSI, 2017). However, the antimicrobial susceptibility testing full profile was missing or not provided for most isolates. Then, it was done in our lab following the same guidelines and instructions. The susceptibility for the following antibiotics: cefoxitin, penicillin G, amoxicillin/clavulanic acid, ceftriaxone, meropenem, vancomycin, erythromycin, clindamycin, gentamicin, SXT, and ciprofloxacin were determined. Isolates were considered to be susceptible isolates when the bacterial zones of inhibition confirmed to

the susceptibility category for a given antibiotic as provided in the CLSI (2017) manual. If isolates were susceptible to cefoxitin, they were excluded from the study. Isolates were defined as multidrug resistance strains (MDR) when they were resistant to at least three different antibiotic groups (non-β-lactam groups) in addition to resistance to the β-lactam antibiotics (Magiorakos et al., 2012). The D-test was performed to test the inducible resistance to clindamycin as needed.

3.4 Freezing of MRSA Isolates

The pure fresh isolates of MRSA were streaked on blood agar to be inoculated, then, in bacterial preserver vials containing 25% glycerol at -80°C until needed.
The freezing solution was prepared as the following: 37.8 gram of brain heart infusion broth (BHI) were weighed and suspended in a graduated cylinder with ddH_2O till 750mL. The solution was mixed well to become homogenous and 250 mL of pure glycerol were added. Then, the mixture was mixed well and autoclaved. After autoclaving, each 1 mL of the prepared freezing mixture was aliquoted in a screw capped sterile eppendrof tubes and freezed at -30°C to be used for the collected isolates.

3.5 DNA Extraction and Quantification

Genomic DNA was extracted from overnight fresh cultures by boiling method as described by Biber and his collegues (Biber et al., 2015) and/or by using the DNA extraction kit "Nucleospin, Macherey-Nagel , Germany" (NucleoSpin®, 2017).

For the extraction by boiling, a loopful suspension of overnight grown cultures on blood agar plates was prepared in a 1.5 mL microcentrifuge tube containing a prepared lysis solution of 95 µL of sterile nuclease free distilled water, 1 µL lysostaphin (1 mg/mL), 1 µL lysosyme (1mg/mL), 1 µL 1M Tris and 2 µL 0.5M EDTA. The suspension was first incubated at room temperature for 5 min. Then, incubated at 37^0C for 30 min and then heated in dry bath incubation at 95^0C for 10 min. The suspension was centrifuged at

10,000 rpm for 5 min; the supernatant was collected in a new clean collection microfuge tube and used as DNA template.

For the extraction by the Nucleospin Kit, it was done according to the manufacturer's instructions with some modifications. Briefly, 2-3 pure colonies were suspended in 180 µL T1 buffer and 25 µL Proteinase K solution. Samples were completely covered with this lysis solution and colonies were lysed by vigorous vortex yielding to a turbid mixture. T1 buffer and Proteinase K mixture are stable for 10–15 min before addition of samples because proteinase K tends to self-digest in T1 buffer without substrate. Thus, it was pre-mixed directly before use only. However, it is important to add proteinase K to digest proteins and remove contamination from preparations of nucleic acid. Then, the mixture was incubated in a shaking incubator overnight at 56°C until complete lysis is obtained. After lysis, 200 µL of B3 buffer were added to each sample, vortexed vigorously and incubated at 70 °C for 15 min. Then, after incubation, samples were vortexed vigorously and adjusted for the DNA binding step. Then, 210 µL absolute ethanol (96–100 %) were added to each sample, vortexed vigorously, and loaded to the NucleoSpin® Tissue Column silica membrane with the collection tube, provided by the kit. Then, samples were centrifuged for 1 min at 11,000 x g and first wash was done by adding 500 µL BW buffer and centrifuged for 1 min at 11,000 x g. After centrifugation, the flow through solution was discarded and columns were placed into new collection tubes. Second wash was done by adding 600 µL B5 buffer to the column and centrifugation for 1 min at 11,000 x g. Then, the flow through solution was discarded and the silica membrane columns were allowed to dry by centrifuging the columns with the collection tubes for 1 min at 11,000 x g. Residual ethanol is removed during this step. The final step, the elution step was modified to yield to high pure and high concentration DNA. Half volume of elution buffer (50 µL) was added to the column and incubated for 10 min at room temperature before centrifugation. The elution buffer was pre-heated at 60°C to dissolve the pellet well. After centrifugation, the second half of the elution buffer (50 µL) was added to the column and incubated, also, for 10 min at room temperature, then centrifuged again. By this, about 85–100 % of bound nucleic acid is eluted in the standard elution volume and at a high concentration.

The extracted DNA by the two methods was stored at –30°C for the molecular analysis. The concentration and purity of DNA were measured by (Nanodrop 1000 spectrophotometer; ThermoScientific). The instrument was adjusted using 1μL distilled water as a blank for the boiling method and the kit elution buffer as a blank for the Nucleospin kit extraction method. The protein/ DNA ratio (260/280 nm) was measured to estimate the purity of DNA samples. Whereas, samples with purity less than 1.2 were not used and re-extracted again. The boiling method was faster and simpler, but the DNA extraction by kit had shown higher purity and better stability of the DNA upon storage for further use.

The DNA concentrations used for molecular typing of all samples ranged between 20-200 ng/μL. However, the volume of template DNA was increased for samples with low concentration and the highly concentrated DNA samples were diluted with nuclease free distilled water for better yield and purity. Figure 3.1 (A) shows a summarized table taken as a snapshot from the Nanodrop 1000 spectrophotometer, for some representative randomly selected extracted samples that were used as template DNA for the genotyping profile. Figure 3.1 (B) shows a representative graph for an extracted DNA sample measured by the Nanodrop.

A

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
7	Default	6/25/2016	10:36 AM	184.22	3.684	3.277	1.12	1.62	50.00	230	2.279	1.804
8	Default	6/25/2016	10:38 AM	295.59	5.912	4.992	1.18	2.24	50.00	230	2.635	0.136
8	Default	6/25/2016	10:40 AM	276.58	5.532	4.657	1.19	1.98	50.00	230	2.801	0.359
10	Default	6/25/2016	10:42 AM	178.73	3.575	3.139	1.14	2.32	50.00	230	1.544	-0.034
25	Default	6/25/2016	10:43 AM	3354.02	67.080	29.389	2.28	3.18	50.00	230	21.086	0.177
20	Default	6/25/2016	10:44 AM	230.38	4.608	3.799	1.21	2.40	50.00	230	1.920	-0.041
15	Default	6/25/2016	10:45 AM	295.35	5.907	4.994	1.18	2.29	50.00	230	2.584	0.119
24	Default	6/25/2016	10:47 AM	251.22	5.024	4.405	1.14	1.90	50.00	230	2.645	1.116

B

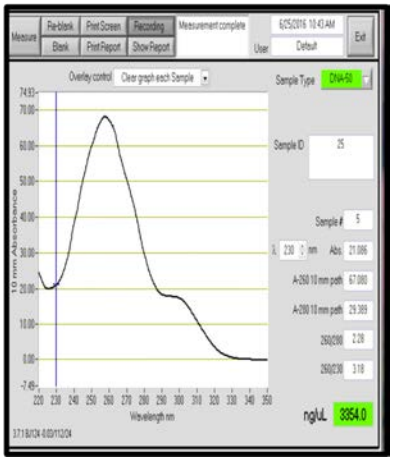


Figure 3.1: A: A table taken as a snapshot from the (Nanodrop 1000 spectrophotometer) for some representative randomly selected extracted samples that were used as template DNA for the genotyping profile. B: A graph of a representative extracted DNA sample as examined on the Nanodrop 1000 spectrophotometer.

3.6 Molecular characterization of the strains: Typing of isolates:

3.6.1 PCR identification of staphylococcal cassette chromosome (SCC*mec*) types:

SCC*mec* types (I–V) were identified by multiplex PCR amplification of the SCC*mec* region as described by others (Boye, Bartels, Andersen, Moller, & Westh, 2007). Table 3.1 shows the primers used in the multiplex PCR for the SCC*mec* typing.

Table 3.1: Primer sequences and target amplicons used for the SCC*mec* typing used in this study.

#	Primer name/target*	Primer sequence (5'-3')	length/bp[3]
1	β/ccrA2F[1]-B	ATTGCCTTGATAATAGCCYTCT	937
	α3/ccrA2R[2]-B	TAAAGGCATCAATGCACAAACACT	
2	ccrCF/ccrC	CGTCTATTACAAGATGTTAAGGATAAT	518
	ccrCR/ccrC	CCTTTATAGACTGGATTATTCAAAATA	
3	1272F1/IS1272	GCCACTCATAACATATGGAA	415
	1272R1/IS1272	CATCCGAGTGAAACCCAAA	
4	5R*mec*AF/IS431	TATACCAAACCCGACAACTAC	359
	5R431R/IS431	CGGCTACAGTGATAACATCC	

*: These primers were used as described by (Boye et al., 2007).
[1]F: Forward primer; [2]R: reverse primer,[3]bp: base pair.

The multiplex PCR mixture was prepared by diluting each stock primer (100µM) described above in table 3.1, as the following: 1:20 to get 5µM from primer β/α3, 1:16 to get 6.25 µM from primer ccrCF/ccrCR, 1:50 to get 2 µM from primer 1272F1/1272R1 and 1:40 to get 2.5 µM from primer 5R*mec*A/5R431.

Multiplex PCR reaction was carried out in a total volume of 25 µL using PCR-Ready Products™ from Syntezza (Syntezza, 2004) or Thermo Scientific PCR Master Mix with 1µL from each diluted primer and 2 µL from template DNA. When using the Thermo Scientific PCR Master Mix, 12.5 µL from this mix were taken with 2.5 µL dd H_2O. However, when Syntezza was used, 15 µL dd H2O with 1µL from each diluted primer were mixed in the Syntezza tubes. The multiplex reaction conditions for SCC*mec* typing using either Syntezza or Thermo Scientific PCR Master Mix are is summarized in table 3.2.

Table 3.2: Multiplex reaction conditions for SCC*mec* typing using either Syntezza or Thermo Scientific PCR Master Mix.

Reagents		Syntezza Mix/µL	Thermo Master Mix/µL
Primers (100 µM)	1:20 primer β/α3	1 µL F[2] + 1 µL R[3]	
	1:16 primer ccrCF[2]/ccrCR[3]	1 µL F + 1 µL R	
	1:50 primer 1272F1/1272R1	1 µL F + 1 µL R	
	1:40 primer 5R*mec*A/5R431	1 µL F + 1 µL R	
Ultra-pure ddH_2O[1]		15 µL	2.5 µL
DNA template		2 µL	
Thermo Master Mix		---	12.5 µL
Final volume/ µL		25 µL	25 µL
The samples were mixed well; then, the tubes were placed in a thermal cycler using program no.2 in table 3.5.			

[1]: Deionized distilled water; [2]F: Forward primer; [3]R: reverse primer.

The SCC*mec* type was determined according to the amplicon banding patterns and length obtained, as described in table 3.3. When the type was not determined or confirmed by multiplex, individual PCR was performed for each primer pair alone. If isolates could not be assigned to types (I–V), they were grouped into SCC*mec* non-typeable (NT). That means that this isolate does not agree to be any of the examined types (I-V).

Table 3.3: Gel band patterns and lengths for the multiplex SCC*mec* typing used in this study.

Multiplex SCC*mec* typing			Types					
#	Primers[1]	Length/bp	I	II	III	IV	V	NT[2]
1	β/ α3	937	--	X	--	X	--	--
2	ccrCF/ccrCR	518	--	--	X	--	X	--
3	1272F1/1272R1	415	X	--	--	X	--	--
4	5R*mec*A/5R431	359	--	--	--	--	X	--

[1]: This multiplex and its interpretation were taken from (Boye et al., 2007).
[2:] Non-typeable.

3.6.2 PCR identification of *spa* types:

All bacterial isolates were characterized by *spa* sequence-based typing as described by (Shopsin et al., 1999), using the following primers: 1095F/*spa*-F (5'-AGACGATCCTTCGGTGAGC -3') and 1017R/*spa*-R (5'-GCTTTTGCAATGTCATTTACTG -3'). All *spa* amplicons were sequenced and analyzed using the *spa* Type Finder/Identifier: (http://spatyper.fortinbras.us/).

3.6.3 Sequencing of *spa* amplicons:

All *spa* positive PCR products detectable by gel electrophoresis were sequenced. DNA sequencing was performed at the Hylabs sequencing service/ Jerusalem, Israel. The chromatograms were checked and the obtained sequences were assembled by the Bioedit software. The *spa* sequences were aligned with each other using the Multalin Multiple sequence alignment tool (http://multalin.toulouse.inra.fr/multalin/).

For clustering of *spa* types and grouping in *spa*-clonal complexes (*spa*-CC), the Based Upon Repeat Pattern (BURP) algorithm of the Staph-Type™ software was used (Ridom GmbH, Münster, Germany). Multi-locus sequence typing (MLST) clonal complexes (CC) corresponding to the respective *spa*-CCs were assessed from the SpaServer website

(www.spaserver.ridom.de) as it was demonstrated that there is a high concordance between BURP and MLST-based clustering results (Biber et al., 2015; Harmsen et al., 2003; Strommenger et al., 2008), this was based on the sequencing results, the *spa* typer server mentioned above and the available MLST data on the MLST database (http://saureus.mlst.net).

The BURP is an automated tool that can provide us with evolutionary signals by clustering the related clones "from the *spa* typing repeat regions" together as clonal complexes (CCs) for many different types (Mellmann et al., 2007). However, this method cannot provide full and optimal data for the MLST-CCs, but can be sufficient for short term and immediate goals.

The Ridom Staphtype is an excellent software tool allowing rapid classification and repeat determination of isolates into the particular clonal lineages. This typing technique is very helpful for short term local epidemiological studies for MRSA as well as for international surveillance studies, long term epidemiological studies and outbreak investigations (Strommenger et al., 2008).

3.6.4 Phylogenetic Analyses

Following DNA amplification and sequencing, a phylogenetic tree was constructed to determine the relationships between MRSA isolates based on the obtained *spa* gene data.

Phylogenetic analyses of the *spa* sequences were performed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) applying the neighbor joining and maximum likelihood algorithms. Phylogenetic tree analysis was conducted all the sequences as a FASTA format (https://www.ebi.ac.uk/Tools/sfc/emboss_seqret/) to the Multiple Sequence Alignment by CLUSTALW; (http://www.genome.jp/tools-bin/clustalw). The reliability of internal branches was assessed by bootstrapping with 1000 pseudo-replicates. Nodes with bootstrap support less than 70% were collapsed.

3.6.5 PCR identification of staphylococcal genes for virulence factors

Genes for virulence factors, namely PVL cytotoxin genes *(lukS* and *lukF*), which produce the PVL cytotoxin causing leukocyte destruction and tissue necrosis, *TSST*-1, ETA, and ACME-*arc*A, were tested for all of the isolates.

All bacterial isolates were characterized for the PVL toxin, using primers: *luk*-PV-1F/PVL (5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3') and *luk*-PV-2R/PVL (5'- GCATCAASTGTATTGGATAGCAAAAGC-3'). For the *TSST-1*, the following primers: G*TSST*R-1F (5'-ACCCCTGTTCCCTTATCATC-3') and G*TSST*R-2R (5'- TTTTCAGTATTTGTAACGCC-3') were used. For the ETA toxin, the following primers: GETAR-1F (5'-GCAGGTGTTGATTTAGCATT-3') and GETAR-2R (5'-AGATGTCCCTATTTTTGCTG-3') were used, and for the ACME-*arc*A toxin using the following primers: *arc*A-F (5'-GAGCCAGAAGTACGCGAG-3') and *arc*A-R (5'-CACGTAACTTGCTAGAACGAG-3'). These toxins were identified by individual PCR amplification yielding to: 433bp, 350bp, 93bp and 724 bp, respectively, as described elsewhere (Lina et al., 1999; Mehrotra et al., 2000; Vento et al., 2013).

The seven target genes, their primer sequences and amplicons length used in the molecular characterization of each MRSA strain in this study are summarized in table 3.4.

Table 3.4: All Target genes and primers used in this study.

#	Gene	Oligo[1]Name	Primer sequence 5'-3'	Length (bp)	Ref.[2]
1	*mecA*	*mec*A1F[3]	GTAGAAATGACTGAACGTCCGATAA	310	(Geha et al., 1994)
		*mec*A2R[4]	CCAATTCCACATTGTTTCGGTCTAA		
2	SCC*mec* multiplex "4 primers"	β/ccrA2F-B	ATTGCCTTGATAATAGCCYTCT	937	(Ito et al., 2001)
		α3/ccrA2R-B	TAAAGGCATCAATGCACAAACACT		
		ccrCF/ccrC	CGTCTATTACAAGATGTTAAGGATAAT	518	(Ito et al., 2004)
		ccrCR/ccrC	CCTTTATAGACTGGATTATTCAAAATA		
		1272F1/IS1272	GCCACTCATAACATATGGAA	415	(Boye et al., 2007)
		1272R1/IS1272	CATCCGAGTGAAACCCAAA		
		5R*mec*AF/IS431	TATACCAAACCCGACAACTAC	359	
		5R431R/IS431	CGGCTACAGTGATAACATCC		
3	*Spa*	1095F/*spa*-F	AGACGATCCTTCGGTGAGC	200-400	(Shopsin et al., 1999)
		1017R/*spa*-R	GCTTTTGCAATGTCATTTACTG		
4	PVL	luk-PV-1F/PVL	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	(Lina et al., 1999)
		luk-PV-2R/PVL	GCATCAASTGTATTGGATAGCAAAAGC		
5	*TSST*-1	G*TSST*R-1F	ACCCCTGTTCCCTTATCATC	350	(Mehrotra et al., 2000)
		G*TSST*R-2R	TTTTCAGTATTTGTAACGCC		
6	ETA	GETAR-1F	GCAGGTGTTGATTTAGCATT	93	(Mehrotra et al., 2000)
		GETAR-2R	AGATGTCCCTATTTTTGCTG		
7	ACME-*arc*A	*arc*A-F	GAGCCAGAAGTACGCGAG	724	(Vento et al., 2013)
		*arc*A-R	CACGTAACTTGCTAGAACGAG		

[1]Oligo: Oligonecleotide;[2]:Reference,[3]F: Forward primer; [4]R: reverse primer.

3.7 PCR Conditions:

All PCR amplification methods were optimized for each target gene in this study. PCR reactions were carried by the (Biometra TProfessional Basic Gradient Thermocycler). In all amplification reactions, both negative controls (mix without DNA) and positive controls (from pervious runs of PCR's in this study) were included.

All thermal cycler programs used for amplification of the target genes and toxins in this study are summarized in table 3.5.

Table 3.5: Thermal cycler programs used for the amplification of the targeted genes and toxins in this study.

#	Target Gene	PCR Program (Temp[1]°C/ Time)*					Cycles
		Initial denaturation**	Denaturation	Annealing	Extension	Final extension	
1	*mec*A	95°C /15 min[2]	95°C /30 sec[3]	58°C/30 sec	72°C/80 sec	72°C/10 min	35
2	SCC*mec*	95°C /15 min	94°C /30 sec	55°C/30 sec	72°C/80 sec	72°C/10 min	35
3	*Spa*	95°C /15 min	95°C /30 sec	58°C/30 sec	72°C/45 sec	72°C/10 min	35
4	PVL	95°C /15 min	95°C /45 sec	55°C/15 sec	72°C/30 sec	72°C/10 min	35
5	*TSST*-1	95°C /15 min	95°C /2 min	54°C/2 min	72°C/2 min	72°C/10 min	35
6	ETA	95°C /15 min	95°C /2 min	54°C/2 min	72°C/2 min	72°C/10 min	35
7	*arc*A	95°C /15 min	94°C /20 sec	55°C/30 sec	72°C/30 sec	72°C/10 min	35

*: These PCR programs were taken as described in their references for each gene mentioned earlier in table 3.4 and optimized to be used in this study too.
**: This initial denaturation time was used for Thermo Master Mix. Otherwise, when Syntezza tubes were used, initial denaturation temperature was modified for 5 min.
[1]Temp: Temperature ,[2]min: Minutes, [3]sec: Seconds.

3.8 Agarose gel electrophoresis:

Amplicons for all the characterized genes were analyzed electrophoretically in 2% agarose gels (SeaKem® LE agarose gel), run at 120 V in 1x -Tris -Acetate –EDTA buffer (TAE), prepared as described in the appendix, containing ethidium bromide (0.5 g/mL) for visualization by UV light using a gel documentation system (the Bio-Imaging Systems MiniLumi transilluminator). A 100 bp or 1Kb molecular weight standard ladders (ThermoScientific GeneRuler) was used as molecular weight standards for each gel.

3.9 Ethical Considerations

The study was approved by the Research Ethical Committee at Al-Quds University. Also, a permission from the Palestinian Ministry of Health (MOH) was obtained to review the clinical reports of patients and to collect the demographic data of patients. Written informed consents were sent for the participating hospitals and clinics.

3.10 Statistical Analysis

Each isolate was coded, and data were entered and analyzed using the Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics for all samples were presented as frequencies and percentages. The distribution of MRSA isolates in clinical samples was analyzed. The associations between the SCC*mec* type, *spa* type and MLST-CCs with the *arc*A, ETA, *TSST*-1 and PVL genes were analyzed using Chi-square test. A p-value of <0.05 was considered to be statistically significant.

Chapter Four:

Results

4.1 Bacterial isolates and Study population

From November 2015 to July 2017, 112 MRSA isolates were collected from ten hospitals and clinics distributed among five different Palestinian regions (Jerusalem, Ramallah, Nablus, Bethlehem and Hebron).

In fact, the highest number of isolates was collected from Al-Makassed Islamic Charitable Hospital (77, 68.8%) and twelve isolates from the Palestine Medical Complex (12, 10.7%). Other isolates were collected from the hospitals as shown in figure 4.1.

Eighty four isolates (75%) were collected from hospitals and clinics in Jerusalem and its suburbs, as the following: (77, 68.8%) from Al- Makassed Islamic Charitable Society Hospital, four isolates from Red Crescent Society Hospital (3.6%), two isolates from Augusta Victoria Hospital (1.8%) and one isolate from Sehat Al-Quds in Bethany (0.9%).

Twelve isolates were collected from the Palestine Medical Complex (10.7%) in Ramallah and seven isolates (6.2%) from Nablus hospitals distributed between three hospitals, as the following: four isolates from Al-Najah Hospital (3.6%), two isolates from Al-Watani/Nablus Hospital (1.8%) and one isolates from Rafidia Hospital (0.9%). Other isolates were collected from Beit Jala Hospital (6, 5.4%) and Hebron Hospital (3, 2.7%). Figure 4.2 displays a map of Jerusalem (Al-Quds) and the West Bank showing the distribution of MRSA isolates among hospitals which are included in this study, indicating the sampling area.

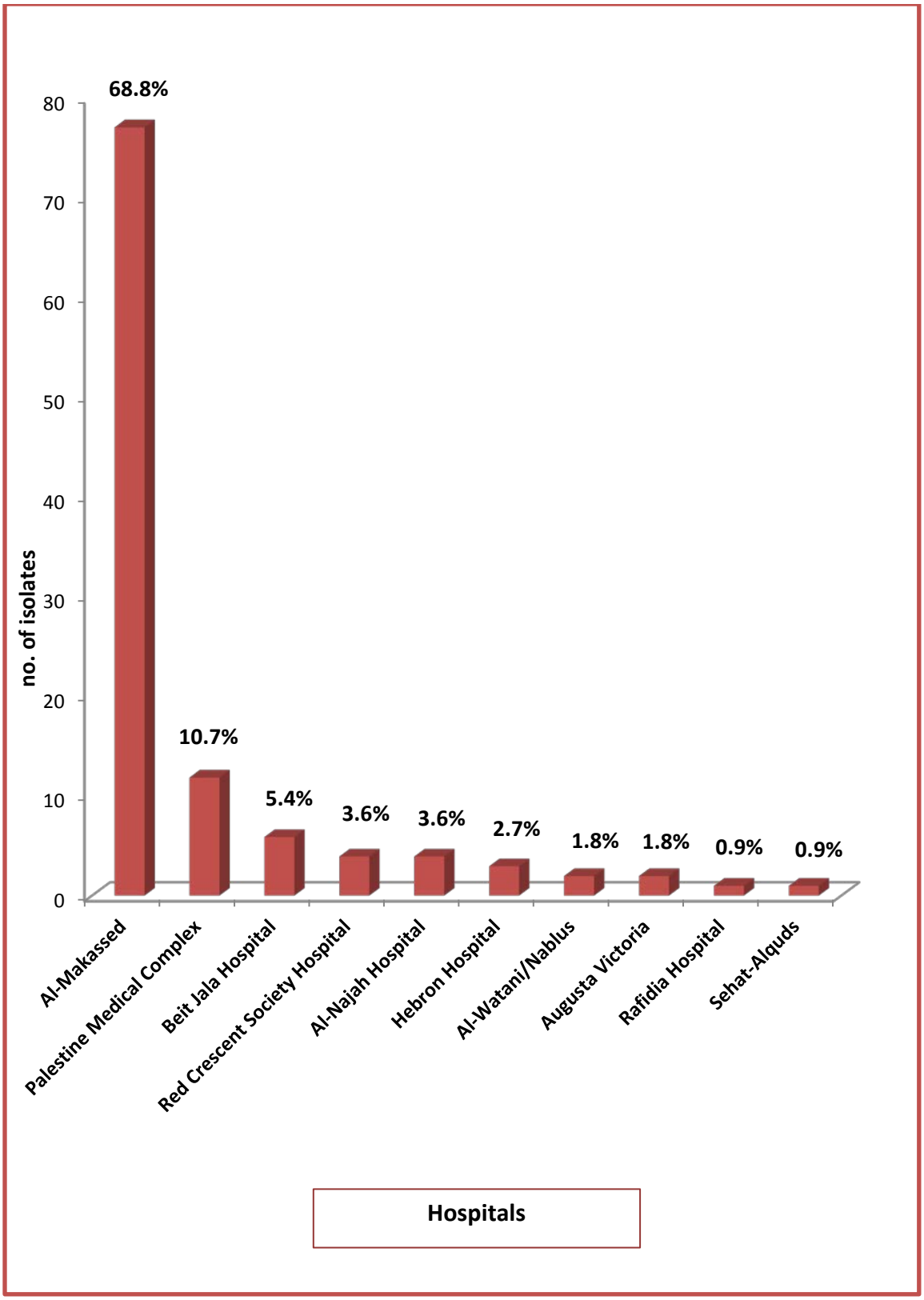


Figure 4.1: Number of isolates and their distribution among hospitals as collected in this study.

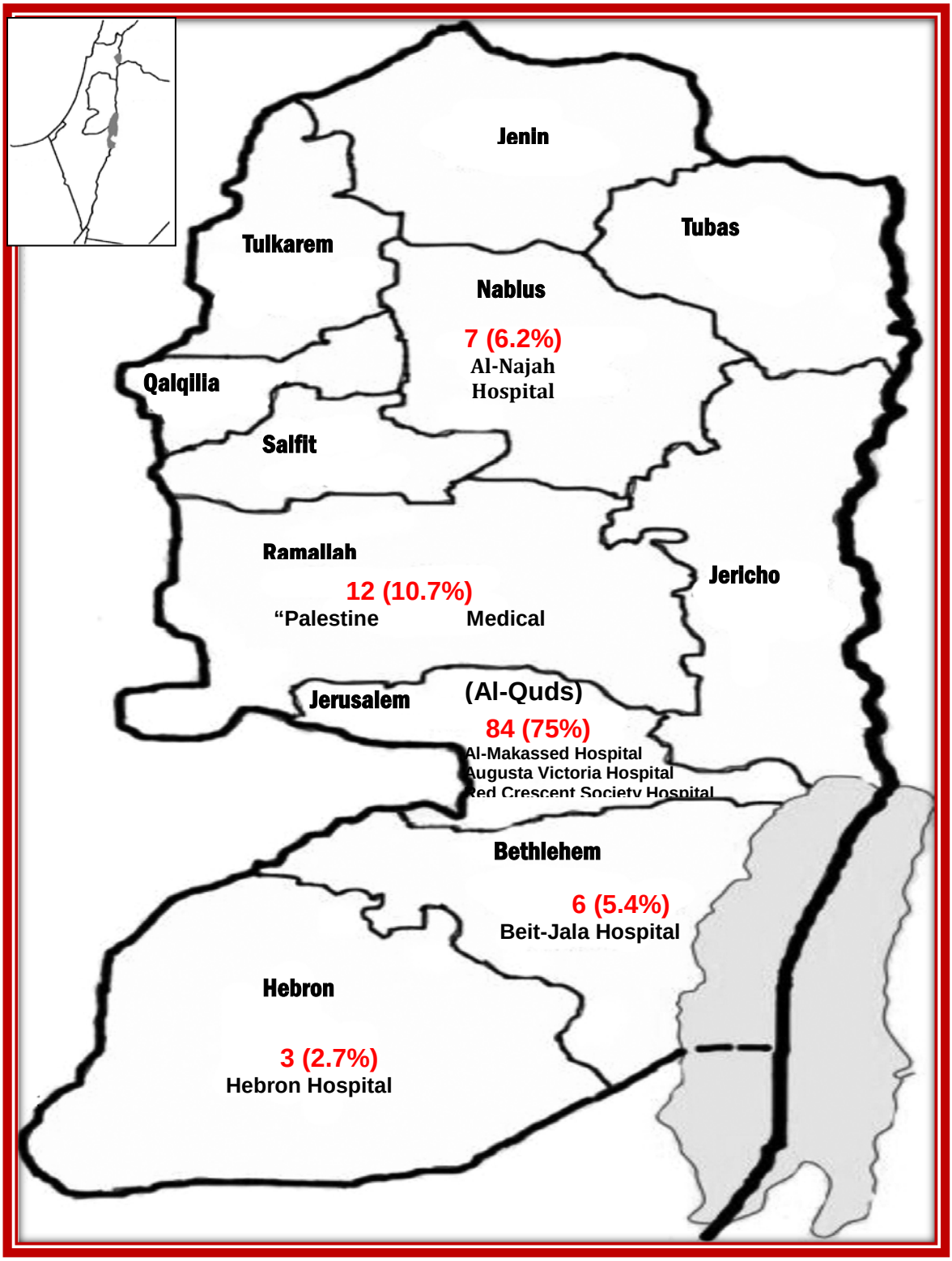


Figure 4.2: A map of Jerusalem (Al-Quds) and the West Bank showing the distribution of MRSA isolates among hospitals which are included in this study (Sampling area).

Full demographic data for each isolate including: the hospitalization period, age group, patient type, gender, place of residence, department and source were available for 82 (73.2 %) of these patients.

Place of residence for all included patients was documented from the archive section. The most frequent regions of the collected isolates were from Gaza and Jerusalem (33, 29.5% for each), followed by the Hebron (15, 13.4%), Nablus (12, 10.7%), Ramallah (12, 10.7%), Bethlehem (5, 4.5%), Tubas (1, 0.9%) and Tulkarem (1, 0.9%), (figure 4.3). However, isolates which were obtained from Gaza's patients were referred to Al-Makassed Islamic Charitable Society Hospital in Jerusalem.

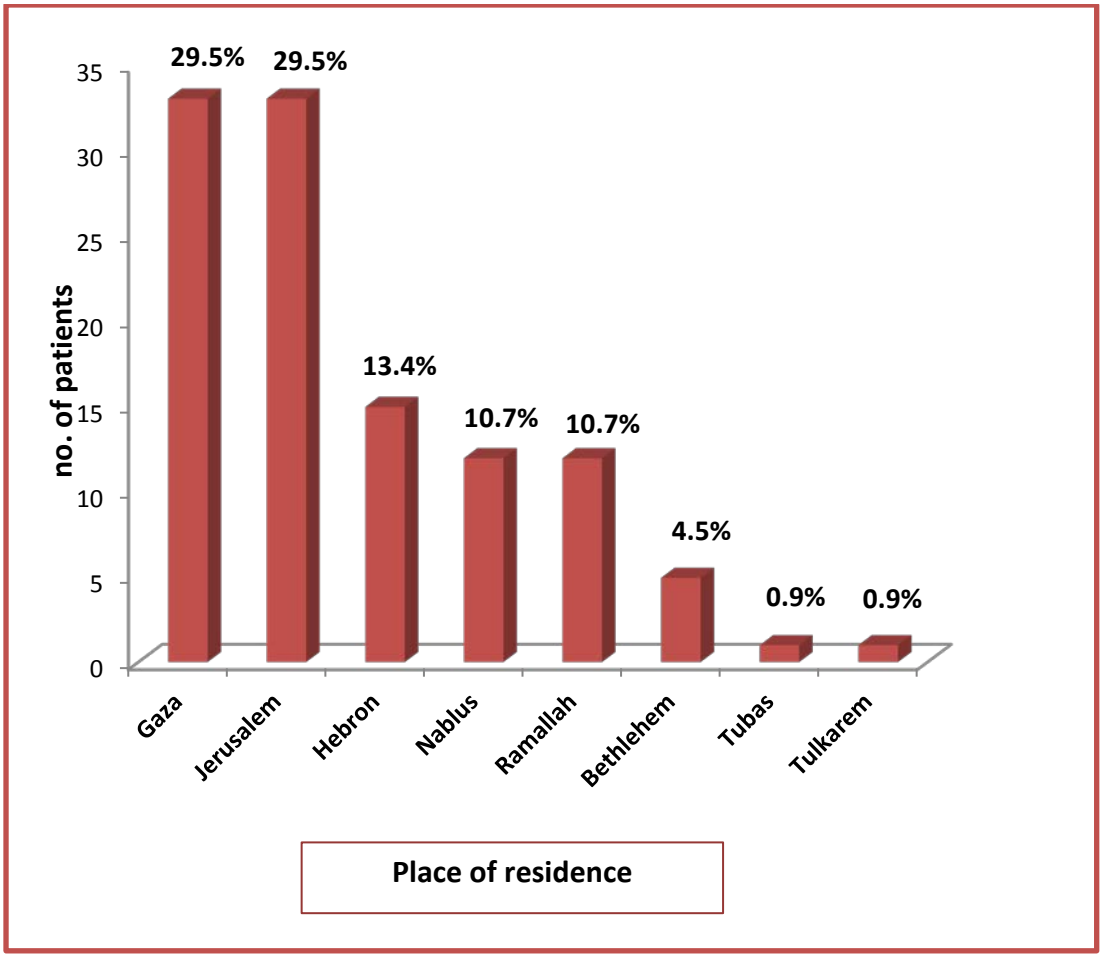


Figure 4.3: Number of patients and their place of residence as collected in this study.

According to gender, of all the 112 cases diagnosed as MRSA, 60 (53.6%) were males and 41(36.6%) were females, with high significant association with gender ($p<0.05$). However, gender of 9.8% (n=11) of the isolates were not found (Figure 4.4).

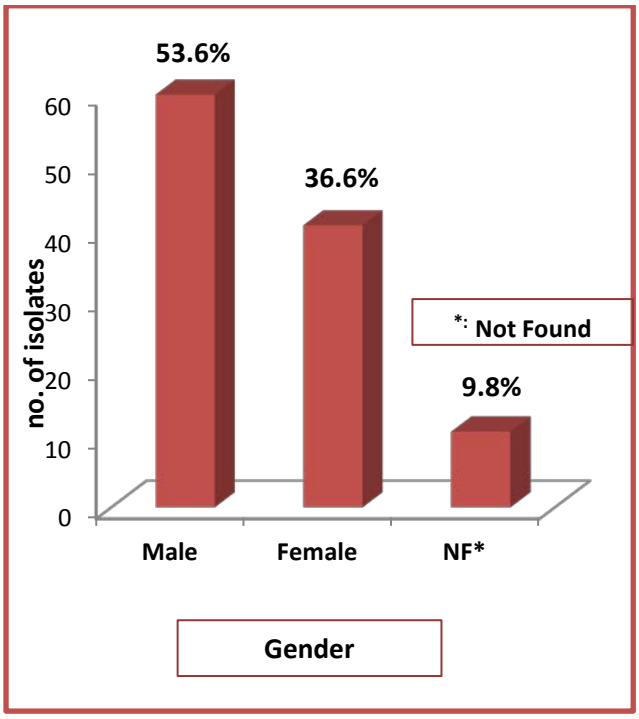


Figure 4.4: MRSA distribution according to gender.

Cases were of all ages. Figure 4.5 shows the age distribution. The mean age was 33 years (median: 30 years; interquartile range: 42 years), with the oldest case being a 85 years old female and the youngest a two months year old female. Age was categorized to four age groups, as the following: Infant; from 0-1 year, children; from 1-10 years, adolescent; from 10-19 years and as adult; more than 19 years.

The most common age group of isolates collected in this study was the adults (68, 60.7%), followed by the adolescents (16, 14.3%), infants (10, 8.9%) and children (8, 7.1%). Age was not found for 8.9% of the samples, (Figure 4.5).

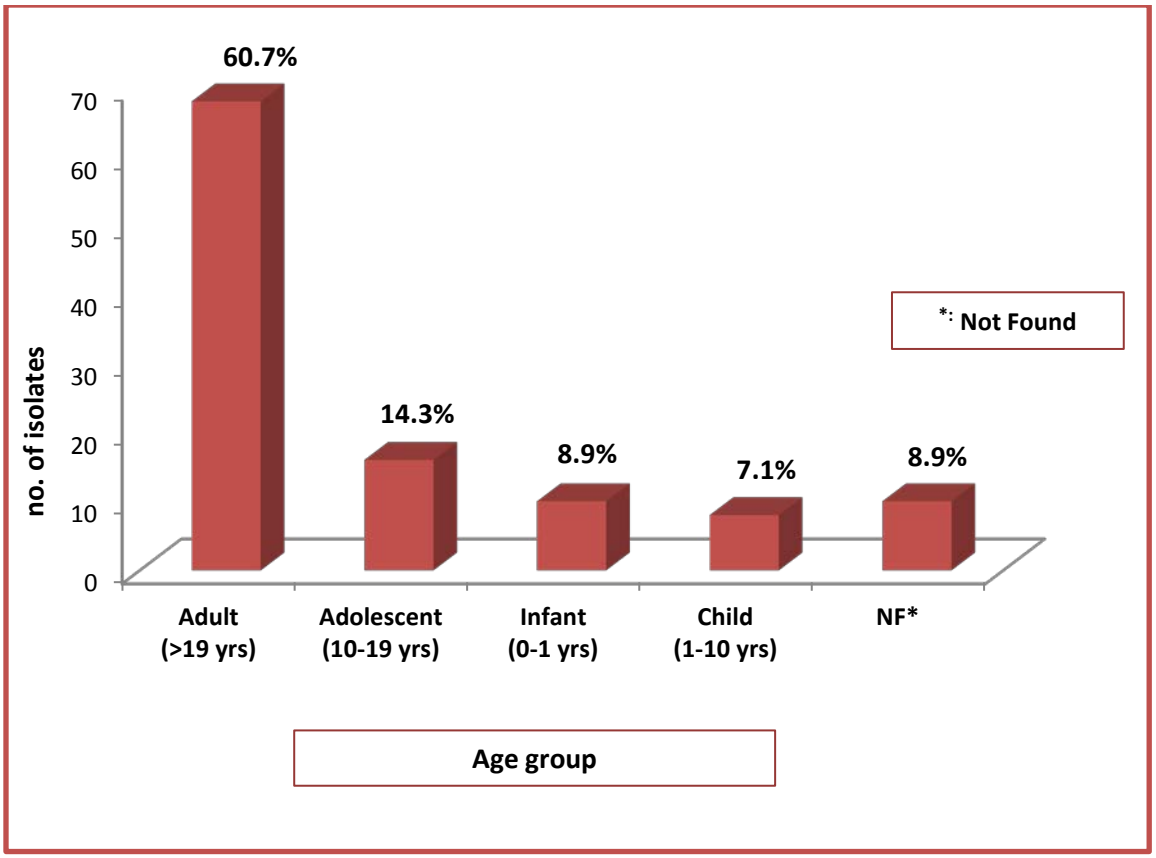


Figure 4.5: Distribution of age groups of patients that isolates were collected from during this study.

Combining gender with age groups showed that adult males (aged>19 years) were significantly at the highest risk ($p<0.05$), followed by adult females, then adolescent males (aged between 10-19 years). (Table 4.1).

Table 4.1: Relationship between gender and age groups of MRSA isolates in this study.

Gender	Age Group					Total
	Adult >19yrs	Adolescent 10-19yrs	Infant 0-1yrs	Children 1-10yrs	Not found	
Male	38	14	3	4	1	60
Female	29	1	6	4	1	41
Not found	1	1	1	0	8	11
Total	68	16	10	8	10	112

More than half of the patients were hospitalized (in-patients, 67.9%). Whereas, (23.3%) were out patients that have been admitted to the emergency or the day care clinics and discharged within the same day. Dialysis patients were considered as in-patients in which they are under healthcare facilities and medical devices exposure. Data for 10 isolates (8.9%) were missing, (Table 4.2).

Table 4.2: Patient types of the study population.

	Patient type	No. of isolates (*n*)	Percentage %
1	In-patient	76	67.9
2	Out-patient	26	23.2
3	NF[1]	10	8.9
	Total	112	100

[1]: Not Found.

The MRSA isolates were collected from different wards and areas in the hospitals. The highest number of cases were from the orthopedic ward (17.9%), followed by the surgery and neurosurgery wards (17.0%), the daycare or the out-clinics (11.6%), the emergency (8.9%), the Intensive Care Unit (ICU) (8.9%), the pediatric ward (8.0%) and other wards (8.9%), as the follows: the medical ward (2.6%), dialysis (1.8%), gynecology (1.8%), holding area (1.8%) and the Cardiac Care Unit (CCU) (0.9%). Data for 21 isolates (18.8%) were missing, (Table 4.3).

Table 4.3: Distribution of the MRSA isolates among the different hospital wards.

	Wards	No. of isolates (*n*)	Percentage %
1	Orthopedic ward	20	17.9
2	Surgery & Neurosurgery wards	19	17.0
3	Daycare	13	11.6
4	Emergency	10	8.9
5	ICU	10	8.9
6	Pediatric ward	9	8.0
7	Others[1]	10	8.9
8	NF[2]	21	18.8
	Total	112	100

[1:] Other wards include: The medical ward, dialysis, gynecology, holding area and the CCU ward, (n=3, 2, 2, 2, and 1) respectively.
[2:] Not Found.

Samples were collected from fifteen different clinical sources. The highest number of isolates were obtained from wound infections (35.7%), followed by: blood culture (12.5%), nasal swabs (8.9%), urine culture (8.9%), pus culture (5.4%), tissue culture (4.5%), abscess (3.6%), ear swabs (2.7%), sputum culture (2.7%) and other sources (5.4%), as the follows: axillary swab (0.9%), CSF culture (0.9%), CVP culture (0.9%), skin swab culture (0.9%), synovial fluid culture (0.9%), and trap (0.9%). Source of eleven isolates (9.8%) was not identified. Figure 4.6 summarizes the distribution of the different sources of MRSA isolates.

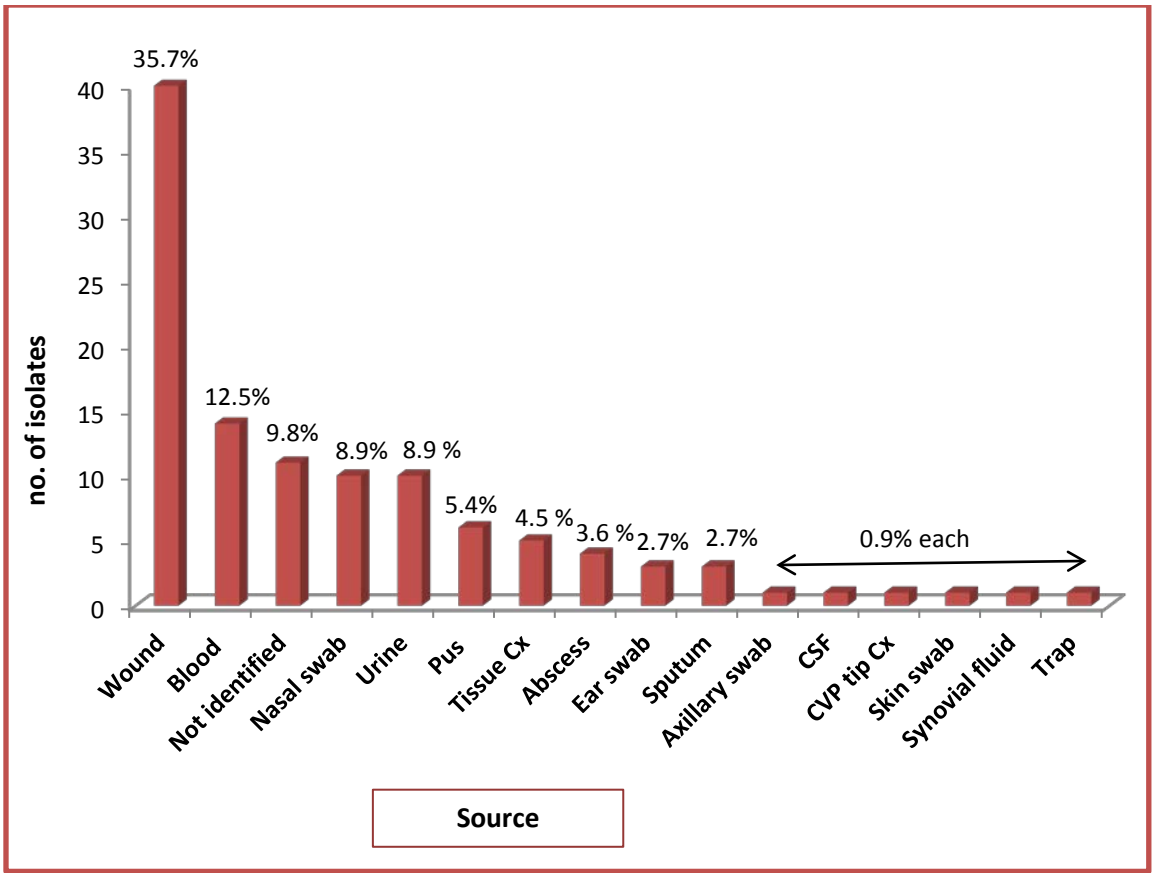
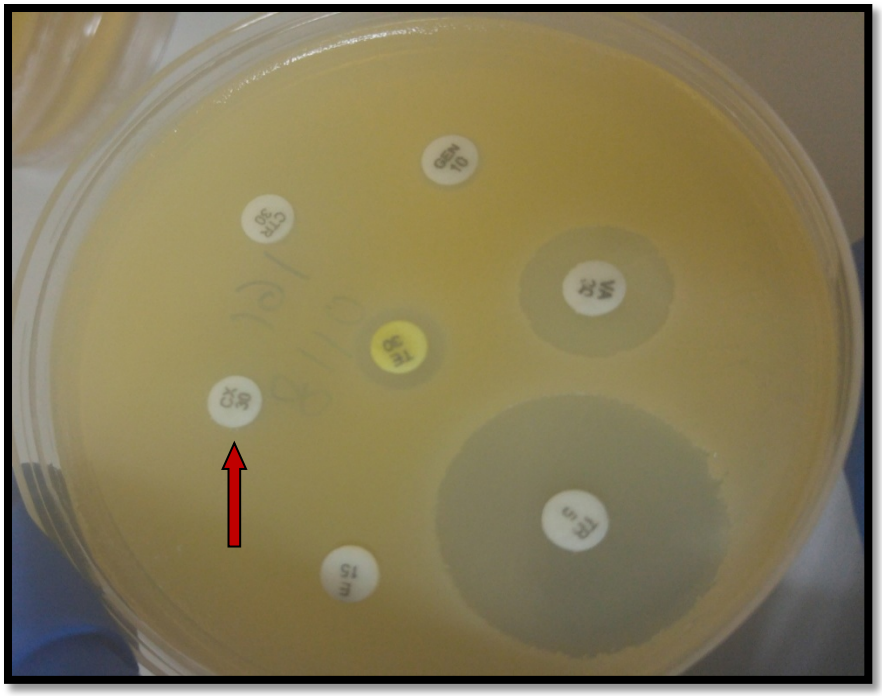


Figure 4.6: The distribution of the different sources of MRSA isolates that have been involved in this study.

4.2 Detection of MRSA by cefoxitin disk diffusion test and *mec*A gene

All of the 112 isolates (100%) were identified as Methicillin Resistant *Staphylococcus aureus* using the cefoxitin disc resistance (<22mm) and confirmed as "MRSA" using PCR targeting the *mec*A gene. Cefoxitin resistance was 100 % correlated with *mec*A gene presence. Figure 4.7 displays representative Mueller Hinton agar plates for the detection of MRSA isolates by the disk diffusion method showing the resistance pattern to cefoxitin. Figure 4.8 is a gel electrophoresis for the detection of the *mec*A gene for representative MRSA isolates in this study.

A



B

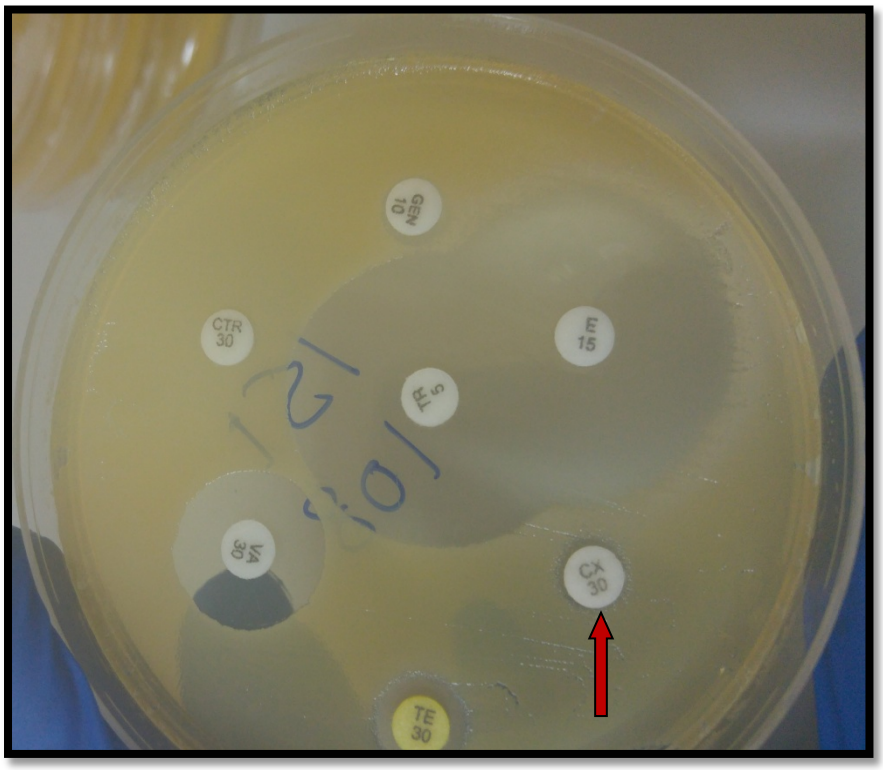


Figure 4.7: The disk diffusion method for the detection of MRSA. Two representative MH plates (A & B) showing the resistance pattern to cefoxitin (red arrows) by MRSA isolates.

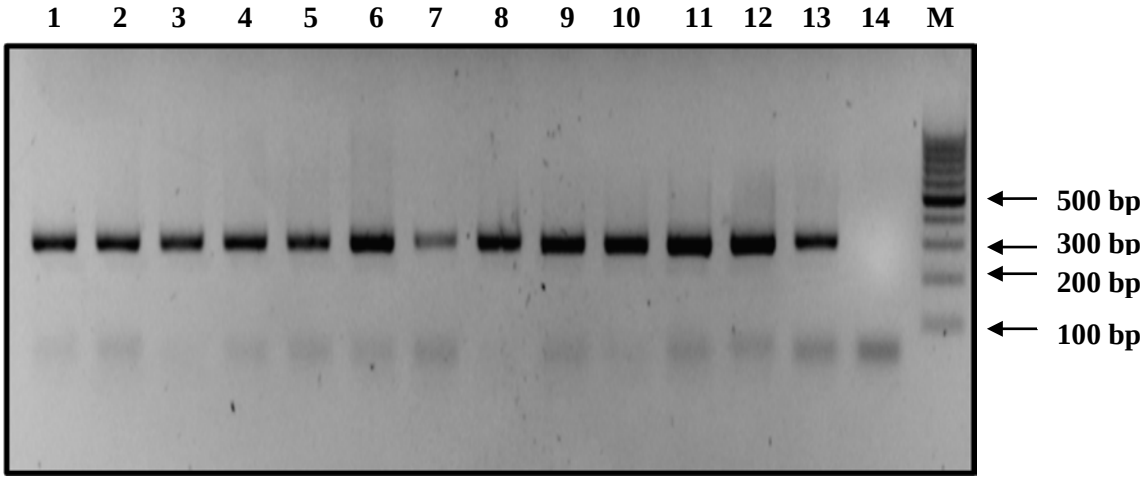


Figure 4.8: A representative agarose gel electrophoresis for the *mec*A gene detection in MRSA. Lanes 1-12: *mec*A positive MRSA isolates. Lane 13: positive control. Lane 14: negative control. M: 100 bp DNA molecular weight marker. Band size is around ~310 bp.

4.3 Antimicrobial susceptibility patterns:

All isolates were tested for susceptibility patterns to the 11 antibiotics. Aside from cefoxitin, high rates of resistance of MRSA isolates against penicillin G, amoxicillin/clavulanic acid, ceftriaxone and meropenem were noticed (100%) and resistance with variable degrees to erythromycin, ciprofloxacin, clindamycin were 63.4%, 39.3%, 34.8%, respectively. On other hand the susceptibility of MRSA isolates to gentamicin and SXT was high (76.8%, 81.3%), respectively. All isolates were susceptible to Vancomycin (100%). Of all the isolates, 32 (28.6%) were multi-drug resistant (MDR). These isolates were resistant to at least three different non-β-lactam antibiotic groups, (Figure 4.9).

Among the 32 MDR isolates, the highest rates were from wound (34%) and blood cultures (16%), followed by: urine (13%), nasal swabs (10%) and tissue cultures (6%). The other MDR isolates were from abscess (3%), axillary swab (3%), pus (3%) and CVP tip culture (3%). Other three isolates were MDR but their source were not available (9%), (Figure 4.10).

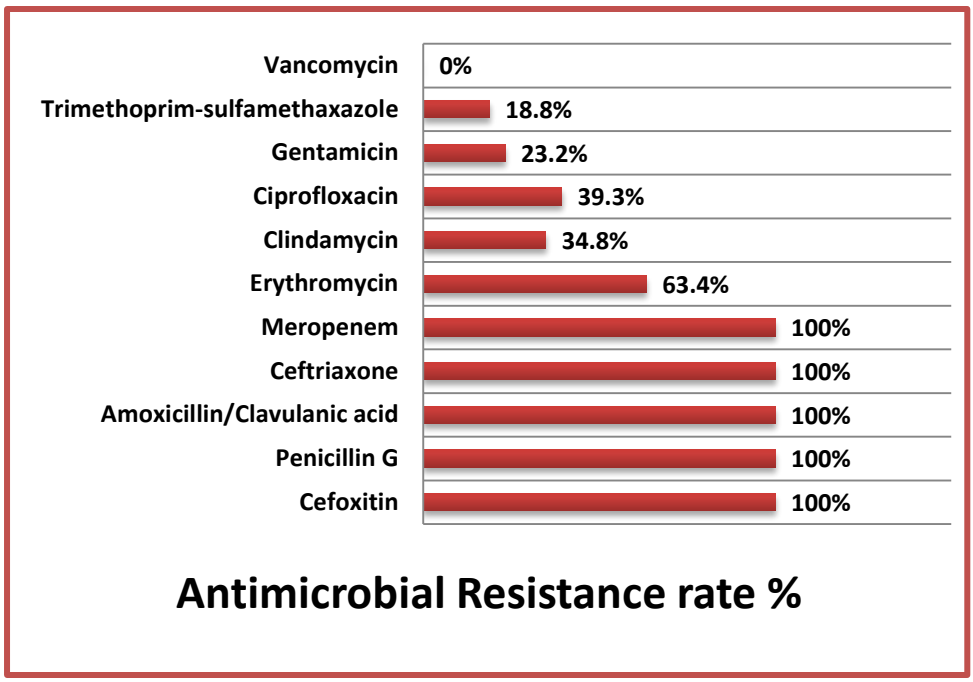


Figure 4.9: Antimicrobial resistance rates among all MRSA isolates in this study.

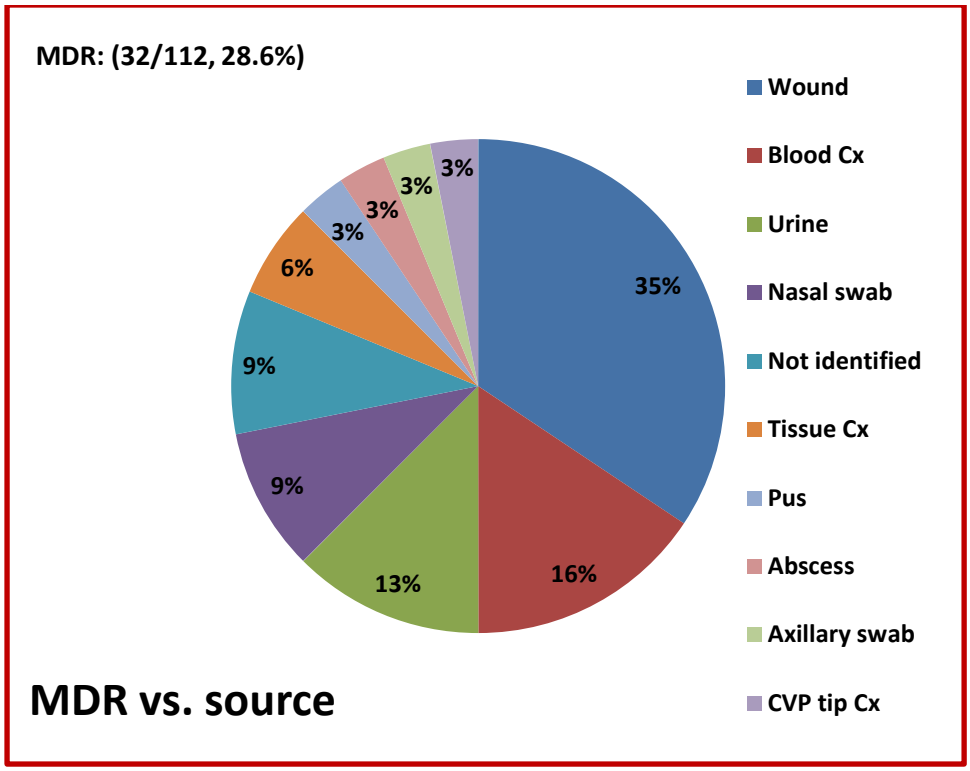


Figure 4.10: Distribution of the MDR isolates according to source.

4.4 Molecular characterization and typing of isolates

4.4.1 SCC*mec* typing:

Strong and easily banding patterns were obtained for most of the SCC*mec* types using the multiplex PCR, (Figure 4.11). Among the 112 MRSA isolates, ninety seven isolates were belonged to SCC*mec* type IV (86.6%), ten isolates were belonged to SCC*mec* type I (8.9%) and one was belonged to SCC*mec* type V (0.9%). Four MRSA isolates (3.6%) could not be SCC*mec* typed and designated as non-typeable (NT). All the NT isolates were retested, using the same primer set of SCC*mec* multiplex PCR, but as individual PCR. Both SCC*mec* type II and SCC*mec* type III were not detected among isolates. SCC*mec* type V was detected only in one isolate by multiplex PCR and more confirmed by individual PCR as shown in figure 4.12.

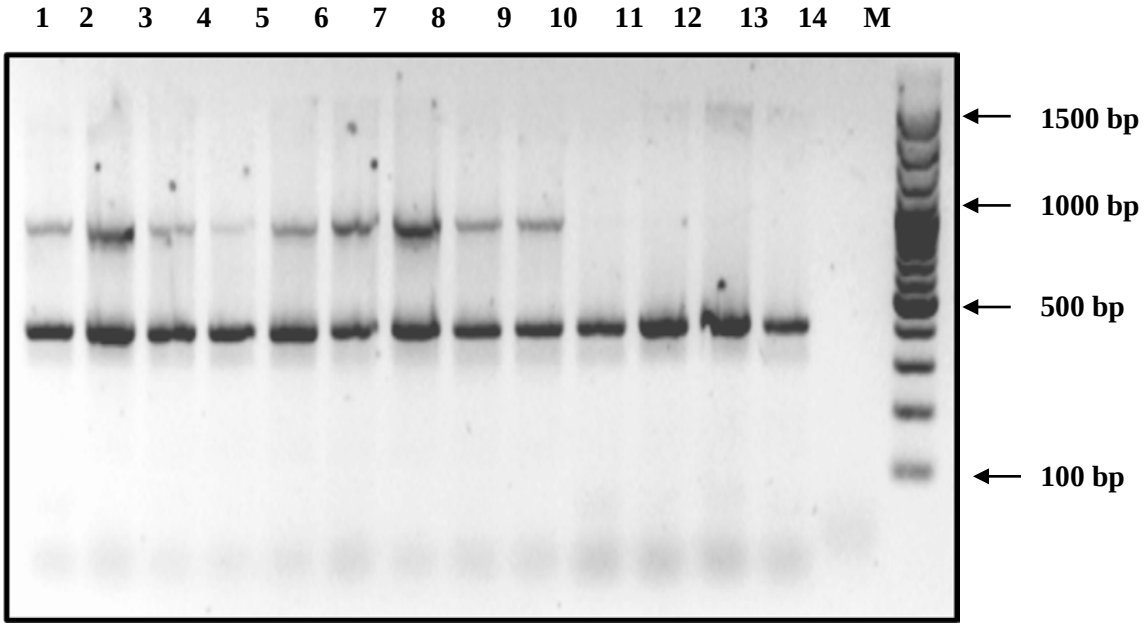


Figure 4.11: A representative agarose gel electrophoresis for the multiplex SCC*mec* typing for some representative isolates involved in this study. Lanes 1-9: SCC*mec* type IV (2 bands: at 937 bp & 415 bp). Lanes 10-13: SCC*mec* type I (1 band at 415 bp). Lane 14: negative control. M: 100 bp DNA molecular weight marker.

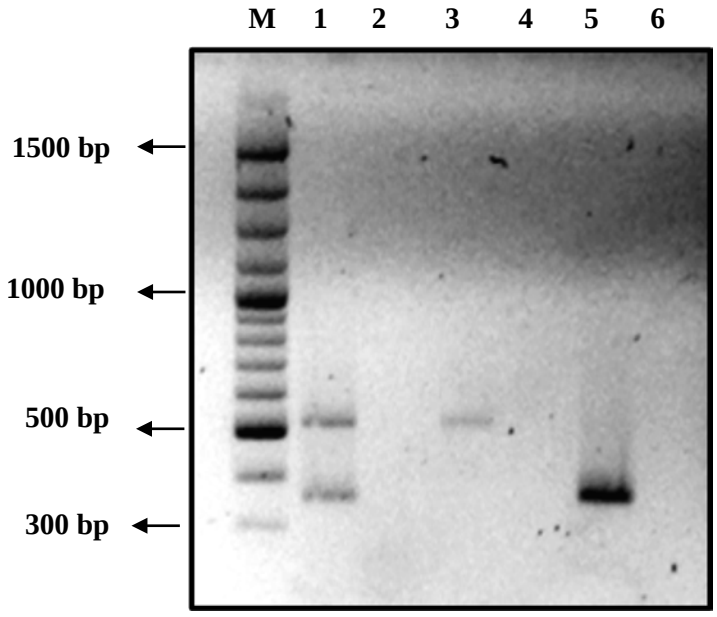


Figure 4.12: The SCC*mec* type V that was detected in only one isolate in this study. Lane 1: the multiplex SCC*mec* PCR (bands at 518 & 359 bp; this is related to SCC*mec* type V. Lane 3: individual PCR for the 518 bp target alone. Lane 5: individual PCR for the 359 bp target alone. Lane 2: the negative control for the multiplex PCR. Both lane 4 & 6 are the negative controls for each individual PCR alone.

Among all community-onset isolates, CA-MRSA was defined as isolates carrying the SCC*mec* types IV or V. In our study, the most common SCC*mec* type was SCC*mec* type IV (86.6%) which is considered as a CA-MRSA type. Ten MRSA isolates were found to be related to the SCC*mec* type I that is considered as a HA-MRSA type (8.9%), Figure 4.13 shows the distribution of the SCC*mec* types as found in this study.

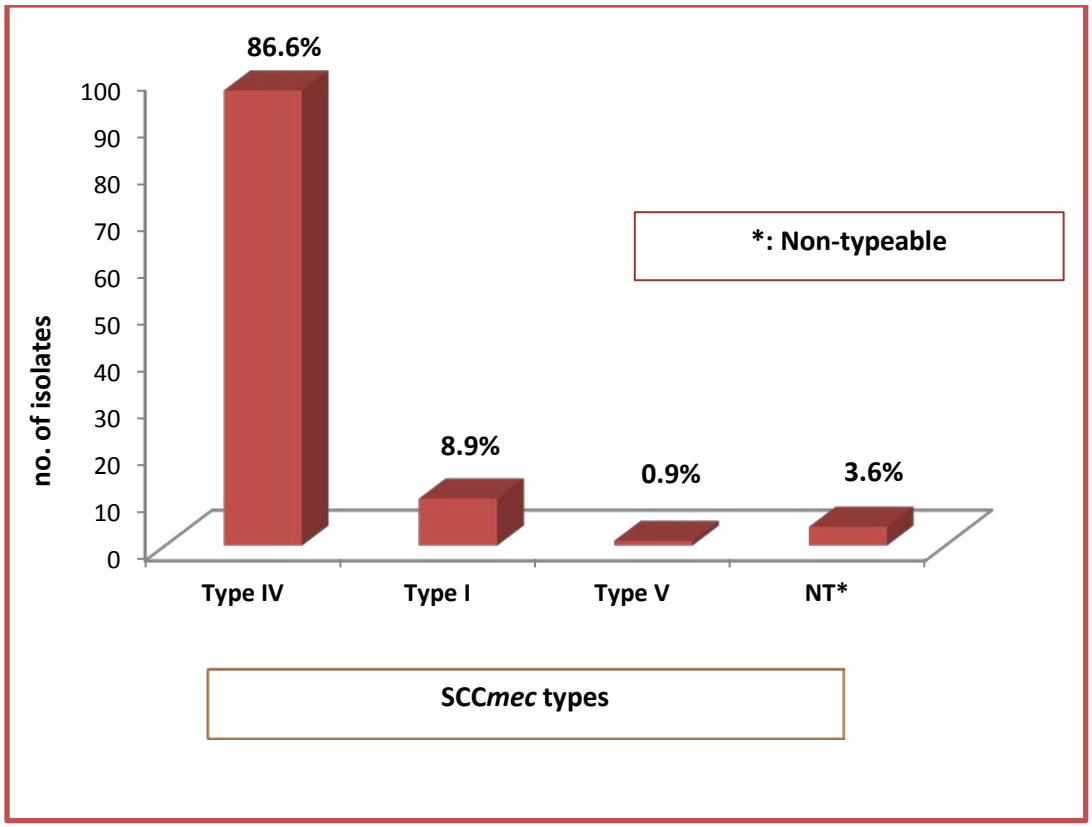


Figure 4.13: The distribution of the SCC*mec* types as found in this study.

4.4.2 *Spa* typing:

A total of twenty nine *spa* types were identified. Four isolates (3.6%) were non-typeable (err) and negative by *spa* typing PCR. One sequenced isolate gave a not identified *spa* type (*) on the *spa* database website (http://spatyper.fortinbras.us/), after sequencing the PCR product from both sides, using forward and reverse primers.

The size of the amplified DNA for *spa* typing ranged between 200-450 bp. The four (err) *spa* negative samples were re-tested from new subcultures and new re-extracted DNA, but remained negative even with different optimizations. In addition, these isolates were confirmed to be negative in laboratories of Sheba Medical Center, Tel-Hashomer, Israel.

Figure 4.14 shows the gel electrophoresis for *spa* PCR for some representative isolates with the bands variation in size as found in this study. It has been found that different

band sizes mostly have different *spa* types; (different short sequence repeats of the polymorphic X region of the staphylococcal protein A gene).

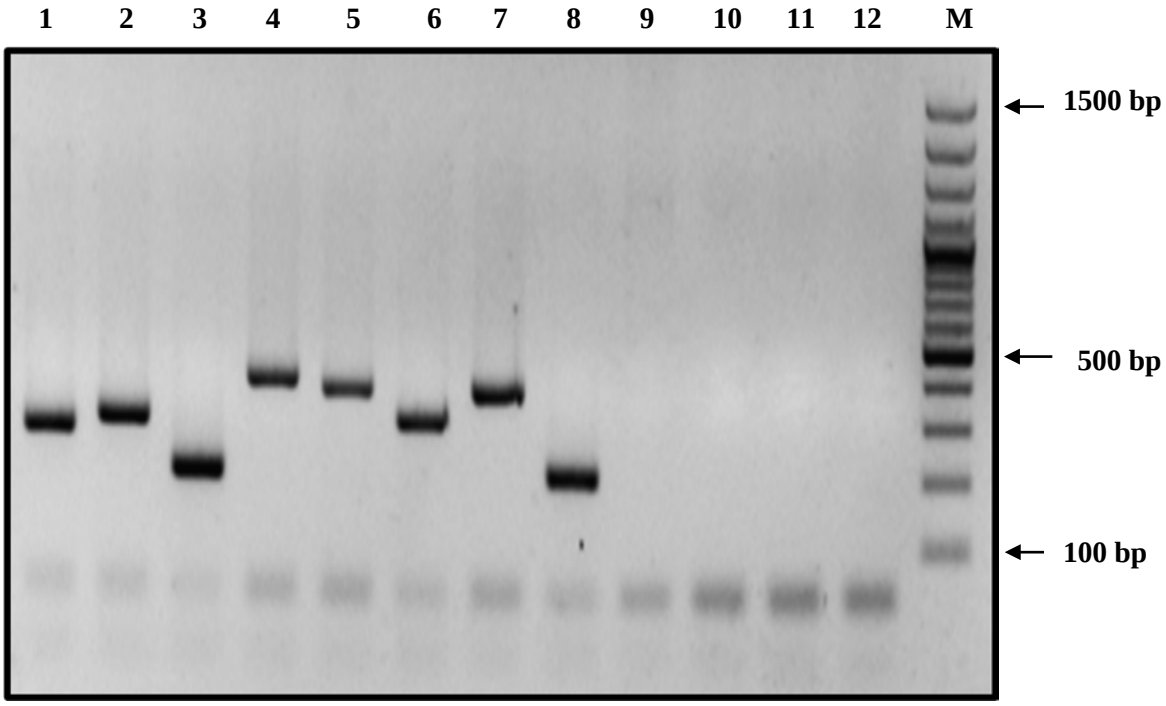


Figure 4.14: A representative agarose gel electrophoresis for the *spa* typing PCR with the different band sizes for some representative samples involved in this study. Lane 1-8: *spa* positive samples. Lanes 9-11: *spa* non-typeable isolates found in this study. Lane 12: negative control. M: 100 bp DNA molecular weight marker.

Five *spa* types: t386, t008, t044, t223 and t037 were predominant and represented 14 (12.5%), 12 (10.7%), 12 (10.7%), 11 (9.8%), 10 (8.9%) isolates, respectively. They were followed by the *spa* types: t021 (7, 6.3%), t991 (6, 5.4%), t002 (4, 3.6%), t1516 (4, 3.6%), t131 (3, 2.7%), t304 (3, 2.7%), t688 (3, 2.7%) and t104 (2, 1.8%). The other sixteen *spa*-types: t005, t011, t018, t084, t085, t1094, t121, t1247, t127, t314, t318, t359, t541, t605, t648 and t932 were found in a frequency of one for each isolate (1, 0.9%), (Table 4.4).

Table 4.4: All the *spa*-types found in this study by *spa* typing.

	spa-type	No. of isolates (*n*)	Percentage %
1	t386	**14**	**12.5**
2	t008	**12**	**10.7**
3	t044	**12**	**10.7**
4	t223	**11**	**9.8**
5	t037	**10**	**8.9**
6	t021	**7**	**6.3**
7	t991	**6**	**5.4**
8	t002	4	3.6
9	t1516	4	3.6
10	t131	3	2.7
11	t304	3	2.7
12	t688	3	2.7
13	t104	2	1.8
14	t005	1	0.9
15	t011	1	0.9
16	t018	1	0.9
17	t084	1	0.9
18	t085	1	0.9
19	t1094	1	0.9
20	t121	1	0.9
21	t1247	1	0.9
22	t127	1	0.9
23	t314	1	0.9
24	t318	1	0.9
25	t359	1	0.9
26	t541	1	0.9
27	t605	1	0.9
28	t648	1	0.9
29	t932	1	0.9
30	(*)?[1]	1	0.9
31	err[2]	4	3.6
	Total	112	100.0

[1](*)?: This *spa*-type was not identified on the *spa* database website; (http://spatyper.fortinbras.us/).
[2:] 4 isolates out of 112 were non-typeable by *spa* typing.

There was a wide range of clonal varieties, with twelve MLST clonal complexes (CCs) were identified. This was done with regard to the BURP analysis. The 12 identified MLST-CCs were: CC22, CC1, CC8, CC80, CC8/239, CC30, CC5, CC913, CC6, CC121, CC126 and CC15. Unfortunately, ten isolates (8.9%) were not found using the BURP analysis.

The CC22 (17, 15.2%), CC1 (15, 13.4%), CC8 (15, 13.4%) and CC80 (15, 13.4%) were the predominant clones in this study, followed by CC8/239 (10, 8.9%), CC30 (9, 8.0%), CC5 (9, 8.0%), CC913 (6, 5.4%), CC6 (3, 2.7%), CC121 (1, 0.9%), CC126 (1, 0.9%) and CC15 (1, 0.9%) as represented in figure 4.15.

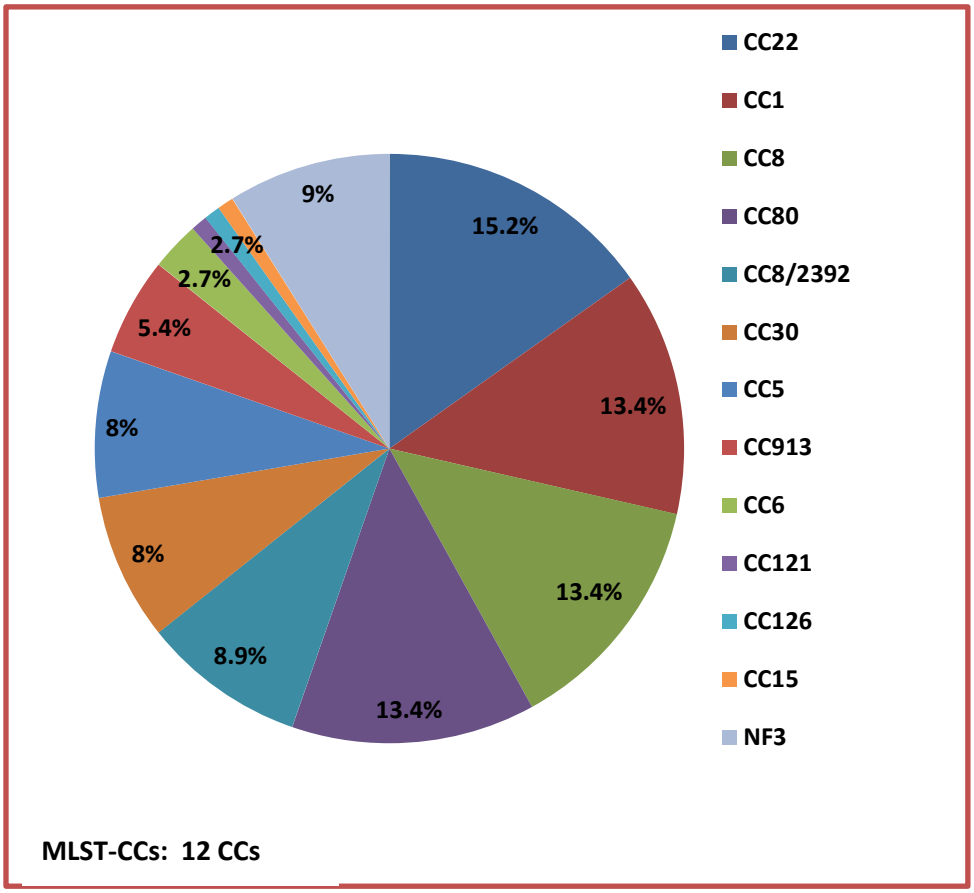


Figure 4.15: All the MLST-CCs found in this study by the BURP analysis.

Here, the 12 found MLST-CCs were distributed among the identified *spa* types as shown in table 4.5.Whereas; a single clonal complex could contain more than one *spa* type.
All of the four *spa* types: t223 (11, 9.8%), t1516 (4, 3.6%), t005 (1, 0.9%) and t541 (1, 0.9%) were belonged to CC22 (17, 15.2%). The *spa* types t386 (14, 12.5%) and t127 (1, 0.9) were belonged to the CC1 (15, 13.4%). For the CC8, it was found in 15 isolates also (13.4%), distributed as the following: t008 (12, 10.7%), t121 (1, 0.9%), t359 (1, 0.9%) and t648 (1, 0.9%).

The *spa* types: t044 (12, 10.7%) and t131 (3, 2.7%) belonged to CC80 (15, 13.4%). The *spa* type t037 was found to be related either to CC8 or CC239 by the BURP and accounted for ten isolates (10, 8.9%).

Also, the *spa* types: t021 (7, 6.3%), t018 (1, 0.9%) and t318 (1, 0.9%) belonged to CC30 (9, 8.0%). The *spa* types: t002 (4, 3.6%), t688 (3, 2.7%) and t104 (2, 1.8%) belonged to CC5 in 9 isolates (8.0%). For the *spa* type t991, it was belonged to CC913 and accounted for six isolates (5.4%). The *spa* type t304 (3, 2.7%) belonged to CC6. For *spa* types: t314, t605 and t084, they were belonged to CC121, CC126 and CC 15, respectively. Each of these was accounted for 1 isolate in its CC (0.9%).

Finally, the CCs using the BURP for the following *spa* types: t011, t085, t1094, t1247 and t329 were not found, (Table 4.5).

Table 4.5: The MLST-CCs and the related *spa* types found in this study by the BURP.

#	MLST-CC[1]s	*spa* types	no. of isolates (%)
1	CC22	t223	11(9.8%)
		t1516	4 (3.6%)
		t005	1 (0.9%)
		t541	1 (0.9%)
2	CC1	t386	14 (12.5%)
		t127	1 (0.9%)
3	CC8	t008	12 (10.7%)
		t121	1 (0.9%)
		t359	1 (0.9%)
		t648	1 (0.9%)
4	CC80	t044	12 (10.7%)
		t131	3 (2.7%)
5	CC8/239[2]	t037	10 (8.9%)
6	CC30	t021	7 (6.3%)
		t018	1 (0.9%)
		t318	1 (0.9%)
7	CC5	t002	4 (3.6%)
		t688	3 (2.7%)
		t104	2 (1.8%)
8	CC913	t991	6 (5.4%)
9	CC6	t304	3 (2.7%)
10	CC121	t314	1 (0.9%)
11	CC126	t605	1 (0.9%)
12	CC15	t084	1 (0.9%)
13	NF[3]	t011	1 (0.9%)
		t085	1 (0.9%)
		t1094	1 (0.9%)
		t1247	1 (0.9%)
		t932	1 (0.9%)
		(*)?	1 (0.9%)

[1:] Clonal Complexes.
[2:] The *spa*-t037 found to be CC8 or CC239 by the BURP, MLST is recommended for finite identification.
[3:] Not Found by BURP.

The antimicrobial resistance rates among the MRSA *spa* types were studied. The resistance rates of MRSA isolates against erythromycin, were significantly high in the *spa* type t386 ($p<0.005$), followed by the *spa* types: t008 (8.9%), t044 (5.4%), t021 (5.4%) and t223 (4.5%). Similarly, the resistance rates of MRSA isolates against clindamycin were high in the *spa* types: t386 (7.1%) and t008 (4.5%), followed by t044 (3.6%) and t021 (2.7%), but with no statistically significance difference.

Moreover, the resistance rates of MRSA isolates against ciprofloxacin and gentamicin were found significantly high ($p<0.05$) in the *spa* type t037 (7.1% for each). Also, for the *spa* types t008 and t044, they have a significant high resistance rate to ciprofloxacin ($p<0.05$).

The MDR MRSA strains were distributed among 16 *spa* types. The rate of MDR (32, 28.6%) were high in 8 isolates of *spa* types t008 and t044 (4 isolates in each) and in 6 isolates of *spa* types t1516 and t037, (3 isolates in each). Two isolates in each of the following *spa* types: t002, t223, t386 were MDR, followed by one isolates for each of the *spa* types: t005, t021, t318, t359, t541, t648, t688, t932 and t991. Moreover, the *spa* (*) isolate and two of the *spa* (err) isolates were MDR. None of the following *spa* types: t011, t018, t084, t085, t104, t1094, t121, t1247, t127, t131, t304, t314, t648 were MDR strains.

Regarding the CCs, the highest MDR isolates were found among CC22 (7/32), followed by 6 isolates related to the CC8, 4 to CC80, 3 to CC5, 3 to CC8/239, 2 to CC1, 2 to CC30 and 1 to CC913. Four MDR isolates CC's were not found by the BURP, (Appendix; table 4, page 102).

4.4.3 Phylogenetic analyses of the *spa* gene

The genetic diversity between MRSA isolates and the phylogenetic relationships in the *spa* repeat region were studied.

A high genetic diversity among MRSA isolates was noted. Phylogenetic analyses were conducted using the neighbor-joining, maximum-likelihood and maximum-parsimony. Based on *spa* typing repeats, the isolates were clustered into two main genotype groups, designated A and B.

Group A consists of 45 MRSA isolates including 10 *spa* types: t688, t127, t131, t1247, t011, t1516, t037, t648, t386, t991 and the *spa* (*) isolate. Within this group, the isolates were clustered into two clades (clade A1 and clade A2). Within each clade, the *spa* types were sub-divided into two sub-clades (A1a, A1b, A2a and A2b).

Clade A1 consists of 25 MRSA isolates out of 45 isolates with 8 *spa* types. The isolates of the *spa* types: t688, t127, t131, t1247 t011 and t1516 belonged to sub-clade A1a, while both the *spa* types t037 and t648 belonged to sub-clade A1b. Notably, the isolate of the *spa* typing (*) was also belonged to sub-clade A1b.

Clade A2 consists of 20 MRSA isolates out of 45 isolates having three repeats. This clade was subdivided into two sub-clades (A2a and A2b). Interestingly, the *spa* type t386 (r07:r23:r13) belonged to sub-clade A2a and the *spa* type t991 (r07:r33:r23) belonged to sub-clade A2b.

Group B consists of 60 MRSA isolates, including 16 *spa* types, as the following: t314, t104, t044, t359, t002, t1094, t084, t085, t008, t304, t121, t005, t223, t021, t318, and t018. Within this group, the isolates were clustered into two clades (clade B1 and clade B2). Within each clade, the *spa* types were also sub-divided into two sub-clades (B1a, B1b, B2a and B2b).

Clade B1 consists of 16 MRSA isolates out of 60 isolates with 4 *spa* types. The isolates of the *spa* types t314 and t104 were belonged to the sub-clade B1a, while the *spa* types t044 and t359 were belonged to the sub-clade B1b.

Clade B2 consists of 44 MRSA isolates out of 60 isolates with 12 *spa* types. Most isolates in clade B2 belonged to sub-clade B2a. Notably, these isolates with *spa* types with more than 8 repeats were clustered into this sub-clade. The isolates of the *spa* types: t002, t1094, t084, t085, t008, 1304, t121, t005 and t223 belonged to the sub-clade B2a, while the *spa* types: t021, t318 and t018 belonged to sub-clade B2b with more than 8 repeats. The branching diagram of MRSA strains based on the *spa* genes is shown in Figure 4.16.

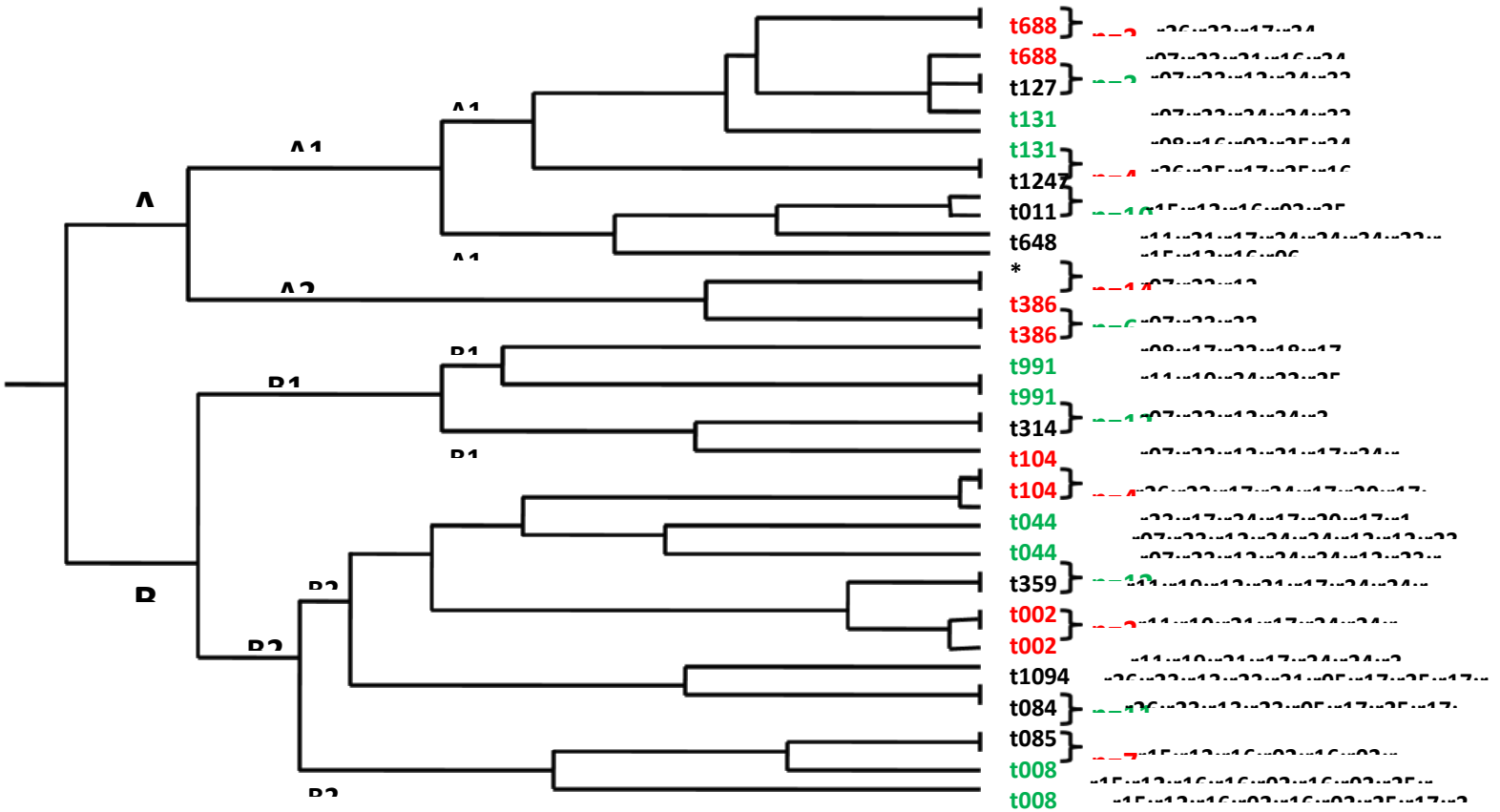


Figure 4.16: A Scheme represents the tree phylogram and the interrelationship between the sequencing derived from the *spa* sequencing and their correlation repeat sequences found through the *spa* typing.

4.4.4 Toxin genes profiling:

4.4.4.1 Detection of PVL toxin:

Of all 112 MRSA isolates, thirty three (29.5%) were positive for PVL toxin gene. The size of the amplified DNA fragments for the PVL toxin gene was around (~433 bp), as shown in figure 4.17.

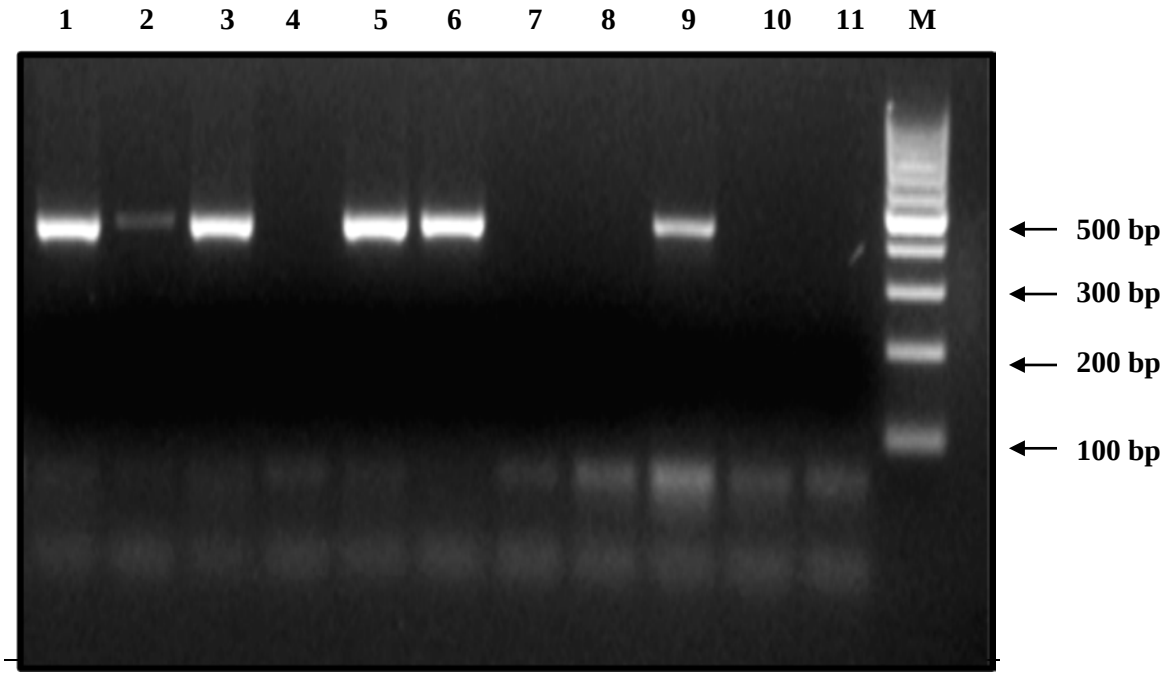


Figure 4.17: A representative agarose gel electrophoresis for the PVL toxin gene. Lanes 1-3, 5-6: PVL positive. Lanes 4, 7-8 &10: PVL negative. Lane 9: positive control. Lane 11: negative control. M: 100 bp DNA molecular weight marker. Band size is around ~433bp.

All the PVL positive MRSA isolates belonged to SCC*mec* type IV. Notably, 34% of the identified SCC*mec* type IV were PVL positive (33/97 type IV). PVL toxin gene was not found nor identified in isolates with SCC*mec* type I or V or the SCC*mec* NT isolates, as shown in table 4.6.

Table 4.6: PVL positive isolates and their SCC*mec* types as found in this study.

PVL & SCC*mec*		**PVL**		Total
		Pos	Neg	
SCC*mec*	Type IV	**33**	64	97
	Type I	0	10	10
	Type V	0	1	1
	NT[1]	0	4	4
Total		33	79	112

[1:] non-typeable.

Panton-Valentine Leukocidin (PVL) toxin gene was detected in eleven *spa* types: t044, t008, t223, t021, t002, t131, t104, t084, t121, t318 and t386.
Two *spa* types: t044 and t008 were predominant and represent (11/12 t044, CC80: PVL +) and (10/12 t008, CC8: PVL +) isolates, respectively, and were associated with SCC*mec* type IV.

Out of 11 isolates of the *spa* type t223, only one isolate was positive for the PVL toxin gene (1/11 t223, CC22: PVL +). Also, among 7 isolates with the *spa* type t021, only one isolate carried the PVL toxin gene (1/7 t021, CC30: PVL+). The two isolates with the *spa* type t002 found in this study were PVL toxin gene positive (2/2 t002, CC5: PVL+).

All th isolates with the *spa* type t131 were PVL positive (3/3 t131, CC80: PVL+). Notably, all the isolates with the *spa* type t386 were PVL negative, except one t386 isolate, which harbored the PVL toxin gene (1/14 t386, CC1: PVL +).
All the other *spa* types, except the mentioned above, were PVL negative, (Table 4.7).

Table 4.7: The distribution of the PVL positive and negative isolates among their *spa* types and CCs as found in this study.

PVL & *spa* types		**PVL**		Total
		Pos	Neg	
***spa* types**	t044 (CC80)	11	1	12
	t008 (CC8)	10	2	12
	t223(CC22)	1	10	11
	t021 (CC30)	1	6	7
	t002 (CC5)	2	2	4
	t131 (CC80)	3	0	3
	t104 (CC5)	1	1	2
	t084 (CC15)	1	0	1
	t121 (CC8)	1	0	1
	t318 (CC30)	1	0	1
	t386 (CC1)	1	13	14
	Others[1]	0	44	44
	Total	33	79	112

[1:] Others: t005, t011, t018, 037, t085, t1094, t1247, t127, t1516, t304, t314, t359, t541, t605, t648, t688, t932 and t991. These *spa* types found to be PVL negative in this study.

Combining all CCs with the 33 PVL-positive isolates, seven different clonal complexes were identified: CC80, CC8, CC5, CC30, CC22, CC15 and CC1. The most common were CC80 (14/33, 42.5%) and CC8 (11/33, 33.3%) followed by CC5 (3/33, 9.1%) and CC30 (2/33, 6.1%). Three PVL positive isolates were related to the CC22 (3.0%), CC15 (3.0%) and CC1 (3.0%), (Figure 4.18).

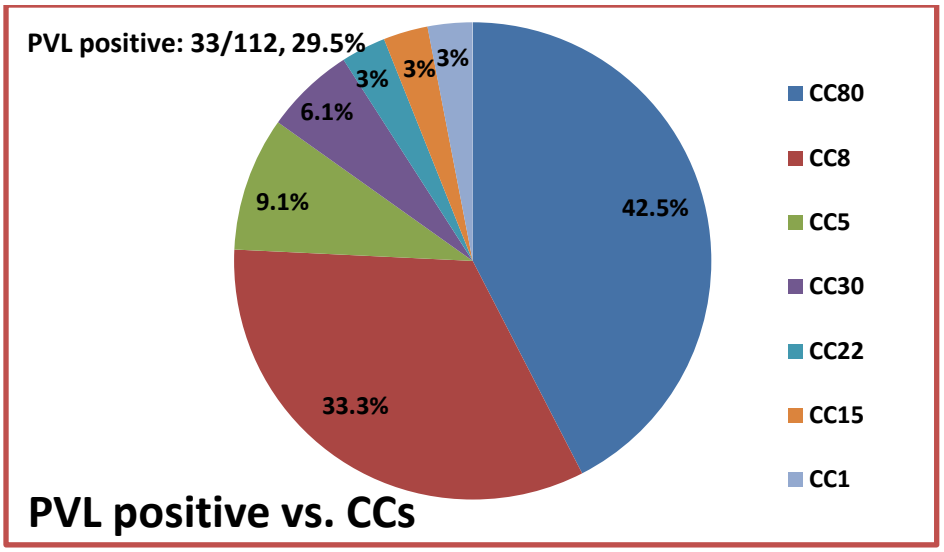


Figure 4.18: The distribution of the PVL positive isolates among their CCs.

4.4.4.2: Detection of ACME-*arc*A toxin gene:

The gene coding for the ACME-*arc*A toxin was detected in 18.8 % (21/112) of isolates. The size of the amplified DNA fragments for the ACME-*arc*A toxin gene was around (~724 bp), as shown in figure 4.19.

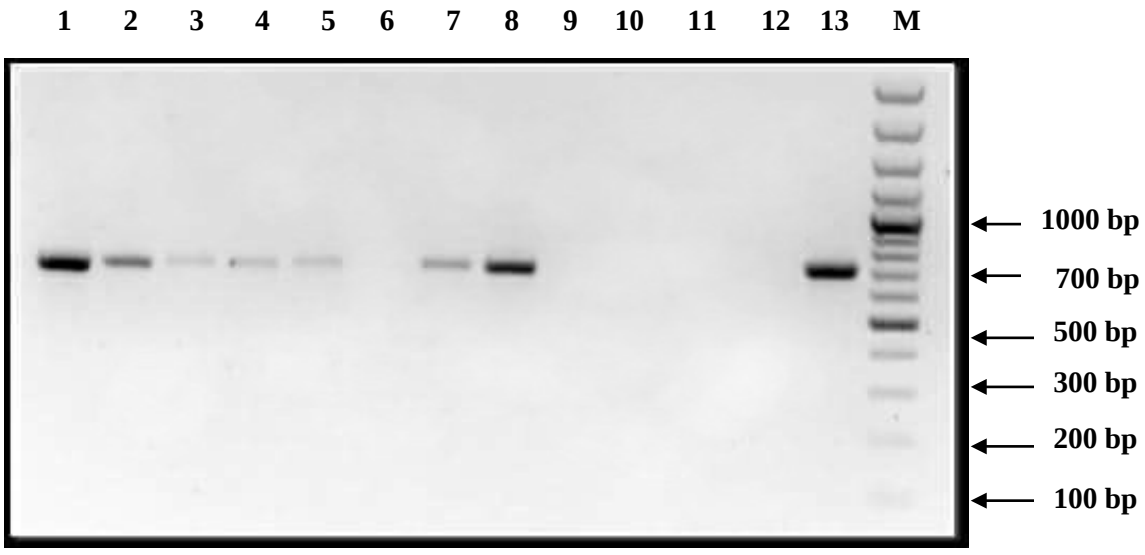


Figure 4.19: A representative agarose gel electrophoresis for the ACME-*arc*A toxin gene. Lanes 1-5 and 7-8: ACME-*arc*A positive. Lanes 6, 9-11: ACME-*arc*A negative. Lane 12: negative control. Lane 13: positive control. M: 100 bp DNA molecular weight marker.

The ACME-*arc*A toxin was detected in 20 isolates harboring the SCC*mec* type IV, and 1 isolate with SCC*mec* type I.

Arginine Catabolic Mobile Element (ACME)-*arc*A gene was detected in nine *spa* types: t008, t991 t037, t104, t1094, t1247, t1516, t223 and t386. This toxin was significantly predominant in the *spa* types t008 ($p<0.05$). Whereas, out of the 12 identified *spa* type t008, nine isolates were ACME-*arc*A positive (9/12 t008, CC8: ACME-*arc*A +), showing that this *spa* type is mostly ACME-*arc*A positive. These nine isolate were also PVL positive and classified as: USA-300 clone. Interestingly, out of the four non-typeable *spa* types (err), three were ACME-*arc*A toxin gene positive.

Out of 10 isolates with the *spa* type t037, only one isolate harbored the ACME-*arc*A toxin gene (1/10 t037, CC8 or CC239: ACME-*arc*A +). The same for the *spa* types t223 and t386 in which only one isolate in each of these *spa* types was carrying the ACME-*arc*A toxin gene, as follows: (1/11 t223, CC22: ACME-*arc*A +) and (1/14 t386, CC1: ACME-*arc*A +), respectively.

Regarding the CCs, out of the 21 ACME-*arc*A positive isolates, six different clonal complexes were identified. The most common were CC8 (9/21), followed by: two isolates related to the CC913 (*spa* type t991), two isolates CC2 (t1516 and t223) and one isolate in each of the following CCs: CC8/CC239 (*spa* type t037), CC5 (*spa* type t104) and CC1 (*spa* type t386). Five ACME-*arc*A positive isolates CCs were not found, (Table 4.8).

Table 4.8: The distribution of the ACME-*arc*A positive and negative isolates among their *spa* types and CCs as found in this study.

ACME-*arc*A & *spa* types (CC)		**ACME-*arc*A**		Total
		Pos	Neg	
***spa* types**	t008 (CC8)	9	3	12
	err[1] (NF)[2]	3	1	4
	t991 (CC913)	2	4	6
	t037(CC8 or CC239)	1	9	10
	t104 (CC5)	1	1	2
	t1094 (NF)	1	0	1
	t1247 (NF)	1	0	1
	t1516 (CC2)	1	3	4
	t223(CC22)	1	10	11
	t386 (CC1)	1	13	14
	Others[3]	0	47	47
	Total	21	91	112

[1]: err are the non-typeable isolates by *spa* typing.
[2]: CCs were not found using the BURP.
[3]: Others: t002,t005, t011, 1018, t021, t044, t084, t085, t121, t127, t131, t304, t314, t359, t318, t541, t605, t648, t688 & t932.These *spa* types found to be *arc*A negative in this study.

4.4.4.3 Detection of *TSST*-1 gene

Similarly, 23% of the MRSA isolates (26/112) tested positive for the presence of the *TSST*-1 gene. The size of the amplified DNA fragments for the *TSST*-1 gene was around (~350 bp), as shown in figure 4.20.

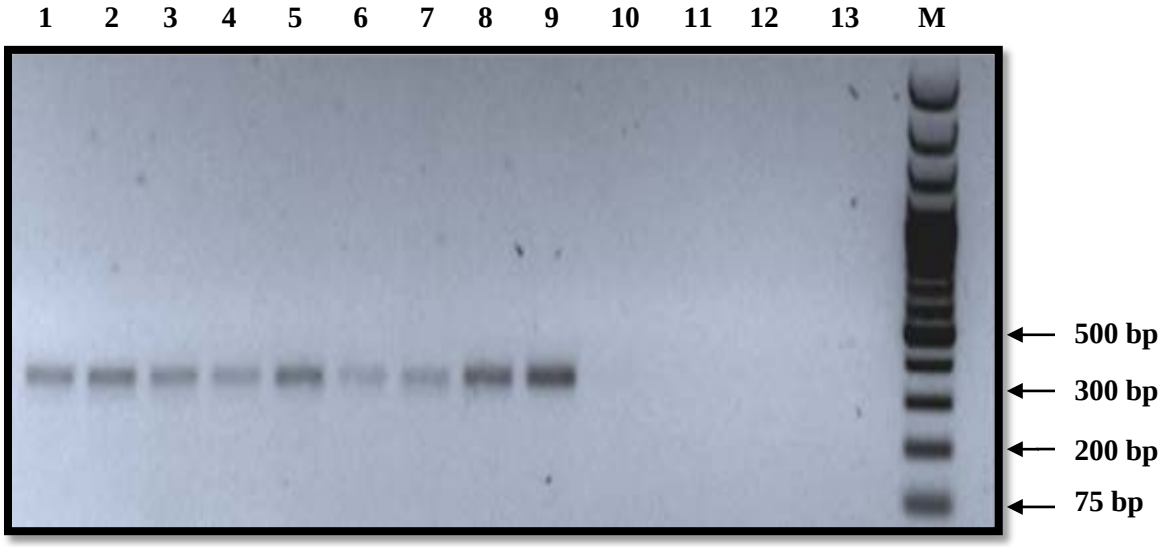


Figure 4.20: A representative agarose gel electrophoresis for the *TSST*-1 gene. Lanes 1-8: *TSST*-1 positive. Lane 9: positive control. Lanes 10-12: *TSST*-1 negative. Lane 13: negative control. M: 1Kb DNA molecular weight marker. Band size is around ~350bp.

Among the 26 positive *TSST-1* gene, 25 MRSA isolates were belonged to the SCC*mec* type IV; the other *TSST*-1 positive isolate was belonged to SCC*mec* type I.

Toxic shock syndrome toxin-1 (*TSST-1*) was detected in ten *spa* types: t223, t021, t1516, t002, 1005, t008, t018, t386, t541 and t605. The two most common *spa* types among *TSST-1* positive isolates were the *spa* type t223 and t021. Out of the 11 identified *spa* type t223, nine isolates were significantly *TSST-1* positive (9/11 t223, CC22: *TSST*-1 +), showing that this *spa* type is mostly *TSST*-1 positive ($p<0.05$).

All the seven *spa* type t021 isolates were *TSST*-1 positive (100%), as shown in table 4.9. *TSST-1* gene was not detected among the other *spa* types found in this study. Notably, all the isolates of the *spa* type t044 (n=12), t037 (n=10), and t991 (n=6) were *TSST-1* negative.

Also, this toxin was not predominant and found rarely in the *spa* types t008 and t386 in which it was found in only one isolate in each of t008 (1/12 t008, CC8: *TSST-1* +) and t386 (1/14 t386, CC1: *TSST-1* +) isolates.

Notably, genes for both PVL and *TSST-1* together were observed only in one isolate belonged to *spa* type t021 (1/7 t021, CC30: PVL & *TSST-1* +).

Regarding the CCs, the *TSST-1* positive isolates were detected predominantly in CC22 (14/26, 53.8%) and CC30 (8/26, 30.8%). The other positive isolates were belonged to one isolates in each of the following CCs: CC126, CC8, CC5 and CC1.

Table 4.9: The distribution of the *TSST-1* positive and negative isolates among their *spa* types and CCs as found in this study.

TSST-1& *spa* types (CC)		*TSST-1*		Total
		Pos	Neg	
spa types	t223 (CC22)	9	2	11
	t021 (CC30)	7	0	7
	t1516 (CC22)	3	1	4
	t002 (CC5)	1	3	4
	t005(CC22)	1	0	1
	t008 (CC8)	1	11	12
	t018 (CC30)	1	0	1
	t386(CC1)	1	13	14
	t541(CC22)	1	0	1
	t605 (CC126)	1	0	1
	Others[1]	0	56	1
	Total	26	86	112

[1]: Others: t011, t037, t044, t084, t085, t104, t1094, t121, t1247, t127, t131, t304, t314, t318, t359, t648, t688, t991 and t932. These *spa* types found to be *TSST-1* negative in this study.

4.4.4.4 Detection of ETA

The gene coding for the exofoliative toxin A (ETA) was detected in 5.4 % (6/112) of all the MRSA isolates harboring the *spa* type t991/CC913. All the other *spa* types were ETA gene negative. The size of the amplified DNA fragments for the ETA gene was around (~93 bp), as shown in figure 4.20.

Among the 6 *spa* type t991, five isolates were of SCC*mec* type IV, and one isolate was belonged to SCC*mec* type I. None of the spa t991 ETA positive isolates were harboring the PVL or *TSST*-1 genes, and only one isolate was ACME-*arc*A toxin positive.

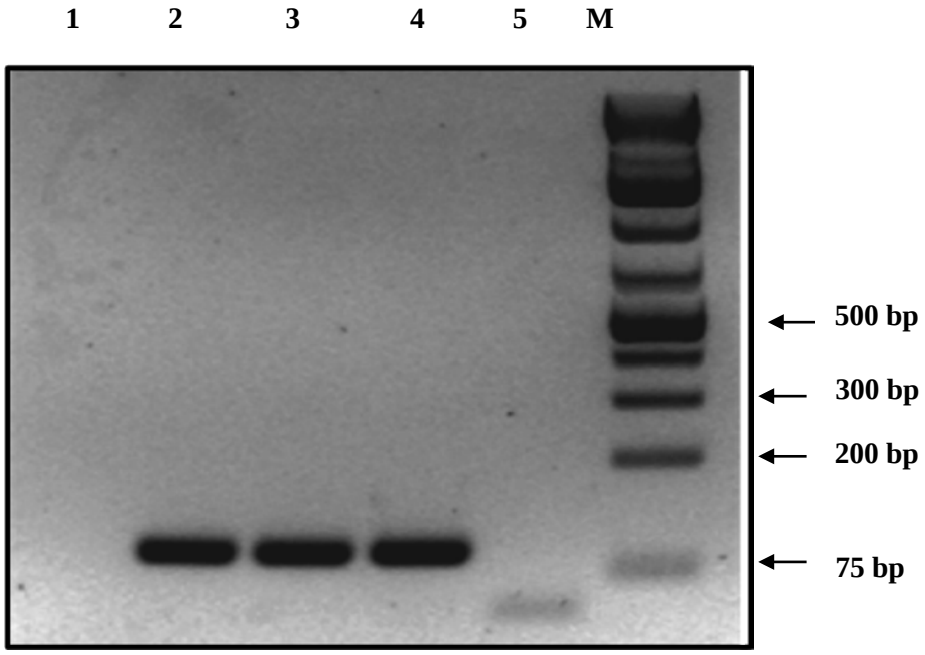


Figure 4.21: A representative agarose gel electrophoresis for the ETA gene. Lane ETA negative. Lanes 2-4: ETA positive (*spa* type t991/CC913). Lane 4: negative control. M: 1Kb DNA molecular weight marker. Band size was around ~93bp.

4.5 Major MRSA clones in this study:

Spa type t386:

This *spa* type was the most predominant type among the all isolates (14, 12.5%) and belonged to CC1.

Out of these fourteen t386 MRSA isolates, twelve isolates were significantly ($p<0.05$) negative for all the four toxin genes: PVL, *TSST-1*, ACME-*arc*A and ETA toxins. One of the other two isolates was negative for *TSST*-1, ACME-*arc*A and ETA toxins but positive for PVL toxin gene, and the second isolate was positive for both *TSST*-1 and ACME-*arc*A but negative for PVL and ETA genes.

All the t386 MRSA isolates belonged to the SCC*mec* type IV. This *spa* type was found in Nablus (5/14), Jerusalem (3), Bethlehem (2), Hebron (2), Ramallah (1) and Tulkarem (1). Notably, this *spa* type was completely absent among isolates obtained from Gaza patients.

Samples were collected mainly from adult males and from different sources. Among the 14 isolates, two were assigned to MDR. All collected samples in t386 MRSA isolates were significantly ($p<0.05$) erythromycin resistant (14/14 t386, 100%). Eight isolates were clindamycin resistant (8/14 t386, 57.1%), three isolates were gentamicin resistant (3/14 t386, 21.4%) and three isolates were ciprofloxacin resistant (3/14 t386, 21.4%). Interestingly, all isolates in this *spa* type were sensitive to SXT, (Table 4.10).

Table 4.10: Genotypic and phenotypic characteristics of *spa* type t386 as found in this study.

***spa* type t386/ CC1 (n=14)**									
	SCC*mec*	PVL	*TSST-1*	*arc*A	Age group	Pt-type[1]	Sex	Department	Source
1	Type IV	neg	neg	neg	Adult	In	M[2]	Neurosurgery	Wound
2		neg	neg	neg	NF[3]	NF	NF	NF	Wound
3		neg	neg	neg	Adult	Out	M	Surgery	Wound
4		neg	neg	neg	Adult	In	M	NF	Blood
5		neg	neg	neg	Adult	In	F[4]	NF	Blood
6		neg	neg	neg	Adolescent	In	NF	NF	Ear
7		neg	neg	neg	NF	NF	NF	NF	Ear
8		neg	neg	neg	Adult	Out	M	Dialysis	Urine
9		neg	neg	neg	Adult	In	M	ICU	NF
10		neg	neg	neg	NF	NF	NF	NF	NF
11		neg	neg	neg	Adult	In	M	Orthopedic	NF
12		neg	neg	neg	Adult	NF	F	NF	Sputum
13		**pos**	neg	neg	NF	Out	M	NF	Sputum
14		neg	**pos**	**pos**	Adult	In	M	Daycare	Pus
MDR (2/14 t386); 100% erythromycin R, 57.1% clindamycin R, 21.4% gentamicin R, 21.4% ciprofloxacin R, 100% SXT S.									

[1:] Patient type, [2:] Male, [3:] Not Found, [4:] Female

***Spa* type t008:**

This *spa* type was predominant among 12 MRSA isolates (10.7 %) and belonged to CC8.

Among the twelve t008 isolates, nine MRSA isolates were identified as: **USA-300 clone** (8% of all isolates), which were SCC*mec* IV and carrying both the PVL and the ACME-*arc*A toxin genes (9/12, 75.0% of the *spa* type t008: USA-300).

Two t008 isolates were SCC*mec* type IV but one was PVL positive and ACME-*arc*A negative. The other one was both PVL and ACME-*arc*A negative. One isolate in this *spa* type harbored the SCC*mec* type I. For *TSST-1*, mostly all isolates were *TSST*-1 negative, except one isolate which was harboring the *TSST*-1 gene.

This *spa* type was mostly isolated from five swabs of wound infections. For the patient type, 6 isolates were obtained from in-patients, 5 isolates from out-patients and one isolate with no data. The *spa* type t008 was predominant in Jerusalem (7/12) followed by Ramallah (4) and Nablus (1). Interestingly, it was not found among the isolates obtained from Gaza patients.

Out of twelve isolates, four t008 isolates were MRSA MDR. Erythromycin resistance were observed in ten isolates (10/12 t008, 83.3%) with a statistically significant resistance rate ($p<0.05$). Clindamycin resistance was detected in five isolates (5/12, 41.7%), and ciprofloxacin resistance was detected in nine isolates (9/12, 75%) with no statistically significance resistance rate for clindamycin ($p>0.05$), but significance resistance rate to ciprofloxacin ($p<0.05$). Notably, one t008 MRSA isolate was resistant to SXT (1/12, 8.0%), while all isolates in this *spa* type were significantly gentamicin sensitive ($p<0.005$), (Table 4.11).

Table 4.11: Genotypic and phenotypic characteristics of *spa* type t008 as found in this study.

***spa* type t008/ CC8 (n=12)**									
#	SCC*mec*	PVL	*TSST*-1	*arc*A	Age group	Pt-type[1]	Sex	Department	Source
1	Type IV	**pos**	neg	**pos**	Adolescent	In	M[2]	Orthopedic	Wound
2		**pos**	neg	**pos**	Adolescent	Out	M	Orthopedic	Wound
3		**pos**	neg	**pos**	Adolescent	In	M	Orthopedic	Wound
4		**pos**	neg	**pos**	Adult	In	M	Orthopedic	Wound
5		**pos**	neg	**pos**	Adult	Out	F[3]	Emergency	Wound
6		**pos**	neg	**pos**	Adult	Out	F	Emergency	Abscess
7		**pos**	neg	**pos**	Adult	Out	M	NF[4]	Pus
8		**pos**	neg	**pos**	Adult	In	F	Surgery	Skin
9		**pos**	neg	**pos**	NF	NF	NF	NF	NF
10		pos	neg	neg	Adult	In	M	NF	Blood
11		neg	pos	neg	Adult	In	F	Neurosurgery	Sputum
12	Type I	neg	neg	neg	Adult	Out	F	Daycare	Urine
MDR (4/12 t008); 83.3% erythromycin R,41.7% clindamycin R, 75.0% ciprofloxacin R, 8.0% SXT R and 100% gentamicin S.									

[1:] Patient type, [2:] Male, [3:] Female, [4:] Not Found.
*: The bold and underlined # (1-9) are the USA-300 clone.

Spa type t044:

This *spa* type was common as the *spa* type t008, in which both were found in 12 MRSA isolates (10.7%). The *spa* type t044 has been found to be related to CC80.

Eleven t044 MRSA isolates (11/12, 91.7%) were PVL positive showing a high significance association between the *spa* type t044 and harboring the PVL toxin gene ($p<0.001$), as expected in all other studies. A single isolate was negative for the PVL toxin gene, which was confirmed in duplicate. All t044 MRSA isolates were SCC*mec* type IV and were all significantly ($p<0.001$) negative for the *TSST*-1, ACME-*arc*A and ETA genes.

Two isolates were obtained from 2 infants, while the others were obtained from adolescent and adults. Notably, PVL positive MRSA isolates were mostly isolated from

wound (6 isolates). For the patient type, most isolates (9/12) were obtained from in-patients, while 3 isolates were obtained from out-patients. This *spa* type were observed commonly ($p<0.05$) in Gaza (7/11) followed by Jerusalem (4) and Hebron (1).

Four t044isolateswere MRSA MDR. Half of these isolates were erythromycin resistant (6/12, 50.0%). Also, ciprofloxacin resistant (41.7%) was relatively common in this *spa* type. About one third (33.3%) were clindamycin resistant, while gentamicin and SXT resistance (2/12 t044, 16.7% for each) were relatively low, (Table 4.12).

Table 4.12: Genotypic and phenotypic characteristics of *spa* type t044 as found in this study.

***spa type* t044/ CC80 (n=12)**									
#	SCC*mec*	PVL	*TSST*-1	*arc*A	Age group	Pt-type[1]	Sex	Department	Source
1	Type IV	pos	neg	neg	Adult	In	M[2]	Neurosurgery	Wound
2					Adult	In	M	Neuro ICU	Wound
3					Adult	In	M	Orthopedic	Wound
4					Adolescent	In	M	Orthopedic	Wound
5					Adult	Out	F[3]	Emergency	Wound
6					Adult	Out	F	Emergency	Wound
7					Adult	Out	M	Emergency	Abscess
8					Adult	In	M	Daycare	Abscess
9					Adult	In	M	ICU	Blood
10					Infant	In	F	ICU	Trap
11					Adult	In	F	Daycare	Tissue
12		neg			Infant	In	F	Pediatric	Nasal
MDR (4/12 t044); 50.0% erythromycin R, 33.3% clindamycin R, 41.7% ciprofloxacin R, 16.7% SXT R and 16.7% gentamicin R.									

[1:] Patient type, [2:] Male, [3:] Female.

Spa type t223:

Eleven patients (9.8% of all isolates) were infected by MRSA isolates belonging to this *spa* type, which was commonly typed, previously, as **Gaza clone** in Gaza region. Interestingly, six patients were from Gaza and five patients were from Jerusalem. The *spa* type t223 has been found to be related to CC22.

This *spa* type is usually PVL negative. Here, most isolates (10/11 t223) were significantly PVL toxin gene negative ($p<0.001$). One isolate was PVL toxin gene positive and was tested in duplicate and confirmed blindly in laboratories of Sheba Medical Center, Tel-Hashomer, Israel. However, the *spa* type t223 with PVL positive toxin gene was considered as a "Unique Gaza strain".

All isolates were significantly positive for the *TSST*-1 gene ($p<0.001$) except two isolates which were negative for this toxin and tested in duplicates. In contrary, all isolates were significantly negative for ACME-*arc*A toxin gene except one isolate ($p<0.005$) that was also positive for the PVL toxin gene (1/11 t223: PVL/ACME-*arc*A +, *TSST*-1 –) and considered as "Unique Gaza Strain". All isolates in this *spa* type were SCC*mec* type IV. Most of the isolates (9/11 t223) were obtained from in-patients, while one isolate was obtained from an out-patient and one isolate with no data.

Two t223 isolates were MRSA MDR. Erythromycin resistance was relatively common (5/11 t223, 45.5%) and significant among t223 isolates ($p<0.005$). Two isolates were clindamycin resistant (2/11 t223, 18.2%), three isolates were SXT resistant (3/11 t223, 27.3%), two isolates were gentamicin resistant (2/11 t233, 18.2%) and only one isolate (1/11 t223, 9.1%) was ciprofloxacin resistant, (Table 4.13).

Table 4.13: Genotypic and phenotypic characteristics of *spa* type t044 as found in this study.

***spa type* t223/ CC22 (n=11) -Gaza clone-**									
#	SCC*mec*	PVL	TSST-1	*arc*A	Age group	Pt-type[1]	Sex	Department	Source
1	Type IV	neg	pos	neg	Child	In	M[2]	Pediatric	Blood
2			pos		Adult	In	F[3]	ICU	Blood
3			pos		Adult	In	M	Neurosurgery	Nasal
4			pos		Adolescent	In	M	Surgery	Nasal
5			pos		Adult	In	M	Medical	Nasal
6			neg		Adult	Out	F	Orthopedic	Pus
7			pos		Adolescent	In	M	Surgery	Urine
8			pos		Adult	In	M	Neuro-ICU	NF[4]
9			pos		Adult	In	M	CCU	NF
10			pos		NF	NF	NF	NF	NF
11*		**pos**	**neg**	**pos**	Adolescent	In	M	Orthopedic	Wound
MDR (2/11 t233); 45.5% erythromycin R,18.2% clindamycin R, 9.1% ciprofloxacin R, 27.3% SXT R and 18.2% gentamicin R.									

[1:] Patient type, [2:] Male, [3:] Female, [4:] Not Found.
*: This isolate is considered as a "Unique Gaza Strain".

***spa* type t037:**

Ten patients (8.9%) were infected with isolates belonged to the *spa* type t037. This *spa* type is belonged to CC8 or CC239 by the BURP. For complete identification, MLST is recommended.

All t037 MRSA isolates were significantly negative for PVL, *TSST*-1, ACME-*arc*A and ETA genes ($p<0.005$), except one isolate which was ACME-*arc*A toxin gene positive. Six isolates were SCC*mec* type IV and 2 isolates were belonged to SCC*mec* type I. Two t037 isolates were non-typeable by both individual and multiplex PCR for the five examined SCC*mec* types I-V.

Mostly, this *spa* type was detected among hospitalized (in patients) patients from Gaza (n=5), Nablus (n=2), Jerusalem (n=1) and Ramallah (n=1).Three isolates were MRSA MDR. Four isolates were erythromycin resistant (4/10, 40.0%) and two isolates were clindamycin resistant (2/10, 20.0%). Notably, this *spa* type has high significant resistance

rates ($p<0.05$) to both ciprofloxacin and gentamicin (80.0%). Moreover, one t037 isolate was SXT resistant (1/10, 10%), (Table 4.14).

Table 4.14: Genotypic and phenotypic characteristics of *spa* type t037 as found in this study

***spa* type t037/ CC8-CC239 (n=10)**									
#	SCC*mec*	PVL	TSST-1	*arc*A	Age group	Pt-type[1]	Sex	Department	Source
1	Type IV	neg	neg	pos	Adult	In	M[2]	Neurosurgery	NF[4]
2				neg	NF	Out	F[3]	Daycare	Urine
3					Adult	In	M	Neurosurgery	Pus
4					NF	NF	NF	NF	Nasal
5					Adult	In	F	Gynecology	Wound
6					Adult	In	M	Orthopedic	Wound
7	Type I				NF	NF	NF	NF	Tissue
8	Type I				Infant	In	M	Pediatric	Blood
9	NT[5]				Adult	In	F	Orthopedic	Wound
10	NT				Adult	In	F	Orthopedic	Tissue
MDR (3/10 t037); 40.0% erythromycin R,20.0% clindamycin R, 80.0% ciprofloxacin 80.0% gentamicin R and 10.0% SXT R.									

[1:] Patient type, [2:] Male, [3:] Female, [4:] Not Found, [5:] Non-typeable.

***spa* type t021:**

This *spa* type was detected in 7 isolates (6.3%). The *spa* type t021 has been found to be related to CC30.

All t021 isolates were significantly carrying the *TSST*-1 gene ($p<0.001$). In contrast, all were significantly negative for the ACME-*arc*A and ETA genes ($p<0.005$). Also, all were PVL toxin gene negative, but one isolate was harboring the PVL toxin gene. All isolates in this *spa* type were related the SCC*mec* type IV.

Most isolates were carried among hospitalized patients, except one isolate which was obtained from an out-patient. All t021 isolates were obtained from males. Sources of these isolates were as the following: 4 isolates were obtained from wounds, two isolates from blood and 1 isolate was obtained from a nasal swab culture. Interestingly, this *spa*

type was predominant in Hebron (4/7), followed by Nablus (2) and Jerusalem (1). Notably, no t021 MRSA isolates were obtained from the patients from Gaza.

One t021 MRSA isolate was MDR in which it was resistant to erythromycin, clindamycin and SXT (in addition to resistance to the β-lactam antibiotics), (Table 4.15).

Table 4.15: Genotypic and phenotypic characteristics of *spa* type t021 as found in this study.

***spa* type t021/ CC30 (n=7)**									
#	SCC*mec*	PVL	*TSST*-1	*arc*A	Age group	Pt-type[1]	Sex	Department	Source
1	Type IV	pos	pos	neg	Adolescent	In	M[2]	Surgery	Wound
2		neg			Adolescent	Out	M	Emergency	Wound
3					Child	In	M	Pediatric	Wound
4					Adult	In	M	Orthopedic	Wound
5					Adolescent	In	M	ICU	Nasal
6					Child	In	M	Pediatric	Blood
7					Adult	In	M	Medical ward	Blood
MDR (1/7 t021, 14.3%).									

[1:] Patient type, [2:] Male.

***spa* type t991:**

As described before, the *spa* type t991 was found in six isolates that were all significantly ETA positive, PVL negative and *TSST*-1 negative ($p<0.001$). For ACME-*arc*A, two isolates were positive, while the other 4 were negative. The *spa* type t991 has been found to be related to CC913.

Among these six t991 MRSA isolates, two isolates were obtained from infants, one from child, one from adolescent and two from adults. Sources of these isolates were mainly wound (3 isolates), ear swab (1) and blood culture (1). One isolate source was not identified. For the patient type, five isolates were obtained from in-patients, while one isolate was obtained from an out-patient. This *spa* type was distributed equally between Gaza, Hebron and Ramallah with two isolates from each region.

Only one MRSA isolate in this *spa* type was MDR in which it was resistant to erythromycin, clindamycin, SXT and ciprofloxacin in addition to resistance to the β-lactam antibiotics, (Table 4.16).

Table 4.16: Genotypic and phenotypic characteristics of *spa* type t991 as found in this study.

***spa* type t991/ CC913 (n=6)**									
#	SCC*mec*	PVL	TSST-1	*arc*A	Age group	Pt-type[1]	Sex	Department	Source
1	Type IV	neg	neg	pos	Infant	In	F[2]	Pediatric	NF[4]
2				neg	Child	In	F	Pediatric	Ear
3				pos	Adult	Out	F	Dialysis	Wound
4				neg	Infant	In	M[3]	Pediatric	Blood
5				neg	Adolescent	In	M	Daycare	Wound
6	Type I			neg	Adult	In	F	Orthopedic	Wound
MDR (1/6 t991, 16.7%)									

[1:] Patient type, [2:] Female, [3:] Male, [4:] Not Found.

4.6 Other MRSA clones:

In addition to the major MRSA clones that were described above, other clones were found to be related to the other 22 *spa* types. Table 4.17 summarizes the other found MRSA clones with their SCC*mec* types, *spa* types, toxin genes profiling and region from where they were isolated. The CCs for the *spa* types were found by BURP. In summary, the molecular and antibiogram items, the phenotypic and genotypic characteristics of all MRSA isolates included in this study are summarized in table 4.18.

Table 4.17: Genotypic and phenotypic characteristics of the other *spa* types as found in this study.

#	*spa* type	SCC*mec* type	(n)	MLST-CCs	PVL	*TSST*-1	*arc*A	Region
1	t002	Type IV	4	CC5	pos	neg	Neg	Hebron
2					pos	neg		Jerusalem
3					neg	pos		Jerusalem
4					neg	neg		Ramallah
5	t1516	Type IV	4	CC22	neg	pos	pos	Jerusalem
6							neg	Ramallah
7							neg	Ramallah
8		Type I				neg	neg	Hebron
9	t104	Type I	2	CC5	neg	neg	neg	Gaza
10		Type IV			pos	neg	pos	Jerusalem
11	t131	Type IV	3	CC80	pos	neg	neg	Gaza
12								Hebron
13								Hebron
14	t304	Type IV	3	CC6	neg	neg	neg	Gaza
15								Gaza
16								Gaza
17	t688	Type IV	3	CC5	neg	neg	neg	Jerusalem
18		Type I						Hebron
19		Type I						Gaza
20	t648	Type IV	1	CC8	neg	neg	neg	Gaza
21	t359		1	CC8	neg	neg	neg	Gaza
22	t121		1	CC8	pos	neg	neg	Nablus
23	t005		1	CC22	neg	pos	neg	Jerusalem
24	t541		1	CC22	neg	pos	neg	Gaza
25	t084		1	CC15	pos	neg	neg	Gaza
26	t127		1	CC1	neg	neg	neg	Jerusalem
27	t318		1	CC30	pos	neg	neg	Nablus
28	t605		1	CC126	neg	pos	neg	Jerusalem
29	t1094		1	NF	neg	neg	pos	Jerusalem
30	t1247		1	NF	neg	neg	pos	Jerusalem
31	t011		1	NF	neg	neg	neg	Gaza
32	t932		1	NF	neg	neg	neg	Hebron
33	t085	Type I	1	NF	neg	neg	neg	Jerusalem
34	t018	Type I	1	CC30	neg	pos	neg	Ramallah
35	t314	NT	1	CC121	neg	neg	neg	Tubas

Table 4.18 The molecular and antibiogram items, the phenotypic and genotypic characteristics of all MRSA isolates included in this study

Age group	*Spa*	*spa* repeat succession	MLST-CC	SCC*mec*	PVL	TSST-1	*arc*-A	MDR	Region
Adult n=68	t386 (9)	r07:r23:r13	CC1	Type IV	neg	neg	neg	Y (2), N(7)	Jerusalem(2),Nablus (2),Hebron (2), Bethlehem, Nablus, Ramallah
	t044 (9)	r07:r23:r12:r34:r34:r33:r34	CC80	Type IV	pos	neg	neg	Y(4), N(5)	Gaza (5), Jerusalem (4)
	t008 (8)	r11:r19:r12:r21:r17:r34:r24:r34:r22:r25	CC8	Type IV (7), Type I (1)	pos (6), neg (2)	pos(1), neg (7)	pos(5), neg (3)	Y(2), N (6)	Ramallah(4), Jerusalem(4)
	t037 (6)	r15:r12:r16:r02:r25:r17:r24	CC8/239	Type IV (4), NT (2)	neg	neg	pos(1),neg(5)	Y(1),N(5)	Gaza (5), Jerusalem
	t223 (6)	r26:r23:r13:r23:r05:r17:r25:r17:r25:r16:r28	CC22	Type IV	neg	pos(5), neg (1)	neg	Y(2), N(4)	Gaza(3), Jerusalem(3)
	t1516(3)	r26:r25:r17:r25:r16:r28	CC22	Type IV	neg	pos	pos(1),neg(2)	Y(2), N(1)	Bethlehem(2),Jerusalem
	t002 (2)	r26:r23:r17:r34:r17:r20:r17:r12:r17:r16	CC5	Type IV	neg	neg (1), pos (1)	neg	Y,N	Ramallah (1), Jerusalem (1)
	t131 (2)	r07:r23:r12:r34:r33:r34	CC80	Type IV	pos	neg	neg	Y,N	Hebron
	t021 (2)	r15:r12:r16:r02:r16:r02:r25:r17:r24	CC30	Type IV	neg	pos	neg	N	Hebron
	t991 (2)	r07:r33:r23	CC913	Type I, Type IV	neg	neg	neg, pos	Y,N	Ramallah
	t688 (2)	r26:r23:r17:r34:r17:r16	CC5	Type I, Type V	neg	neg	neg	N	Gaza, Jerusalem
	t011	r08:r16:r02:r25:r34:r24:r25	NF	Type IV	neg	neg	neg	N	Gaza
	t304	r11:r10:r21:r17:r34:r24:r34:r22:r25	CC6	Type IV	neg	neg	neg	N	Gaza
	t005	r26:r23:r13:r23:r31:r05:r17:r25:r17:r25:r16:r28	CC22	Type IV	neg	pos	neg	Y	Jerusalem
	t018	r15:r12:r16:r02:r16:r02:r25:r17:r24:r24:r24	CC30	Type I	neg	pos	neg	N	Ramallah
	t084	r07:r23:r12:r34:r34:r12:r12:r23:r02:r12:r23	CC15	Type IV	pos	neg	neg	N	Gaza
	t085	r07:r23:r12:r34:r34:r12:r23:r02:r12:r23	NF	Type I	neg	neg	neg	N	Jerusalem
	t104	r11:r10:r34:r22:r25	CC5	Type I	neg	neg	neg	N	Gaza
	t1094	r23:r17:r34:r17:r20:r17:r12:r17:r16	NF	Type IV	neg	neg	pos	N	Jerusalem
	t121	r11:r19:r21:r17:r34:r24:r34:r22:r25	CC8	Type IV	pos	neg	neg	N	Nablus
	t1247	r07:r23:r34:r34:r33:r34	NF	Type IV	neg	neg	pos	N	Jerusalem
	t127	r07:r23:r21:r16:r34:r33:r13	CC1	Type IV	neg	neg	neg	N	Jerusalem
	t359	r07:r23:r12:r21:r17:r34:r34:r33:r34	CC8	Type IV	neg	neg	neg	Y	Gaza
	t605	r07:r23:r13	CC126	Type IV	neg	pos	neg	N	Jerusalem
	t932	r12:r16:r02:r25:r17:r24	NF	Type IV	neg	neg	neg	Y	Hebron
	(*)?	_____	NF	NT	neg	neg	neg	Y	Gaza
	Err	_____	NF	Type I	neg	neg	pos	Y	Ramallah
	Err	_____	NF	Type IV	neg	neg	pos	N	Bethlehem
Adolescent n=16	t008 (3)	r11:r19:r12:r21:r17:r34:r24:r34:r22:r25	CC8	Type IV	pos	neg	pos	Y	Jerusalem
	t021 (3)	r15:r12:r16:r02:r16:r02:r25:r17:r24	CC30	Type IV	pos (1), neg (2)	pos	neg	N	Nablus (2), Jerusalem
	t223 (3)	r26:r23:r13:r23:r05:r17:r25:r17:r25:r16:r28	CC22	Type IV	pos (1), neg (2)	pos (2), neg (1)	pos (1), neg (2)	N	Gaza(2),Jerusalem
	t304 (2)	r11:r10:r21:r17:r34:r24:r34:r22:r25	CC6	Type IV	neg	neg	neg	N	Gaza
	t044	r07:r23:r12:r34:r34:r33:r34	CC80	Type IV	pos	neg	neg	N	Gaza
	t386	r07:r23:r13	CC1	Type IV	neg	neg	neg	N	Bethlehem

	t648	r11:r21:r17:r34:r24:r34:r22:r25	CC8	Type IV	neg	neg	neg	Y	Gaza
	t314	r08:r17:r23:r18:r17	CC121	NT	neg	neg	neg	N	Tubas
	t991	r07:r33:r23	CC913	Type IV	neg	neg	neg	N	Gaza
Children	t021 (2)	r15:r12:r16:r02:r16:r02:r25:r17:r24	CC30	Type IV	neg	pos	neg	Y,N	Hebron
n=8	t104	r11:r10:r34:r22:r25	CC5	Type IV	pos	neg	pos	N	Jerusalem
	t131	r07:r23:r12:r34:r33:r34	CC80	Type IV	pos	neg	neg	N	Gaza
	t1516	r26:r25:r17:r25:r16:r28	CC22	Type I	neg	neg	neg	Y	Hebron
	t223	r26:r23:r13:r23:r05:r17:r25:r17:r25:r16:r28	CC22	Type IV	neg	pos	neg	N	Gaza
	t541	r26:r17:r25:r17:r25:r16:r28	CC22	Type IV	neg	pos	neg	Y	Gaza
	t991	r07:r33:r23	CC913	Type IV	neg	neg	neg	N	Hebron
Infant	t991 (2)	r07:r33:r23	CC913	Type IV	neg	neg	pos	N	Gaza, Hebron
n=10	t002 (2)	r26:r23:r17:r34:r17:r20:r17:r12:r17:r16	CC5	Type IV	pos	neg	neg	Y,N	Hebron, Jerusalem
	t044 (2)	r07:r23:r12:r34:r34:r33:r34	CC80	Type IV	neg, pos	neg	neg	N	Gaza, Hebron
	t037	r15:r12:r16:r02:r25:r17:r24	CC8/239	Type I	neg	neg	neg	N	Gaza
	t688	r26:r23:r17:r34:r17:r16	CC5	Type IV	neg	neg	neg	Y	Hebron
	err (2)	_____	NF	Type IV	neg	neg	neg, pos	Y,N	Ramallah, Jerusalem
NF	t386 (4)	r07:r23:r13	CC1	Type IV	neg(3), pos (1)	neg	neg	N	Nablus (3),Jerusalem
n-10	t037 (3)	r15:r12:r16:r02:r25:r17:r24	CC8/239	Type IV (2), Type I	neg	neg	neg	Y (2), N	Nablus (2), Ramallah
	t008	r11:r19:r12:r21:r17:r34:r24:r34:r22:r25	CC8	Type IV	pos	neg	pos	N	Nablus
	t223	r26:r23:r13:r23:r05:r17:r25:r17:r25:r16:r28	CC22	Type IV	neg	pos	neg	N	Jerusalem
	t318	r15:r12:r16:r16:r02:r16:r02:r25:r17:r24	CC30	Type IV	pos	neg	neg	Y	Nablus

Chapter Five:

Discussion, Conclusions, Limitations and Recommendations

5.1 Discussion

MRSA is a major pathogen in hospitals and among healthy populations, characterized by different resistance mechanisms and associated with a broad variety of mild skin and soft tissue infections to serious life threatening and invasive diseases with severe morbidity and mortality rates (Cardona & Wilson, 2015; Gardam, 2000; Gordon & Lowy, 2008; Reddy, Srirama, & Dirisala, 2017; Simor, Loeb, & Committee, 2004; Stryjewski & Chambers, 2008).

Our knowledge of the major MRSA types and clones in Palestine is not well established; most reports mainly describe the prevalence and the SCC*mec* types of MRSA among community and health care workers. This study aimed to investigate the epidemiological and genetic diversity of MRSA isolates in healthcare settings in Palestine, from 2015 to 2017.

This study was conducted over a period of 2 years (2015-2017) to characterize the molecular and the antimicrobial profile of MRSA isolates in Palestinian regions. Most isolates were obtained from Al- Makassed Islamic Charitable Society Hospital (68.8% of all isolates). Al-Makassed Islamic Charitable Society Hospital is one of the most important and leading medical institutions in Palestine. It is located in East Jerusalem and has been established since 1956. Al-Makassed Islamic Charitable Society Hospital currently has about 250 beds and a staff of 750 employees. This hospital provides medical services to all Palestinians in the West Bank, Gaza Strip and East Jerusalem. It is considered as a referral hospital, receiving patients from all over the nation in Palestine.

Here, the antibiogram and resistance rates to different antibiotics among MRSA isolates were investigated. In this study, it has been noticed that the resistance rate to erythromycin was high (63.4%) in comparison to other local and nearby countries studies. However, a study conducted in Palestine by Kaibni et al has shown a low resistance rate to erythromycin (18.5%) (Kaibni, Farraj, Adwan, & Essawi, 2009). Another study conducted in Gaza strip has reported a resistance rate of 37.9% to erythromycin (Biber et al., 2012). In a study conducted in Jordan, resistance rate to erythromycin was reported to be 42.8% (Aqel, Alzoubi, Vickers, Pichon, & Kearns, 2015). Recently, in Egypt, MRSA resistance rate among to erythromycin was in a percent of 52.9% (Khairalla, Wasfi, & Ashour, 2017). Similarly, a high resistance rate to erythromycin (58.6%) was reported in Iran (Goudarzi et al., 2017). Higher resistance rates to erythromycin were reported in Israel and Germany (70.0%, 77.8%), respectively (Biber et al., 2015; Cuny et al., 2015). Interestingly, in a study conducted in the northern region of Palestine, a resistance rate to erythromycin was as high as 96% among MRSA isolates (Adwan et al., 2013).

For clindamycin, 34.8% of our MRSA strains were resistant. That, similarly, agrees with a study conducted in Palestine in 2013 (52%) and in Iran (46.9%) (Adwan et al., 2013; Goudarzi et al., 2017). A notable higher clindamycin resistance rates (61.7%, 69.9%) were reported in Israel and Germany, respectively (Biber et al., 2015; Cuny et al., 2015). On the other hand, a lower MRSA clindamycin resistance rates were reported in Egypt (26.5%) and Jordan (5.35%) (Aqel et al., 2015; Khairalla et al., 2017).

Regarding gentamicin, 23.2% of our MRSA isolates were resistant. Similarly, 20% of MRSA isolates were resistant to gentamicin in a study conducted in the Primary Hospital in Gaza (Al Laham et al., 2015). A higher gentamicin resistance rate was reported in the neighboring countries; such as: Israel (37.9%) (Biber et al., 2015) and Egypt (73.5%) (Khairalla et al., 2017). However a low resistance rate (3.5%) to gentamicin was reported in Germany (Cuny et al., 2015). Interestingly, no gentamicin resistant MRSA isolates were reported in a recent study conducted in Jordan (Aqel et al., 2015).

For trimethoprim-sulfamethoxazole, in the last few years, it has been known and reported that MRSA isolates are usually susceptible to this antibiotic in a percent of (97-99%) (Aguadero, Gonzalez-Velasco, Vindel, Gonzalez-Velasco, & Moreno, 2015; Berla-Kerzhner et al., 2017; Biber et al., 2012; Biber et al., 2015; Cuny et al., 2015)

Interestingly, in this study, most isolates were sensitive to SXT (81.3%), but 18.8% of MRSA isolates were SXT resistant. In a recent study conducted in India, SXT resistance rate among MRSA isolates was very similar to ours (18.9%) (Pramodhini, 2017). A study conducted in Gaza has shown a slightly higher resistance rate to SXT (20.0%) among MRSA isolates (Al Laham et al., 2015), while a study conducted in Germany/North Rhine has revealed that only 0.2% of 1952 MRSA isolates were SXT resistant (Cuny et al., 2015). Moreover, a high resistance rate to SXT (33.6%) was reported recently in Iran (Goudarzi et al., 2017). Also, a notable high SXT resistance rate (59.0%) was reported in Pakistan (Idrees, Jabeen, Khan, & Zafar, 2009).

For ciprofloxacin, resistance rate among our MRSA strains was (39.3%), which was in disagreement with a study conducted in Israel in 2015, where the resistance rate to ciprofloxacin was extremely high (83.9%) (Biber et al., 2015). Another much higher resistance rate to ciprofloxacin (95.9%) was reported in Germany (Cuny et al., 2015). A study conducted in 2003 on the CA-MRSA in Palestine revealed a resistance rate to ciprofloxacin of 29.6%, similar to our findings (Kaibni et al., 2009). Lower resistance rates to ciprofloxacin were reported in Jordan and Israel (3.5%, 0.3%), respectively (Aqel et al., 2015; Biber et al., 2012). These differences in the resistance rates among different countries could be due to the misuse and abuse of ciprofloxacin and other next generations of quinolones; such as levofloxacin (Fair & Tor, 2014). Also, it could be due the over prescription and selection pressure on this antibiotic for the treatment not only for MRSA infections, but also other infections caused by different bacterial strains, even gram negative organism since ciprofloxacin is a broad-spectrum synthetic antibiotic (King, Malone, & Lilley, 2000). However, ciprofloxacin resistance can develop rapidly and this may limit the usefulness of quinolones for treating MRSA in some destination (Blumberg, Rimland, Carroll, Terry, & Wachsmuth, 1991; Hershow, Khayr, & Schreckenberger, 1998).

According to the MDR definition proposed by Magiorako et al, MDR isolate is usually resistant to at least three different non β lactam antibiotic group in addition to be resistant to β-lactam antibiotics (i.e., penicillins and cephalosporins) (Magiorakos et al., 2012). In our study, 32 MRSA isolates were MDR (28.6%). In comparison with other MRSA MDR reports based on the same above definition, a high MDR resistance rate (60.0%) was reported in Israel (Biber et al., 2015), while a similar MDR resistance rate (25.0%) was reported in Jordan by Bazzoun et al (Bazzoun et al., 2014). However, no MDR strains (according to the same definition), were reported in a state wide surveillance of antibiotic resistance patterns study conducted on 1952 MRSA isolates in North Rhine-Westphalia (Cuny et al., 2015).

This broad and diverse antimicrobial resistance patterns among MRSA isolates between different regions could be due to the different antibiotic regimens between countries and the selection pressure on certain antibiotics by the physicians. This diversity is an alarm to the crucial need to refer MRSA antimicrobial therapy to culture results which are the only ideal predictors for appropriate treatment approaches in which appropriate susceptibility reports are really necessary for optimum and lifesaving therapy of MRSA infections.

In this study, highest susceptibility patterns were noticed among SXT (81.3%), gentamicin (76.8%) and ciprofloxacin (65.2%). This may aid medical laboratory technologist to test the susceptibility of these antibiotics to be used as therapeutic options for the treatment of MRSA infections. Also, as it has been found that no VRSA strains were reported in this study, physicians can still prescribe this antibiotic based on empirical therapy when needed, especially for urgent infections, while lower susceptibility patterns were found among macrolides (erythromycin) and lincosamides (clindamycin).

These findings support the need of antibiotic stewardship and wise antibiotic selection to eliminate any further development of more resistant MRSA strains; this may preserve the still effective antibiotics as valuable therapeutic resources, especially when we analyze

the molecular characteristics and the toxins carriage by the MRSA isolates that emerged in our country and elsewhere.

Each strain was characterized by: staphylococcal chromosomal cassette *mec* (SCC*mec*), *Staphylococcus aureus* protein A (*spa*) and a panel of toxin genes, including: Panton-Valentine Leukocidin (PVL), ACME-*arc*A, Toxic Shock Syndrome Toxin-1 (*TSST*-1) and Exfoliative Toxin A (ETA). The SCC*mec* type IV is the smallest structural type among the SCC*mec* types and is believed to be the most mobile version that is associated with CA-MRSA and having characteristic of some nosocomial MRSA clones of HA-MRSA infections. This type is mainly associated with the CA-MRSA based on the molecular and genetic characteristic (Milheirico, Oliveira, & de Lencastre, 2007a; Robinson & Enright, 2003). However, the CA-MRSA clones are getting more implicated in nosocomial infections with increased colonization and transmissibility characteristics which may ultimately displace HA-MRSA clones (Otto, 2012). Currently, MRSA strains distinctions based on the hospitalization days and clinical epidemiology are beginning to blur and becoming less relevant and confusing as CA-MRSA is being transmitted in the health care related-setting. This disagreeing in favor of genotyping and molecular characterization of MRSA strains (Gonzalez et al., 2006; Mediavilla, Chen, Mathema, & Kreiswirth, 2012).

Nowadays the accurate definition of the CA-MRSA and the HA-MRSA is problematic and confused based on the hospitalization days and the traditional risk factors eliminations, such as: recent hospitalization, residence in a long-term care facility or medical devices, history of MRSA carriage, dialysis, or surgery within the last year (Rachel J. Gorwitz, 2006). However, the CDC has defined the CA-MRSA either as an infection of an outpatient that has not been hospitalized or an infection that has been isolated before 72 hrs or within the first 48 hrs of hospitalization (David & Daum, 2010; David et al., 2008; Rachel J. Gorwitz, 2006).

Thus, on the basis of the hospitalization days, not all the CA-MRSA are truly community infections and the molecular typing should be included for accurate definition for the type of MRSA infections, either CA-MRSA or HA-MRSA.

Interestingly, our findings were consistent with the previous studies showing the shift in MRSA strains among the Palestinian populations to be more community acquired (SCC*mec* types IV and V). However, many studies have shown shifts in MRSA strains to be community associated with new emerged clones among different populations. This shift of MRSA infections have been associated with the detection of new MRSA strains that are different from the previous HA-MRSA strains which can spread easily and rapidly among healthy people in the community that lack risk factors for exposure or contact to the health care system (Boswihi, Udo, & Al-Sweih, 2016; David & Daum, 2010; Udo, Pearman, & Grubb, 1993).

This study revealed that the predominant MRSA strains in our region are community acquired, with a high diversity among the MRSA clones which were harboring different toxins and virulence genes that are related to severity and infectivity of MRSA infections and can affect the response to the treatment approaches.

Even though most isolates in this study were obtained from in-patients (67.9%), but the molecular analysis showed that most MRSA strains isolated from these in-patients are mostly community acquired, (type IV, 86.6%). This can give a good indication that we have a high carriage of MRSA among healthy population or the health care workers in our region. These MRSA isolates could be carried as a normal flora in skin, hands, and groin or in the nasal cavity of the health care workers or the patients themselves, but when these patients are hospitalized or have an opened wound or surgery, their immunity may fall down allowing this normal flora or colonization to cause secondary infections. Therefore, hand hygiene and infectious control programs must be applied well in our hospitals. So, the MRSA infection may be caused from the patients themselves as they were normally colonized with MRSA before hospitalization or from the visitors or the health care workers. This interface may serve as a causative agent of cross contamination of hospital acquired and community acquired MRSA infections.

Also, it has been reported that carriage of MRSA is a major risk factor for transmission and subsequent infections that may develop to systemic or severe infections (Kuehnert et al., 2006; Piechowicz, Garbacz, Wisniewska, & Dabrowska-Szponar, 2011). A recent study conducted at Al-Shifa hospital in Gaza Strip for the nasal carriage of MRSA has shown a carriage rate of MRSA among the health care workers equal to 25.5%. This highlights that there is a high carriage rate of MRSA among the Palestinian populations, especially the health care workers who are in contact with the vulnerable patient in the hospitals (El Aila, Al Laham, & Ayesh, 2017). Moreover, a study conducted by Adwan and his colleagues has shown a nasal carriage rate of *S. aureus* of 24% among Palestinian students with 9% MRSA carriage (Adwan et al., 2013).

Our results regarding the SCC*mec* typing and the shift to CA-MRSA infection (86.6% type IV) highly agree with a study done in Copenhagen (Bartels, Boye, Rhod Larsen, Skov, & Westh, 2007), where Bartels and his colleagues have reported that there is a rapid increase of genetically diverse strains with shift to CA-MRSA, where SCC*mec* type IV was found in (86%)of isolates.

Moreover, in a large study about the epidemiology of MRSA in the southern-eastern Mediterranean countries, it has been shown that there is a significant presence of MRSA in Egypt, Jordan, Algeria, Malta, Morocco, Tunisia, Cyprus, Lebanon and Turkey. In this study, 62 hospitals were included showing an evidence of endemicity of MRSA in these regions (Borg et al., 2007). Also, it has been reported that there is an introduction of several MRSA strains with intercontinental exchange of new MRSA clones in the Middle East (Tokajian, 2014).

In a recent study conducted in Gaza by Al Laham and his colleagues, similar results were obtained with a high frequency of the SCC*mec* type (type IV), which accounted for (79.3%) of MRSA isolates. This study has revealed that the most dominant *spa* types were t223 and t044. From seven isolates in this study with the *spa* type t223, one isolate was called as a “unique Gaza strain” with PVL toxin gene positive, while all the other isolates with the *spa* type t223 did not carry the PVL toxin gene (Al Laham et al., 2015).

In this present study, also, one isolate has shown a pattern as similar as the unique Gaza strain with *spa* type t223, SCC*mec* type IV, PVL positive and *TSST*-1 negative.

Notably, in this study, the Gaza clone (t223) with *TSST*-1 positive, CC22 and SCC*mec* type IV was detected in the isolates collected from Al- Makassed Islamic Charitable Society Hospital only. Six isolates were from Gaza patient and five isolates were from patients living in Jerusalem and its suburbs. Interestingly, this could be explained due the transmission of the Gaza clone to other Palestinian populations as Gaza patients were referred to Al- Makassed Islamic Charitable Society Hospital in Jerusalem.

Also, the *spa* type t223 was identified as the most dominant MRSA lineage among isolates from health care workers, adults and children in the community of the Jordanian population (Aqel et al., 2015). Moreover, the *spa* type t223 was also reported in other countries, such as: Kuwait, Egypt and Saudi Arabia (Abou Shady, Bakr, Hashad, & Alzohairy, 2015; Khairalla et al., 2017; Udo & Al-Sweih, 2017; Udo, Boswihi, & Al-Sweih, 2016). This transmission of MRSA strains could be due to national travels between these countries.

In our study, the *spa* type t008 was predominant in 12 MRSA isolates. Nine of them were related to the USA-300 clone, harboring both the PVL and the ACME-*arc*A toxin genes (8% of all isolates). This clone is the most widespread CA-MRSA clone in the United States and had emerged in the late 1990s. It is a common cause of SSTIs. Also, it is widely spread in Canada and Europe. This clone is a significant and dramatic epidemic clone due to its carriage of virulence and resistance determinants that may enhance severity and pathogenicity of the isolated strain (Alam et al., 2015; Diep et al., 2006).
The carriage of PVL toxin gene among the CA-MRSA strains, especially the USA-300 clone, is crucial and make these strains hyper-virulent and considered as a potential cause of increased CA-MRSA virulence which may cause occasionally fatal infections (Otto, 2010). This pore-forming toxin induces polymorphonuclear cell death by apoptosis, increasing the pathogenicity of the MRSA isolates that are carrying this potent cytotoxin factor (Genestier et al., 2005; Loffler et al., 2010).

To the best of our knowledge, our study is the first study that has characterized the presence of the USA-300 clone in Palestinian regions. Whereas, we have detected this clone among nine t008 MRSA isolates, while this clone and the PVL toxin gene were not detected among the *spa* type t008/CC8 isolates in a study done on Palestinian Gaza patients (Al Laham et al., 2015). Also, the ACME-*arc* toxin gene presence was not characterized by Al-Laham et al, while it was found in 18.8% of MRSA isolates in our study. In contrast, the *TSST*-1 gene was highly harbored (27.4%) among MRSA isolates (Al Laham et al., 2015).

The *spa* type t044, which is mostly PVL positive, was predominant in our study. A study was conducted in Middle East including Jordan and Lebanon has revealed the significance and the spread of this *spa* type in the Middle East with a high prevalence of both the PVL and *TSST*-1 (73% and 19%, respectively). This study was in agreement with our results regarding the genetic diversity of MRSA strains (Harastani & Tokajian, 2014). They have explained that this could be due to genetic mutations and differences in the antibiotic descriptions between countries and this may led to the development of heterogeneous strains (Harastani & Tokajian, 2014).

For the toxin genes profiling we found the PVL, *TSST*-1, and ACME-*arc*A toxin genes in 29.5%, 23.2% and 18.8% of all isolates, respectively. While a similar study conducted in China has detected the PVL and *TSST*-1 toxin genes in 11, 3% and 18.8%, respectively (Wang et al., 2017). Another previous study conducted in Armenia has not detected the PVL toxin gene in any of MRSA isolates and detected the ACME-*arc*A in 15% of all MRSA isolates (Mkrtchyan et al., 2017). This indicates that there is a high carriage of PVL, ACME-*arc*A, *TSST*-1 and ETA these toxin genes among MRSA strains in Palestine.

In summary, this phenotypic and genotypic diversity in the antimicrobial resistance rates and harboring several toxin genes by MRSA isolates in Palestine is crucial and may be contributed by the surge of more difficult MRSA infections which may be associated with more social and economic burdens. Thus, there is an urgent need to develop better measurements among clinical microbiology laboratories for proper detection and reporting of MRSA isolates and deep knowledge among physicians toward antibiotic

prescription practices. This may control and limit the development and spread of new clones and more complicated MRSA infections.

5.2 Conclusion

Our results provide important insights into the epidemiology of MRSA strains in Palestine with high carriage of different toxin. We report a diversity of MRSA strains in hospitals in Palestine, with frequent SCC*mec* type IV carriage. Knowledge of the dominant MRSA clones in our region with the antimicrobial susceptibility profile is necessary for selection of the appropriate empirical antimicrobial treatment of MRSA infections.

5.3 Limitations and strengths:

This study had limitations concerning the cooperation from hospitals regarding the provision of sample, whereas most of the samples were from Al-Makassed Islamic Charitable Society Hospital. Also, some of the demographic data were nor found or accessible for some isolates, especially the clear categorization of CA-MRSA or HA-MRSA according to hospitalization days and exposure to health care facilities.

This study has two strong points. First, the sample size (112 isolates) was relatively large regarding other cross sectional studies conducted in Palestine on MRSA carriage or molecular characterization (Essawi et al., 1998; Kaibni et al., 2009; Sabri, Adwan, Essawi, & Farraj, 2013). The second point is the relatively deep and diverse analysis of the molecular profile and the toxin genes for each isolate. To the best of our knowledge, this study is the first study that analyze the antibiograms, the SCC*mec* type, the *spa* type (107 isolates were sequenced by *spa* typing) and four associated toxin genes related to MRSA.

5.4 Recommendations

Due to the shift of MRSA infections to the community and the high carriage among populations, no individual or group can be considered not at risk for CA-MRSA infection. This worthy point needs further attention because interesting and meaningful results and control programs can be achieved. Control measures are needed to minimize and control the spread of the MRSA in general and the dramatic clones specifically, such as the (USA-300) found throughout this study.

Regarding the appropriate treatment approaches, culturing is recommended for the MRSA infections because it is the only ideal predictor of the appropriate antibiotics therapy. Also, the appropriate empirical antibiotic therapy is essential for saving patients and reducing the mortality rates in the complicated hospitalized patients. Thus, educational and awareness programs are recommended for both the physicians and the medical laboratories for reliable appropriate diagnosis and proper antibiotic therapy decisions. This can help to minimize the development for more complicated resistant MRSA strains in hospitals and may save many immunocompromized hospitalized patients.

All together, these points suggest the need for efficient future surveillance studies and infection control strategies. Also, more *spa* typing and sequencing based studies are needed to associate the antimicrobial resistance patterns with the *spa* types and their corresponding CCs.

5.5 Future plan:

We are planning to do the MLST for some of representative isolates with the unique or rare strains and compare it with the CCs we were able to conclude through the algorithm BURP. Also, to concern on the *spa* or the SCC*mec* non-typeable isolates we found throughout our research and to identify the sub-types of SCC*mec* IV since it was the most dominant type in this study. Currently, among this study SCC*mec* type IV isolates, the most common SCC*mec* subtype was found to be mostly SCC*mec* subtype IVa. However, this SCC*mec* sub-typing is still under testing and investigations.

Chapter Six:

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Chapter Seven:

Appendices

Table 1: Bacterial Culture Media and freezing solutions

#	Item	Manufacture
1	Blood Agar Base (Infusion Agar)	HIMEDIA (India)
2	Muller Hinton Agar	HIMEDIA (India)
3	Coagulase Mannitol Salt Agar Base	HIMEDIA (India)
4	Brain Heart Infusion Broth	HIMEDIA (India)
5	Pure Glycerol	EMPROVE (Germany)

Table 2: Materials and reagents used in this study

#	**Reagent**	**Manufacture**
1	Antibiotic Disks	Oxoid (UK)
2	Gram Stain Kit	SIGMA
3	DNA Molecular weight marker (1Kb/ 100 bp ladders)	ThermoScientific, (USA)
4	DNA extraction kit	Nucleospin, Macherey-Nagel, (Germany)
5	lysostaphin 1 mg/mL	PROSPEC
6	lysosyme 1mg/mL	PROSPEC
7	Primers	Hylabs, Israel
8	PCR-Ready Products	Syntezza, Israel
9	PCR Master Mix	ThermoScientific, (USA)
10	PCR tubes	4titude, EU
11	Ultra-Pure Water	Fisher
12	Freezing tubes	Nunc Cryotube Vials, Denmark
13	Etidium Bromide	AMRESCO
14	Agarose Gel	SeaKem® LE agarose gel

Table 3: Apparatus and special equipments used in this study

#	Item	Manufacture
1	Autoclave	__
2	Petri Dishes	Miniplast/ Ein-Shermer
3	Hot plate	Stuart
4	Sensitive Balance	Sartorius
5	Research pipettes	Eppendrof
6	Nanodrop 1000 spectrophotometer	ThermoScientific
7	Thermal cycler	Biometra TProfessional Basic Gradient Thermocycler
8	Microwave oven	LG
9	Electrophoresis set-up	Bio-Rad
10	Electrophoresis Power supplier	Consort
11	Spectrafuge	Labnet
12	UV light	Bio-Imaging Systems MiniLumi transilluminator

- **TAE buffer preparation:**

The pre-diluted 1x TAE buffer was prepared by suspending 20 ml from 50x concentrated buffer to 1000 mL dd H_2O. The 50x concentrated TAE buffer was prepared by dissolving 242 gm Tris base and 57.1 gm acetic acid in 100 ml 0.5M EDTA with dd H_2O to 1L. Mixture was shaked vigorously and PH was adjusted to 8.5 using KOH.

Table 4: Antimicrobial resistance rates (%) and MDR isolates associated with the *spa* types and CCs found using BURP among 112

studied MRSA isolates.

t008	t011	t018	t021	t037	t044	t084	t085	t104	t1094	t121	t1247	t127	t131	t1516	t223	t304	t314	t318	t359	t386	t541	t605	t648	t688	t932	t991
8	NF	30	30	8/239	80	15	NF	5	NF	8	NF	1	80	22	22	6	121	30	8	1	22	126	8	5	NF	913
10.7	0.89	0.89	6.30	8.9	10.7	0.89	0.89	1.8	0.89	0.89	0.89	0.89	2.7	3.6	9.8	2.7	0.89	0.89	0.89	12.5	0.89	0.89	0.89	2.7	0.89	5.4
10.7	0.9	0.9	6.3	8.9	10.7	0.89	0.89	1.8	0.89	0.89	0.89	0.89	2.7	3.6	9.8	2.7	0.89	0.89	0.89	12.5	0.89	0.89	0.89	2.7	0.89	5.4
10.7	0.9	0.9	6.3	8.9	10.7	0.89	0.89	1.8	0.89	0.89	0.89	0.89	2.7	3.6	9.8	2.7	0.89	0.89	0.89	12.5	0.89	0.89	0.89	2.7	0.89	5.4
10.7	0.9	0.9	6.3	8.9	10.7	0.89	0.89	1.8	0.89	0.89	0.89	0.89	2.7	3.6	9.8	2.7	0.89	0.89	0.89	12.5	0.89	0.89	0.89	2.7	0.89	5.4
10.7	0.9	0.9	6.3	8.9	10.7	0.89	0.89	1.8	0.89	0.89	0.89	0.89	2.7	3.6	9.8	2.7	0.89	0.89	0.89	12.5	0.89	0.89	0.89	2.7	0.89	5.4
8.9	0.0	0.0	5.4	3.6	5.4	0.89	0.0	1.8	0.0	0.89	0.89	0.89	2.7	2.7	4.5	0.0	0.0	0.89	0.89	12.5	0.89	0.0	0.89	0.9	0.89	1.8
4.5	0.0	0.0	2.7	1.8	3.6	0.0	0.0	0.0	0.0	0.0	0.89	0.0	1.8	1.8	1.8	0.0	0.0	0.89	0.89	7.1	0.0	0.0	0.89	0.89	0.89	0.0
8.0	0.89	0.0	0.0	7.1	4.4	0.0	0.0	0.0	0.89	0.89	0.0	0.89	0.89	0.89	0.89	0.0	0.0	0.89	0.89	2.7	0.0	0.0	0.0	0.89	0.89	0.89
0.0	0.0	0.89	0.0	7.1	1.8	0.0	0.0	0.0	0.89	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.89	2.7	0.89	0.0	0.0	0.0	0.89	1.8
0.89	0.89	0.00	0.89	0.89	1.80	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	2.7	0.0	0.0	0.0	0.0	0.0	0.89	0.89	0.89	1.8	0.0	0.89
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0	0	1	3	4	0	0	0	0	0	0	0	0	3	2	0	0	1	1	2	1	0	1	1	1	1
12	1	1	7	10	12	1	1	2	1	1	1	1	3	4	11	3	1	1	1	14	1	1	1	3	1	6

Data are represented in percentage (%) of the resistance rates for each of the following antibiotics: FOX: Cefoxitin, Pen G: Penicillin G, AMC: Amoxicillin/Clavulanic acid, CRO: Ceftriaxone, MEM: Meropenem, E: Erythromycin, C: Clindamycin, CIP: Ciprofloxacin, CN: Gentamicin, SXT: Trimethoprim-sulfamethoxazole, V: Vancomycin. The 32 MDR isolates were distributed among 16 *spa* types, the *spa* (*) isolate and 2 *spa* (err) isolates (red line). The last column (red) indicates the total percentage of the antimicrobial resistance rate for each antibiotic distributed among different *spa* types, while the last line indicates the total number of isolates tested within each *spa* type and CCs found in this study.

- **Ethical Approval**

Al-Quds University
Faculty of Medicine
Abu-Dies, Jerusalem

جامعة القدس
كلية الطب
أبوديس – القدس

التاريخ: 11.12.17

حضرة الدكتور رفيق الحسيني المحترم
مدير مستشفى المقاصد
القدس

الموضوع: طلب الوصول لملفات بعض المرضى في قسم الأرشيف لاستكمال بحث بعنوان:

Molecular characterization and antimicrobial profile of Methicillin Resistant *Staphylococcus aureus* in Palestinian regions

تحية طيبة و بعد،

نرجو من حضرتكم السماح للطالبة عطاف سليمان ابراهيم هادية وهي طالبة دراسات عليا في جامعة القدس الوصول ملفات المرضى في قسم الأرشيف للحصول على معلومات لأغراض البحث وذلك استكمالاً لعينات تم تجميعها من قسم البكتيريا والزراعة في مختبر مستشفى المقاصد الخيرية مع مراعاة السرية التامة، وما زال تجميع العينات والتعاون معكم مستمراً.

شاكرين لكم حسن تعاونكم.

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