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# Prevalence and Molecular Typing of Methicillin Resistant *Staphylococcus aureus* among Veterinary Doctors in Palestine

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# Prevalence and Molecular Typing of Methicillin Resistant *Staphylococcus aureus* among Veterinary Doctors in Palestine

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Thesis Approval

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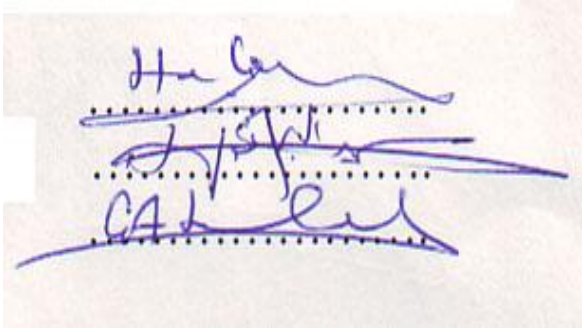

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**Declaration**

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

Signed:

Rozan Romel Fawzi Attili

Date: 8.01.2013

# Dedications

To my Father Romel Attili my mother Wejdan, my brother Fawzi, my two sisters Fatima and Lilian. To my Husband Adnan Fayyad and my son Yazan.

A great thank for your support.

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## Abbreviations and Definitions

**CA-MRSA** Community-associated methicillin-resistant *Staphylococcus aureus*

**CDC** Centers for Disease Control and Prevention

**CLSI** Clinical Laboratory Standards Institute

**g** Grams

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

**l** Litre

**Kb** Kilo base

**mg** Milligram

**MIC** Minimum Inhibitory Concentration

**mL** Milliliter

**MRSA** Methicillin-resistant *Staphylococcus aureus*

**MSA** Mannitol Salt Agar

**NHLS** National Health Laboratory Service

**PFGE** Pulsed-Field Gel Electrophoresis

**UPGMA** Unweighted-Pair Group Matching Analysis

# Abstract

**Objectives** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health problem due to its multiple antibiotic resistance properties. Recently the emergence of MRSA have been described and investigated in animals. Through this study we aim to determine the prevalence of MRSA among Palestinian veterinary doctors, determine the molecular characteristics of these MRSA isolates and evaluate the risk of nasal MRSA carriage among those with direct contact with livestock .**Methods** Nasal swabs were obtained from 200 subjects including veterinary students and doctors from An-Najah National university and veterinary doctors working at the Ministry of Agriculture. at (). Data regarding animal exposure, information about professional contact and known MRSA risk factors were obtained from participants by designed questionnaire. Participants were screened for MRSA by standard microbiological techniques. Molecular analysis was done using Pulse Field Gel Electrophoresis (PFGE) in order to characterize the *S. aureus* isolates. **Results:** Nasal carriage of *S. aureus* was found in 28 of 200 specimens (14%) of which MRSA accounted for 4% (8 isolates). All 28 strains of *S. aureus* were sensitive to vancomycin.

MRSA resistances to other antibiotics used in this study were as follows: 89.2% to erythromycin, 100% to ampicillin, 28.5% to Oxicillin, 14% to chloramphenicol, 39.2% to gentamycin. Four major types of PFGE patterns were identified (A, B, C and D) among MRSA strains. Three predominant PFGE types were recognized, Type A (37.5%). Type B (33.8%) and Type C (33.8%). One isolate with a unique PFGE pattern Type D (12.5%) was identified as a different clone.

**Conclusions** MRSA colonization may be an occupational risk for veterinary professionals. As MRSA spread among community, changes in its epidemiology are certain. The micro flora of

humans and animals, are closely intertwined. MRSA is now a pathogen of domestic animals that can be transmitted between animals and humans. Accordingly, further scrutiny of the roles of animals in MRSA infection and colonization is required. The effect of routine contact with household pets on the global epidemiology of MRSA is still unknown.

**Key terms** Antibiotic Resistance*;* MRSA; Veterinary Doctors.

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# Chapter One

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## INTRODUCTION

*Staphylococcus aureus* is a Gram-positive, coagulase positive coccus, non-motile, non-spore forming bacteria. The golden appearance of this bacterium on blood agar is the etymological root of the bacteria's specific name as *aureus* means "gold" in Latin (Lowy, 1998). *Staphylococcus aureus* has been commonly noted to occur as a commensal organism, which usually exists without likely harmful effects, on the body surface or in the nasal passages of healthy humans and animals. Under certain conditions, such as weakness to immune system or injury to skin surfaces, a commensal organism may become opportunistic and be capable to causing infection. Clinical signs of infection in humans may range from light skin infections (pimples, boils and impetigo), to much more serious conditions such as post-operative wound infections and cellulites. *S. aureus* may also cause bacteremia, sepsis, meningitis and pneumonia (Kluytmans et al., 1997).

In addition to human colonization by *S. aureus*, the organism can colonize many other animals, including horses, cats, birds, dogs, pigs, cattle and chickens (Weese. 2005). Antibiotics have been the first line of defense in treating clinical infection in both man and animals. Bacteria that are resistant to antibiotic treatment are a public health concern.

During the past four decades, many strains of *S. aureus* have been evolved from controllable problem into a serious public health concern since they have become Methicillin-Resistant (David et al., 2010). The resistant bacteria produced penicillinase, an enzyme that breaks down

penicillin. By 1961 the first case of MRSA infection was reported in England (Jevons et al., 1961). Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains are now prevalent worldwide in both human and veterinary medicine (Barber , 1990).

The rapid spread of bacteria resistant to antimicrobials, although it is a global phenomenon, is seen to be higher in developing countries attributed mainly to misuse of antibiotics including use of prophylaxis (Palavecino, 2004).

MRSA is a major cause of hospital-acquired infections worldwide (Jarvis et al. 2006), that's why MRSA must be recognized now as one of the most common causes of infections acquired in the community (Graham et al., 2006). Transmission of MRSA from animals to staff tending to these animals appears to be an international problem, creating a new reservoir for community-acquired MRSA (CA-MRSA) in humans (Seguin et al., 1999).

The rise of a new zoonotic source of MRSA could have a severe impact on the epidemiology of CA-MRSA, and may have effects for the control of MRSA, especially in those countries that maintain a low prevalence by means of search and destroy policies (Lee, 2003).

The objectives of this study were to evaluate the prevalence of MRSA among veterinary doctors in Palestine, and to determine the molecular characteristics of MRSA isolates by Pulsed-Field Gel Electrophoresis.

## Aims and Objectives

1. To evaluate the prevalence and molecular characteristics of (MRSA) among veterinary doctors and to estimate the impact of animal reservoir on human healthcare.

2. To investigate if those in professional contact (veterinary doctors) with livestock are at higher risk for (MRSA) nasal carriage.

# Chapter Two

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**LITERATURE REVIEW**

## 2. History of *Staphylococcus aureus*

*Staphylococcus aureus* was discovered in the 1880s. During this time. *S. aureus* infection commonly caused painful skin and soft tissue conditions such as boils, scalded-skin syndrome, and impetigo. *S. aureus* is an important mammalian pathogen that has long been recognized for its ability to cause serious and invasive diseases. In 1878, Koch first noted that different diseases were caused by Gram-positive cocci depending on whether they formed pairs or chains.

*S. aureus* was isolated in 1884 by German scientist Anton Rosenbach, who grews the two strains, *S. aureus* ("golden staph," for the golden colonies that forms on bacterial medium and *S. albus* (white colonies), in pure culture. In 1894, Van de Velde worked on the virulence determinants of *S. aureus* and on the protective immunogenicity effect of leucocytes and "humor", an old word for serum.

## 2.1Characteristics

*S. aureus* is a member of the Staphylococcaceae, a taxomic group which contains 33 other members (Freney, 1999). *S. aureus* can be distinguished from other staphylococcal species on the basis of the gold pigmentation of colonies and positive results of coagulase, mannitol-fermentation, and deoxyribonuclease tests (Wilkinson, 1997). The staphylococcal genome consists of a circular chromosome with prophages, plasmids, and transposons. Genes governing virulence and resistance to antibiotics are found on the chromosome, as well as the extrachromosomal elements **(**Clink et al., 1987**).**

*S. aureus* is normally found on the skin or in the nostrils of 20-30% of healthy individuals. *S. aureus* most commonly colonizes in the nostrils. Colonization is the state of *S. aureus* being present without causing any symptoms. When symptoms are present, it is called an infection (Graham et al., 2006).

## 2.2 *S. aureus* Carriage and Disease

Up to 20% of the human population is colonized by *S. aureus* as a commensal on the squamous epithelium of the anterior nares at any one time. However, it has been estimated that *S. aureus* can transiently colonize up to 60% of the human population (Foster, 2004). Nasal carriage of *S. aureus* is one of the major risk factors for *S. aureus* infection (Kluytmans, 1997).

Human is a natural reservoir of *S. aureus*. Thirty to fifty percent of healthy adults are colonized, with 10 to 20 percent being persistently colonized (Roman et al., 1997).

In healthy individuals, the carrier rate of *S. aureus* range between 15% to 35% and certain groups of individuals are more susceptible to *S. aureus* colonisation than others including health-care workers, nursing home inhabitants, prison inmates, military recruits and children (Sequin et al., 1999).

*S. aureus* is frequently isolated from the nostrils (anterior nares) and nose picking has been associated with *S. aureus* nasal carriage. Wertheim (Wertheim et al., 2004) investigated the possible association between nose picking and *S. aureus* nasal carriage in a study of 238 outpatients and 86 healthy hospital volunteers at a tertiary-care hospital's ear, nose, and throat (ENT) clinic between June 2001 and May 2003 (Wertheim et al., 2004).

*S. aureus* is a major pathogen in both nosocomial (hospital-acquired) and community-acquired infections worldwide, and according to the Centers for Disease Control and Prevention (CDC), is

one of the most common causes of human skin and soft tissue infections in the United States. Clinical signs range from minor skin conditions (e.g., pimples, boils and impetigo) to more severe disease such as cellulitis and postoperative wound infections. *S. aureus* can also cause pneumonia, bacteremia, meningitis, sepsis, and pericarditis. *S. aureus* is one of the most commonly identified bacteria that cause food poisoning and toxic shock syndrome (Wenzel and Perl, 1995). Individuals colonized with *S. aureus* are at increased risk for subsequent infections (Musher, 1994).

*S. aureus* infection is a major cause of skin, soft-tissue, respiratory, bone, joint, and endovascular disorders. The majority of these infections occur in persons with multiple risk factors for infection (Lowry , 1998).

*S. aureus* commonly causes boils, carbuncles, furuncles and impetigo, but after gaining access to the blood, may also be a major cause of endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and septicemia (Klevens et al., 2007).

## 2.3 Occurrence of Antibiotic Resistances to Beta-Lactam Drugs

Many people assumed with the discovery of antibiotics that the problems associated with infectious diseases were ended, but a new problem of antibiotic resistance emerged. Mutated forms of bacteria develop resistance to antibiotics and give rise to newer strains of infections (Aubrey et al., 2004).

Many antimicrobial agents inhibit bacterial cell wall synthesis. These agents include β-lactam compounds such as penicillins (e.g. penicillin G, ampicillin and methicillin), cephalosporins and carbapenems.

*S. aureus* has also developed resistant mechanisms to β-lactam methicillin which was considered the drug of choice for treatment of *s. aureus* infections (Tenover, 2006).

Methicillin is a semi-synthetic penicillin derivative and the resistance to this β-lactam drug among *S. aureus* is of great concern to medical and scientific personnel. MRSA has been reported as the most common cause of hospital-acquired infection for a number of years. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a mutated form of bacteria resistant to antibiotics, known as beta-lactams, such as methicillin, oxacillin, penicillin, and amoxicillin, and the cephalosporins, such as cephalexin, and ceflactor (Tortora etal., 2003).

Resistance to methicillin confers resistance to all penicillinase-resistant penicillins and cephalosporins. This high level of resistance requires the presence of the *mec* gene that encodes penicillin-binding protein 2a (Chambers, 1997).

Although many methicillin-resistant strains appear to be descendants of a limited number of clones, some appear to be multiclonal in origin, suggesting the horizontal transfer of *mec* DNA (Archer et al., 1994). The description "methicillin-resistant" was first used in 1961, based on the discovery of a human *S. aureus* infection in the United Kingdom that was resistant to methicillin (Moran et al., 2006).

Penicillin was introduced in the 1940s and was used to treat *S. aureus* infections. Shortly after its introduction isolates of penicillin-resistant *S. aureus* were identified. Methicillin was first used in 1959 to treat these penicillin-resistant strains of *S. aureus* and by 1961 methicillin-resistant strains were also being reported (Deresinski, 2005). The first methicillin-resistant case in Canada was reported by Low *et al.* in 1981 (Simor et al., 2001). For all MRSA infections, the annual incidence ranged between 18 and 25.7 cases per 100,000 population. The majority of these,

approximately 75% were skin and soft tissue infections. There is only limited information on morbidity and mortality of CA-MRSA (Hill and M., 2006).

## 2.4 Factors Contributing to Increased Resistance

Of the factors contributing to the rise in antibiotic resistant bacteria human misuse and abuse plays a critical role. Misuse includes indiscriminate prescription and dispensing of antibiotics and failure of patients to complete full course of antibiotic treatment. The microenvironment created by the constant use of antibiotics generate a selective pressure that selects for organisms able to survive and propagate in such an environment as the result of novel mutations, newly acquired genetic elements or inherent resistance as a result of genetic diversity within a population (Tenover et al., 2001).

The use of antibiotics in food animals is thought to contribute heavily to the emerging resistance patterns in human pathogens. Antibiotics are used in animal husbandry to improve growth and maintain the health of livestock. Antibiotic use falls neatly into three main categories: therapeutic, prophylactic and nutritive (Teuber, 1999).

Many of the broad and narrow spectrum antibiotics used for therapeutic treatment of infected animals are the same as those used in humans. Since animals living so closely together are at risk of spreading disease, many livestock companies require their growers to feed animals low doses of antibiotics as a preventative measure. In fact, some 70% of antibiotics used in the United States are fed to animals that are not sick (Wallinga, 2004.).

## 2.5 Hospital Acquired *S. aureus*

Hospital-associated MRSA (HA-MRSA) infections occur most commonly in immunocompromised individuals in hospitals and healthcare centers. Risk factors for HA-MRSA include hospitalization, surgery, dialysis, long-term care, indwelling devices, and history

of previous MRSA infection. To date, the majority of clinically significant MRSA infections are HA-MRSA (Klevens et al., 2007). Hospitalized patients and health-care workers have higher colonization rates than the general population (Parker and M., 1972).

These gram-positive cocci can withstand environmental factors for extended periods allowing susceptible individuals to become infected through contact with contaminated objects, but direct contact with persistently or transiently colonized people is the more important route of transmission (Goldmann et al., 1996).

Such characteristics have made *S. aureus* the most common hospital acquired (nosocomial) pathogen with a wide array of virulence and resistance strategies (Sanford et al., 1994).

## 2.6. Epidemiology of MRSA

Epidemiological data on MRSA in Africa are scarce. The prevalence of MRSA was determined in eight African countries between 1996 and 1997 and was relatively high 50 % in Nigeria, Kenya, and Cameroon (Kesah et al., 2003) and below 10% in Tunisia and Algeria (Kesah et al., 2003).

In Algeria, the rate of MRSA increased to 14% in 2001 (Ramdani-Bouguessa et al., 2001) and the prevalence of PVL-positive MRSA has increase in 2006, these strains were resistant to multiple antibiotics, including gentamicin and ofloxacin (Ramdani-Bouguessa, et al., 2006). Approximately 1.5% of the U.S. population is currently thought to be nasally colonized by MRSA (Gorwitz et al., 2004).

Farmers are also at risk of MRSA reservoirs. In a survey done on pig farmers in the Netherlands, 23% (6/26) of pig farmers were colonized with MRSA, a rate that was 760 times higher than the general Dutch population; however, only 2 of 40 pigs tested were MRSA positive (Wulf et al., 2006). Veterinary personnel are at increased risk of being MRSA reservoirs and zoonotic

transmission of MRSA and subsequent MRSA colonization should be considered an occupational risk for members of the veterinary healthcare team, particularly those in large animal practices. At American College of Veterinary Internal Medicine (ACVIM) Forum 2005, 6.5% of the attending veterinary personnel who volunteered to be tested were found to be colonized with MRSA (Weese et al., 2006).

In that study, the volunteers provided a nasal swab and completed a questionnaire that identified potential risk factors for MRSA colonization. None of those who tested positive had a recent history of hospitalization or previous diagnosis of MRSA. Large animal practice was the only significant risk factor for MRSA colonization, with 12/271 (4.4%) small animal practice personnel colonized, and 15/96 (15.6 %) of large animal practice personnel colonized (Weese, et al., 2006).

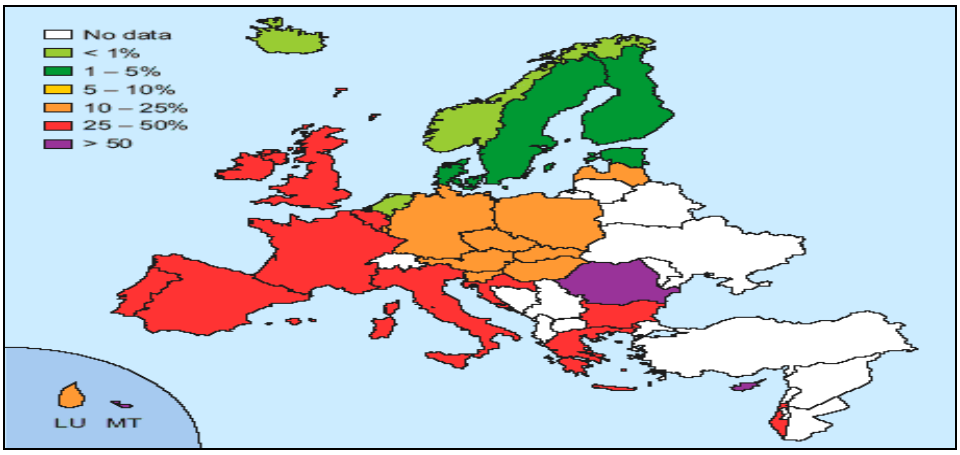


Figure 2.1: **MRSA in Europe.** *Staphylococcus aureus* proportion of invasive isolates resistant to oxacillin (MRSA) in 2005 from European countries. Adapted from (EARSS Annual Report, 2005)

As it can be seen in figure 2.1, Scandinavia and the Netherlands are amongst the regions with the lowest MRSA prevalence. Holland has long been renowned for having a very stringent

MRSA control plan known as “search and destroy”. The isolation of MRSA positive patients, barrier nursing, high standards of hygiene and regular MRSA screening of patients and staff are the centre of this strategy, which has also been adopted by the Danish health service. The low prevalence of MRSA cases in these countries is thought to be attributed to this practical approach (Wertheim et al., 2004). The highest prevalence of MRSA were reported by Jordan, Egypt and Cyprus, where more than 50% of the invasive isolates were methicillin-resistant (Klevens et al., 2007).

## 2.7 Community Acquired MRSA (CA-MRSA)

Until recently MRSA infections were primarily confined to the health care setting (Seybold et al., 2006). The first report of community onset infections with MRSA came from Australia in 1993 (Udo, 1993). Unlike HA-MRSA, which spread throughout the world slowly in the past 20-30 years, CA-MRSA emerged only in the 1990’s and has rapidly moved throughout the world (Hawkey, 2008).

Community-associated MRSA (CA-MRSA) infections occur in otherwise healthy people without a recent history of hospitalization or medical procedures, and are usually associated with skin and soft tissue infection. Risk factors for CA-MRSA include crowding, frequent contact, compromised skin, contaminated surfaces, shared items, and poor hygiene. To date the MRSA strain most commonly associated with CA-MRSA in humans is USA 300 (Naimi, 2003).

In fact, CA-MRSA infections in U.S. outpatients increased seven-fold from 1999-2006. CA-MRSA may be adding to the overall number of infections in hospital populations rather than replacing HA-MRSA (Nicolle, 2006). CA-MRSA strains are SCC*mec* type IV, V, or VI, and

they contain the *pvl* gene. They are resistant to clindamycin and fluoroquinolones (Deurenbergand Stobberingh, 2008).

CA-MRSA infections have been identified often in the context of dramatically rising prevalence of MRSA in hospitals with MRSA isolation rates approaching 50% of S. aureus infections. Some features differentiate CA-MRSA from endemic hospital MRSA. HA-MRSA tends to be multiresistant, whereas CA-MRSA tends to be susceptible to narrow-spectrum non-beta-lactams such as clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX), and tetracyclines (Adcock etal., 1998). Another distinguishing genetic feature of CA-MRSA is that a high percentage of strains carry genes for Panton-Valentine leukocidin (PVL). Genes for PVL are largely absent from HA-MRSA strains. Severe invasive diseases, such as necrotizing pneumonia and necrotizing fasciitis, appear to be more common with CA-MRSA than HA-MRSA. Furthermore, unlike HA-MRSA, which is considered an opportunistic pathogen, CA-MRSA causes infection in healthy, predominantly young hosts who have no predisposing co-morbidities (Fridkin et al., 2005)

**2.8 MRSA carriage in veterinary staff**

There are various reports that have associated the emergence of antimicrobial resistance with the use of antimicrobials in companion animals, particularly fluoroquinolones and cephalosporins (Bywater , 2004). Several recent investigations have found that repeated exposure to antimicrobials may predispose human patients to infection with or carriage of MRSA (Lloyd, 2006). It is unlikely that daily antimicrobial exposure of veterinary staff when treating their patients *i.e.* through handling or applying topical and oral antimicrobial preparations, spillage, drawing up fluid antimicrobial suspensions into syringes can lead to the development of resistant strains amongst *S. aureus* commensal to human skin. Carriage rates of

*MRSA* in veterinary surgeons and related staff are high when such staff are sampled whilst on duty (Weese, 2004).

## 2.9 Characterization of MRSA by Typing Techniques

Strain typing is epidemiologically important for recognizing outbreaks of infection, detecting the cross-transmission of nosocomial pathogens, determining the source of the infection, and recognizing virulent strains. At present, there is no clear consensus regarding the best method to use for typing MRSA. In order to be effective, it should be highly discriminatory, reproducible, standardized, based on a stable feature, widely available, inexpensive and have performed satisfactorily in an epidemiological investigation (Tenover et al., 1994).

Phenotypic methods such as biotyping, antibiogram, serotyping, phage typing and others frequently show lower discriminatory power ( Olive and Bean,. 1999). The shortcomings of phenotypically based typing methods have led to the development of typing methods based on the microbial genotype which minimize problems with typeability, reproducibility, and (in some cases) enable the establishment of large databases of characterized organisms.

## 2.10 Pulsed Feild Gel Electrophoresis of whole chromosomal DNA

Pulsed Feild Gel Electrophoresis (PFGE) is one of the genotypic techniques which is often considered the "gold standard" of molecular typing methods ( Olive and Bean 1999). PFGE has proven to be superior to most other methods for biochemical and molecular typing. It is highly discriminatory and superior to most methods for analysis of *Escherichia coli*, vancomycin-resistant enterococci, *S. aureus*, *Acinetobacter* species, *Pseudomonas aeruginosa*, and *Myobacterium avium* (Arbeit et al., 1990, Arbeit et al., 1993, Barbier et al., 1996). PFGE was more efficient at differentiating strains of methicillin-resistant *S. aureus* than other methods tested (Prevost et al., 1992, Saulnier et al., 1993).

This study is the first study to determine the prevalence, and molecular characterization of MRSA among Veterinarians in Palestine.

# Chapter Three

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## Materials and Methods

### 3.1 Study Design

A cross sectional study was designed to find out the prevalence of MRSA nasal carriage among veterinary doctors as well as veterinary students in Palestine and then to determine the antimicrobial susceptibility testing and to characterize the Pulse-field gel electrophoresis genotype of the MRSA isolates.

### 3.2 Clinical Specimen and Questionnaire information

Two hundred nasal swab were obtained from healthy veterinarians attached to the Ministry of Agriculture as well as final year students at the Department of Veterinary Medicine at AN-Najah University, Palestine. About 200 nasal swabs were collected between June 2011 and October 2011. The swabs were collected from all veterinarians whether they were in the administrative or field work. Along with the nasal swabs a questionnaire was filled by each participant prior to the sampling which included information about professional contact with specific animal species and information on recognized risk factors for MRSA carriage, such as previous hospitalization (within the last 6 months), antimicrobial therapy (within the last 6 months) and residence with a human health care worker.

To collect the nasal specimen, a sterile cotton-tipped swab (BD Diagnostics, Sparks MD) was moistened with the solution in the transport container and then rotated for 10-15 seconds in the anterior nares (nostril). The swab was not inserted deeper than 1cm. The procedure was repeated

with the same swab on the other nostril then the swabs were placed in tubes of sterile culture medium and were then put in biohazard bags. The samples were stored overnight in the refrigerator and were plated the following morning.

Samples were distributed as follows; in Tulkarem, the nasal swabs were collected from veterinary doctors of Ministry of Agriculture and AN- Najah University, veterinary Doctors and students were in the age group between 18 and 60 years. In Nablus, Hebron, Bethlehem, Ramallah, Qalqilia, Jericho, and Jenin, the nasal swabs were collected from veterinarians working in the units of veterinary and veterinary clinics.

## 3.3 Microbiological identification of *S. aureus*

The *S. aureus* isolates were identified based on standard laboratory phenotypic methods.

All media were prepared in laboratory depending on the manufacturers instruction, this included mannitol salt agar (Himedia ,India), muller hinton agar (Biomark, India) and nutrient broth (Himedia, India) and blood agar prepared (Biomark, India).

The specimens were plated on mannitol salt agar. Plates were observed for the appearance of characteristic yellow colonies. Distinct, easily seen, yellow colonies were produced and visible after 18-hour of incubation at 37 $^{0}$C (aerobically)

*S. aureus* was identified based on its Gram stain morphology, colonial morphology, and production of catalase and coagulase that are specific for *S. aureus*. (Gould et al., 2010).

## 3.4 Antimicrobial Susceptibility Testing of MRSA Isolates

The antimicrobial susceptibilities were tested by the disk diffusion method after overnight incubation at 37 °C on mueller-hinton agar plates according to the guidelines recommended by

the CLSI. The antimicrobials tested included Oxacillin (1μg), Vancomycin (30μg), Ampicillin (10μg), Erythromycin (15μg), Gentamycin (10μg), Ciprofloxacin (5μg), and Chloramphenicol (30μg) all from Oxoid, UK.

Isolates with zone sizes 10 mm or less were considered as methicillin resistant.

## 3.5 Storage of *S. aureus* isolates and MRSA

All confirmed *S. aureus* isolate where stored in 16%v/v glycerol broth at -80 $^0$C. the glycerol broth was prepared by adding 16 ml glycerol in 84 ml nutrient broth and distributed to 5 ml screw cap tubes. These tubes were then autoclaved at 115 $^0$C for 20 minutes. The pH of the medium ranged from 7.2-7.6 at room temperature. Using a sterile swab, the entire growth of an overnight pure cultures was transferred to 5-ml of sterile glycerol broth and immediately stored at -80 $^0$C. After 24 hours, the viability of the organism was checked by thawing the suspension at 35 $^0$C and inoculation of blood agar plates.

## 3.6 Pulsed-Field Gel Electrophoresis for Typing *MRSA*

The PFGE protocol was tried according to different methods such as the Matushek technique (Matushek et al., 1996), the standardized European technique (Murchan et al. 2003), and the Canadian standardized protocol (Mulvey et al. 2001). The Matushek has been validated and used for the present study, as it produced more consistent separation of the bands and better migration down the agarose gel. The PFGE protocol described briefly as follows:

### 3.6.1 Extraction of DNA

A single colony of MRSA was grown in overnight in trypticase soy broth at 37 $^0$C in a shaking incubator. Bacteria were harvested by centrifugation at 3000 r/min for 10 min. Cells were resuspended in 2.5 ml PIV buffer (1 mol/L NaCl, 10 mmol/L Tris–HCl, pH 7.4). A volume of 0.5 mL of the cell suspension was mixed with 0.5 ml of 1.6% low-melting-point agarose (Sigma)

and pipetted into a 300 ml plug mold. The gel was solidified at 4 $^0$C. Plugs were released into 1 mL lysis solution (0.5 mg lysozyme, 10 mg RNase, 100 mg lysostaphin, 6 mmol/L Tris–HCL, 1 mol/L NaCl, 10 mmol/L EDTA, 0.5% *m*/*v* Brij 58, 0.2% *m*/*v* deoxycholate, 0.5% *m*/*v* sodium lauryl sarcosine, pH 7.5) and incubated overnight at 37 $^0$C. Lysis solution was replaced with 1 mL ESP (100 mg proteinase K, 10 mmol/L Tris–HCl, 1 mmol/L EDTA, 1% SDS, pH 7.4) and plugs were incubated overnight at 50 $^0$C. Plugs were washed twice in TE buffer (10 mmol/L Tris–HCl, 0.1 mmol/ L EDTA, pH 7.4) for 30 min each. They were then stored in fresh TE at 4$^0$C. A slice of the plug 2–4 mm wide was placed in 30 units of *Sma*I enzyme at 25$^0$ C overnight. Plug slices were washed for 1 h. in TE at 37$^0$ C. *Sma*I digests the genome of *S. aureus* into 15–20 restriction fragments with sizes ranging from 10 to 700 kb. The enzyme recognition sequence is CCC GGG.

**3.6.2 Electrophoresis and Imaging**

Plugs were inserted into the wells of a 1% agarose prepared in 0.5× TBE buffer (0.9 mol/ L Trizma base, 0.9 mol/ L boric acid, 20 mmol/ L EDTA, 1 LdH2O). Lambda PFG markers (50 mg/mL) (50–1000 kb) (New England BioLabs, USA) were inserted into appropriate wells in the gel. Inter gel comparison was done via the Bionumerics 3.0 software. Wells were over layered with low-melting-point agarose and allowed to solidify at 4 $^0$C. Gel was placed in the PFGE machine (CHEF DR III) (Bio-Rad Laboratories, Hercules, California, USA) adjusted with the following conditions: temperature, 14 $^0$C;run time, 21 h; volts, 200 V (6 V/cm); and pulse time, initial switch time 1 s and final switch time 20 s. Gels were stained with 0.5 mg/mL ethidium bromide and gel images were digitized through a UV gel image acquisition camera (Gel Doc XR; Bio-Rad Laboratories).

DNA fragments on each gel were normalized using the molecular weight standards run on the gel. Dendrogram were generated for combined gel images. A 1.00% band tolerance and an optimization of 1% were selected for use during comparisons of DNA profiles. Cluster analysis was performed by the unweighted pair-group method using arithmetic averages and DNA relatedness was calculated based on Dice coefficient (Struelens etal. 1992).

The established criteria or guidelines proposed by Tenover et al. were used for the interpretation of PFGE (Tenover et al, 1997). With these guidelines, a banding pattern difference of more than three bands were considered different strains.

# Chapter Four

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## Results

## 4.1 Epidemiological Data

A total of 200 veterinarian and student from 8 agriculture units participated in this project. Data were collected using designed questionnaire that include information regarding the participant health and work history, job description, and exposure to animals. 196 subjects were males and 4 females as shown in table 1. Only 3% of veterinarians reported to have lung problems, 2.5% heart problems and 1.5% having chronic medical problems. None of the participants were diagnosed with clinical MRSA infection in the past 12 months.

Different variables seemed to be associated with carrying MRSA such as examining and treating cattle, sheep, horses, and the number of animals they contact with daily. On the other hand, length of employment did not seem to be associated with carrying MRSA, even among respondents who had worked more than 18 years on field.

The prevalence of MRSA among veterinary doctors has been found to be 4%. Among as 200 samples 28 samples were positive for *S. aureus* (14 %) out of which 8 were MRSA. In Nablus; 34 (17%) nasal swabs were collected from veterinary doctors, 11 (5.5%) of them were colonized with *S. aureus* and 5 (2.5%) of them were MRSA. In Tulkarem; 45 (22.5%) nasal swabs were collected from veterinary doctors and students and of these 7 (3.5%) were colonized with *S. aureus* and none of them were MRSA. In Jenin 14 (7%) nasal swabs were collected from veterinary doctors none of them were *S. aureus* nor MRSA. In Hebron 45 (22.5%) nasal swabs were collected from veterinary doctors 10 (5%) were colonized with *S. aureus* and from them 3

(1.5%) were MRSA. In Qalqilia 15 (7.5%) nasal swabs were collected from veterinary doctors and none of them were *S. aureus* nor MRSA. In Bethlehem 14 (7%) nasal swabs were collected from veterinary doctors and none of them were *S. aureus* nor MRSA. From Ramallah 20 (10%) nasal swabs were collected from veterinary doctors and none of them were *S. aureus* nor MRSA and from Jericho 13 (6.5%) nasal swabs were collected from veterinary doctors and none of them were *S. aureus* nor *MRSA*. The highest prevalence of MRSA isolates were seen in Nablus (15%) and Hebron (10.3%) districts. However, the prevalence of MRSA was absent in Bethlehem, Ramallah, Jenin, Tulkarem, Jericho, and Qalqilia. The average age of the participants was 42.7 years old. Antibiotic use was reported in 3% of veterinary doctors and 97% of them did not use any antibiotic within 7 days before collecting nasal swabs as shown in Table 4.1

Table 4.1: **Characteristics of the Veterinary doctors Population and MRSA Prevalence**

| **Variable** | **Total Number of Veterinarians** | | **Percentage of MRSA positive** |
|---|---|---|---|
| **Gender** | **Male**<br>**Female** | **196**<br>**4** | **4%**<br>**0%** |
| **Lung problems** | **Yes**<br>**No** | **6**<br>**194** | **0%**<br>**4%** |
| **Heart problem** | **Yes**<br>**No** | **5**<br>**195** | **0%**<br>**4%** |
| **Chronic medical problem** | **Yes**<br>**No** | **3**<br>**197** | **0%**<br>**4%** |
| **antibiotics in the past 7 days** | **Yes**<br>**No** | **6**<br>**194** | **0**<br>**4%** |
| **Length of employment (years)** | **< 3**<br>**4-18 years**<br>**> 18 years** | | **0%**<br>**2.5%**<br>**1.5%** |
| **Spent time in a jail** | **Yes**<br>**No** | **4**<br>**196** | **0%**<br>**4%** |
| **Diagnosed with MRSA in the past 12 months** | **Yes**<br>**No** | **0**<br>**200** | **0%**<br>**4%** |

| | | |
|---|---|---|
| **City:** | | |
| **Nablus** | **N=34** | **14%** |
| **Tulkarem** | **N=45** | **0%** |
| **Jenin** | **N=14** | **0%** |
| **Hebron** | **N=45** | **6.6%** |
| **Qalqilia** | **N=15** | **0%** |
| **Bethlehem** | **N=14** | **0%** |
| **Ramallah** | **N=20** | **0%** |
| **Jericho** | **N= 13** | **0%** |

All MRSA carriers in this study have had recent or regular contact with cows and sheep and all of them were males.

## 4.2 Antibiotic Sensitivity Profile of the *S. aureus* Isolates

The resistant, susceptible, and intermediate isolates to the most common used antibiotics against *S. aureus* are shown in Table 4.2. Eight out of 28 *S. aureus* isolates were resistant to oxacillin and considered to be MRSA. Twenty five (89.28%) of *S. aureus* isolates showed resistant to erythromycin, one was sensitive and two were intermediate to erythromycin. All the twenty eight *S. aureus* isolates were resistant to ampicillin. Eleven showed resistant to chloramphenicol, fifteen were sensitive and 2 were intermediate. All were sensitive to vancomycin. Six of *S. aureus* isolates were gentamycin resistant, twenty two were gentamycin sensitive.

Table 4.2: **Antibiotic profile of the twenty eight *S. aureus* isolates, and percentage of isolates sensitivity and resistant**.

| **Antibiotic** | **Resistant** | | **Susceptible** | | **Intermediate** | |
|---|---|---|---|---|---|---|
| | **number** | **%** | **number** | **%** | **number** | **%** |
| **Oxacillin** | **8** | **28.5** | **19** | **67.8** | **0** | **0** |
| **Erythromycin** | **25** | **89.2** | **1** | **3.5** | **2** | **7.1** |
| **Ampicillin** | **28** | **100** | **0** | **0** | **0** | **0** |
| **Chloramphenicol** | **11** | **39.2** | **15** | **53.5** | **2** | **7.1** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Vancomycin** | **0** | **0** | **28** | **100** | **0** | **0** |
| **Gentamycin** | **6** | **21.4** | **22** | **78.5** | **0** | **0** |

All the eight MRSA isolates tested exhibited resistance to oxacillin, ampicillin and erythromycin. The 8 MRSA isolates were assigned to 1 of 4 antibiotic profiles based on their susceptibilities to the various antibiotics used.

Type 1 comprised of three isolate and were resistant to oxacillin, ampicillin, erythromycin and gentamycin, Type 2 comprised of three isolates and were resistant to Oxacillin, ampicillin, erythromycin, gentamycin and chloramphenicol. Type 3 had 1 and showed a resistant pattern of Oxacillin, ampicillin, erythromycin and type 4 had one which showed a resistant pattern of oxacillin, ampicillin, erythromycin and chloramphenicol as shown in Table 4.3.

Table 4.3: **Resistant pattern of MRSA to various antibiotic groups isolates**

| Resistant Pattern | Number of isolates | Percentage of isolates | Antibiogram type |
|---|---|---|---|
| **Oxa,Amp,Ery,Gen, Chl** | **3** | **1.5%** | **1** |
| **Oxa, Amp, Ery** | **1** | **0.5%** | **2** |
| **Oxa, Amp, Ery, Gen** | **3** | **1.5%** | **3** |
| **Oxa, Amp, Ery, Chl** | **1** | **0.5%** | **4** |

**Oxa=** oxacillin**, Amp=** ampicillin**, Ery=**erythromycin, **Gen=** gentamycin**, Chl=** chloramphenicol**, Vanco**= vancomycin

Most MRSA isolates were multi-resistant to more than three antibiotics including; ampicillin, erythromycin, oxacillin, and gentamycin as seen in Figure 5.

Figure 4.1**: Antibiotic susceptibility testing showing oxacillin resistant and multi drug resistance**

## 4.3 Molecular Typing

## Pulsed Field Gel Electrophoresis Results

Genetic investigation using Pulsed field gel electrophoresis were carried out for typing MRSA isolates. PFGE grouped the 8 isolates into 4 types A, B, C and D (Figure 4.2), All the MRSA isolates generated about 14-18 amplification fragments ranging from50-1000 bp and were easily comparable

PFGE type A was the most common pattern seen in Nablus which includes 3 isolates PFGE type B included two isolates; one was from Nablus and the other from Hebron. PFGE type C had two isolates from Hebron. PFGE type D was seen in 1 isolate only.

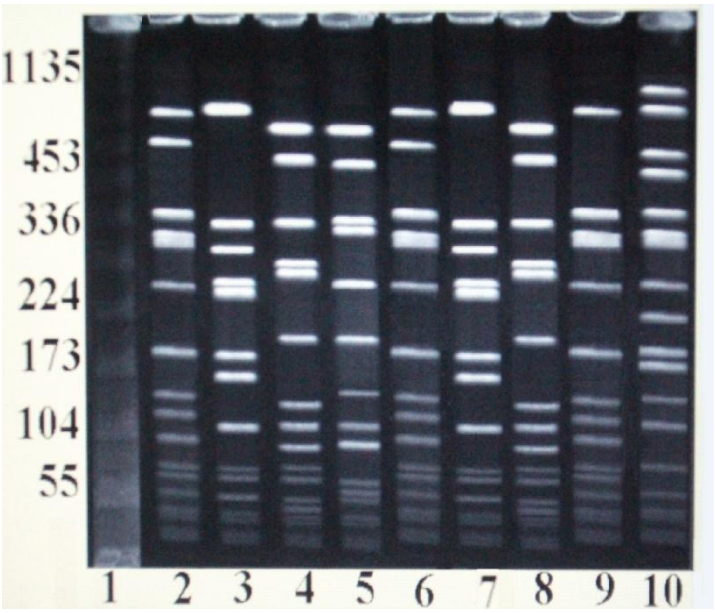


Figure 4.2: **PFGE types of 8 MRSA isolates**
Lane 1. Molecular weight marker, lane 2-9 MRSA isolates, PFGE type A (2, 6, 9), PFGE type B (3, 7), PFGE type C (4, 8), PFGE type D (5). Lane 10 positive control ATCC (25923).

A dendrogram showing estimates of the percent similarities among the 4 types identified by PFGE as shown in figure 4.3.

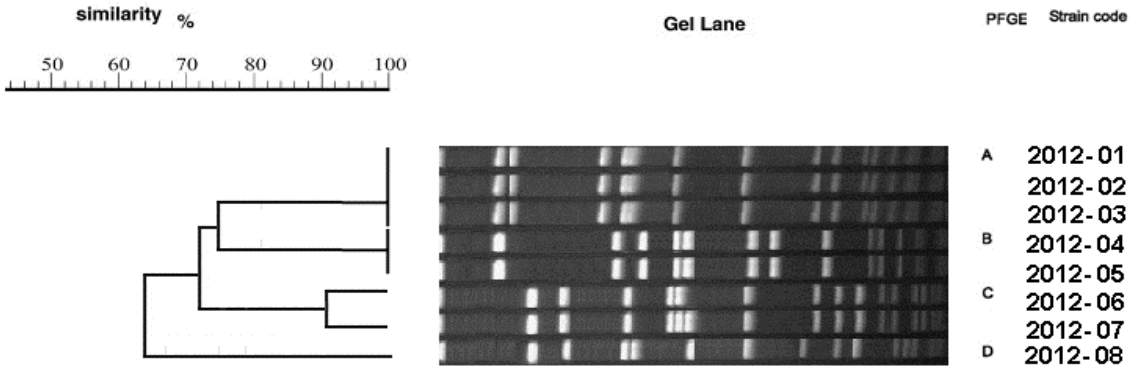


Figure 4.3: **PFGE dendrogram showing percentage similarities of MRSA isolate.** Data was analyzed using BioNumerics Version 3.0. Dendrogram were derived from the
Unweighted pair group method using arithmetic averages (UPGMA) and based on Dice coefficients. Band position tolerance and optimization were set at 1.00%.

# Chapter Five

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## Discussion

*S. aureus* is one of the most commonly diagnosed pathogens. It causes diseases ranging from self-limiting food poisoning to life-threatening septicemia and pneumonia. Control and treatment measures for this organism are complicated by its remarkable adaptation ability to its environment especially through the acquisition of antibiotic resistance determinants. The most alarming trend in recent years was the acquisition of methicillin resistance that turned the use of β-lactam antibiotics into useless for controlling *S. aureus* infections. Among Hospital-based infection by MRSA, the ratio is continuously increasing in most countries but there are considerable differences in MRSA prevalence among individual countries. The MRSA rate was low (2.0%) in Netherlands (de Sousa et al. 1998) and 1.8% in Switzerland (Kotilainen et al. 2003) and high MRSA rates > 50% in Italy, Portugal, and > 30% in Greece and Turkey (Gosbell et al. 2001). These variations of MRSA may be attributed to variations in patient populations, the biological characteristics of the *S. aureus* strains and infection control practices (Orrett and Land ,2006). In a study carried out in Palestine by Adwan et al in 2005 that include a total of 321 clinical isolates of *S. aureus* from different patients, have found that the prevalence of MRSA was 8.7 % and in another study done on 72 health-care workers found that 10/72 (13.9%) where colonized by MRSA (Kaibni et al., 2009).

Among community based colonization, the rate of MRSA is considered low (Salgado et al., 2003). In the Netherlands, the prevalence of MRSA upon admission to a hospital was 0.03% (Wertheim et al., 2004). Even in countries with a high prevalence of MRSA, e.g. the USA and

Portugal, carriage rates in the general population are only 0.2–3% [Wertheim et al., 2004). Recently, a shift in the epidemiology of MRSA infection has been documented, whereby community-associated (CA)-MRSA infections have become more common (Faria et al., 2005; Zetola et al., 2005).

In a study done in Palestine by Kaibni et al, 2009 843 participant without a history of hospitalization found that 2.0% where colonized by MRSA.

This study represents the first study in Palestine that studied the MRSA colonization in veterinary personnel. Although a control group was not included, the prevalence in veterinary personnel (4%) was higher than previously reported rates for community-based colonization (Kaibni et al, 2009). All 8 MRSA carriers in our study have had recent or regular contact with large animals such as cows and sheep which suggest that they could have acquired MRSA from the animals they deal with. Five MRSA isolates were from Nablus and 3 from Hebron. Our results suggest an increased risk for veterinary professionals. Further investigation is required to more accurately identify the occupational risk. The prevalence of MRSA usually varies among different geographical regions and between different institutions in a given area (Durmaz et al., 1997).

All of the 28 *S. aureus* isolates including the 8 MRSA were resistant to ampicillin which is in concordance with previously reported studies. (Hamid et al., 2011; Albrich,and Harbarth, 2008**.** ). MRSA are also resistant to other group of antibiotics such as erythromycin. About 89% of the *S. aureus* isolates were resistant to erythromycin including the 8 MRSA (100% MRSA resistant to erythromycin) and about 7% were reported to be intermediate (Table 2 and 3). In a study carried out by Udo etal., they have reported that the prevalence of erythromycin resistance MRSA increased from 66% in 1994 to 88% in 2004 (Udo et al., 2006).

The MRSA strains have variable sensitivity to other antimicrobials such as chloramphenicol, and gentamicin. Half of MRSA isolates were resistant to chloramphenicol and (75%) resistant to gentamycin (Table 7). In a study done in India they found out that about 40–50% of MRSA were resistant to chloramphenicol and gentamicin (Vidya et al., 2010). All *S. aureus* including MRSA isolates were sensitive to vancomycin.

The 8 MRSA isolates were assigned to 1 of 4 antibiotic profiles based on their susceptibilities to the various antibiotics used. All MRSA isolates were resistant to at least 3 different antibiotics including oxacillin and were considered multi drug resistant.

The PFGE type distribution was as follows: Type A from Nablus included 3 MRSA isolates, type B comprised 2 isolates from Hebron and interestingly type C had 2 isolates; one from Hebron and the other from Nablus. This similarity between the isolates could be explained by the fact that regular gathering takes place between Palestinian veterinarians such as workshops and other official meetings and transmission could have taken place during those gatherings and not through common animal source. In comparison to our study, a common PFGE similarity patterns of MRSA were found in a study carried out on veterinarians attendees at an international veterinary conference (Beth et al., 2006).

A comparison of PFGE results with antibiograms indicated that considerable agreement obtained between both techniques, i.e. PFGE type A (strain numbers 2, 6 and 9 Figure 3,4) had same antibiogram pattern (Type 3), and were resistant to oxacillin, ampicillin, erythromycin and gentamicin (Table 7). PFGE type C (strain numbers 4 and 8 Figure 3,4) had same antibiogram pattern (Type 1), and were resistant to oxacillin, ampicillin, erythromycin, gentamicin and chloramphenicol (Table 2).

As MRSA prevalence increase in the community, changes in its epidemiology are certain. The lives of humans and animals, and their microflora, are closely intertwined. MRSA is now a pathogen of domestic animals that can be transmitted between animals and humans. Accordingly, further studies should be carried out on the prevalence of MRSA in animals as well as veterinaries to find out the extent of MRSA transmission between them. While job related exposure to animals may be a risk factor for MRSA colonization, the effect of regular contact with house pets on the global epidemiology of MRSA is still unknown.

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# Appendices

## Enrollment Questionnaire

Thank you for participating in this study. This questionnaire is intended to gather information on your current and past contact with animals and other potential risk factors for infection with methicillin-resistant *Staphylococcus aureus* (MRSA), a bacterium that can cause a variety of diseases, and should only take a few minutes to complete. Please answer the questions to the best of your knowledge and check the appropriate boxes. All responses will remain confidential.

**DEMOGRAPHICS**

1. What is your gender?

Male ☐

Female ☐

2. Your age 18-34 ☐ 35-59 ☐ > 60 ☐

3. What is your city?

**Tulkarem** ☐ **Jenin** ☐ **Nablus** ☐ **Kalkilia** ☐ **Ramallah** ☐

**Jericho** ☐ **Betlahem** ☐ **Hebron** ☐

**MEDICAL HISTORY AND EXPOSURES**

4. Do you have a medical history of any chronic lung problems such as asthma or emphysema?

Yes ☐ No ☐

5. Do you have a medical history of any heart disease or vascular disease?

Yes ☐ No ☐

6. Do you have a medical history of any other chronic medical problems including diabetes, kidney disease, cancer, blood disease, or diseases that weakens the immune system?

Yes ☐ No ☐

7. Do you take medications such as anti-cancer drugs, corticosteroids like prednisone, or other drugs that weaken the immune system?

Yes ☐ No ☐

8. Have you taken antibiotics in the past 7 days?

Yes ☐ No ☐

9. Have you spent time in a jail or other correctional facility in the previous 6 months?

Yes ☐ No ☐

10. Have you or any family members visited a patient in the hospital in the previous 12 months?

Yes ☐ No ☐

11. Do you or any immediate family members work in a hospital or long-term care facility?

Yes ☐ No ☐

12. Have you previously been diagnosed with a methicillin-resistant *Staphylococcus aureus* infection?

Yes ☐ No ☐

13. Do you have regular contact with those animals?

Cattle ☐ sheep ☐ horses ☐ Pigs ☐

14. Have you been diagnosed with a skin or soft tissue infection (such as infection of the muscle) in the previous 12 months?

Yes ☐ No ☐

15. How long have you been working as veterinarian?

<3 years ☐ 3-18 years ☐ 18<years ☐

16. What is the animal type which you have great contact with it?
Large animal ☐ Small animals ☐

17. Do you were masks? Yes ☐ NO ☐

**Bacterial culture media**

Different bacterial culture media were used during this study. These media are presented in Table1

**Table1:**

| **Media** | **Manufacture** |
|---|---|
| Mannitol salt agar | Himedia (India) |
| Muller Hinton agar | Biomark ( India) |
| Transport media | Aptaca (USA) |
| Tryptic Soy broth. | Himedia (India) |
| Thioglycollate broth | Himedia (India) |

**Reagents**

Reagents are presented in Table2

Table2**: Reagents and materials employed in the study**

| **Reagent** | **Manufacture** |
|---|---|
| Gram stain reagents | Sigma (USA) |
| Hydrogen peroxide | Sigma (USA) |
| DNA molecular weight marker (50bp ladder | Promega (USA) |

| | |
|---|---|
| Master Mix | Promega (USA) |
| Primers | TIB MOLBIOL (Germany ) |
| Antibiotic disks | Himedia( India), Oxoid (UK |
| Ethidium bromide | Sigma (USA) |

**Equipments**

Table3**: Apparatus and special equipments that were used in the study**

| | |
|---|---|
| Thermal cycler | Eppendorf |
| Research pipettes | Eppendorf |
| Microfuge tube – 1.5 ml | Eppendorf |
| PCR microfuge tube, 0.2 ml | Eppendorf |
| Microwave oven | LG |
| Hoefer Shortwave UV light Table, (Trans illuminator) | Hoefer (USA) |
| Digital Camera) | Cannon (Japan |
| Power supply | Bio-Rad (USA) |
| Micro-Centrifuge | Sanyo (UK) |
| Electrophoresis set-up | Bio-Rad (USA) |

دراسه توضح التشخيص الجزيئي لبكتيريا المكورات الذهبيه العنقوديه المقاومه للميثيسيلين وانتشارها بين اطباء البيطره في فلسطين

**اعداد**: روزان رومل فوزي عتيلي

**اشراف** : د. حاتم عيده

## ملخص:

**الاهداف:** تمثل المكورات العنقودية الذهبية المقاومة للميثيسيلين مشكلة صحية رئيسية نتيجه لخاصيه هذا النوع من البكتيريا في مقاومه المضادات الحيويه. قمنا ببحث انتشار وخطر انتقال المكورات العنقودية الذهبية المقاومة للميثيسيلين من انف الاشخاص الذين لديهم اتصال مهني مع المواشي.

الطريقه والاسلوب : تم الحصول على مسحات من انوف الاطباء البيطرين ومعلومات عن الحيوانات التي يتعرضون اليها و عوامل الخطوره المعروفة للمكورات العنقودية الذهبية المقاومة للميثيسيلين من المشاركينحيث تم جمع (N = 200) عينه من جامعة النجاح ووحدات الزراعة المنتشره في 8 مدن فلسطينيه. كذلك تم فحص المشاركين بواسطة تقنيات الميكروبيولوجية القياسية للكشف عن المكورات العنقودية الذهبية المقاومة للميثيسيلين ووصفها .

**النتائج**: وجدنا 8 اشخاص حاملين للمكورات العنقودية الذهبية المقاومة للميثيسيلين بنسبة انتشارقدرها 4٪. استخدمنا تقنيه المضادات الحيويه ونتج عنها ان نسب البكتيريه العنقوديه المقاومه كانت 89.2 % للاريثروميسين, و 100 % للامبيسيلين, و 28.5 % للاوكساسيلين, و 14% للكلورمفينيكول, و 39.2% من العينات كانت مقاومه للجينتاميسن. استخدمنا تقنية نبض الكهربائي هلام فيلد التي نتج عنها اربع انواع كان الاكثر شيوعا هو نوع أ (37.5٪) الذي انتشر بنابلس، نوع **ب** (12.5) الذي انتشر عند طبيب بيطري واحد في نابلس وطبيب واحد في الخليل . واعتبر نوع **ج**(25٪) الاكثر انتشارا بين اطباء بيطرة الخليل اما النوع **د** فقد انتشر عند طبيب واحد في نابلس.

قد تكون البكتيريا العنقوديه المضاده للميثيسلين تستعمر انوف البياطره وهي من المخاطر المهنيه للعاملين في مهنة الطب البيطري. كما ادى انتشار البكتيريا العنقوديه المضاده للميثيسلين في المجتمع الى حدوت تغيرات في علم الأوبئة. البكتيريا العنقوديه المضاده للميثيسلين الممرضة للحيوانات المنزلية و التي يمكن أن تنتقل بين الحيوانات والبشر. وفقا لذلك، هنالك حاجة إلى مزيد من التمحيص لدور الحيوانات في نقل البكتيريا العنقوديه المضاده للميثيسلين ودراسة تأثير الاحتكاك مع الحيوانات المنزلية الأليفة وذلك حسب الاحصائيات الوبائيه العالمية, دراسة تاثير انتقال المكورات العنقوديه المقاومه للميثيسيلين من الحيوانات الاليفه والمنزليه لا زال مجهولا.