An Najah National University

Faculty of Graduate Studies

Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Hospitals Surfaces, Equipments and Patients in Northern Palestine

By

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This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Environmental Sciences, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

2014

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This thesis was defended Successfully on 24/04/2014 and approved by:

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Dedication

I dedicate This Thesis to my Mother, Father, Sisters, Brothers, all the family members and friends, my fiancé Ahmad and SITTCO team for their help and support to accomplish this work.

To my Grandmother’s Soul …

Acknowledgements

I wish to express my deepest gratitude to my thesis supervisor, Dr. Ghaleb Adwan for his supervision, constant encouragement, indispensable guidance throughout this work, constructive comments and for his valuable criticism. Special thanks goes to Dr. Ali Barakat for his statistical consultations.

Many thanks for all who made the lab a friendly environment for working, especially the technicians in the Department of Biology and Biotechnology at An-Najah National University for their help and cooperation.

Deep appreciation goes to Mr. Haytham Shaka, without him I couldn't have the time to achieve this success.

I would thank my family, specially my mother and father for their continuous love and support in my decisions and my life.

Thank you all …

الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Hospitals Surfaces, Equipments and Patients in Northern Palestine

أقر بأن ما شملت عليه هذه الرسالة إنّما هو نتاج جهدي الخاص، باستثناء ما تمّت الإشارة إليه حيثما ورد، وأنّ هذه الرسالة ككل، أو أيّ جزء منها لم يقدّم من قبل لنيل أيّ درجة أو لقب علميّ لدى أيّ مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted else where for any other degree or qualification.

Student's Name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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Abbreviations

agr	accessory gene regulator
CA-MRSA	Community Acquired Methicillin Resistant *Staphylococcus aureus*
CPAP	Continuous positive airway pressure
dNTPs	deoxynucleotide triphosphates
EDTA	Ethylendiaminetetraacetic acid
ERIC PCR	Enterobacterial Repetitive Intergenic Consensus PCR
HA-MRSA	Health Acquired Methicillin Resistant *Staphylococcus aureus*
MHA	Meuller Hinton Agar
MLST	Multilocus Sequence Typing
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
MSSA	Methicillin Susceptible *Staphylococcus aureus*
NA	Nutrient Agar
NB	Nutrient Broth
PBP	Penicillin-binding protein
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
PVL	Panton-Valentine leukocidin
REP	Repetitive Extragenic Palindromic
S.aureus	*Staphylococcus aureus*
SCC*mec*	Staphylococcal Cassette Chromosome *mec*
Spa	*Stapylococcus* protein A
TSB	Tryptone Soy Broth

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Abstract

Staphylococcus aureus is considered one of the most important pathogens to humans and animals. Infections of this pathogen can be acquired through both hospital and community settings.

This study aimed to investigate the contamination of environmental surfaces with MRSA in two hospitals in Northern Palestine, Rafidia Hospital (Nablus) and Thabet Thabet hospital (Tulkarm). In addition, to investigate molecular characterization of MRSA strains isolated from the patients and their environment in these hospitals and to use molecular methods to study the identity among these strains.

Two hundred sixty five swabbed samples were collected from two hospitals (Rafidia Hospital, 100 environmental samples and 34 clinical and nasal swab samples; Thabet Thabet Hospital, 96 environmental samples and 35 clinical and nasal swab samples).

Results showed that, 71 and 79 samples were mannitol fermenters from Rafidia hospital and Thabet Thabet hospital, respectively. Incidence of *S. aureus* in Rafidia hospital and Thabet Thabet hospital was 23.1% and

37.4%, respectively. The number of MRSA among *S. aureus* in both hospitals was 13 strains, Rafidia hospital 29% (n=9) and Thabet Thabet hospital 8.2% (n=4).

Results of this research showed that these 13 strains were resistant to oxacilllin using Disk diffusion method. All strains showed *mecA* gene by PCR technique. These strains carried different antibiotic resistance genes including *aacA-aphD* (Gentamicin), *erm*(C) (Erythromycin and clindamycin), *tetK* (Tetracyclin).

SCC*mec* typing identified that the majority (84.6%) of isolates carried SCC*mec* type II (*n* = 11), which is traditionally associated with HA-MRSA. Types I and III were not detected in this study. Strain No. 64T (7.7%) harbored SCC*mec* type IVa, which is traditionally associated with CA-MRSA. Strain 3HPR (7.7%) was non-typeable. Panton–Valentin leukocidin (PVL) was detected in 2 (14.3%) samples (3HPR and 4HPR) isolated from patients; PVL that was detected in 4HPR sample was associated with SCC*mec* type II while that was detected in 3HPR SCC*mec* was non-typeable. PVL genes of these positive samples were sequenced.

The results also showed that ERIC PCR analysis revealed to that all the 13 MRSA had 12 different patterns. Samples 48BT and 99AT had the same pattern that were no bands under these conditions of ERIC PCR. Both samples were isolated from Thabet Thabet Hospital from environment

(patient bed) and patient (nasal swab) for strains 48BT and 99AT, respectively. Three clinical and nasal swab isolates (3HPR, 4HPR and 99AT), were investigated by *spa* typing, indicating that the 3 isolates were clonally non related. Strains 4HPR and 99AT were belonged to clones t044 and t386, respectively, while the third strain 3HPR is new clone. The nucleotide sequences reported in this study were further registered at the GenBank database under the accession numbers (KJ544514, KJ544515, KJ544516, KJ544517 and KJ544518).

This study characterized MRSA burden in environmental surfaces, clinical samples and nostrils of some patients using conventional environmental sampling methods (e.g., swab cultures). Special attention to infection control policies, work practices, and cleaning techniques are necessary to reduce the risk potential of MRSA transmission in hospital staff and patients. The association between environmental contamination and the epidemiology of *S. aureus* nosocomial infections is complex and thus further investigations are needed to reach a better understanding of this relationship.

Chapter One

Introduction

1.1 General Background

Bacteria of the *Staphylococcus* genus comprise various species and subspecies that are widely distributed in nature and found mostly in the skin and mucous membranes of birds and mammals. They are Gram-positive cocci, grapelike structures. These bacteria are non spore-former, and most of the species are facultative anaerobic organisms. Species of the *Staphylococcus* genus are the most common pathogens in hospital environments, and they are the etiological agents of a wide variety of infections (Tenover, 2000). *Staphylococcus aureus* (*S. aureus*) is considered one of the most important pathogen to humans and animals. *S. aureus* infections can be acquired through both hospital and community settings. This pathogen causes the majority of all hospital-acquired infections. It is the most common cause of surgical wound infections and pneumonia, and the second most common cause of bacteraemia. Other infections caused by this pathogen include endocarditis, septicaemia, osteomyelitis, meningitis, various types of skin infections, gastroenteritis, and toxic shock syndrome (Elliot *et al*., 2001; Hartmann *et al*., 2004). However, many of these infections are difficult to treat because of evolved resistance to antimicrobial drugs. About 30% of individuals carry this organism in the anterior nares at any given time (Ryan, 2004). Carriage occurs mostly on the skin and the organism is found in large quantities on the mucous membranes of the anterior nares and vagina. Carriers serve as a source of infection to themselves and others via direct contact or contamination of fomites (Elliot *et al*., 2001).

1.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. It is a common and often indispensable technique used in medical and biological research labs for a variety of applications. There are three major steps involved in the PCR technique: denaturation, annealing, and extension. PCR is useful in the investigation and diagnosis of a growing number of diseases (Joshi *et al.,* 2011).

1.3 Resistance to Antibiotic and SCC*mec* Types

Antibiotic resistance occurs when a microbe acquires a gene, which allows the microbe to inactivate the antimicrobial agents by a different mechanisms. Acquisition of these genes may occur spontaneously, due to genetic mutation or involve acquisition of a mobile genetic element such as a plasmid, transposon, integron, or gene cassette (Muto *et al*., 2003). Methicillin Resistant *Staphylococcus aureus* (MRSA) are resistant to all penicillins, including semi synthetic penicillinase-resistant congeners, penems, carbapenems, and cephalosporins. The most important mechanism of resistance to penicillin is caused by the acquisition of an exogenous gene, *mecA*, that encodes an additional β-lactam-resistant penicillin-binding protein (PBP), termed PBP-2' (or PBP-2a). Another gene involved in penicillin resistance in staphylococci is *blaZ* which encodes β-lactamase (Malachowa and DeLeo, 2010). *mecA* gene, which is located on

Staphylococcal cassette chromosome *mec* (SCC*mec*). Eight different types of SCC*mec* (I–VIII) have been defined up to date, but only type I–V are globally distributed. SCC*mec* typing accompanied with overall genotyping has already provided strong evidence for the independent origins of health-care associated MRSA and community-acquired (Ghaznavi-Rad *et al*., 2010). The emergence of MRSA strains has become a major concern, especially in the hospital environment due to few therapeutic options, MRSA increases length of hospitalization, hospital costs, attributable morbidity and mortality as compared with those associated with methicillin sensitive *S. aureus* (MSSA) (Sydnor and Perl, 2011). The prevalence of methicillin resistance among clinical *S. aureus* isolates in Palestine was 56.4% (Adwan *et al*., 2009).

1.4 Panton-Valentine leukocidin (PVL) Acquisition

Practically all of the emerging community-associated MRSA (CA-MRSA) strains, carry Panton-Valentine leukocidin (PVL) virulence genes and possess a novel small mobile staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V genetic element which harbors the methicillin resistance (*mecA*) gene and which is more easily transferred to other strains of *S. aureus* than the larger SCC*mec* types (types I to III) that are prevalent in hospital-acquired MRSA (HA-MRSA) strains. The PVL is a bicomponent leukocidin encoded by two cotranscribed genes, namely, *lukS-PV* and *lukF-PV* (*lukS/F-PV*), which cause leukocyte destruction and tissue necrosis. The presence of PVL virulence genes in *S. aureus* is

associated with increased disease severity, ranging from cutaneous infection to severe chronic osteomyelitis and deadly necrotizing pneumonia (McClure *et al*., 2006).

1.5 Molecular typing of *S. aureus*

Numerous molecular typing methods have been developed for *S. aureus*, including Pulsed-Field Gel Electrophoresis (PFGE), MultiLocus Sequence Typing (MLST), but also typing based on sequence polymorphisms of the following loci: the Staphylococcal Cassette Chromosome *mecA* (SCC*mec*), the accessory gene regulator (*agr*) and the X region encoding protein A (*spa* gene) (Harmsen et al., 2003; AL-Tam et al., 2012). In the last ten years, the *spa*-typing based on the X region of the protein A gene has become a standard method for *S. aureus* (Valentin-Domelier et al., 2011; Basset et al., 2012;). The X region of *spa* locus is composed of variable numbers of repeats of 21 to 27 base pairs (24-bp repeat being the most common one). Its diversity is attributed to deletions or duplications of these repeats and, more seldom, to point mutations (Shopsin et al., 1999; Kahl et al., 2005). Numerous papers showed the usefulness of this region to characterize both micro- and macro-variation in *S. aureus*. Nowadays more than 500 repeats and more than 10000 *spa*-types were described according to the sequence and organization of repeats, considering repeat polymorphism, association of repeats and number of repeats. The *spa* typing can be used to investigate both the molecular evolution and hospital outbreaks of MRSA. The main advantage of *spa* typing over MLST is its

simplicity, since it involves sequencing of only a single locus (AL-Tam et al., 2012).

ERIC PCR is a PCR-fingerprinting technique but it is not arbitrary because the primer was designed to known target sequence. The primers used in ERIC PCR are complementary to repetitive sequence that highly conserved in the genome DNA. There are few repetitive sequences have been reported in bacteria genome include enterobacterial repetitive intergenic consensus (ERIC) sequences, repetitive extragenic palindromic (REP) sequences, and BOX elements. The enterobacterial repetitive intergenic consensus (ERIC) sequences are present in many copies in the genomes of different enterobacteria such as *Escherichia coli*, *Salmonella typhimurium* (Hulton *et al*., 1991; Zulkifli *et al*., 2009). These elements are highly conserved at the nucleotide level, 126 bp long, and include a central core inverted repeat. The position of ERIC elements in enterobacterial genomes varies between different species and has been used as a genetic marker to characterize isolates within a bacterial species (Versalovic *et al*., 1991; Son *et al*., 2002). In ERIC-PCR a band pattern is obtained by amplification of genomic DNA located between ERIC elements or between ERIC elements and other repetitive DNA sequences. This technique uses consensus primers in the PCR to amplify DNA sequences located between successive repetitive elements such as ERIC sequence for subtyping Gram-negative enteric bacteria (Hulton *et al*., 1991; Zulkifli *et al*., 2009).

1.6 Literature Review

The major reservoirs for MRSA are colonized or infected patients and, occasionally, personnel in the hospital (Boyce, 1992). Environmental surfaces frequently touched by health care workers are commonly contaminated in the rooms of patients colonized or infected with MRSA. Nosocomial infections result in severe health and financial difficulties for patients and healthcare facilities. Multidrug-resistant strains of *S. aureus*, particularly MRSA, pose a major clinical and epidemiological problem in hospitals, as they are easily transferred among hospital staff and patients (Neely *et al*., 2005). It was also found that contamination of the inanimate environment with MRSA occurred when either infected or colonized individuals were present in hospital rooms. It was demonstrated how the hands (gloved or otherwise) of healthcare workers can become contaminated, presumably by touching surfaces in the immediate vicinity of an infected patient (Boyce, 1997; Bhalla *et al*., 2004). More clearly, it was found that 65% of nursing staff that had directly treated an infected individual contaminated their gowns/uniforms with the organism. MRSA contamination of gloves was also observed in 42% of personnel who had no direct contact with the patient, but had touched surfaces in infected patient's rooms (Boyce *et al*., 1997). The proportional of hospital surfaces contaminated with MRSA has varies considerably in published studies, ranging from 1%-27% surfaces in patient rooms or regular hospital wards, and from a few percent to 64% of surfaces in burn units with MRSA patient (Boyce, 2007). Rates of environmental contamination also vary on

the basis of the site of infection in source patients: contamination is more common in the rooms of patients with infected urine or wounds than it is in the rooms of patients with bacteremia only (Boyce *et al*., 1997). In other study, contamination is more common in the rooms of patient with heavy gastrointestinal colonization by MRSA and concomitant diarrhea than it is in the rooms of patients with MRSA at other body sites, but not in their stool (Otter *et al*., 2006). Also, The inanimate environment of burn units tends to be more heavily contaminated than that of non-burn units: MRSA contamination rates range from 1% to 18% in non-burn wards and up to 64% in burn units (Boyce *et al*., 1997). Hydrotherapy rooms associated with burn units have a particularly high contamination rate (Boyce *et al*., 1992). *S. aureus* has been isolated from hospital mattresses during an outbreak. Moist mattress padding and leaks in mattress covers are common findings during outbreaks (Ndawula et al., 1991).

It was found that at least 16% of patients were colonized with MRSA. A significant factor contributing to the transmission of microorganisms is their ability to survive on environmental surfaces (Hails et al., 2000). It also found that keyboards and mice might serve as a source for the transmission of microorganisms. Computer keyboards and mice might serve as a source for the transmission of microorganisms in the intensive care unit (ICU) (Hartmann *et al*., 2004; Anastasiades et al., 2009). Qualitative bacteriological sampling was used to show that the colonization rate for keyboards and mice with potentially pathogenic bacteria was greater than that of other surfaces in the ICU (Hartmann *et al*., 2004).

Studies have shown contamination of common hospital surfaces such as room door handles (Oie et al., 2002), sterile packaging (Dietze et al., 2001), gowns and gloves (Boyce et al., 1997), mops (Oie and Kamiya, 1996), ward fabrics and plastics (Neely and Maley, 2000), healthcare workers pens (Banerjee et al., 1999), keyboards and taps (Bures et al., 2000; Hartmann *et al*., 2004; Anastasiades et al., 2009), curtains (Trillis et al., 2008), stethoscopes (Cohen et al., 1997), ultrasonic nebulizers (Schultsz et al., 2003), ventilation grills (Cotterill et al., 1996; Kumari et al., 1998), blood pressure cuff (de Gialluly et al., 2006) and telephones and mobiles (Ciragil et al., 2006; Trivedi et al., 2011) by MRSA. In addition to this, there is mounting indirect evidence of a link between contaminated surfaces and nosocomial infection (Boyce et al., 1997; Talon, 1999; Bhalla et al., 2004). It was shown by molecular methods that identical or closely related isolates were recovered from the patient and their environment, suggesting possible environmental contamination of the isolation rooms, possibly contributing to endemic MRSA (Sexton et al., 2006).

In Palestine, molecular characterization of MRSA strains isolated from the patients and their environment in hospitals and to use molecular methods to study the identity among these strains and to identify the identity between clones of *S. aureus* circulating in hospitals including environmental surfaces in Northern Palestine has not been investigated previously.

1.7 Objectives

This study aims to:

1. Investigate the contamination of environmental surfaces with MRSA in two hospitals in Northern Palestine.

2. Investigate molecular characterization of MRSA strains isolated from the patients and their environment in hospitals and to use molecular methods to study the identity among these strains.

Chapter Two

Materials and Methods

2.1 Study area and site of samples analysis

The Samples were collected from Two government hospitals in northern West Bank which are Rafidia Hospital-Nablus and Thabet Thabet Hospital-Tulkarm.

The collected Samples were cultured, identified and analyzed in Department of Biology and biotechnology, Science college, An-Najah National University, Nablus-Palestine.

2.2 Media preparation

2.2.1 Tryptone Soy Broth (TSB):

Tryptone Soy Broth (OXOID) was prepared according to manufacturer's instructions labeled on the bottle. In a 0.5 L bottle, 250 ml deionized water were mixed and boiled with 7.5 gm TSB to dissolve. The broth was then distributed into tubes to have 5 ml each and plugged with cotton. The tubes were autoclaved at 121 °C for 15 minutes, allowed to cool and then refrigerated.

2.2.2 Nutrient Broth (NB):

Nutrient broth (ACUMEDIA) was prepared according to manufacturer's instructions labeled on the bottle. In a 0.5 L bottle, 250 ml deionized water were mixed and boiled with 2 g of NB to dissolve. The broth was then distributed into tubes to have 5 ml each and plugged with cotton. The tubes

were autoclaved at 121 °C for 15 minutes, allowed to cool and then refrigerated.

2.2.3 Nutrient Agar (NA):

Nutrient agar (ACUMEDIA) was prepared according to manufacturer's instructions labeled on the bottle. In a 1 L bottle, 500 ml deionized water were heated and mixed with 11.5 g NA until the agar dissolved. The solution was allowed to boil for 1 minute, and then autoclaved at 121 °C for 15 minutes. After that it was allowed to cool to about 45 °C. The agar was poured into sterile Petri dishes to have 20 ml each and left overnight at room temperature. The following morning the Petri dishes were turned upside down and refrigerated.

2.2.4 Mannitol salt agar (MSA):

BBL™ Mannitol agar (BD) was prepared according to the manufacturer's instructions labeled on the bottle. In a 0.5 L bottle, 250 ml deionized water were heated and mixed with 27.75 g MSA until the agar dissolved. The solution was allowed to boil for 1 minute, and then autoclaved at 121 °C for 15 minutes. After that it was allowed to cool to about 45 °C, and poured into sterile Petri dishes to have 20 ml each, then left overnight at room temperature. The following morning the Petri dishes were turned upside down and refrigerated.

2.2.5 Meullar Hinton agar (MHA):

Meullar agar (BD) was prepared according to manufacturer's instructions labeled on the bottle. In a 2 L bottle, 1 L of deionized water were mixed with 38 g MHA and 20gm NaCl, heated and stirred until the agar dissolved. The solution allowed to boil for 1 minute, and then autoclaved at 121 °C for 15 minutes. After that it was allowed to cool to about 45 °C, and the agar was poured into sterile Petri dishes to have (25-30) ml each that was left overnight at room temperature. The following morning the Petri dishes were turned upside down and refrigerated.

2.3 Samples collection and Bacterial identification

Samples were collected between October and November 2013. Collection of samples was performed from two hospitals in Northern Palestine. Rafidia hospital in Nablus City (Table 2.1) and Thabet Thabet hospital in Tulkarm City (Table 2.2), with total number of beds 215 and 114, respectively, for the year 2013. A total of 196 samples from different environmental surfaces and equipments (n=100 from Rafidia hospital and n=96 from Thabet Thabet hospital) have been collected. Other 69 samples from Patients (n=34 from Rafidia hospital and n=35 samples from Thabet Thabet hospital) have been also collected. The surfaces included in this study were incubators of new born, breathing masks, bed side cabinets, floors, door knobs, bed rails, faucets, light buttons and other items are mentioned in Tables 2.1 and 2.2. These surfaces were sampled with a saline moistured sterile cotton swab, immediately transferred into 5 mL TSB and

incubated for 18-24 h at 37ºC. Thereafter, 10 µL from each TSB tube were subcultured on MSA using platinum loop to get isolated colonies. Yellow colonies (mannitol fermentors) were then subcultured on NA and furthermore they were identified by Gram Stain, Catalase test and Coagulase test both slide & tube methods.

Samples from patients were obtained by swabbing nares and processed as mentioned before.

Table 2.1: Hospital wards, source and number of samples collected from Rafidia Hospital

Hospital ward	Sample source	Number of samples
Neonatal	Baby incubator	7
	Water tap	2
	Medication trolley	1
	Ambu bag buff	3
	Masks	2
	CPAP machine (tubes)	1
	Patient monitors	2
	Ventilator	2
	Floor	1
	Window	1
	Milk preparing trolley	1
	Refrigerator door handle	2
	Door	1
	Chairs	1
	Staff keyboard	2
	Staff mouse	1
	Staff telephone	1
Pediatric	patients beds	15
	Floor	2
	Door handle	6
	Masks	5
	Light button	2
	TV button	1
	Pulse oximeter sensor	1
	Water tap	5
	Bed side cabinet	1
	Window	2
	Wall	1
	Food trolley	1
	Drug trolley	1
	Staff keyboard	1
	Staff mouse	1
	Linen trolley	1
	Chairs	2
	Refrigerator door handle	1
	patients	9
Men's and Urology	Patients (males and females)	20
	Patients beds	20
Hospital Lab	Patients	5
Total number of the collected samples		**134**

Table 2.2. Hospital wards, source and number of samples collected from Thabet Thabet Hospital.

Hospital Ward	Sample source	Number of samples
Neonatal	Baby closed incubator	5
	Water tap	2
	Floor	2
	Chair	1
	Door handle	1
	Balance	1
	Window	1
	Drug trolley	2
	Phototherapy unit	3
	Bed Side cabinet	1
	Nurse telephone	1
	table	1
	Light button	1
	Drug cabinet	1
	New born mask	2
	Infusion pump	1
Pediatric	Bed	11
	Bed side cabinet	5
	Water tap	4
	Door Handle	4
	Floor	1
	Wall	1
	Light button	1
	Window	1
	Bed side table	1
	Patient female	1
	Mask	5
	TV button	1
	Patient monitor/button	1
	Drug trolley	1
	Nurse central telephone	1
	Chair	1
	Scale	1
	Patient archiving trolley	1
	Cleaning trolley	1
Surgical and Internal Medicin	Beds of clinical patients	28
	Patients	28
Hospital Lab	Patient samples	6
Total number of the collected samples		**131**

2.3.1. Identification of *S. aureus*:

S. aureus isolates recovered in this study were identified and confirmed by the following tests:

2.3.1.1. Mannitol fermentation:

Aseptically a single line of inoculation of test organism was cultured on MSA. The plate culture was incubated for 24 hours at 37ºC (Cappiccino and Sherman 1996).

2.3.1.2. Gram staining:

Gram staining of bacteria was performed from NA as described by Cappiccino and Sherman (1996).

2.3.1.3. Catalase test:

Catalase test was carried out by addition 1-2 drops of 3% hydrogen peroxide (3 ml of 30% Stock hydrogen peroxide concentration with 97 ml sterile water) on bacterial colony cultured on NA (Cappiccino and Sherman 1996).

2.3.1.4. Slide coagulase test:

One Staphylococcal colony from NA and one drop of diluted citrated plasma (1 ml citrated plasma with 3 ml sterile normal saline) were mixed on a slide. Agglutination or clumping of cocci within 1 minute was

considered as positive (Cappiccino and Sherman 1996). Negative samples were further tested by tube coagulase test.

2.3.1.5. Tube coagulase test:

This test was done by inoculating 1 ml of diluted (1:4) fresh citrated human plasma with a catalase positive colony from NA in a tube. The tube was then incubated for 18-24 hours at 37ºC and inspected from time to time for the presence of clumping or agglutination (Cappiccino and Sherman 1996).

2.4. Antibiotic resistance

2.4.1 Oxacillin disk diffusion test

Oxacilllin (1 μg) antibiotic disks (Oxoid) was used to detect methicilline resistant *S. aureus*. Zones of inhibition was determined in accordance with procedures of the Clinical and Laboratory Standards Institute (CLSI, 2011), isolates were categorized as susceptible and resistant. According to Oxacilllin, *S. aureus* isolates were considered susceptible if inhibition zones were ≥13 mm after incubation on 2% NaCl MHA at 35ºC for 24 hours.

2.5. DNA extraction and polymerase chain reaction

2.5.1. DNA extraction:

S. aureus DNA was prepared for PCR according to method described previously (Adwan et al., 2013a). Briefly, cells were scraped off an overnight nutrient agar plate with a sterile loop, washed twice with 1 ml of

1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), then the pellet was resuspended in 0.5 ml of sterile distilled H_2O, and boiled for 10-15 min. The cells then were incubated on ice for 10 min. The debris pelleted by centrifugation at 11,500 X *g* for 5 min. DNA concentration was determined using spectrophotometer and the samples stored at -20ºC until use for further DNA analysis.

2.5.2. PCR assay for PVL detection:

The primer sequences for the PVL gene were described previously (McClure et al., 2006), for *luk-PV-1*, (5-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCCA-3); for *luk-PV-2*, (5-GCA TCA AGT GTA TTG GAT AGC AAA AGC-3). The PCR reaction mix with a final volume of 25 µL , was performed with 12.5 µL of PCR premix with $MgCl_2$ (ReadyMix™ 1.5 U Taq PCR Reaction Mix with 0.2 mM dNTP and 1.5 mM $MgCl_2$, Sigma), 0.4 µM of each primer and 2 µL of template DNA. The amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: denaturation at 94 ºC for 30 s, annealing at 50 ºC for 30 s, extension at 72 ºC for 1 min (30 cycles), with a final extension step at 72ºC for 2 min. The PCR products were resolved by electrophoresis through 1.5% agarose gels to determine the size of amplified fragment (433 bp) after ethidium bromide staining (0.5 µg/ml) .

2.5.3. Multiplex PCR assay for SCC*mec* typing:

The primer sets used for SCC*mec* typing were described previously (Ghaznavi-Rad et al., 2010), Table 2.3. Each PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix with $MgCl_2$ (ReadyMix^{TM} 1.5 U Taq PCR Reaction Mix with 0.2 mM dNTP and 1.5 mM $MgCl_2$, Sigma), 0.2 µM of each primer, and 2 µL DNA template. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 4 min at 94ºC was followed by 35 cycles of denaturation 94ºC for 30 s, followed by annealing at 48ºC for 30 s and extension at 72ºC for 2 min, with a final extension step at 72ºC for 4 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel after ethidium bromide staining (0.5 µg/ml). The SCC*mec* type was determined on the basis of the band pattern obtained. The expected fragments size for the subtypes I, II, III, IVa, IVb, IVc, IVd, IVh and V would be 613, 287, 243, 776, 1000, 677, 1242, 663 and 325 bp, respectively.

Table 2.3. Primers used for the simultaneous identification and SCC*mec* typing of MRSA by multiplex PCR (Ghaznavi-Rad et al., 2010).

Primer	Orientation	sequence	Size (bp)
Type I	Forward	5-GCT TTA AAG AGT GTC GTT ACA GG-3	613
	Reverse	5-GTT CTC TCA TAG TAT GAC GTC C-3	
Type II	Forward	5- GAT TAC TTC AGA ACC AGG TCA T-3	287
	Reverse	5- TAA ACT GTG TCA CAC GAT CCA T-3	
Type III	Forward	5- CAT TTG TGA AAC ACA GTA CG-3	243
	Reverse	5- GTT ATT GAG ACT CCT AAA GC-3	
Type IVa	Forward	5- GCC TTA TTC GAA GAA ACC G-3	776
	Reverse	5- CTA CTC TTC TGA AAA GCG TCG-3	
Type IVb	Forward	5- AGT ACA TTT TAT CTT TGC GTA-3	1000
	Reverse	5- AGT CAT CTT CAA TAT GGA GAA AGTA-3	
Type IVc	Forward	5- TCT ATT CAA TCG TTC TCG TAT T-3	677
	Reverse	5- TCG TTG TCA TTT AAT TCT GAA CT-3	
Type IVd	Forward	5- AAT TCA CCC GTA CCT GAG AA-3	1242
	Reverse	5- AGA ATG TGG TTA TAA GAT AGC TA-3	
Type IVh	Forward	5- TTC CTC GTT TTT TCT GAA CG-3	663
	Reverse	5- CAA ACA CTG ATA TTG TGT CG-3	
Type V	Forward	5- GAA CAT TGT TAC TTA AAT GAG CG-3	325
	Reverse	5- TGA AAG TTG TAC CCT TGA CAC C-3	

2.5.4. PCR assay for ERIC:

ERIC (Enterobacterial repetitive intergenic consensus) PCR was performed using Primer ERIC1: 5`-ATG TAA GCT CCT GGG GAT TCA C-3 and Primer ERIC2: 5-AAG TAA GTG ACT GGG GTG AGC G-3 according to Zulkifli *et al* (Zulkifli *et al*., 2009). Each PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix with $MgCl_2$ (ReadyMix^{TM} 1.5 U Taq PCR Reaction Mix with 0.2 mM dNTP and 1.5 mM $MgCl_2$, Sigma), 1 µM of each primer, 3 µL DNA template. In addition, the master mix was

modified by increasing the concentration of dNTPs to 400 μM , 3 mM MgCl2 and 2 U of Taq DNA polymerase. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 2 min at 94ºC was followed by 30 cycles of denaturation 94ºC for 1 min, annealing at 25ºC for 2 min and extension at 72ºC for 3 min, with a final extension step at 72ºC for 8 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel after ethidium bromide staining (0.5 μg/ml), and photographed for further analysis. Fingerprints were compared visually.

2.5.5. *Spa* amplification:

The primer sequence for amplification *Staphylococcus* protein A (*spa*) repeat region were described previously (Prosperi et al., 2013). The primers used for amplification are spa-1113f (5'- TAA AGA CGA TCC TTC GGT GAG C-3') and spa-1514r (5'- CAG CAG TAG TGC CGT TTG CTT-3'). Each PCR reaction mix (25 μL) was performed using 12.5 μL of PCR premix with $MgCl_2$ (ReadyMix^{TM} 1.5 U Taq PCR Reaction Mix with 0.2 mM dNTP and 1.5 mM $MgCl_2$, Sigma), 0.2 μM of each primer, and 2 μL DNA template. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation (4 min at 94°C), followed by 35 cycles of denaturation 94°C for 40 s, annealing 55°C for 40 s, and extension 72°C for 90 s, with a final extension 72°C for 5 min. Amplified

PCR products were purified from gel by the MinElute PCR purification kit (Qiagen, Hilden, Germany) and sequenced by dideoxy chain termination method using ABI PRISM sequencer, model 3130 (Hitachi Ltd, Tokyo, Japan), Bethlehem University, Bethlehem, Palestine. Sequence information was further submitted for accession number in primary bioinformatics web servers.

2.5.6. Multiplex PCR For the detection of selected antibiotic resistance genes

The PCR primers used to detect 9 antibiotic resistant genes in a multiplex PCR approach are listed in Table 2.4 (Strommenger et al., 2003). Multiplex PCR amplifications were carried out in a 25µl volume contained approximately 3µl of template DNA, 1X PCR buffer, 4 U Taq DNA polymerase, 0.2 µM of each of the 18 primers, 4 mM MgCl2,. The DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf), after initial denaturation step at 94ºC for 2 min, followed by 30 cycles of initial denaturation 94ºC for 30 s, 55 ºC for 30 s and 72ºC for 30 s ending with a final extension step at 72 ºC for 4 min. Amplification Products (15 µl) were analyzed on a 2.0% agarose gel.

Table 2.4. Features of the primers used for the detection of antibiotics resistance genes (Strommenger et al., 2003).

Primer pair	Target gene	Resistance phenotype [a]	Sequence (5`→ 3`)	Amplicon size (bp)
mecA 1 mecA 2	*mecA*	P, OX	AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C	532
aacA-aphD 1 aacA-aphD 2	*aacA-aphD*	CN	TAA TCC AAG AGC AAT AAG GGC GCC ACA CTA TCA TAA CCA CTA	227
ermA 1 ermA 2	*erm*(A)	E, CLI	AAG CGG TAA ACC CCT CTG A TTC GCA AAT CCC TTC TCA AC	190
ermC 1 ermC 2	*erm*(C)	E, CLI	AAT CGT CAA TTC CTG CAT GT TAA TCG TGG AAT ACG GGT TTG	299
tetK 1 tetK 2	*tetK*	TE	GTA GCG ACA ATA GGT AAT AGT GTA GTG ACA ATA AAC CTC CTA	360
tetM 1 tetM 2	*tetM*	TE	AGT GGA GCG ATT ACA GAA CAT ATG TCC TGG CGT GTC TA	158
vatA 1 vatA 2	*vat*(A)	QD	TGG TCC CGG AAC AAC ATT TAT TCC ACC GAC AAT AGA ATA GGG	268
vatB 1 vatB 2	*vat*(B)	QD	GCT GCG AAT TCA GTT GTT ACA CTG ACC AAT CCC ACC ATT TTA	136
vatC 1 vatC 2	*vat(C)*	QD	AAG GCC CCA ATC CAG AAG AA TCA ACG TTC TTT GTC ACA ACC	467

[a]P, penicillin; OX, oxacillin; CN, gentamicin; E, erythromycin; CLI, clindamycin; TE,tetracyline; QD, quinupristin-dalfopristin

2.6. Data analysis

2.6.1. Statistical analysis:

Statistical analysis was done using Minitab software, version 15 by applying Chi Square dependency Test to check if the prevalence of *S.aureus* is depending on site or not in the two hospitals. A *P* value of <0.05 was considered to be dependent.

2.6.2. *Spa* Sequence Analysis

The software spaTyper software (http: //fortinbras.us/cgi-bin/spatyper/spaTyper.pl) was used for spa sequence analysis to determine the clonal types.

Chapter Three

Results

Two hundred sixty five swabbed samples were collected from two hospitals (Rafidia Hospital, 100 environmental samples and 34 clinical samples; Thabet Hospital, 96 environmental samples and 35 clinical samples). Results showed that, 71 and 79 samples were mannitol fermenter from Rafidia hospital and Thabet Thabet hospital, respectively. Incidence of *S. aureus* in Rafidia hospital and Thabet Thabet hospital was 23.1% and 37.4%, respectively. The number of MRSA among *S. aureus* in both hospitals was 13 strains, Rafidia hospital 29% (n=9) and Thabet Thabet hospital 8.2% (n=4). Data are presented in Tables 3.1 and 3.2. Statistical analysis showed that the prevalence of *S. aureus* is depending on the site, in the two hospitals using Chi Square Test (Minitab software, version 15) and the *P*-value was less than 0.05.

Table 3.1. Number of mannitol fermenter isolates, *S. aureus*, MRSA isolates recovered from Rafidia hospital and Thabet Thabet hospital.

Samples Category	Rafidia Hospital	Thabet Hospital	Total
Environmental	100	96	196
Clinical and nasal swab	34	35	69
Mannitol fermentor isolates	71	79	150
Coagulase +ve (*S. aureus*)	(23.1%)	(37.4%)	
Environmental	22	36	58
Clinical and nasal swab	9	13	22
MRSA	(29.0%)	(8.2%)	
Environmental	4	3	7
Clinical and nasal swab	5	1	6

Table 3.2. Source of MRSA isolates, SCC*mec* type, PVL gene for MRSA isolates recovered from both Rafidia hospital and Thabet Thabet hospital.

Ward	Hospital	Type of sample	Sample No.	SCC*mec* type	PVL
Neonatal	Rafidia	Environment (Medication Trolley)	39R	II	-ve
Pediatric	Rafidia	Environment (Patient bed)	8R	II	-ve
	Rafidia	Environment (Light button)	18R	II	-ve
	Thabet Thabet	Environment (Bed side cabinet)	64T	IVa	-ve
	Thabet Thabet	Environment (Bed side table)	40AT	II	-ve
Men and Urology	Rafidia	Nasal swab	70AR	II	-ve
	Rafidia	Nasal swab	70BR	II	-ve
	Rafidia	Environment (Patient bed)	102R	II	-ve
Hospital Lab	Rafidia	Clinical	2HPR	II	-ve
	Rafidia	Clinical	3HPR	Non-typeable	+ve
	Rafidia A	Clinical	4HPR	II	+ve
Surgical and Internal Medicin	Thabet Thabet	Environment (Patient bed)	48BT	II	-ve
	Thabet Thabet	Nasal swab	99AT	II	-ve

Results showed that these 13 strains were resistant to oxacilllin using Disk diffusion method. All strains showed *mecA* gene by PCR technique. These strains carried different antibiotic resistance genes including *aacA-aphD* (Gentamicin), *erm (C)* (Erythromycin and clindamycin), *tetK* (Tetracyclin). Results are presented in Figure 3.1 and Table 3.3.

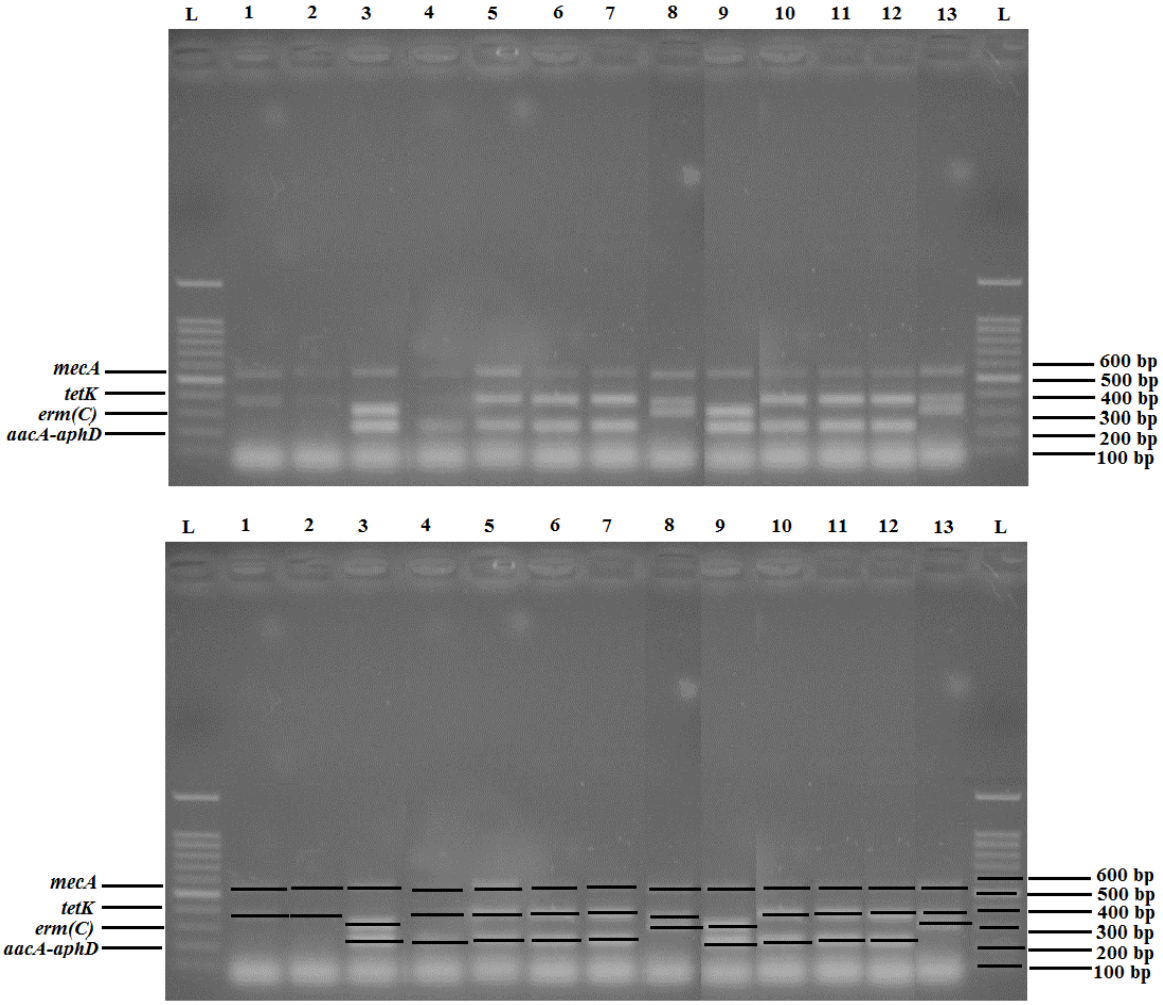

Figure 3.1. Profile of antibiotic resistance genes detected by multiplex PCR technique of 13 MRSA isolates recovered from environmental surfaces, clinical and nasal swabs on 1.5% agarose gel. Lanes: L, 100 bp DNA ladder. Lanes numbered with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 are referring to samples 8R, 18R, 39R, 40AT, 48BT, 64T, 70AR, 70BR, 102R, 2HPR, 3HPR, 4HPR and 99AT, respectively.

Table 3.3 Antimicrobial resistance patterns of 13 MRSA strains isolated from hospital environmental surfaces, clinical isolates and nasal swabs.

No.	Resistance pattern	Multi drug resistance
1	P, OX, TE[a]	2 Strains (8R, 18R)
2	P, OX, E, CLI, CN	2 Strains (39R, 102R)
3	P, OX, TE, CN	7 Strains (40AT, 48BT, 64T, 70AR, 2HPR, 3HPR, 4HPR)
4	P, OX, TE, E, CLI	2 Strains (70BR, 99AT)

[a]P, penicillin; OX, oxacillin; CN, gentamicin; E, erythromycin; CLI, clindamycin; TE, tetracycline.

All the 13 MRSA isolates were positive for *mecA* and a certain type of SCC*mec*. SCC*mec* typing identified that the majority (84.6%) of isolates carried SCC*mec* type II (n = 11), which is traditionally associated with HA-MRSA. Types I and III were not detected in this study. Strain No. 64T (7.7%) harbored SCC*mec* type IVa, which is traditionally associated with CA-MRSA. Strain 3HPR (7.7%) was non-typeable. Panton–Valentin leukocidin (PVL) was detected in 2 (14.3%) samples (3HPR and 4HPR) isolated from patients; PVL that was detected in 4HPR sample was associated with SCC*mec* type II, while that was detected in 3HPR SCC*mec* was non-typeable. PVL genes of these positive samples were sequenced. Representative of MRSA sample with SCC*mec* types and PVL is shown in Figure 3.2.

In this study, ERIC PCR analysis revealed that all the 13 MRSA had 12 different patterns. Samples 48BT and 99AT had the same pattern that were

no bands under these conditions of ERIC PCR (Figure 3.3). Both samples were isolated from Thabet Thabet Hospital from environment (patient bed) and patient (nasal swab) for strains 48BT and 99AT, respectively. Three clinical isolates (3HPR, 4HPR and 99AT) were investigated by *spa* typing, indicating that the 3 isolates were clonally non related. Strains 4HPR and 99AT were belonged to clones t044 and t386, respectively, while the third strain 3HPR is new clone and does not exist in this database. Data are presented in Table 3.4. The nucleotide sequences reported in this study were further registered at the the GenBank database under the accession numbers (KJ544514, KJ544515, KJ544516, KJ544517 and KJ544518).

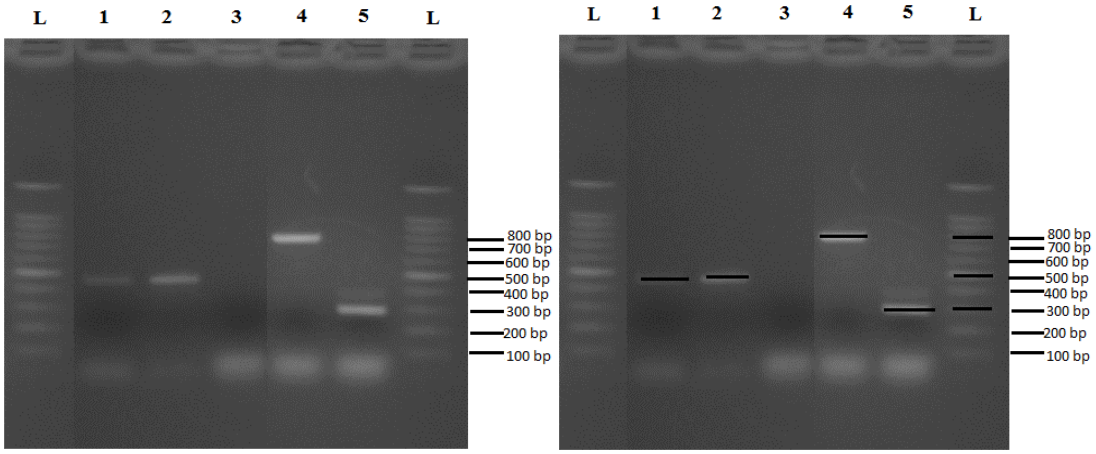

Figure 3.2. Representative image showing detection of Panton–Valentin leukocidin (PVL) and SCC*mec* types in MRSA isolates by PCR**.** Lanes: L, 100 bp DNA ladder; 1 and 2, PVL positive strains; 3 negative control, 4, *SCCmec* type IVa and 5, type II.

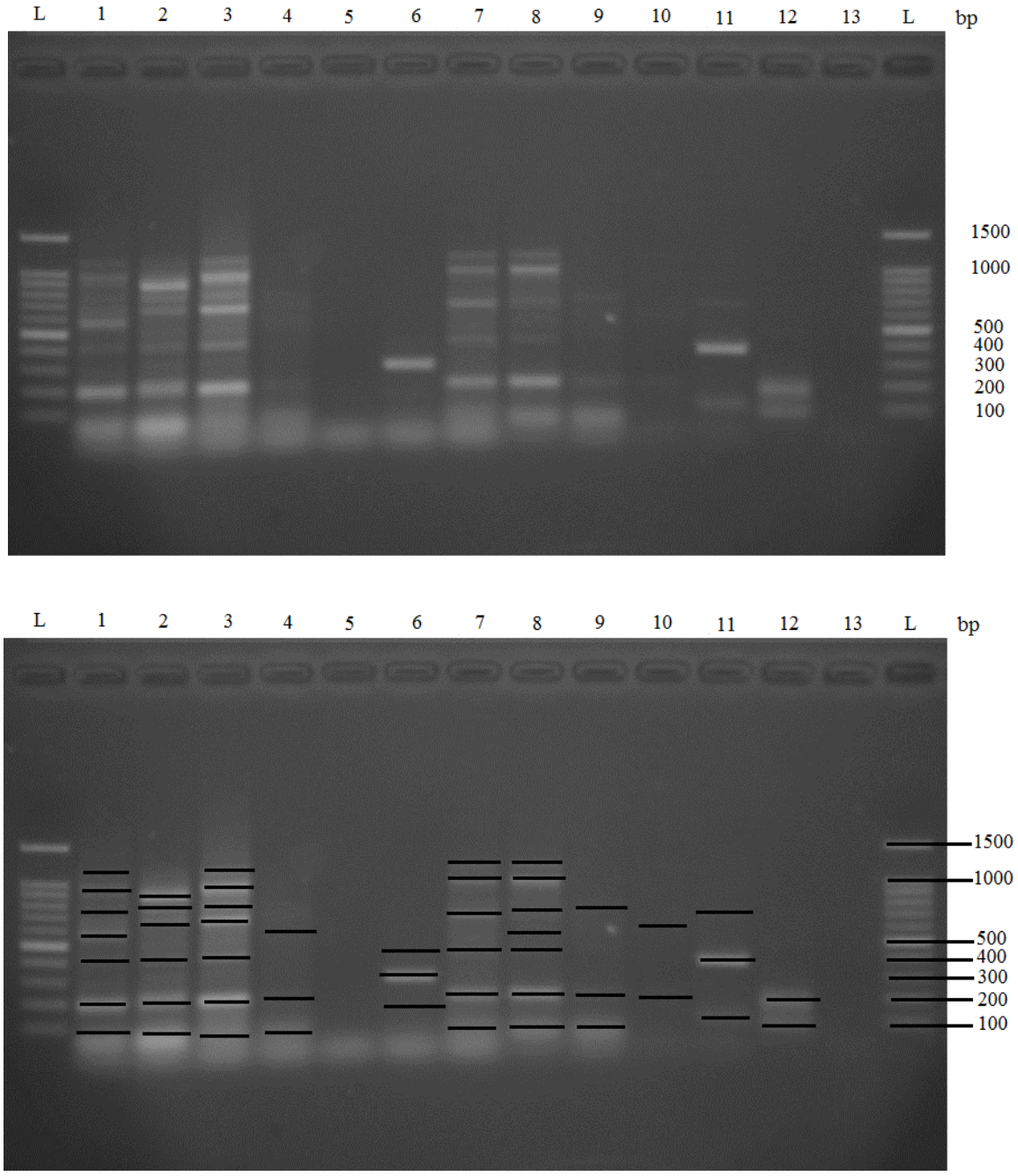

Figure 3.3. DNA fingerprints generated by ERIC PCR analysis of 13 MRSA isolates recovered from clinical and environmental surfaces on 1.5% agarose gel. Lanes numbered with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 are referring to samples 8R, 18R, 39R, 40AT, 48BT, 64T, 70AR, 70BR, 102R, 2HPR, 3HPR, 4HPR and 99AT, respectively.

Table 3.4. Number of repeat units, length of variable number tandem repeat (VNTR), Kreiswirth, Ridom nomenclature and Ridom type name.

sequence	start pos[1]	repeat units[2]	length in bp[3]	repeat seq[4]	ridom type[5]	SCC *mec* type
3HPR	51	9	216	I2:G1:E1:K1:M1:M1:J1:H2:M1 14:12:13:16:17:17:23:18:17	*	type II
4HPR	51	7	168	U1:J1:G1:B1:B1:P1:B1 07:23:12:34:34:33:34	t044	type II
99AT	75	3	72	U1:J1:E1 07:23:13	t386	type II

[1]Starting coordinate of repeats in sequence; [2] Number of repeat units; [3] Length of entire VNTR; [4] Kreiswirth, Ridom nomenclature, but where ***** indicates a sequence that is a likely *spa* repeat, but does not exist in this database and [5] Ridom type name.

Chapter Four

Discussion

Results of this research showed that 3.6% (7/196) of environmental surfaces in hospitals in Northern Palestine were contaminated with MRSA. The proportion of hospital surfaces contaminated with MRSA have varied considerably in published reports, ranging from 0.5% to 44% of surfaces in patients rooms on regular hospitals wards and from few percent to 64% of surfaces in burn units with MRSA patients (Boyce *et al*., 1997; Fitzpatrick *et al*., 2000; Rampling *et al*., 2001; Asoh *et al*., 2005). It was found that 22% of hospital privacy curtains were contaminated with MRSA (Trillis et al., 2008). Rates of environmental contamination also vary on the basis of the site of infection in source patients: contamination is more common in the rooms of patients with infected urine or wounds than it is in the rooms of patients with bacteremia only (Boyce *et al*., 1997). In other study, contamination is more common in the rooms of patient with heavy gastrointestinal colonization by MRSA and concomitant diarrhea than it is in the rooms of patients with MRSA at other body sites, but not in their stool (Otter *et al*., 2006). Environmental contamination may contribute to transmission of healthcare associated pathogens when healthcare workers contaminate their hands or gloves by touching contaminated surfaces, or when patients come into direct contact with contaminated surfaces. Transmission of healthcare-associated pathogens depend on many factors such as viability of pathogens on these surfaces, frequency of contact

between patients and healthcare workers and contaminated surfaces, and number of transmitted pathogens (Boyce, 2007). Several studies showed that nurses or volunteers who touched objects in the room of patients contaminated their gloves or hands with *S. aureus* or MRSA (Boyce et al., 1997; Bhalla et al., 2004). It was found ample evidence that a wide range of pathogens are not only present in the environment of care around the patient, but also survive for days or even months. Organism survival is related to several variables, such as the ambient temperature, relative humidity, concentration of the initial inoculum, and surface substrate (Kramer *et al*., 2006). MRSA has been isolated from different environmental surfaces, approximately 16 sites within 51 ambulances, and 49% of the ambulances had at least one area testing positive for MRSA (Brown et al., 2010).

Methicillin-susceptible *S. aureus* (MSSA) become MRSA through the acquisition and insertion into their genomes of a large DNA fragment known as staphylococcal chromosome cassette *mec* (*SCCmec*), which contains the methicillin resistance determinant, *mecA* (Hiramatsu et al., 2001). Several variants of *SCCmec* have been described, which differ with respect to the composition of their recombinase genes and mec gene complex (containing the *mecA* gene) (Oliveira et al., 2006; Nickerson et al., 2009). In spite of changes in the epidemiology of MRSA strains, they

remain one of the most important causes of nosocomial infections (Durlach et al., 2012; Köck et al., 2011). Many reports have been published about the molecular epidemiology of MRSA in different geographical regions and online databases (www.mlst.net, www.spaserver.ridom.de) allow tracking their distribution. It is clear that some strains are found worldwide while others are found geographically restrained, some of these strains are more successful than others in causing outbreaks (Székely et al., 2012). Molecular epidemiologic analysis of the isolate from the environment and the patient is much more informative than simple recovery of microbes from environmental surfaces or patient care equipment or other things in hospital (Bartley and Olmsted, 2008). SCC*mec* types (I–III) were detected more usually in hospital settings or in patients with a history of hospitalization. In this study results showed that majority of isolates carried SCCmec type II whether these strains were isolated from hospital environmental surfaces, clinical and nasal swabs. These results were in contrast to reports published recently from Palestine (Adwan et al., 2013a, b), which showed that SCC*mec* type III were detected from clinical strains. These results were consistent with a previous report that SCC*mec* type I was not detected while both types II and IV were presented. SCC*mec* type II detected from patients Nasal swabs this may gives indication that these strains were obtained from hospital environmental surfaces. Results of this

study were in contrast to other report published recently (Sabri *et al*., 2013), which showed that 95.5% and 4.5% of MRSA from Palestine, Jordan and Iraq were belonged to SCC*mec* type III and V, respectively. ERIC PCR confirmed that samples 48BT (patient bed) and 99AT (nasal swab) isolated from Thabet Thabet Hospital were belonged to the same clone. Identical or closely related isolates were recovered from the patients and their environment previously, suggesting possible environmental contamination of the isolation rooms, possibly contributing to endemic MRSA (Asoh *et al*., 2005; Sexton et al., 2006). The epidemiology of MRSA can differ from one hospital to another, even if they are from the same town. Based on these facts it seems to be important for each hospital, to assess their own epidemiological situation for the establishment of adequate infection control programmes, and to monitor their MRSA strains evolution (Székely *et al*., 2012). The data confirms the tendency of SCCmec types IV and V which are traditionally associated with CA-MRSA to spread in hospital settings as mentioned previously (Strande'n *et al*., 2009; Valsesia *et al*., 2010; Adwan *et al*., 2013b). Type IVa was isolated from bed side cabinet from Thabet Thabet Hospital.

Panton–Valentin leukocidin (PVL) was detected in 2 (14.3%) samples (3HPR and 4HPR) isolated from patients; PVL that was detected in 4HPR sample was associated with SCC*mec* type II while that was detected in

3HPR SCC*mec* was non-typable. Few MRSA isolates (14.3%) carried the PVL-toxin genes, this could be explained by the fact that our MRSA isolates were originally hospital acquired (Sabri *et al*., 2013). Detection of PVL gene in some of these strains indicate that these strains might produce a potent toxin, which is responsible for unusual virulence forming pores in leukocytes (Kaneko et al., 2004). PVL-producing strains can cause complicated chronic deep-skin infections, or necrotising pneumonia with an extremely high mortality, even in young and previously healthy patients (Lina et al., 1999; Boussaud *et al*., 2003; van der Flier *et al*., 2003, Boubaker *et al*., 2004; Diep *et al*., 2004; Francis *et al*., 2005). Diagnostic procedures are needed that allow the detection of PVL-positive strains, as well as their assignment to clonal groups, in order to monitor epidemiological developments. *spA* gene from three isolates was sequenced and these showed that belonged to different clones, t386, t044 and one does not exist in this database. Clone t386 was detected in Palestine in addition to clone t932 (Sabri *et al*., 2013).

This study characterized MRSA burden in environmental surfaces and nostrils of some patients using conventional environmental sampling methods (e.g., swab cultures). Special attention to infection control policies, work practices, and cleaning techniques are necessary to reduce the risk potential of MRSA transmission in hospital staff and patients. The

association between environmental contamination and the epidemiology of *S. aureus* nosocomial infections is complex and thus further investigations are needed to reach a better understanding of this relationship.

References

- Adwan G, Abu-Shanab B, Odeh M. **Emergence of Vancomycin-Intermediate Resistant *Staphylococcus aureus* in North of Palestine** . Asian Pac J Trop Med. 2009;2(5):44-48.
- Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A. **Prevalence of *seg, seh* and *sei* genes among clinical and nasal *Staphylococcus aureus* isolates**. Br Microbiol Res J. 2013a; 3(2): 139-149.
- Adwan K, Jarrar N, Abu-Hijleh A, Adwan G, Awwad E, Salameh Y. **Molecular analysis and susceptibility patterns of methicillin-resistant *Staphylococcus aureus* strains causing community- and health care-associated infections in the northern region of Palestine.** Am J Infect Control. 2013b;41(3):195-198.
- AL-Tam F, Brunel AS, Bouzinbi N, Corne P, Bañuls AL, Shahbazkia HR. **DNAGear--a free software for spa type identification in *Staphylococcus aureus***. BMC Res Notes. 2012;5:642.
- Anastasiades P, Pratt TL, Rousseau LH, Steinberg WH, Joubert G. ***Staphylococcus aureus* on computer mice and keyboards in intensive care units of the Universitas Academic Hospital, Bloemfontein, and ICU staff's knowledge of its hazards and cleaning practices**. South Afr J Epidemiol Infect 2009; 24(2):22-26.

- Asoh N, Masaki H, Watanabe H, Watanabe K, Mitsusima H, Matsumoto K, Oishi K, Nagatake T. **Molecular characterization of the transmission between methicillin resistant *Staphylococcus aureus* to human and environmental contamination in geriatric long-term care wards**. Intern Med 2005;44:41-45.

- Banerjee D, Fraise A, Chana K. **Writing pens are an unlikely vector of crossinfection with methicillin resistant *Staphylococcus aureus* (MRSA)**. J Hosp Infect, 1999, 43(1), 73–75.

- Bartley JM, Olmsted RN. **Reservoirs of Pathogens Causing Health Care-Associated Infections in the 21st Century: Is Renewed Attention to Inanimate Surfaces Warranted?** Clinical Microbiological Newsletter, 2008; 30(5):113-117.

- Basset P, N¨ ubel U, Witte W, Blanc DS: **Evaluation of adding a second marker to overcome *Staphylococcus aureus spa* typing homoplasies.** *J Clin Microbiol* 2012, 50(4):1475–1477.

- Bauer AW, Kirby WM, Sherris JC, Truck M. **Antibiotic susceptibility testing by a standardized single disk method**. Am J Clin Pathol. 1966; 45: 493-496.

- Bhalla A, Pultz NJ, Gries DM, Ray AJ, Eckstein EC, Aron DC, Donskey CJ. **Acuisition of nosocomial pathogens on hands after contact with**

environmental surfaces near the hospitalized patients. Infect Control Hosp Epidemiol. 2004;25(2):164-167.

- Boubaker K, Diebold P, Blanc DS, Vandenesch F, Praz G, Dupuis G, Troillet N. **Panton–Valentine leukocidin and staphyloccoccal skin infections in school children**. Emerg Infect Dis 2004; 10: 121–124.
- Boussaud V, Parrot A, Mayaud C, Wislez M, Antoine M, Picard C, Delisle F, Etienne J, Cadranel J. **Life-threatening hemoptysis in adults with community-acquired pneumonia due to Panton–Valentine leukocidin-secreting *Staphylococcus aureus*.** Intens Care Med 2003; 29: 1840–1843.
- Boyce JM, Potter-Bynoe G, Chenevert C, King T. **Environmental contamination due to methicillin-resistant *Staphylococcus aureus*: Possible infection control implications.** Infect Control Hosp Epidemiol. 1997;18(9):622-627.
- Boyce JM. **Environmental contamination makes an important contribution to hospital infection**. J Hosp Infect. 2007 Jun;65 Suppl 2:50-54.
- Boyce JM. **Methicillin-resistant *Staphylococcus aureus* in hospitals and long-term care facilities: microbiology, epidemiology, and preventive measures.** Infect Control Hosp Epidemiol 1992; 13:725–737.

- Brown R, Minnon J, Scheider S, Vaughn J. **Prevalence of methicillin-resistant *Staphylococcus aureus* in ambulances in southern Maine.** Prehosp Emerg Care, 2010; 14:176–181.
- Bures S, Fishbain JT, Uyehara CFT, Parker JM, Berg BW. **Computer keyboards and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit.** Am J Infect Control. 2000; 28(6), 465–471.
- Cappuccino, J.G. and Sherman, N. (1996). Microbiology: A laboratory Manual. 4th ed. **The Binjamin/Cummings Publishing Company**. Inc. Californai. USA. pp: 477.
- Ciragil P, Gul M, Aral M. **Bacterial contamination of computers and telephones in a university hospital in Turkey**. J Hosp Infect. 2006;62(2):247-248.
- Clinical and Laboratory Standards Institute (CLSI), Performance standards for antimicrobial susceptibility testing, informational supplement M100-S21. Wayne, PA, USA; 2011.
- Cohen HA, Amir J, Matalon A, Mayan R, Beni S, Barzilai A. **Stethoscopes and otoscopes - a potential vector of infection**?, Fam Pract, 1997; 14(6), 446–449.
- Cotterill S, Evans R, Fraise AP.**An unusual source for an outbreak of methicillin-resistant *Staphylococcus aures* on an intensive therapy unit**. J Hosp Infect. 1996;32(3):207-16.

- de Gialluly C, Morange V, de Gialluly E, Loulergue J, van der Mee N, Quentin R. **Blood pressure cuff as a potential vector of pathogenic microorganisms: a prospective study in a teaching hospital**. Infect Control Hosp Epidemiol. 2006;27(9):940-943.
- Diep BA, Sensabaugh GF, Somboona NS, Carleton HA, Perdreau-Remington F. **Widespread skin and soft-tissue infections due to two methicillin resistant *Staphylococcus aureus* strains harboring the genes for Panton–Valentine leucocidin**. J Clin Microbiol 2004; 42:2080–2084.
- Dietze B, Rath A, Wendt C, Martiny H. **Survival of MRSA on sterile goods packaging**, J Hosp Infect, 2001; 49(4), 255–261.
- Durlach R, McIlvenny G, Newcombe RG, Reid G, Doherty L, Freuler C, Rodríguez V, Duse AG, Smyth ET. **Prevalence survey of healthcare-associated infections in Argentina; comparison with England, Wales, Northern Ireland and South Africa**. J Hosp Infect. 2012; 80:217-23.
- Elliot T, Hastings M, Desselberger U. **Lectures Notes on Medical Microbiology**. 3rd ed. Oxford: Blackwell publishing, 2001.
- Fitzpatrick F, Murphy OM, Brady A, Prout S, Fenelon LE. **A purpose built MRSA cohort unit**. J Hosp Infect. 2000;46:271-279.
- Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, Cai M, Hansel NN, Perl T, Ticehurst JR, Carroll K, Thomas DL, Nuermberger E, Bartlett JG. **Severe community-onset pneumonia in healthy adults**

caused by methicillin-resistant Staphylococcus aureus carrying the Panton–Valentine leukocidin genes. Clin Infect Dis 2005;40: 100–107.

- Ghaznavi-Rad, E. Shamsudin, M. Sekawi, Z. Belkum, A. and Neela, V. "**A simplified multiplex PCR assay for fast and easy discrimination of globally distributed staphylococcal cassette chromosome mec types in meticillinresistant *Staphylococcus aureus*".** J Med Microbiol. 2010; 59: 1135–1139.

- Hails J, Kwaku F, Wilson AP, Bellingan G, Singer M. **Large variation in MRSA policies, procedures and prevalence in English intensive care units: a questionnaire analysis**. Intensive Care Med 2000; 29: 481-483.

- Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U. **Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management**. J Clin Microbiol. 2003;41(12):5442-5448.

- Hartmann B, Benson M, Junger A, Quinzio L, Röhrig R, Fengler B, Färber UW, Wille B, Hempelmann G. **Computer keyboard and mouse as a reservoir of pathogens in an intensive care unit.** J Clin Monit Comput. 2004; 18: 7-12.

- Hiramatsu K, Cui L, Kuroda M, Ito T: **The emergence and evolution of methicillin-resistant *Staphylococcus aureus*.** Trends Microbiol 2001; 9:486-493.
- Hulton CSJ, Higgins CF, Sharp PM. **ERIC sequences: a novel family of repetitive elements in the genomes of *Escherichia coli, Salmonella typhimurium* and other enterobacteria**. Mol Microbiol, 1991;5: 825-834.
- Joshi M, Deshpande J D. **Polymerase Chain Reaction: Methods, Principles and Application.** Int J Biomed Res. 2011; 2(1): 81-97.
- Kahl BC, Mellmann A, Deiwick S, Peters G, Harmsen D. **Variation of the polymorphic region X of the protein A gene during persistent airway infection of cystic fibrosis patients reflects two independent mechanisms of genetic change in *Staphylococcus aureus*.** J Clin Microbiol. 2005;43(1):502-5.
- Kaneko J, Kamio Y. **Bacterial two-component and heteroheptameric pore-forming cytolytic toxins: structures, poreforming mechanism, and organization of the genes**. Biosci Biotechnol Biochem 2004; 68: 981-1003.
- Köck R, Mellmann A, Schaumburg F, Friedrich AW, Kipp F, Becker K: **The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Germany**. Dtsch Arztebl Int. 2011; 108:761-767.

- Kramer A, Schwebke I, Kampf G. **How long do nosocomial pathogens persist on inanimate surfaces? A systematic review**. BMC Infect Dis. 2006;6:130.
- Kumari DN, Haji TC, Keer V, Hawkey PM, Duncanson V, Flower E.**Ventilation grills as a potential source of nethicillin-resistant *Staphylococcus aureus* causing an outbreak in an orthopaedic ward at a district general hospital**. J Hosp Infect. 1998;39(2):127-133.
- Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J. **Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia.** Clin Infect Dis. 1999; 29(5):1128-1132.
- Malachowa, N. and F.R. DeLeo, 2010. **Mobile genetic elements of *Staphylococcus aureus*.** Cell Mol. Life Sci. 67: 3057-3071.
- McClure, J. Conly, J. Lau, V .Elsayed, S. Louie, T. Hutchins, W. and Zhang, K. "**Novel Multiplex PCR Assay for Detection of the Staphylococcal Virulence Marker Panton-Valentine Leukocidin Genes and Simultaneous Discrimination of Methicillin-Susceptible from – Resistant Staphylococci".** J. Clin. Microbiol. 2006; 44 (3):1141-1144.
- Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, Farr BM. **SHEA guideline for preventing nosocomial transmission**

of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus*. Infect Control Hosp Epidemiol. 2003; 24(5):362-386.

- Ndawula EM, Brown L. **Mattresses as reservoirs of epidemic methicillin-resistant *Staphylococcus aureus*** . Lancet 1991; 337:488.
- Neely AN, Maley MP. **Survival of enterococci and staphylococci on hospital fabrics and plastic**, J Clin Microbiol, 2000, 38(2), 724–726
- Neely AN, Webber JM, Daviau P, MacGregor A, Miranda C, Nell M, Bush P, Lighter D; **Shriners Hospitals for Children task force. Computer equipment used in patient care within a multihospital system: recommendations for cleaning and disinfection**. *Am J* Infect Control Epidemiol 2005;33(4):233-237.
- Nickerson EK, West TE, Day NP, Peacock : ***Staphylococcus aureus* disease and drug resistance in resource-limited countries in South and East Asia.** Lancet Infect Dis 2009, 9:130-135.
- Oie S, Hosokawa I, Kamiya A. **Contamination of room door handles by methicillinsensitive/methicillin-resistant *Staphylococcus aureus***. J Hosp Infect. 2002;51(2):140-143.
- Oie S, Kamiya A. **Survival of methicillin-resistant *Staphylococcus aureus* (MRSA) on naturally contaminated dry mops,** J Hosp Infect. 1996, 34(2), 145-149.

- Oliveira DC, Milheirico C, de Lencastre H: **Redefining a structural variant of staphylococcal cassette chromosome mec, SCCmec type VI**. Antimicrob Agents Chemother 2006, 50:3457-3459.
- Otter J, Havill N, Adams N, Boyce J. **Extensive environmental contamination associated with patients with loose stools and methicillin-resistant *Staphylococcus aureus* colonization of the gastrointestinal tract. Presented at the 16th Annual Scientific Meeting of the Society for Healthcare Epidemiology of America,** Chicago, IL, 2006; abstract 159.
- Prosperi M, Veras N, Azarian T, Rathore M, Nolan D, Rand K, Cook RL, Johnson J, Morris JG Jr, Salemi M. **Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in the genomic era: a cross-sectional study**. Sci Rep. 2013;3:1902.
- Rampling A, Wiseman S, Davis L, Hyett AP, Walbridge AN, Payne GC, Cornaby AJ. **Evidence that hospital hygiene is important in the control of methicillin-resistant *Staphylococcus aureus*.** J Hosp Infect. 2001;49:109-116.
- Ryan KJ. Staphylococci. In: Ryan KJ, Ray GC., editors. **Sherris Medical Microbiology: An introduction to infectious disease**. 4th ed. New York: McGraw-Hill**,** Medical Publishing Division; 2004.

- Sabri I, Adwan K, Essawi TA, Farraj MA. **Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolates in three different Arab world countries**. Eur J Microbiol Immunol (Bp). 2013;3(3):183-7.
- Schultsz C, Meester HH, Kranenburg AM, Savelkoul PH, Boeijen-Donkers LE, Kaiser AM, de Bree R, Snow GB, Vandenbroucke-Grauls CJ. **Ultrasonic nebulizers as a potential source of methicillin resistant *Staphylococcus aureus* causing n outbreak in a university tertiary care hospital**. J Hosp Infect. 2003;55(4):269-275.
- Sexton T, Clarke P, O'Neill E, Dillane T, Humphreys H. **Environmental reservoirs of methicillin-resistant *Staphylococcus aureus* in isolation rooms: correlation with patient isolates and implications for hospital hygiene**. J Hosp Infect. 2006;62(2):187-194.
- Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. **Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains**. J Clin Microbiol.1999;37(11):3556-3563.
- Son, R., Micky, V., Kasing, A., Raha, A.R., Patrick, G.B. and Gulam, R. 2002. **Molecular characterization of Vibrio cholerae O1 outbreak strains in Miri, Sarawak (Malaysia)**. Acta Tropica 83: 169-176.

- Strande'n A, Frei R, Adler H, Flückiger U, Widmer A. **Emergence of SCCmec type IV as the most common type of methicillin-resistant *Staphylococcus aureus* in a university hospital.** Infection 2009;37:44-48.
- Strommenger B, Kettlitz C, Werner G, Witte W. **Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus***. J Clin Microbiol 2003. 41: 4089-4094.
- Sydnor ER, Perl TM. **Hospital epidemiology and infection control in acute-care settings**. Clin Microbiol Rev. 2011; 24(1):141-173.
- Székely E, Man A, Mare A, Vas KE, Molnár S, Bilca D, Szederjesi J, Toma F, Lőrinczi L. **Molecular epidemiology and virulence factors of methicillin-resistant *Staphylococcus aureus* strains in a Romanian university hospital.** Romanian Review of Laboratory Medicin, 2012; 20(4): 371-382.
- Talon D. **The role of the hospital environment in the epidemiology of multi-resistant bacteria**. J Hosp Infect. 1999;43(1):13-17.
- Tenover FC, Gaynes RP. 2000. **The epidemiology of *Staphylococcus aureus* infections. In Gram-positive Pathogens**. Edited by Fischetti, V.A.. Washington (D.C): ASM Press, 414–421.

- Trillis F III, Eckstein EC, Budavich R, Pultz MJ, Donskey CJ. **Contamination of hospital curtains with healthcare-associated pathogens.** Infect Control Hosp Epidemiol. 2008; 29(11):1074-1076.
- Trivedi HR, Desai KJ, Rivedi LP, Malek SS, Avdekar TB. **Role of Mobile Phone in Spreading Hospital Acquired Infection: A Study in Different Group of Health Care Workers.** Natl J Integr Res Med. 2011; Vol. 2(3):61-66
- Valentin-Domelier AS, Girard M, Bertrand X, Violette J, Francois P, Donnio PY, Talon D, Quentin R, Schrenzel J, van der Mee-Marquet N; Bloodstream Infection Study Group of the Réseau des Hygiénistes du Centre (RHC). **Methicillin-susceptible ST398 *Staphylococcus aureus* responsible for bloodstream infections: an emerging human-adapted subclone?** *PLoS One* 2011, 6(12):e28369.
- Valsesia G, Rossi M, Bertschy S, Pfyffer GE. **Emergence of SCCmec Type IV and SCCmec Type V methicillin-resistant *Staphylococcus aureus* containing the Panton-Valentine leukocidin genes in a large academic teaching hospital in central Switzerland: external invaders or persisting circulators?** J Clin Microbiol. 2010;48:720-727.
- van der Flier M, van Dijk NB, Fluit AC, Fleer A, Wolfs TF, van Gestel JP. **Fatal pneumonia in an adolescent due to community-acquired**

methicillin-resistant *Staphylococcus aureus* positive for Panton-Valentine-leukocidin. Ned Tijdschr Geneeskd. 2003;147(22):1076-1079.

- Versalovic J., Koueth T. Lupski JR. **Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes**. Nucleic Acid Res 1991;19: 6823-6831.
- Zulkifli Y, Alitheen NB, Son R, Raha AR, Samuel L, Yeap SK and Nishibuchi M. **Random amplified polymorphic DNA-PCR and ERIC PCR analysis on Vibrio parahaemolyticus isolated from cockles in Padang, Indonesia.** Int Food Res J. 2009. 16: 141-150.

Appendices

Chi-Square Test: Rafidia, Thabet

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	Rafidia	Thabet	Total
1	31	49	80
	40.45	39.55	
	2.209	2.259	
2	103	82	185
	93.55	91.45	
	0.955	0.977	
Total	134	131	265

Chi-Sq = 6.401, DF = 1, P-Value = 0.011

بسم الله الرحمن الرحيم

An - Najah National University

Faculty of Medicine & Health Sciences
Department of Graduate Studies

جامعة النجاح الوطنية

كلية الطب وعلوم الصحة
دائرة الدراسات العليا

IRB Approval letter

Study title:
Molecular characterization of methicillin resistant Staphylococcus aureus strains isolated from hospitals surfaces, equipments and patients in Northern Palestine

Submitted by:
Hala "Mohammad Shuaib" Abd Alqader Shaheen

Date Reviewed:
Nov 10, 2013

Date approved:
Nov 28, 2013

Your study titled: " Molecular characterization of methicillin resistant Staphylococcus aureus strains isolated from hospitals surfaces, equipments and patients in Northern Palestine " Was reviewed by An-Najah National University IRB committee & approved on Nov 28, 2013 .

Samar Musmar,MD, FAAFP

IRB

IRB Committee Chairman,
An-Najah National University

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National University
Vice President Office for Academic Affairs

جامعـــة
النجـــاح الوطنيـــة
مكتب نائب الرئيس للشؤون الاكاديمية

الرقم: ن ك ص/ ٣١اول/٢٠١٣
التاريخ: ٢٠١٣/٩/٢٦

حضرة الدكتوره أمل ابو عوض المحترمه
ق.أ.مدير عام التعليم الصحي

تحية طيبة وبعد،

الموضوع: تسهيل مهمة

تهديكم جامعة النجاح أطيب التحيات ونعلمكم بأن الطالبة هلا شاهين طالبة ماجستير تخصص علوم بيئية ترغب في عمل بحث لإتمام رسالة الماجستير الخاصة بها والتي بعنوان **الوصف الجزيئي للمكورات العنقودية الذهبية المقاومة للمثلين المعزوله عن سطح اجهزة ومرض في مستشفيات شمال فلسطين**

Molecular Characterization to methicillin resistant Staphylococcus aurous strains isolated from hospitals surfaces equipments patients in northern Palestine

وتحتاج الطالبة إلى جمع عينات من مستشفى رفيديا الحكومي نابلس ومستشفى ثابت ثابت في طولكرم يرجى من حضرتكم الايعاز للمعنيين في المستشفيات تسهيل مهمة الطالبة علما بأن المعلومات ستستخدم لاغراض البحث العلمي وستقوم الطالبة بتزويدكم بملخص عن الرسالة والتوصيات المطلوبه.

شاكرين لكم حسن تعاونكم.

مع الاحترام.

نائب الرئيس للشئون الأكاديمية

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State of Palestine
Ministry of Health - Nablus
General Directorate of Higher & Continuing Education

دولة فلسطين
وزارة الصحة- نابلس
الإدارة العامة للتعليم الصحي

Ref.: .....
Date:.....

الرقم: ٢٠١٣/١٢٧٤/١٦٠٤
التاريخ: ٢٠١٣/٩/٢٦

الأخ ق.أ. مدير عام الادارة العامة للمستشفيات المحترم،،،

تحية واحترام،،

الموضوع: تسهيل مهمة طلاب طب - جامعة النجاح

تماشياً مع سياسة وزارة الصحة المتعلقة بتعزيز التعاون مع الجامعات والمؤسسات الأكاديمية بإتاحة فرص التدريب أمام الطلبة والخريجين والباحثين في المؤسسات الوطنية وإسهاماً في تنمية قدراتهم.

يرجى تسهيل مهمة الطالبة هلا شاهين- ماجستير احياء/ جامعة النجاح، في عمل بحث بعنوان "الوصف الجزيئي للمكورات العنقودية الذهبية المقاومة للمثلين المعزولة عن سطح اجهزة ومرضى في مستشفيات شمال فلسطين " وذلك من خلال السماح للطلبة بأخذ عينات من المستشفيات التالية ومن ممرضى بعد موافقتهم، علما بانه سيتم الالتزام بمعايير البحث العلمي والحفاظ على سرية المعلومات. وذلك في:

- مستشفى رفيديا / نابلس
- مستشفى ثابت ثابت / طولكرم

مع الاحترام،،

د. أمل ابو عوض
ق. أ. مدير عام التعليم الصحي

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جامعة النجاح الوطنية

كلية الدراسات العليا

الوصف الجزيئي للمكورات العنقودية الذهبية المقاومة للمثيسيلّين المعزولة من أسطح، أجهزة ومرضى في مستشفيات شمال فلسطين

إعداد

هلا "محمد شعيب" عبد القادر شاهين

إشراف

د. غالب عدوان

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2014

الوصف الجزيئي للمكورات العنقودية الذهبية المقاومة للمثيسيلّين المعزولة من أسطح، أجهزة ومرضى في مستشفيات شمال فلسطين

إعداد

هلا "محمد شعيب" عبد القادر شاهين

إشراف

الدكتور غالب عدوان

الملخص

تعتبر المكورات العنقودية الذهبية واحدةً من أهم مسببات الأمراض للإنسان والحيوان. من الممكن أن تتم العدوى بهذا الممرض من خلال المستشفى و العديد من الأماكن والمرافق العامة في المجتمع.

هدفت هذه الدراسة إلى البحث في تلوث الأسطح البيئية بهذه الجرثومة في مستشفيين في شمال فلسطين، مستشفى رفيديا (نابلس) و مستشفى ثابت ثابت (طولكرم). إضافةً إلى البحث في الوصف الجزيئي للكشف عن (أنواع جين SCC*mec*، جين PVL، ERIC ومعرفة تسلسل الحمض النووي) من سلالة الجرثومة المعزولة من المرضى وبيئتهم في هذه المستشفيات و استخدام التقنيات الجزيئية لدراسة هذه السلالات.

تم جمع مائتين وخمسة وستين عينة من هذه المستشفيات (مستشفى رفيديا، 100 عينة بيئيّة و 34 عينة تشمل مسحات سريرية ومن الأنف؛ مستشفى ثابت ثابت، 96 عينة بيئيّة و 35 عينة تشمل مسحات سريرية ومن الأنف).

وقد أظهرت النتائج أن 71 و 79 عينة كانت مخمّرة للمانيتول من مستشفى رفيديا و مستشفى ثابت ثابت، على التوالي. وكانت نسب ظهور المكورات العنقودية الذهبية في كل من مستشفى رفيديا و مستشفى ثابت ثابت هي 23.1 ٪ و 37.4 ٪، على التوالي. وقد تبين أنّ ثلاث عشرة عيّنة من عينات البكتيريا العنقودية الذهبية التي تم عزلها في كلا المستشفيين هي من البكتيريا العنقودية الذهبية المقاومة للمثيسيلّين بنسبة 29 ٪ (ن = 9) في مستشفى رفيديا و8.2 ٪ (ن = 4) مستشفى ثابت ثابت.

وأظهرت نتائج هذا البحث أنّ هذه السلالات الثلاثة عشرة كانت مقاومة للأوكسيسيلّيين باستخدام طريقة Disk Diffusion. وقد أظهرت جميع السلالات وجود جين *mecA* بواسطة تقنية تفاعل البلمرة المتسلسل - PCR . كما تبين أن هذه السلالات تحمل العديد من الجينات المقاومة للمضادات الحيوية بما في ذلك جين *aacA-aphD* (جينتاميسين)، جين *erm*(C) (ايريثروميسين و كليندامايسين) وجين *tetK* (تيتراسيكلين).

في حين أنّ تجربة الكشف عن أنواع SCC*mec* قد بيّنت أن الغالبية 84.6 ٪، (ن = 11) من العزلات هي من نوع (II) SCC*mec*، الذي يرتبط تقليديا مع المكورات العنقودية الذهبية المقاومة للمثيسيلّيين المكتسبة من المستشفيات. في حين أن الأنواع (I) و (III) لم تظهر خلال هذه الدراسة. وقد تبين أن السلالة رقم 64T (7.7 ٪) تحمل SCC*mec* من النوع (IVa)، والذي يرتبط تقليديا مع المكورات العنقودية الذهبية المقاومة للمثيسيلّيين المكتسبة من الأماكن والمرافق العامة في المجتمع. في حين أنّ السلالة رقم 3HPR (7.7 ٪) لم تظهر تحت أي من التصنيفات المعروفة non-typable وقد تم الكشف عن جين (PVL) في عينتين (14.3 ٪) والتي هي (3HPR و 4HPR) المعزولة من المرضى; حيث كان مرتبطاً مع نوع (II) SCC*mec* في العينة 4HPR في حين أنه لم يرتبط مع أي نوع في العينة 3HPR نظراً لكونSCC*mec* فيها non-typeable. وقد تم الكشف عن التسلسل الجيني لجين (PVL) في هذه العينتين.

وأظهرت النتائج أيضا من خلال تحليل ERIC PCR إلى أنّ جميع الثلاثة عشرة سلالة للمكورات العنقودية الذهبية المقاومة للمثيسيلّيين تنتمي إلى اثنتي عشرة نمطاً مختلفاً باستخدام ERIC primers. و أنّ العينتين رقم 48BT و 99AT كانتا تنتميان لنفس النمط حيث أنه لم تظهر أيّ علامة في مسار العينتين في ظل هذه الظروف من ERIC PCR .حيث تم عزل العينات من مستشفى ثابت ثابت من سرير أحد المرضى و مسحة أنف لإحدى المرضى للسلالات رقم 48BT و99AT، على التوالي. وقد تم إجراء تسلسل النيوكليوتيدات للجين *Spa* لثلاث عزلات، والتي هي مسحات سريريّه و مسحة من الأنف (3HPR، 4HPR و99AT)، على التوالي، حيث تبين أن هذه العزلات الثلاثة تنتمي كل منها إلى نمط مختلف وأنه لا يوجد صلة بينها.

وتنتمي السلالتيّن 4HPR و 99AT للنّمطين t044 و t386، على التوالي، في حين أنّ السلالة الثالثة 3HPR تنتمي لنمط جديد. لقد تم تسجيل تسلسل النيوكليوتيدات الذي تم الكشف عنه في هذه الدراسة في قاعدة بيانات بنك الجينات تحت أرقام الانضمام (KJ544514،KJ544515، KJ544516، KJ544517 و KJ544518) .

بحثت هذه الدراسة في انتشار المكورات العنقودية الذهبية المقاومة للمثيسيلّيين، في السطوح البيئية ومسحات سريريّه ومن الأنف لبعض المرضى باستخدام الطرق التقليدية لأخذ العينات (مثل، زراعة المسحات). إنّ الاهتمام بسياسات مكافحة العدوى بشكل خاص، وممارسات العمل، و تقنيات التنظيف، من الأمور الواجب إتّباعها للتقليل من احتمال خطر انتقال هذه الجرثومة بين العاملين في المستشفى والمرضى. إنّ العلاقة بين تلوث البيئة و دراسة عدوى المستشفيات المرتبطة بالبكتيريا المكورة العنقودية الذهبية المقاومة للمثيسيلّيين معقّدة، لذلك هناك حاجة ملّحة لعمل المزيد من الدراسات والأبحاث للتوصل إلى فهم أفضل لهذه العلاقة.