



**Molecular Genotyping and Antibigram of *Staphylococcus aureus*  
Isolated from Patients Admitted to Ramallah Hospital, Palestine.**

**By**

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**This Thesis was submitted in partial fulfillment of the requirements for  
the Masters degree in clinical Laboratory Science from the Faculty of  
Graduate Studies at Birzeit University – Palestine.**

**September, 2006**

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This Thesis was defended successfully on 7/9/2006

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This thesis is dedicated to:

My beloved Father and Mother

My brothers Shukri and Shadi

My sister Abeer

My aunts Widad and Hiyam

The lovely children Yaqoub, Hanna, Amir and Nizar

All My Friends

### *Acknowledgements*

I would like to extend my deep thanks to my supervisor Dr. Tamer Essawi for his tremendous efforts in guiding and helping me to accomplish this work. I appreciate deeply his support and patience, and hope that our joint efforts will be successful. Also I would like to thank him in behalf of all the students in this program for providing us with suitable research facilities.

I would like to thank Dr. Mohammad Farraj for his help and guidance through this work.

I am most grateful to Dr. Mahmoud Srour for the valuable discussions, help, encouragement and advice mainly in the molecular biology part of this study.

Thanks to Shadi Rifae for his friendship and technical assistance.

I would like to thank the laboratory staff in Ramallah Hospital for their cooperation.

Special, warm and deep thanks to my family for their encouragement and support.

Thanks to Hiba Abu Zayyad for her help and support.

Finally, I would like to thank all my friends and colleagues for their support.

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**List of Abbreviations**

MSSA - Methicillin Susceptible *Staphylococcus aureus*

MRSA - Methicillin Resistant *Staphylococcus aureus*

HA-MRSA - Hospital Acquired Methicillin Resistant *Staphylococcus aureus*

CA-MRSA - Community Acquired Methicillin Resistant *Staphylococcus aureus*

ICU - Intensive Care Unit

CSICU - Cardiac Surgery Intensive Care Unit

SW - Surgical ward

PW- Pediatric Ward

OBS - Obstetric Ward

MLST- Multilocus Sequence Typing

MLRFT - Multilocus Restriction Fragment Typing

RFT - Restriction Fragment Typing

PCR - Polymerase Chain Reaction

PFGE - Pulsed Field Gel Electrophoresis

ST- Sequence Typing

Ox – Oxacillin

Da – Clindamycin

Cn - Gentamycin

Cip – Ciprofloxacin

E – Erythromycin

Kf - Cephalothin

Te - Tetracycline

P - Penicillin

Amp - Ampicillin

Va – Vancomycin

Tec - Teicoplanin



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## Abstract

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are increasingly considered as a main health concern worldwide for hospitalized patients. The prevalence of community-acquired infections has risen continuously during the last few years. Therefore, the control of MRSA spread is now more important than ever.

This study was performed to investigate the prevalence of *S. aureus* nasal carriage among 843 patients admitted to Ramallah hospital and 72 health care workers (HCW) between October 2003 and October 2004, and to determine phenotypic (antibiogram) and genotypic (Multilocus Restriction Fragment Typing) characteristics of MRSA isolates.

The prevalence of *S. aureus* nasal carriage among patients and HCW was 25.8 and 20.8 % respectively. The prevalence of MRSA isolates among *S. aureus* from patients and HCW was 6.8 and 66.6 % respectively. Eleven antibiogram types were characterized for the 28 MRSA isolates by using 10 different antibiotics. The most predominant antibiogram was antibiogram I and was observed among 46.4 of all MRSA isolates. All isolates were completely resistant to penicillin and ampicillin, while all isolates were 100

% susceptible to teicoplanin and vancomycin. However, the susceptibility to ciprofloxacin, Erythromycin, gentamycin, and clindamycin was 71.5 %, 82.2 %, 85.8 % and 89.3 % respectively. By using MLRFT method, the 28 MRSA isolates were differentiated into eight restriction fragment types (RFTs). Twenty-two (78.5 %) of the 28 isolates were grouped into four RFTs. The remaining six MRSA isolates were assigned to four additional RFTs.

Four of the common MRSA RFTs observed in this study could be provisionally identified as belonging to sequence types of known clonal lineages. Where the RFTs genotypes – CAAACAC, AAACCAA , BBBB BAB and BAAACAC correspond to the archaic/Iberian/clone V group, the NewYork/Pediatric/Japan group, the epidemic MRSA type 16 (EMRSA – 16) group, and the Brazilian clone respectively. The other RFTs retrieved in this study did not correspond to any of the major lineages that spread internationally.

## XII

تستحوذ المكورات العنقودية المقاومة للمضاد الحيوي ميثيسيلين اهتماما خاصا لما تسببه من مشاكل صحية في العالم اجمع, اذ ان وجودها لم يقتصر على المرضى في المستشفيات وانما اصبحت تعزل الآن من الناس في المجتمع.

تمت هذه الدراسة للتحقق من نسبة وجود العنقوديات الذهبية بما فيها المضادة للميثيسيلين في 843 مريض دخل مستشفى رام الله في الفترة الواقعة ما بين تشرين 2003 وتشرين 2004 وكذلك تحديد نسبة مقاومة هذه العزلات لعدة مضادات حيوية ومن ثم تحليلها جينيا باستعمال multilocus restriction fragment typing.

كانت نسبة وجود العنقوديات الذهبية في انف المرضى والممرضين الذين تم دراستهم 25.8 و 20.8 تتابعيا. في حين ان نسبة العنقوديات الذهبية المقاومة للمضاد الحيوي ميثيسيلين في المرضى والممرضين هي 6.8 و 66.6 تتابعيا ايضا.

وقد كانت نسبة حساسية العزلات المقاومة للمضاد الحيوي ميثيسيلين للمضادات الحيوية الاخرى كما يلي: : 71.5 %، 82.2 %، 85.8، 89.3 لكل من سيبروفلوكساسين، ايروثرومايسين، جنتاميسين و كلنداميسين تتابعيا. جميع العينات كانت غير مقاومة للفانكوميسين، غير ان جميعها كانت مقاومة للبنسلين.

باستخدام ال MLRFT فقد توزعت ال 28 عينة التي تم تحليلها الى ثماني Restriction fragment types. اثنا وعشرون عزلة من الثماني والعشرون تنتمي الى اربع Restriction fragment types، اما العزلات الست الباقية فتتوزع على اربع Restriction fragment types اضافية.

من الممكن ان اربعة من ال MRSA RFTs السابقة ان تنتمي وبصفة مؤقتة الى سلالة معينة. البنية الوراثية ل RFTs هي -CAAACAD, AAACCAA, BBBBBAB and BAAACAC والتي تتوافق مع مجموعة Archiac/Iberian/cloneV، مجموعة New york/pediatric/ Japanese group، مجموعة وباء MRSA نوع 16 (EMRSA-16)، والمجموعة البرازيلية بالترتيب. اما بقية ال RFTs المكتشفة في هذه الدراسة لم تتطابق مع أي من السلالات المنتشرة عالميا.

# Chapter 1

## 1. Introduction

The genus *Staphylococcus* is a member of the family Micrococcaceae. It is composed of 33 species, 17 of which may be encountered in human clinical specimens. *Staphylococcus aureus* subsp. *aureus* is by far the most important human pathogen among the staphylococci (13). *S. aureus* is regarded as one of the most significant pathogens, causing both nosocomial and community-acquired infections (28).

The incidence of community-acquired and hospital acquired *S. aureus* infections has been rising with increasing emergence of drug-resistant strains referred to as methicillin-resistant *S. aureus* (MRSA). MRSA is an established pathogen in most health care facilities. Previously limited to hospitals, MRSA infections have been increasingly reported in the community (31).

## **1.1 *Staphylococcus aureus***

### **1.1.1 Laboratory diagnosis**

*Staphylococcus aureus* grows as gram positive cocci in clusters; it is catalase and coagulase positive facultative anaerobe that shows beta hemolysis on sheep blood agar (13).

Laboratory diagnosis of *S. aureus* is based on phenotypic characteristics and biochemical tests: typical colonial morphology appears as large yellow or grey colonies, coagulase positive, fermentation of mannitol and the production of heat stable nucleases. Coagulase production is considered the most reliable characteristic for identifying *S. aureus*. A four-hour tube coagulase test is a definitive test. A slide test is used as a screening method for the detection of the clumping factor. Latex agglutination tests are also used as rapid identification tests (13).

### **1.1.2 Diseases**

*Staphylococcus aureus* is a major pathogen, responsible for a wide spectrum of diseases (28), ranging from relatively benign infections to life threatening systemic illnesses. *S. aureus* is a common cause of skin infections such as folliculitis, impetigo, furuncles and carbuncles. It is also commonly associated with wound infections, Toxic Shock Syndrome (TSS) and food poisoning. More serious infections may be also caused by *S. aureus* such as



pneumonia, mastitis, meningitis, endocarditis, osteomyelitis and bacteremia (13, 53).

### **1.1.3 Colonization**

*S. aureus* presents as normal flora of different body sites (13). Colonization may be transient or persistent at a single site or multiple body sites (53). The anterior nares are the most common site of colonization; other sites include intertriginous skin folds, perineum, axillae and the vagina (13, 53). Some patients are more often colonized than others by *S. aureus* such as newborns, intravenous drug users, diabetics, patients with skin diseases and hemodialysis patients.

As with methicillin susceptible *S. aureus*, most persons who are colonized with MRSA are not infected (53). However, significant proportions – at least 30 % - of hospitalized patients who become colonized with MRSA eventually have an MRSA infection such as pneumonia, bacterimia, or wound infection (46, 53).

Common risk factors for the acquisition of MRSA include increasing age, admission to intensive care units, previous hospitalization, invasive procedures and over use of antibiotics (53).

### **1.1.3.1 Nasal carriage of *S. aureus***

Although *S. aureus* can be cultured from multiple sites of the skin and mucosal surfaces of carriers, the primary reservoir of staphylococci is thought to be the anterior nares (26). Nasal carriage rates of 25 % - 40 % have been reported, and are known to be influenced by ethnicity, age, exposure to antibiotics and the hospital environment (34).

Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection (16, 26). Additionally, nasal carriage of *S. aureus* has been identified as a risk factor for the development of infections in various settings (26).

Several studies suggested that MRSA carriage constitutes a greater risk for the development of *S. aureus* infection than does MSSA carriage. This could be a result of the resistance itself or an increased intrinsic virulence of MRSA compared with MSSA or of a more vulnerable category of patients being colonized by MRSA (26, 29, 35). Elimination of nasal carriage would theoretically reduce the infection rates in populations in which it has been identified as a risk factor (26).

#### **1.1.4 Mode of transmission**

*S. aureus* including MRSA strains are primarily spread from patient to patient via the transiently colonized hands of health-care workers during patient contact or handling of contaminated materials (46, 53).

Environmental surfaces are not thought to play a major role in transmission except in special populations such as patients in burn units or intensive care units. Airborne transmission of *S. aureus* has been reported but does not appear to be an important mode of spread, except possibly in burn units (53).

The cohort of colonized or infected MRSA patients usually constitutes the most significant in-hospital reservoir from which MRSA is transmitted to other individuals (46). Because spread of MRSA in health care settings is often clonal, hand hygiene and barrier precautions are often effective in interrupting its spread. Targeted surveillance for MRSA is also a useful aid for infection control (61).

#### **1.1.5 Treatment**

Most strains of *S. aureus* are treated with penicillin –type antibiotics, such as flucloxacillin, cloxacillin, dicloxacillin and methicillin. Alternative antibiotics, such as erythromycin, may be used in persons who are allergic to penicillin. Treatment of MRSA infections remains difficult due to the multiple resistant of these strains. Vancomycin is the drug of choice.

Teicoplanin and daptomycin are two investigational antibiotics related to vancomycin in structure and in spectrum of activity (10). Mild to moderately severe MRSA infections may be treated with trimethoprim-sulfamethoxazole or minocycline if the organism is susceptible to these agents. Although most strains are currently susceptible to rifampin and fusidic acid, these agents should not be used alone because of the risk for selecting resistant mutants during treatment (53).

## **1.2 Methicillin Resistant *S. aureus***

### **1.2.1 The evolutionary origin of MRSA:**

After methicillin was introduced into clinical practice in 1959, resistant strains of *Staphylococcus aureus* (MRSA) appeared, bearing a newly acquired resistant gene, *mecA* that encodes an altered penicillin binding protein. The origin of the *mec* element is still unclear (8). A *mecA* like gene with more than 80 % sequence homology to the MRSA *mecA* is found in all strains of the animal species *Staphylococcus scuri*. Therefore, leading to the hypothesis that *S. scuri* *mecA* gene may be an evolutionary relative of the PBP- encoding *S. aureus* *mecA*, although the native *S. scuri* *mecA* gene does not confer methicillin resistance (8, 11).

The mode of transfer of the *mec* gene from an unknown donor to *S. aureus* is poorly understood (17). However, two hypotheses have been raised to

explain this issue (21): The single clone hypothesis which suggests that *mecA* entered the *S. aureus* population on one occasion and resulted in the formation of a single MRSA clone that has since spread around the world (27). While the second hypothesis proposes that MRSA have emerged on various occasions giving different clonal lineages (18).

### **1.2.2 Methicillin Resistance in *Staphylococcus aureus***

Methicillin was synthesized to treat infections caused by penicillin-resistant *staphylococcus aureus*. Unfortunately, as soon as it was used clinically, methicillin-resistant *S. aureus* strains were isolated (11, 56, 18). Resistance was termed intrinsic because it was not due to the production of beta-lactamase (11, 56).

The main mechanism of methicillin resistant in *S. aureus* is through the expression of a foreign PBP, PBP2a that is encoded by *mecA* gene (56, 62). Normally, *staphylococcus aureus* strains produce four major penicillin binding proteins (PBPs), PBP1, 2, 3 and 4 with approximate molecular masses of 85, 81, 75, 45 KDa, respectively (11). These proteins are the enzymes that catalyze the transpeptidation reaction that cross-links the peptidoglycan of the bacterial cell wall (11, 56). PBPs are also the first targets of beta-lactams (8). PBPs 1, 2 and 3 that have high affinity for most

beta-lactam antibiotics are essential for cell growth and for the survival of susceptible strains, and binding of beta-lactams to these PBPs is lethal (11).

In methicillin resistant cells PBP2a is expressed in addition to the usual PBPs (11, 56, 24). PBP2a has low affinity for beta-lactam antibiotics, so that it can substitute for the essential functions of high affinity PBPs at concentrations of antibiotic that are otherwise lethal (11, 56, 7).

PBP2a is encoded by *mecA* gene, which is located on a mobile genetic element, Staphylococcal chromosomal cassette *mec* (SCC*mec*). The SCC*mec* is horizontally transferable among staphylococcal species and has no allelic equivalent in susceptible strains (8, 25). The *mec* element is always found near the *pur-nov-his* gene cluster on the *S. aureus* chromosome (11). In addition to the structural gene *mecA*, the *mec* element contains *mecI* and *mecR1* which are regulatory elements controlling *mecA* transcription and the additional *mec* associated DNA (11, 8). The *mec* element also carries attachment sites for transposons and at least one IS 257 (IS431*mec*). The later sequence acts as a trap for the capture of further IS257- linked resistant determinants and resistance plasmids. This leads to clustering of multiple resistant in that part of the DNA (62).

Several genes have been implicated in regulating *mecA* gene transcription. These include the beta-lactams regulatory sequences, *blaR1-blaI*, and the

analogous and partially homologous chromosomal sequences; *mecR1-mecI* (40, 11, 56, 8). *mecA* gene expression varies among strains (11) and the strain's genetic background profoundly influences the methicillin resistance phenotype (67). Chromosomal genes independent of SCCmec, for example, determine whether a strain is homogeneous or heterogeneous in its pattern of resistance (25). Moreover, environmental conditions such as pH, temperature and salt concentration can influence the expression of the *mecA* gene (methicillin resistance).

Other mechanisms with low-level of methicillin resistance have been described. These mechanisms are distinct from true methicillin resistance, and can result from the production of large amounts of beta-lactamase, or increased production and /or modified penicillin binding capacity of normal PBPs (11).

### **1.2.3 Types of resistance**

Homogeneous resistance: it refers to a cell population where all the cells are resistant to high concentrations of methicillin (56, 40). The nature of the chromosomal mutations that give rise to homogeneous resistance are not known but mutations in the newly described *hmr* loci may be involved in some cases (56).

Heterogeneous resistance: The majority of cells in heterogeneous strains (typically 99.9 or more) are susceptible to low concentrations of beta-lactam antibiotics, with only a small proportion of cells growing at high methicillin concentrations (11, 40, 8, 56). The small minority of cells that exhibit high level resistance in the heterogeneous population are due to an additional chromosomal mutation that occurs outside the mec element (56).

#### **1.2.4 Community acquired MRSA:**

A significant increase in the occurrence of infections caused by community – acquired MRSA (CA-MRSA) has been reported in different areas of the world during the past ten years (14). CA-MRSA has been shown to cause infections in children and young adults who did not present with classical risk factors for nosocomial infections (50, 61, 12). Frequently, these isolates were associated with skin and soft tissue diseases. However, more severe infections such as highly lethal necrotizing pneumonia have been reported (50, 14).

CA-MRSA isolates are not clonally related to hospital acquired MRSA (HA-MRSA) international clones (50). A novel genetic mobile element designated SCCmec type IV has been identified in these isolates (14, 42, 12). In contrast to HA-MRSA, CA-MRSA tends to be susceptible to most non beta- lactam antibiotics (14, 12, 50).



The origins of these community-acquired strains of MRSA are subject to debate. One possibility is that MRSA strains of nosocomial origin may be transmitted in the community through discharged hospital patients or health care workers. Another possibility could be of horizontal transfer of the methicillin resistance determinant into a formerly susceptible background (12).

### **1.2.5 Prevalence of MRSA:**

The prevalence of MRSA among the *S. aureus* isolates differs widely among different countries as well as from one hospital to another in the same country (55). Its prevalence is consistently higher in the United States, Japan and Southern Europe than in other countries. More than 30 % of individuals in these countries are infected, compared with less than 2 % in Scandinavia, the Netherlands and Switzerland (22).

The actual prevalence of community acquired MRSA cannot be accurately determined but it is estimated that 40 % of adult cases may be acquired outside the hospital (12, 70).

### **1.3 MRSA Typing:**

Nosocomial infections caused by methicillin resistant strains of *S. aureus* (MRSA) belong to the most important multiresistant pathogens world-wide (57). The increase of the frequency of MRSA and the possibility of

emergence of resistance to vancomycin demands a quick characterization of isolates and identification of clonal spread within hospitals (63).

Bacterial strain typing distinguishes related or clonal isolates from unrelated isolates (52). It has become an important clinical tool to investigate suspected outbreaks and to evaluate nosocomial transmission (52). It is also needed for providing information on changes in the MRSA population during long-term surveillance and to deduce the evolution and global spread of these strains.

Numerous typing methods focus on discriminating MRSA isolates. These methods can be characterized in terms of typeability, reproducibility, discriminatory power and ease of interpretation (60, 32). The choice of the typing method varies depending on the application (44).

Typing methods fall into two broad categories: phenotypic methods and genotypic methods (as shown in the next sections).

### **1.3.1 Phenotypic methods:**

Before the era of molecular typing, phenotypic techniques such as biotyping, serotyping, antimicrobial susceptibility and phage typing have been used (63). These methods characterize the products of a certain gene in order to differentiate strains (60). Such systems are inherently limited by the capacity of bacteria to alter the expression of the characteristic being

assessed. Thus, independent isolates of the same strain can vary phenotypically. In addition, some of these approaches such as phage typing technique are limited by the relatively large fraction of strains that appear phenotypically null and consequently are non-typeable (32).

Although, the application of the phenotypic techniques has been decreased, they may be still useful for discrimination of clinical isolates (57).

### **1.3.2 Genotypic methods:**

Genotyping methods are those based on the analysis of the genetic structure of an organism (60, 52). These methods are less subject to natural variation than phenotypic methods, although they can be affected by insertions or deletions of DNA into the chromosome, the gain or loss of extrachromosomal DNA or random mutations that may create or eliminate restriction endonuclease sites (60).

With the advent of molecular biology, several genotypic techniques have been developed. Initial techniques compared restriction endonucleases patterns of chromosomal or plasmid DNA. The second generation of genotyping methods included southern blot hybridization using gene-specific probes, ribotyping, polymerase chain reaction (PCR) based approaches and pulsed field gel electrophoresis (52, 59, 32). Recently PCR

and DNA sequencing methods such as multilocus sequence typing and single locus sequence typing have been advanced (17, 52).

#### **1.3.2.1 Multilocus Restriction Fragment Typing (MLRFT):**

Molecular typing of *S. aureus* has been used to examine both long-term or global epidemiology and short-term or local epidemiology (44). Pulsed field gel electrophoresis (PFGE) is generally regarded as the most discriminatory technique for strain identification, particularly in the context of identifying strains involved in local outbreaks (5, 17, 36, 44). A major disadvantage of PFGE and all methods that depend on comparison of DNA fragment patterns on gels is the difficulty of comparing the results from different laboratories (17, 5, 30). Moreover, there is no convenient metric scale that reliably measures genetic relationships among strains with substantially different PFGE patterns (5). These features limit the value of PFGE as a tool for investigating the population genetics and global epidemiology of *S. aureus* (5, 17). These problems are overcome through the use of multilocus sequence typing (MLST) and Spa typing (5, 18, 30).

Multilocus sequence typing (MLST) is a highly discriminatory method of characterizing bacterial isolates on the basis of the sequences of 450 bp internal fragments of seven house-keeping genes (30). Sequence data are

portable and are easily analyzed to provide measures of genetic relationships and population structures (5)

Unfortunately, neither PFGE nor the sequence-based approaches are conveniently applied in a clinical setting. Both require specialized equipment and are relatively costly and time consuming (5).

MLRFT is a rapid, low cost strain typing technique based on restriction fragment (RF) pattern analysis of the seven loci used in MLST. It captures about 95 % of the between-strain genetic variability detected by MLST. Moreover, by basing MLRFT on the same seven loci used in MLST, it is possible to systematically link MLRFT results to the MLST sequence database. MLRFT thus has value both as a convenient stand-alone technique for strain typing and as a rapid screening technique to categorize strains for targeted PFGE and/or MLST analysis (5).

## 1.4 Aims of the study

Nosocomial infections due to MRSA are continuing to be a major health concern world-wide. Furthermore, reports of infections caused by *S. aureus* with reduced susceptibility to vancomycin (the drug of choice for MRSA infections) have increased these concerns (54).

In these terms, monitoring and limiting the spread of MRSA strains remains a primary focus of most hospital infection control programs. Increased surveillance, including the screening of high risk patients has been recognized as an important component of effective hospital infection control programs (34).

In Palestine, clinical isolates of *Staphylococcus aureus* resistant to methicillin and other antibiotics are present; however, data describing their prevalence, patterns of resistance, genetic and epidemiological relatedness have not been investigated. Therefore, the aims of this study are to:

1. Investigate the prevalence of MRSA among isolates collected from the anterior nares of 843 patients admitted to Ramallah hospital and 72 HCW between October 2003 and October 2004.
2. Characterize methicillin-resistant *S. aureus* isolates by using phenotypic (antibiogram) and genotypic (MLRFT) methods.

3. Determine the resistance profiles of MRSA strains against several antibiotics.
4. Compare the common MRSA strains in Palestine with the internationally recognized strains.

## Chapter 2

### 2. Materials and methods

#### 2.1 Collection of *S. aureus* isolates

Ramallah hospital is the main general hospital in Ramallah. It has 136 beds distributed in six wards: medical, surgical, obstetrics, pediatrics including the neonatal unit, intensive care unit and the cardiac surgery intensive care unit.

#### Study design and data collection:

A total of 843 patients admitted to Ramallah hospital were screened for nasal carriage of *S. aureus* at their admission between October 2003 and October 2004. A second nasal swab has been taken from patients who stayed more than 5 days in the hospital. Isolates of *S. aureus* recovered from patients who had enrolled in the study and developed an infection later were also collected. Moreover, a group of 72 health care workers from Ramallah hospital were screened for nasal carriage of *S. aureus*.

#### 2.2 Identification of *S. aureus*

Carriage of *S. aureus* was determined by obtaining nasal swab specimens from both anterior nares of patients. The swabs were streaked on mannitol salt agar, and the plates were incubated at 35°C and examined after 24 hours and 48 hours of incubation. Colonies that have been suspected to be *S.*



*aureus* were cultured on blood agar and tested for production of catalase, coagulase and DNase. Isolates that have been confirmed to be *S. aureus* were stored at  $-70^{\circ}\text{C}$  in tryptic soy broth containing 15 % glycerol for further investigation (44).

## **2.3 MRSA Determination**

### **2.3.1 Oxacillin susceptibility**

Screening for methicillin resistant *S. aureus* isolates were detected by using the disk diffusion method outlined by the National Committee for Clinical Laboratory Standards (NCCLS) (38). By using this method 1 $\mu\text{g}$  oxacillin disks were placed on Muller-Hinton agar (oxid, United Kingdom) supplemented with 4 % NaCl. The inoculum size was adjusted to a final concentration of  $10^5$  CFU/ml. Zones of inhibition were measured following incubation at  $35^{\circ}\text{C}$  for 24 hours. A zone diameter of  $< 10$  mm was considered as indicative of resistance.

### **2.3.2 Detection of mecA gene**

Resistance to methicillin was confirmed by PCR-based detection of the *mecA* gene as described previously (37). PCRs were carried out in 25  $\mu\text{l}$  reaction volumes. Each reaction contained 2.5  $\mu\text{l}$  of 10X Taq buffer, 0.2 mM of each dNTP (Invitrogen; UK), 0.5  $\mu\text{M}$  of each sense and anti sense primer, 1.25 U of Taq DNA polymerase (Invitrogen; UK) and 2.5  $\mu\text{l}$  of

chromosomal DNA. Thermal cycling was performed in PTC-150 DNA engine, with an initial 5 min denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension step at 72°C for 5 min. The primers that have been used are listed in Table 1.

## **2.4 Antimicrobial Susceptibility Testing**

The susceptibilities of all *S. aureus* isolates to different antibiotics were tested by the agar disk diffusion method on Muller- Hinton agar (oxid, Uk) as standardized by the National Committee for Clinical Laboratory Standards (NCCLS) (38). Tested antibiotics included: ciprofloxacin 5 µg, penicillin 10 µg, gentamycin 10 µg, tetracycline 30 µg, clindamycin 2 µg, ampicillin 10 µg, teicoplanin 30 µg, erythromycin 15 µg, vancomycin 30 µg, oxacillin 1 µg and cephalothin 30 µg. The inoculum size was adjusted to a final concentration of  $10^5$  CFU/ml and zones of inhibition were measured after 24 hours of incubation at 35°C.

## **2.5 Multilocus Restriction Fragment typing (MLRFT)**

### **2.5.1 DNA Extraction**

DNA extraction was carried out by using the rapid lysis method (65): Bacteria were harvested from blood agar plates (one loopful, by using a 1 µl loop). Cells were resuspended in 50 µl of lysostaphin (100 µg/ml in water; Sigma chemical Co., USA). Cell suspensions were incubated at 37°C. After 10 min, 50 µl of proteinase K solution (100 µg/ml, Sigma, USA) and 150 µl buffer (0.1 M Tris, PH 7.5) were added. Cell suspensions were incubated for an additional 10 min at 37°C and then placed in a boiling water bath for 5 minutes. The mixture was centrifuged at 2000 rpm for 1 minute and the supernatant containing the chromosomal DNA was used for PCR.

### **2.5.2 PCR**

PCRs were carried out with 50 µl reaction volumes. Each reaction contained 5 µl of 10X Taq buffer, 0.2 mM of each dNTP (Invitrogen; United Kingdom), 1 µM of each sense and antisense primer, 1.25 U of Taq DNA polymerase (Invitrogen; United Kingdom) and 5 µl of chromosomal DNA. Thermal cycling was performed in PTC-150 DNA engine, with an initial 5 min denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension step at 72°C for 5 min.

### **2.5.2 Multilocus Restriction Fragment Typing (MLRFT)**

Amplicons were directly subjected to digestion with restriction endonucleases by adding 10 µl of DNA amplicon to 20 µl of a reaction mixture containing 3 µl of 10x appropriate digestion buffers and 5 U of restriction enzyme. The restriction reaction was incubated at the optimal temperature for each respective restriction enzyme, as recommended by the manufacturer, for 2-4 hours. Complete digestion was achieved without prior purification of the PCR amplicon. The restriction enzyme or combination of restriction enzymes used for each locus are listed in table (2). CfoI and DdeI were purchased from Roche, Switzerland, while all other restriction enzymes were purchased from Fermentas, Lithuania.

RFs were separated by electrophoresis on 4.0 % agarose gel (Sigma, USA), and were sized against a 50-bp DNA ladder (Roche, Switzerland). The gels were visualized under UV illumination.

Table 1. Sequences of PCR primers

Target gene	Primer name	Sequence (5'-3')
Carbamate Kinase (arcC)	arcC-Up arcC-Dn	TTGATTCACCAGCGCGTATTGTC AGGTATCTGCTTCAATCAGCG
Shikimate dehydrogenase (aroE)	aroE-Up aroE-Dn	ATCGGAAATCCTATTTTCACATTC GGTGTTGTATTAATAACGATATC
Glycerol Kinase (glpF)	glpF-Up glpF-Dn	CTAGGAACTGCAATCTTAATCC TGGTAAAATCGCATGTCCAATTC
Guanylate Kinase (gmk)	gmk-Up gmk-Dn	ATCGTTTTATCGGGACCATC TCATTA ACTACAACGTAATCGTA
Phosphate acetyltransferase (pta)	pta-Up pta-Dn	GTTAAAATCGTATTACCTGAAGG GACCCTTTTGTTGAAAAGCTTAA
Triosephosphate isomerase (tpi)	tpi-Up tpi-Dn	TCGTTTCATTCTGAACGTCGTGAA TTTGCACCTTCTAACAATTGTAC
Acetyl coenzyme A acetyltransferase (yqiL)	YqiL-Up YqiL-Dn	CAGCATA CAGGACACCTATTGGC CGTTGAGGAATCGATACTGGAAC
mecA gene	MecA-Up MecA-Dn	AAAATCGATGGTAAAGGTTGGC AGTTCTGCAGTACCGGATTTGC

Table 2. Restriction endonucleases used in MLRFT

Locus	Size of product (pb)	No. of MLST alleles/locus	Restriction enzyme(s)	No. of MLRFT Alleles/locus
arcC	570	37	HinfI	3
aroE	536	66	AluI & CfoI	5
glpF	543	44	Tsp5091	9
gmk	488	35	CfoI	5
pta	575	44	RsaI	7
tpi	475	58	BbuI & MboI	4
yqiL	598	52	VspI & DdeI	4

## Chapter 3

### 3. Results

#### **Bacteriological screening:**

A total of 843 patients admitted to Ramallah hospital were enrolled in the study. Nasal swabs were obtained from patients and screened for the presence of *S. aureus*. The presence of *S. aureus* in nasal swab samples was confirmed by recovery of the *S. aureus* from mannitol salt agar and a positive test for catalase, coagulase and DNase. From the 843 patient samples, 218 isolates of *S. aureus* were isolated. Nasal carriage percentage of *S. aureus* differs among wards with a range of 17.4 % to 31.1 %. The medical ward showed the highest rate of 31.1 % while the surgical ward showed the lowest rate of 17.4% as shown in Table 3.

From the 218 isolates of *S. aureus*, a total of 15 specimens were found to be positive for MRSA in all wards of the hospital. The prevalence of MRSA isolated from the different wards ranged from 0 % to 33.3 %. The medical ward showed the highest rate of 33.3 % while no MRSA was isolated from OBS as shown in Table 3.

As shown in Table 4, a total of 72 health care workers (HCW) were screened for *S. aureus* carriage. *S. aureus* was isolated from 15 HCWs giving a

prevalence of 20.8 %. Among the 15 HCW positive for *S. aureus*, 10 isolates were found to be positive for MRSA giving a percentage of 66.6 %.

Eight patients enrolled in this study have developed *S. aureus* infection after the first sample. Two of these patients were carriers for the organism, whereas the others were not. One of these eight clinical isolates was methicillin resistant *S. aureus* (Data not shown).

Another swab has been taken for 65 patients after one week of their admission to the hospital. Forty three of these patients were not *S. aureus* carriers at the time of admission. After one week four patients have a positive result, in which one of them was MRSA. The other 22 patients were carriers at their admission to the hospital and after one week, 13 of them have negative results mostly because of antibiotic treatment, while the other 9 patients remained carriers to the organism in which one of them was MRSA carrier (data not shown).

### **Resistance to antibiotics:**

Figure 2 shows the antimicrobial susceptibilities of MRSA strains. All isolates were completely resistant to penicillin and ampicillin, while all isolates were 100 % susceptible to teicoplanin and vancomycin. However, the susceptibility to ciprofloxacin, erythromycin, gentamycin, and clindamycin was 71.5 %, 82.2 %, 85.8 %, 89.3 % respectively.



The different antibiotic-resistance patterns encountered for the isolates are shown in Table 5. Eleven distinct patterns were identified, where 13 isolates were found to match antibiotic type I, 2 isolates for antibiotic type IV, 3 isolates for each antibiotic types (III and X) and 1 for each of the following antibiotic types: II, V, VI, VII, VIII, IX and XI. The distribution of MRSA isolates with respect to their antibiotic types is shown in Fig. 1.

As shown in Table 6, the majority of isolates from patients and HCW showed antibiotic type I with 8 and 5 isolates, respectively. The other isolates were scattered among the remaining antibiotic types.

**mecA gene:**

All the MRSA isolates that were detected for the presence of mecA gene showed a positive result. Clear bands of 533 bp were visualized under UV illumination (Data not shown).

**MLRFT:**

MLRFT differentiated the 28 isolates into 8 RFTs. The distribution of the isolates in the 8 RFT is shown in Table 8. Twenty-two (78.5 %) of the 28 isolates were grouped into four RFTs. The remaining six MRSA isolates were scattered among additional four RFTs.

## **Correlation between antibiogram types and RFTs**

As shown in Table 10, three of six isolates belonging to the restriction fragment type AAAAAAA had the antibiogram type I, the other 3 had the antibiotype X. All the isolates belonging to the types AAABCBC, AAJBCBC, BBBBAB had antibiotype I. However, the isolates that belong to the type AAACCAA had different antibiotypes. Three of the 4 isolates that belong to the type CAACAC had the antibiotype III; the last isolate had the type VI.

Table 3: Nasal carriage of methicillin susceptible and methicillin resistant *S. aureus* among patients admitted to different units of Ramallah hospital. Samples were collected between October 2003 and October 2004.

Patients in Ramallah hospital					
Ward	No. of specimens	No. of <i>S. aureus</i>	MRSA	% of <i>S. aureus</i>	% of MRSA
Medical	299	93	5	31.1	33.3
ICU	108	25	4	23.1	26.6
CSICU	88	22	1	25	6.6
SUR	132	23	4	17.4	26.6
P.W	112	27	1	24.1	6.6
OBS	104	28	0	26.9	0.00
Total	843	218	15	25.8	6.8

\* % of *S. aureus* = (No. of *S. aureus* isolates (ward)/ no. of specimens (ward)) X 100

\* % of MRSA = (No. of MRSA isolates (ward) / total no. of MRSA isolates) X 100

\*Other three MRSA isolates were collected from patients, one of them is a clinical isolate (caused an infection to a patient) and the other 2 were isolated from the nares of 2 patients who are screened after one week of admission to the hospital.

Table 4: Nasal carriage of methicillin susceptible and methicillin resistant *S. aureus* among health care workers at Ramallah hospital. Samples were collected from 72 HCW working at different units at the hospital.

Healthcare Workers					
Ward	No. of specimens	No. of <i>S. aureus</i>	MRSA	% of <i>S. aureus</i>	% of MRSA
Medical	11	4	1	36.36	10
ICU	15	4	4	26.6	40
CSICU	22	6	5	27.27	50
SUR	4	0	0	0	0
P.W	12	1	0	8.3	0
OBS	8	0	0	0	0
Total	72	15	10	20.8	66.6

\* % of *S. aureus* = (No.of *S. aureus* isolates (ward)/ No. of specimens (ward)) X 100

\* % of MRSA = (No. of MRSA isolates (ward)/ total No. of MRSA isolates) X 100

Table 5: Antibigram types of the 28 MRSA isolates retrieved in this study.

Isolates		Susceptibility to the following antibiotics										
Type	No.	Ox	Tec	Cn	Cip	E	Amp	Va	Te	P	Kf	DA
I	13	R	S	S	S	S	R	S	S	R	S	S
II	1	R	S	R	S	S	R	S	S	R	S	S
III	3	R	S	S	R	S	R	S	S	R	S	S
IV	2	R	S	S	R	R	R	S	S	R	R	R
V	1	R	S	R	R	R	R	S	R	R	R	R
VI	1	R	S	S	R	S	R	S	S	R	R	S
VII	1	R	S	R	R	S	R	S	S	R	R	S
VIII	1	R	S	S	S	R	R	S	S	R	S	S
IX	1	R	S	R	R	R	R	S	R	R	R	S
X	3	R	S	S	S	S	R	S	S	R	R	S
XI	1	R	S	S	S	S	R	S	R	R	S	S

Ox: oxacillin, Tec: teicoplanin, Cn: gentamicin, Cip: ciprofloxacin,  
 E: erythromycin, Amp: ampicillin, Va: Vancomycin, Te: tetracycline,  
 P: penicillin, Kf: cephalothin, Da: clindamycin.  
 S: Sensitive, R: Resistance

Table 6: Distribution of the 11 antibiogram types among patients and health care workers

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Patients	8	0	2	2	1	0	1	1	1	2	0
HCW	5	1	1	0	0	1	0	0	0	1	1

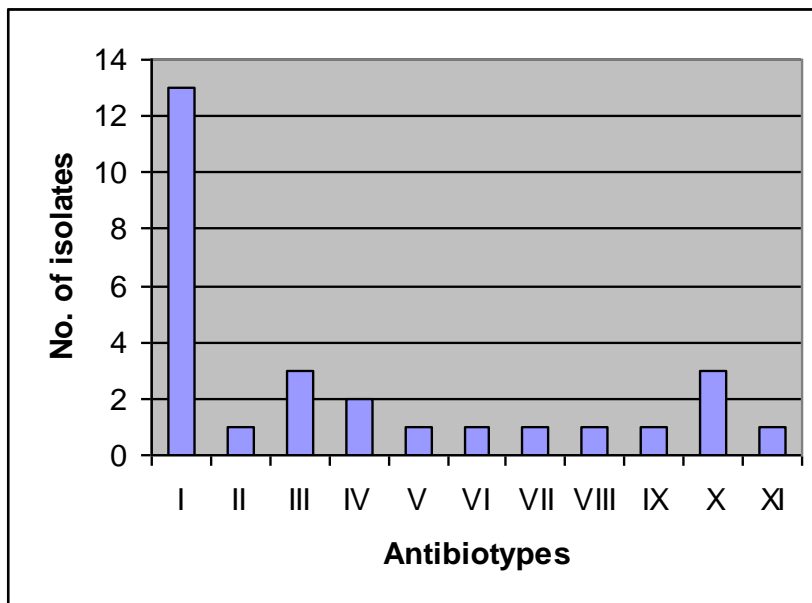


Figure 1: Distribution of the 28 MRSA isolates among the different 11 antibiotypes. The antibiotic susceptibility of all isolates was performed by the disk agar diffusion method on Muller- Hinton agar. Zones of inhibition were determined after incubation at 35°C for 24 hrs. The following antibiotics were tested: ciprofloxacin 5 µg, penicillin 10 µg, gentamycin 10 µg, tetracycline 30 µg, clindamycin 2 µg, ampicillin 10 µg, ticoplanin 30 µg, erythromycin 15 µg, vancomycin 30 µg, oxacillin 1 µg, cephalothin 30 µg.

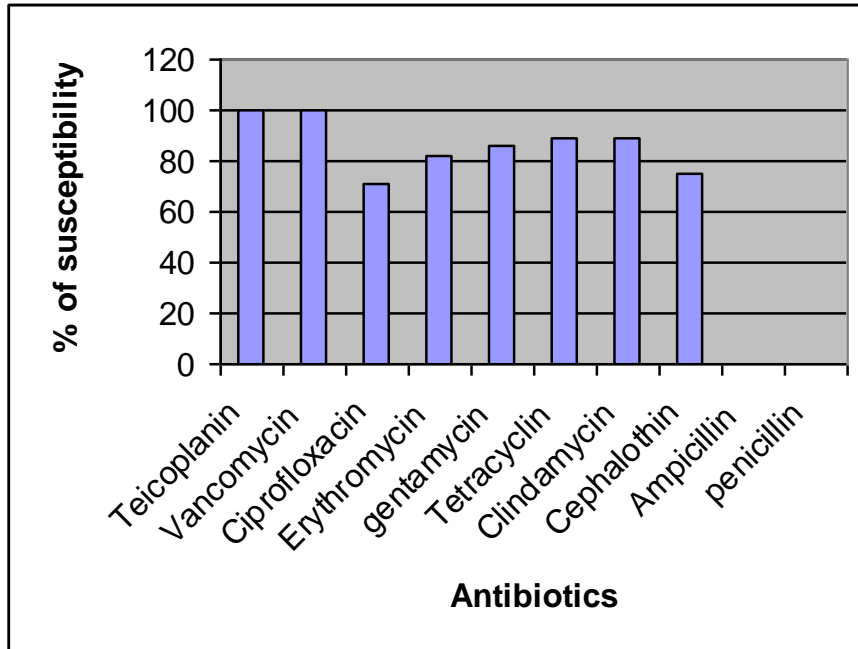


Figure 2: Susceptibility of the MRSA isolates to the different antibiotics used for preparation of antibiogram. The antibiogram was performed as described in the legend of Figure 1.

Table 7: Distribution of MRSA antibiogram types among the different hospital units (patients and health care workers).

Unit	Antibiogram Type											Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
Medical	3	0	1	0	0	0	1	1	1	1	0	8
ICU	3	0	1	2	1	1	0	0	0	1	0	9
CSICU	4	1	0	0	0	0	0	0	0	1	1	7
SUR	2	0	1	0	0	0	0	0	0	0	0	3
PW	1	0	0	0	0	0	0	0	0	0	0	1
OBS	0	0	0	0	0	0	0	0	0	0	0	0
Total	13	1	3	2	1	1	1	1	1	3	1	28

Table 8: MLRFT genotypes of the MRSA isolates retrieved in this study. Only genotypes that match the isolates retrieved in this study are shown.

No. of isolate	arcC	aroE	glpF	gmk	pta	Tpi	yqiL
6	A	A	A	A	A	A	A
8	A	A	A	C	C	A	A
2	A	A	J	B	C	B	C
4	C	A	A	A	C	A	C
1	A	A	A	A	C	A	A
4	A	A	A	B	C	B	C
1	B	B	B	B	B	A	B
1	B	A	A	A	C	A	C
* 1		A	A	A	C	A	C

MLRFT was defined by the combination of alleles at the seven loci (e.g. RFT-AAACCAA in the order RFT-arcC-aroE-glpF-gmk-pta-tpi-yqiL). RFTs retrieved in this study are AAAAAAA, AAACCAA, AAJBCBC, CAAACAC, AAAACAA, AAABCBC, BBBBBAB and BAAACAC

\* isolate no. 28 has not been classified, the arcC gene in this isolate cannot be amplified, this may be referred to a mutation that may occurred in this house keeping gene.( This may be confirmed by sequencing)



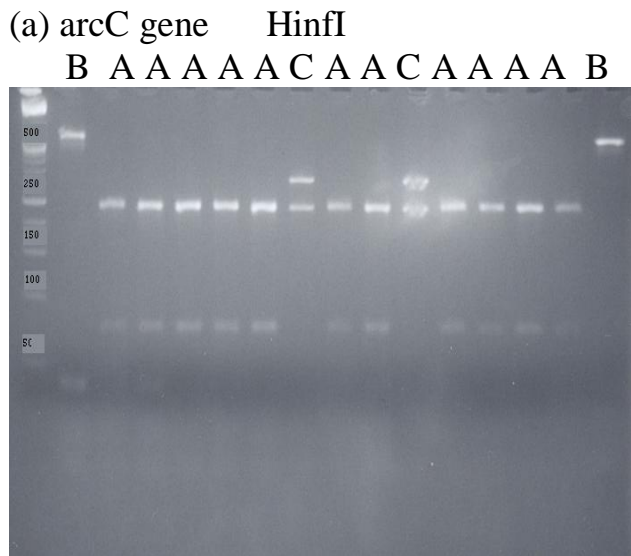
Table 9: Distribution of RFTs among patients and health care workers

RFTs	patients	HCW
AAAAAAA	5	1
AAACCAA	5	3
AAJBCBC	1	1
CAAACAC	2	2
AAAACAA	0	1
AAABCBC	3	1
BBBBBAB	0	1
BAAACAC	1	0
total	17	10

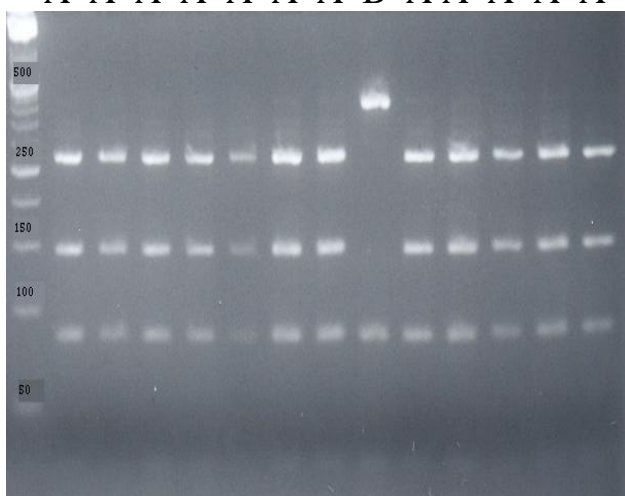
Table 10: Correlation between antibiogram and MLRFT types of MRSA.

RFT genotype	No. of MRSA isolates	Antibiotype										
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
AAAAAAA	6	3									3	
AAAACAA	1											1
AAABCBC	4	4										
AAACCAA	8	3	1		2			1	1			
AAJBCBC	2	2										
BBBBBAB	1	1										
BAAACAC	1									1		
CAAACAC	4			3			1					
Total	27	13	1	3	2	0	1	1	1	1	3	1

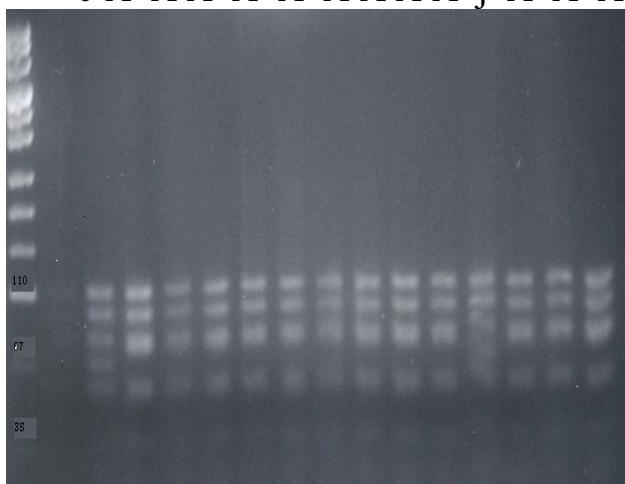
Figure 4: MLRFT genotypes. A fragment of about 450 bp from seven housekeeping genes in MRSA were PCR-amplified using different sets of primers. Followed by digestion of 10 µl of PCR products with one or two restriction enzymes specific for each fragment (a-g). Restriction fragments were analyzed by electrophoresis on a 4 % agarose gel. The size of fragments was determined by comparison to a 50 bp ladder ( shown in the first left lane of each gel) and then the genotype was identified by comparison to MLFRT genotype patterns described by (5). The gene name and restriction enzymes are shown below along with each gel photograph. Only a representative gel for each genotype is shown. All PCR and restriction experiments were performed in duplicates.



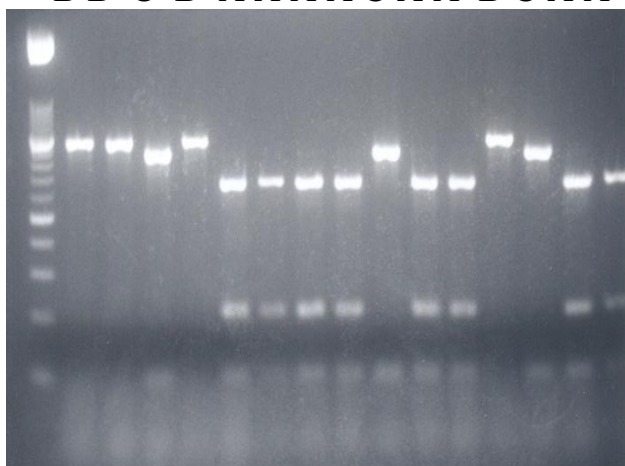
(b) *aroE* gene      AluI & CfoI  
A A A A A A A B A A A A A



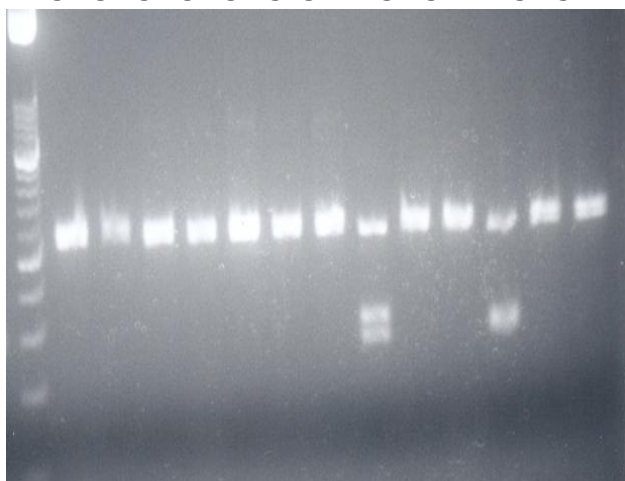
(c) *glpF* gene      Tsp5091  
J A A A A A A A A j A A A



(d) gmk gene CfoI  
B B C B A A A C A A B C A A

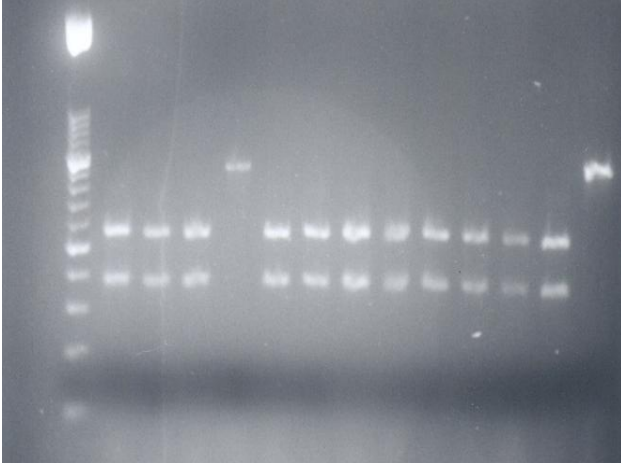


(e) pta gene RsaI  
C C C C C C C B C C A C C



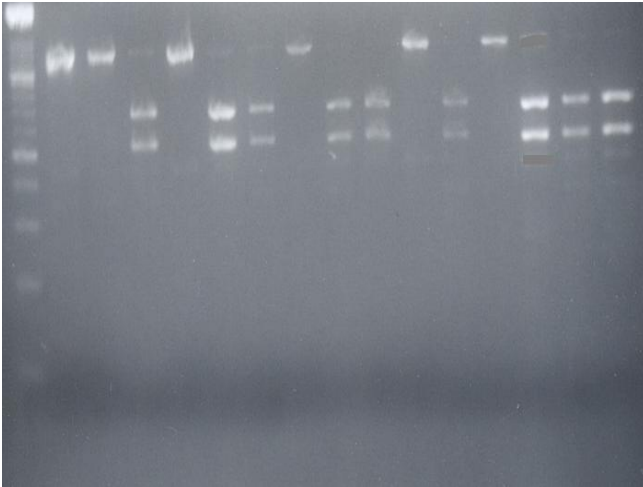
(f) tpi BbuI & MboI

A A A B A A A A A A B



(g) yqil VspI & DdeI

C C A C A A C A A C A C A A A



## Chapter 4

### Discussion

Methicillin resistant *Staphylococcus aureus* (MRSA) is a growing problem both in hospitals and in the community. Upon its introduction, MRSA quickly became known for its ability to cause large hospital outbreaks. Most strains of MRSA are sporadic (recovered only from a few patients) but a few strains have the ability to spread very rapidly throughout an institution and reach the epidemic level (1).

In general, most hospital-acquired infections caused by MRSA (HA-MRSA) are associated with a relatively small number of epidemic clones spread over continents including Iberian (ST247-SCCmec IA), Brazilian ( ST239-III), New York/Japan (ST5-II), Pediatric (ST5-IV), EMRSA-16 (ST36-II), EMRSA-15(ST22-IV), and Berlin ( ST45-IV) clones (2, 50). Community acquired MRSA (CA-MRSA) has emerged as a new pathogen, it is noted that these isolates are not clonally related to HA-MRSA international clones. Moreover, CA-MRSA are susceptible to antimicrobial drugs (50).

In order to control the spread of MRSA a clear picture of the variety and distribution of the local types is necessary. This can only be based on strain typing studies (43).

In this study, we investigated the prevalence of MRSA among patients recently admitted to Ramallah hospital. The prevalence of nasal carriage of *S. aureus* was 25.8 % among these patients. Whereas, five of the six hospital wards studied showed a prevalence of *S. aureus* carriage that ranged from 23.1-31.1%, the sixth ward (surgical ward) showed a prevalence rate of 17.4%. The high prevalence rate observed in this study was within the prevalence ranges reported in previous surveys of patients on admission from other countries such as USA, UK, Israel, Spain and Netherlands (26, 49, 69, 64, 4). In this study, the prevalence of MRSA nasal carriage was 6.8% among patients recently admitted to Ramallah Hospital. However, only the medical and the ICU units showed a consistently high prevalence rate of *S. aureus* and MRSA. These figures are worrying, but are still within the prevalence ranges reported for patients on admission from other countries (4, 3, 64). Considering the unrestricted, inappropriate use of antibiotics and the lack of antibiotic policies in Palestine, the increasing spread of MRSA is expected.

The health care workers at Ramallah hospital showed a high incidence of colonization with strains of MRSA (66.6 %). This high carriage rate of HCW indicates that the hospital lacks a screening and control program for the employees. This is extremely dangerous for other patients served by

these carriers. In fact, they are probably the most significant source for transmitting MRSA to their patients as well as to other health care workers. The highest prevalence rate of *S. aureus* and MRSA carriage among HCWs was in the CSICU (50 %), ICU (40 %) and medical unit (10 %). However, the CSICU and ICU showed a consistently high prevalence rate of *S. aureus* and MRSA but no such correlation could be observed regarding the medical ward. Moreover, from the six hospital wards, only the ICU unit showed a consistently high prevalence rate of both *S. aureus* and MRSA and in both HCWs and patients (Tables 3 & 4).

Our bacterial collection of MRSA strains showed a broad range of antibiotic resistance patterns (Table 5). The antibiogram typing revealed that antibiogram type I (resistant to oxacillin, penicillin and ampicillin) was predominant among 46.4 % of all the nasal isolates (in patients and health care workers). This result indicates that the hospital environment may represent a reservoir for this type. Additionally, each of antibiotype III and X was found in 10.7 % of the isolates and the remaining isolates were scattered among the rest eight antibiotypes. Moreover, the antibiotypes tend to be scattered among the different hospital wards and no clustering of any antibiotype could be observed in any hospital ward.



Some strains were multiresistant, i.e., they were resistant to beta-lactam antibiotics as well as to Cip, Da, Ery, Cn and variably resistant to other antibiotics (Table 5). Unexpectedly, most of our MRSA isolates were variably susceptible to several, or virtually all non Beta-lactam antibiotics, this may be due to the lack of genes encoding resistance to these drugs that are usually conserved within *mecA* DNA. Multisusceptible MRSA strains have gradually been reported in the European region, Singapore, Greek and Australia (48, 47, 23, 41). Gentamycin-sensitive methicillin resistant *S. aureus* (GS-MRSA), as either a nosocomial or community – acquired infection phenomenon, is worldwide now. GS-MRSA with increased susceptibility to other antimicrobials has recently been reported in six widely dispersed hospitals in France and one in the West Indies. Nimmo *et al* (41) reported that Community acquired GS-MRSA strains were not related to nosocomial GR-MRSA. However phage typing results suggest that they are related to GS-MRSA as previously reported in New Zealand (41).

Molecular typing, complemented by conventional methods provides a sensitive and specific approach for outbreak tracking, and its usefulness in nosocomial epidemiology is very well documented (26, 55). MLRFT is a useful tool for the characterization of *S. aureus* strains (5). Besides the low cost, rapidity and simplicity of this technique it possesses two important

virtues of MLST, the strain characterization approach that is highly discriminating and it is portable. Moreover, because both MLRFT and MLST rely on the same sequence database, it is possible to work back and forth between the typing systems. Indeed MLRFT captures about 95 % of the discriminating power of MLST that makes it appear counterintuitive, given that MLRFT detects far fewer alleles per locus than MLST (5). These virtues make the technique widely applicable, particularly in clinical research settings for strain screening purposes and in the developing world, where the sequencing technology required for MLST is not readily available. In our study, the 28 MRSA isolates were distributed among eight RFTs (Table 8). Because of the translational property between MLRFT and MLST, four of the common MRSA RFTs observed in this study could be provisionally identified as belonging to STs of known clonal lineages. Where the RFTs genotypes – CAAACAC, AAACCAA , BBBBBAB and BAAACAC correspond to the archaic/Iberian/clone V group, the NewYork/Pediatric/Japan group, the epidemic MRSA type 16 (EMRSA – 16) group, and the Brazilian clone, respectively (5, 68).

The other four MRSA RFTs (AAAAAAA, AAJBCBC, AAAACAA, AAABCBC) retrieved in this study, did not correspond to any of the major

lineages that have been determined previously, so that they may be regarded as sporadic strains.

The Iberian clone was first reported in Spain in 1989 and since then has been reported in Portugal, Italy, the UK, Germany, Belgium, Switzerland, France, Czech Republic, Poland, and the USA (6, 33). The Brazilian clone was first described in 1992 in Brazil and then Portugal, Argentina, Uruguay, Chile and Czech Republic (58). The New York/Japan clone was identified as the dominant MRSA in Hospitals in metropolitan, New Jersey, Pennsylvania, and Connecticut, and also in one hospital in Tokyo, Japan. The pediatric clone was first reported in a pediatric hospital in Portugal in 1992 and later reported in Poland, USA, Argentina, and Colombia . E-MRSA 16 is most frequently isolated from UK hospitals (43).

According to Oliveria *et al* (43); most isolates of the Iberian clone, NewYork /Japan, and Hungarian clones are resistant to most commonly used antimicrobial agents, with the exception of the co-trimoxazole. While the Brazilian clone was only susceptible to septomycin and the pediatric clone was only resistant to Oxacillin, Penicillin, Gentamycin, and occasionally Erythromycin. But all clones were susceptible to Vancomycin (43).

Although reports indicated that Iberian and New York/Japan Clones usually show multidrug resistance, our strains that confirmed to be from these clones by MLRFT typing were susceptible to most of the antibiotics.

Some RFTS exhibited a clear association with specific antimicrobial susceptibility patterns; others did not as shown in Table 10. The 13 MRSA isolates of antibiotic type I were scattered among five RFT genotypes (Table 10) and did not show preference for a specific RFT genotype. In contrast the 3 isolates in each antibiotic type III and X were clustered into RFT genotypes CAAACAC and AAAAAAA, respectively. For the remaining MRSA isolates, each one or two isolates were associated with a different antibiotic type and thus no specific clustering with RFT genotypes could be observed. Despite the low number of MRSA isolates grouped into antibiotic types III and X (3 isolates each), their correlation with specific RFT genotypes is interesting and should be further investigated on a large sample of MRSA isolates. However, the lack of a correlation between the antibiotic types and the RFT genotypes could be explained by the fact that the seven genes used to establish the RFT genotypes are housekeeping genes and are probably not involved directly with virulence of the isolate. But a possible correlation between the antibiotic types and the RFT genotypes cannot be ruled out based on our study, partly because of low sample number. Furthermore, such a

correlation can be established only by investigating a larger sample of MRSA isolates and probably by analyzing some genes directly involved in the virulence of *S. aureus* and MRSA in particular such as the *mecA* gene and PVL gene (70, 50)..

In conclusion, the prevalence of average nasal carriage of *S. aureus* among patients admitted to Ramallah hospital was 25.8 %, whereas the prevalence of MRSA was 6.8 %. By using MLRFT genotyping method 14 MRSA isolates belong to the epidemic clones: Iberian, Brazilian, NewYork/Japan, EMRSA-16. The other 13 isolate do not correspond to any of the major lineages that spread internationally. Most of our MRSA clones are susceptible to non beta-lactam antibiotics.

## Recomendations

- Antibiotic resistant MRSA are increasingly isolated and are a serious problem in Palestine indicating the need for continuous surveillance programs to generate accurate local antimicrobial susceptibility data and studying alternative antibiotic therapies.
- Wise use of antibiotics based on monitoring programs in the health care systems will drastically decrease the incidence of multidrug resistant MRSA isolates.
- Annual routine screening for MRSA should be implemented in order to evaluate the size of the problem in palestine.
- Combination of results for more than one genotyping is more powerful in differentiating between epidemiologically related and unrelated MRSA strains.

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