ANTIBIOTIC RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTION STUDIES IN HOSPITALS

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ABSTRACT

ANTIBIOTIC RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTION STUDIES IN HOSPITALS

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Clinical *S. aureus* strains were gathered from four hospitals, two in Turkey (Hacettepe hospital 200 strains and Ankara Hospital 106 strains) and the other two from Libya (Aljalla Hospital 88 strains and Jamahyria Hospital 62 strains). The clinical specimens were collected form different sources including blood, urine, wound, pus, burn, sputum, semen, catheter and aspiration. Patients were aged between 0 to 84 years and from both sexes.

Resistance to Methicillin was determined by measuring the Oxacillin MIC; this was done by using the oxacillin E-test, with resistance defined as an MIC of >2 µg ml. In this study all isolates displayed an Oxacillin MIC of ≥256µg/ml. The MRSA strains were (56%) in Turkish hospitals, and (59%) in Libyan hospitals.

The percentage of the VRSA and VISA in Libyan hospitals was (7%) and (26%) respectively, although the percentage of VRSA in Turkish hospitals was only 2% and there were no intermediately susceptible *Staphylococcus aureus* (VISA). Besides the MRSA isolates, Coagulase Negative *Staphylococcus* showing Methicillin resistance was collected from clinical isolates in thirteen patients in Turkish hospitals.

In both countries, the majority MRSA isolates were multiresistant to more than five classes of antibiotics including; Ampicillin, Amoxicillin, Tetracycline, Erythromycin and Ciprofloxacin. Most of the MRSA isolates were from blood (68%), wounds (57%) and pus (50%).

The results of genetic investigations indicated that the mecA gene was present in the majority of isolates in both countries; the community acquired MRSA type (ccr-BIV) was present in three samples out of thirty in Turkish hospitals and in one case out of twenty in Libyan hospitals; There was no case out of fifty specimens that carry the hospitals acquired MRSA type (ccr-BI, II, III) in both countries. Besides the Methicillin resistance gene, the incidence of Tetracycline resistance gene was quite high (tetM and tetK 50%) in Turkish hospitals isolates, and the prevalence of Panton-Valentine Leukocidin gene was high (PVL 70%) in Libyan hospitals specimens.

Keywords: Antibiotic resistance, *S. aureus*, MRSA, mecA gene and PVL.

ÖZ

ANTİBİYOTİK DİRENÇLİ *STAPHYLOCOCCUS AUREUS* ENFEKSİYON ÇALIŞMALARI

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Klinik *S.aureus* soş, ikisi Türkiye'de (200'ü Hacettepe Hastanesi'nden, 106'sı Ankara Hastanesi'nden), ikisi ise Libya'da (88'i Aljalla Hastanesi'nden, 62'si Jamahyria Hastanesi'nden) olmak üzere, dört farklı hastaneden elde edilmiştir. Klinik örnekler kan, idrar, püy, balgam, semen, sonda(katater) ve solunum gibi farklı kaynaklardan toplanmıştır. Hastalar, her iki cinsiyetten, 0 - 84 yaş (45±20) arasında dağılım göstermektedir.

Methicillin'e direnç oxacillin MIC ile tespit edilmiştir; bu işlem de oxacillin E-testi ile gerçekleştirilmiştir, ki 2 µg/ml den yüksek değerler direncin varlığını işaret etmektedir. Bu çalışmadaki tüm soşler 256 µg/ml'den büyük oxacillin MIC değerleri göstermiştir. Türk Hastanelerindeki MRSA soyları %56 iken, Libyadakilerinde %59'dur.

Türk Hastanelerindeki VRSA oranı yalnızca %2 iken ve orta düzeyde hassas *Staphylococcus aureus* (VISA) gözlenmezken, Libya hastanelerindeki VRSA ve VISA yüzdeleri sırasıyla %7 ve %26 dır. MRSA soşlarının yanı sıra, Türk Hastanelerindeki 13 hastadan alınan örneklerde koagulaz negatif *Staphylococcus* gözlenmiştir.

Her iki ülkede, MRSA soşlarının çoğunluğu ampicillin, amoxicillin, tetracycline, erythromycin ve ciprofloxacin'den oluşan beşden fazla antibiyotiğe karşı çoklu-direnç göstermektedir. MRSA soylarının çoğu (68%), (57%) ve (50%) oranlarında olmak üzere sırasıyla kan, yara ve irin örneklerinden elde edilmiştir.

Genetik araştırmaların sonuçları her iki ülkeden elde edilen örneklerde mecA geninin varlığını göstermektedir: Türk Hastanelerindeki 30 örneğin 3'ünde ve Libya Hastanelerindeki 20 örneğin 1'inde MRSA tip (ccr-BIV) gözlenmiştir. Buna karşılık, her iki ülke hastanelerinin sağladığı 50 örneğin hiçbirinde MRSA tip (ccr-BI, II, III) gözlenmemiştir. Methicillin-dirençli genin yanı sıra, Türk Hastanelerinden gelen örneklerdeki tetracycline-direnci gösteren vaka oranı oldukça yüksektir (tetM ve tetK 50%); ve Libya hastanelerindeki örneklerdeki Panton-Valentine Leukocidin geninin yaygınlığı da oldukça yüksektir (PVL 70%).

Anahtar Kelimeler: Antibiyotik direnci, *S. aureus*, MRSA, mecA geni ve PVL.

TO MY PARENTS,

WHO ALWAYS SUPPORT ME IN ALL ASPECTS OF MY LIFE

TO MY WIFE

FOR HER PATIENCE AND SUPPORT IN MY STUDY

AND TO MY CHILDREN

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AMP	Ampicillin
AMX	Amoxicillin
C	Chloramphenicol
CA-MRSA	Community- associated methicillin resistant *S. aureus*
CIP	Ciprofloxacin
CoNS	Coagulase-negative *staphylococci*
CTT	Cefotetan
DA	Clindamycin
E	Erythromycin
ESBLs	Extended-spectrum beta-lactamases
F	Nitrofurantoin
G	Gentamycin
HA-MRSA	Hospital-acquired methicillin resistant *S. aureus*
MIC	Minimum Inhibitory Concentration
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
OX	Oxacillin
PBPS	Penicillin-binding proteins
PCR	Polymerase Chain Reaction
PVL	Panton-Valentine Leukocidin
SCC*mec*	Staphylococcal chromosomal cassette *mec*
TE	Tetracyclin
VISA	Vancomycin intermediately susceptible S. aureus
VIRSA	Vancomycin-resistant Staphylococcus aureus

CHAPTER 1

INTRODUCTION

1.1 History of *Staphylococcus aureus*

S. aureus is an important mammalian pathogen that has long been recognized for its propensity 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, chains or clusters. The *staphylococci* were initially identified as grape-like clusters of bacteria isolated from the pus of human abscesses by Ogston in 1881 (1). In 1884, Rosenbach differentiated species of *staphylococci* based on pigmentation (2). The disease-causing *Staphylococcus aureus* produced a golden yellow pigment, whereas the non-disease causing strain *S. albus*, (later renamed *S. epidermidis*), was generally white. Before the advent of antibiotics, invasive *S. aureus* disease was a significant cause of mortality; however the introduction of penicillin in the 1940s eliminated many deaths from this organism. Early in the 1950s, strains were isolated that were resistant to penicillin and then later, to semi-synthetic penicillin derivatives such as methicillin. Most recently, methicillin-resistant *S. aureus* (MRSA) strains exhibiting intermediate and complete resistance to vancomycin have been isolated in hospitals and some MRSA strains are now endemic in various community niches. The significant events in modern history of *S. aureus* are given in Table 1.1.

Table 1.1: Significant events in modern history of *S. aureus*

Date	Event	References
1881	Ogeston identifies grape-like clustering bacteria in human pus	(1)
1884	Rosenbach differentiates staphylococcal species based on pigment	(2)
Pre-1940s	Surgeons fear staphylococcal wound infections; significant mortality from invasive infections observed	(3,4)
1950s	Multi-drug resistant strains of *S. aureus* emerge, resistance spread by phage 80a	(5)
1959	Development of methicillin to treat penicillin-resistant *S. aureus*	(4)
1961	Barber induces methicillin-resistance in *S. aureus* laboratory strains	(5)
1963	Jevons describes the first naturally occurring methicillin-resistant *S. aureus* (MRSA)	(6)
1960s to 2000s	Resistance to macrolides, tetracyclines, chloramphenicol, aminoglycosides and fluoroquinolones reported	(7,8)
Mid-1980s	Genetic basis for methicillin-resistance described; PBP2a characterize	(9,10,11)
2002	Glycopeptide-intermediate *S. aureus* strains isolated	(12)
2000 to Present	Increased occurrence of community-acquired *S. aureus* reported among athletic teams and compromised population	(13,14,15)

1.2 Distinguishing characteristics of *S. aureus*

S. aureus is a member of the *Staphylococcaceae*, a taxomic group that contains 33 other members (16). Based on 16S ribosomal RNA sequencing, the *Staphylococcaceae* is taxonomically placed between the *Bacilliaceae* and *Listeriaceae*. The genome of *S. aureus* is approximately 2.8 Mbp and may include prophages, transposons and extra-chromosomal elements such as plasmids. S. aureus forms morphologic grape-like clusters and is a non-motile, facultatively aerobic bacterium. The staphylococcal cell wall is defined by a thick layer of peptidoglycan, in which a specific pentaglycine interbridge between the tetrapeptide, serves to crosslink the glycans, N acetylmuraminic acid and TV-acetylglucosamine. The presence of this pentaglycine bridge distinguishes S. aureus from other members of the staphylococci. Many strains of *S. aureus* also produce one of eleven different polysaccharide capsules (PC), and strains producing types 5 and 8 PC are responsible for most human infections.

S. aureus produces coagulase, an enzyme that induces plasma coagulation by activating prothrombin. The coagulase test is commonly used in clinical microbiology laboratories to distinguish between stains of *S.aureus* and the coagulase-negative s*taphylococci* (CoNS), which generally less invasive and pathogenic.

1.3 *S. aureus* carriage and disease

S. aureus is found as a commensal organism on the squamous epithelium of the anterior nares up to 20% of the population at any one time, however, it has been estimated that *S. aureus* can transiently colonize up to 60% of the human population (17). It causes staphylococcal infections and staphylococcal food poisoning.

A breach in the skin, such as through a cut or a wound, may lead to *S.aureus* infection. *S. aureus* can cause a wide range of infections ranging from minor skin abscesses to more serious invasive diseases (18). *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 (19). Many invasive staphylococcal infections are correlated with nasal carriage of infecting strains (20). Although immunocompromised patients may be at greater risk for developing an invasive staphylococcal infection, healthy individuals may be also susceptible, especially if they are carriers.

Nosocomial *S. aureus* infections have been steadily increasing for the past two decades. This increase is due, in part, to the common use of implanted, intravascular medical devices. Additionally, there has been a significant increase in infections caused by MRSA and these strains have evolved resistance to other antimicrobials such as vancomycin (21).

1.4 *S. aureus* virulence factors

The remarkable ability of *S. aureus* to cause an enormous range of infections is due, in part, to its ability to produce multiple virulence factors. *S. aureus* can express proteins to bind fibrinogen (22), fibronectin (23), laminin (24), vitronectin (25), collagen (26), elastin (27) and thrombospondin (28) to promote adherence and attachment to endothelial cells and basement membranes. Collectively, these proteins are known as MSCRAMMs for microbial-surface components recognizing adhesive matrix molecules. *S. aureus* also expresses Protein A, on its surface, which binds to the F portion of immunoglobulin, and is required for full virulence of *S. aureus* (29).

MSCRAMMs are generally expressed during exponential growth and their expression is controlled by the Agr system. In stationary phase, *S. aureus* produces large numbers of membrane-damaging exotoxins and proteases to promote tissue damage. Tissue invasion is mediated by proteases, nucleases, lipases and staphylokinase, a fibrin-specific thrombolytic enzyme (30). In addition, some toxemic strains of *S. aureus* produce superantigens, such as toxic shock syndrome toxin I (TSST-I), to activate large numbers of T cells resulting in proliferation and cytokine release (31). Figure 1.1 shows virulence factors of *S. aureus*.

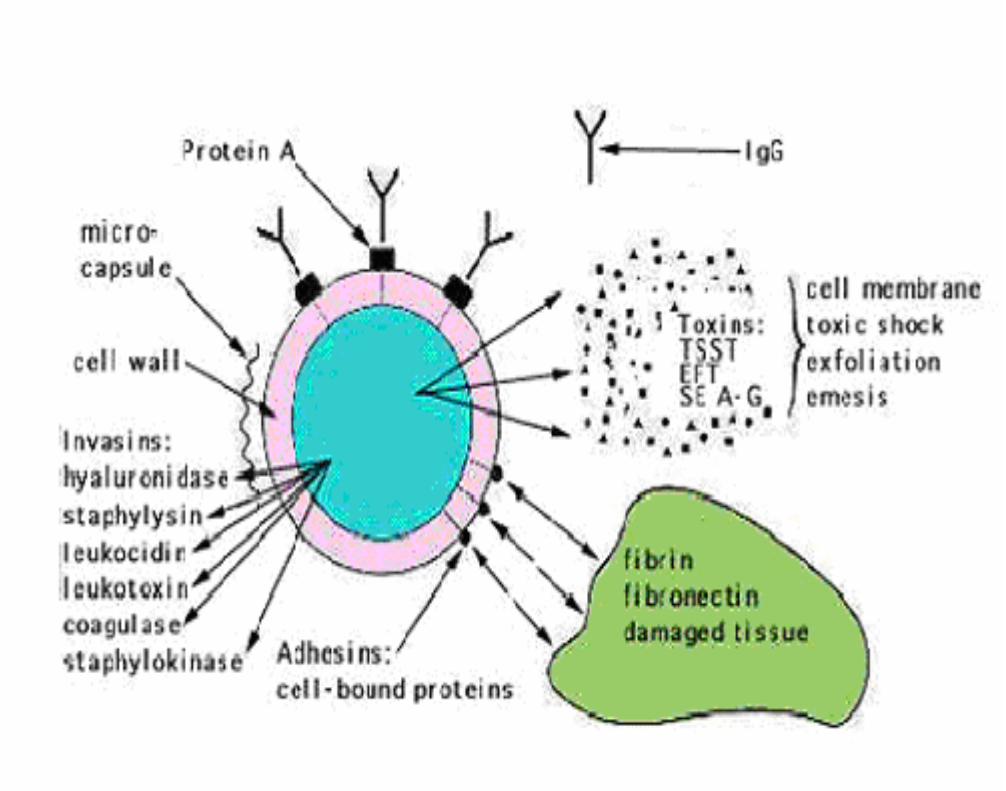


Figure 1.1 virulence factors of *S. aureus* (136).

1.5 *Staphylococcus aureus*: A Nosocomial Pathogen

S aureus is a significant human pathogen that is unsurpassed in the number of virulence factors it produces and in the wide range of infections and toxinoses it causes from innocuous skin lesions to life-threatening systemic infections (32,33). Genetic plasticity and diversity, especially regarding antibiotic resistance, are hallmarks of *S. aureus*. As a consequence, in the 50 years that antibiotics have been used clinically, some strains have acquired resistance to all antimicrobial agents except vancomycin (34, 35). Methicillin, a β-lactamase-resistant derivative of penicillin, subdivides the species into sensitive and resistant subgroups. Often, methicillin-resistant *S. aureus* (MRSA) strains are involved in hospital epidemics (36).

The incidence of MRSA infections is increasing world wide and in some locations exceeds the incidence of methicillin sensitive *S. aureus* (MSSA) infections. Among the many species of antibiotic-resistant bacteria, MRSA is one of the most important causes of antibiotic treatment failure, increased morbidity and mortality. *S. aureus* has a remarkable ability to colonize skin and mucous membranes. Hospitalized patients and health-care workers have higher colonization rates than the general population (37, 38, and 39).

These gram-positive cocci can withstand harsh environments 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 (40,41). Such characteristics have made *S. aureus* the most common hospital acquired (nosocomial) pathogen with a formidable array of virulence and resistance strategies (32).

1.6 Coagulase-Negative *Staphylococci*

The coagulase-negative *staphylococci* encompass over 30 species, of which 12 have been isolated from humans (42). These bacteria make up a significant proportion of the normal aerobic flora in humans. Many of these species are known to cause disease in humans; the pathogens in this group include *S. epidermidis, S. saprophyticus and S. haemolyticus.*

S. epidermidis, although less pathogenic than *S. aureus*, is also a leading cause of hospital-acquired infections (43). In particular, its ability to form biofilms ensures that *S. epidermidis* is a major source of infections involving indwelling devices (44), such as intravascular catheters, pacemaker leads, prosthetic heart valves and shunts. In addition, *S. epidermidis* is often associated with surgical wound infections.

S. saprophyticus is commonly associated with acute urinary tract infections in female outpatients. Besides *E. coli*, *S. saprophyticus* is the second most common cause of urinary tract infections in young sexually active females (45). In addition, *S. saprophyticus* has also been implicated in prostatitis

S. haemolyticus can be regarded as an emerging hospital pathogen. It has been shown to cause various human infections, including endocarditis, septicemia, peritonitis, and infections of bones, joints, and wounds (42). *S. haemolyticus* has a tendency to develop resistance to antibiotics and therefore is regarded as a serious threat for the future.

1.7 The problem of Antibiotic Resistance

In recent years, antibiotics resistant bacteria have become a great concern to the medicals community. There has been amerced increase in the number of species that have acquired resistance to antibiotics, as well as an increase in the kinds of antibiotics (46, 47).

1.7.1 Antibiotic Resistance Overview

Antibiotics work in variety of ways, (Table 1.2). Some 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, as well as monolactams and β-lactamase inhibitors. β-lactams inhibit the final stage of murein synthesis. This, by some undetermined mechanism, triggers murein hydrolases to lyse the cell. A related group of antibiotics that prevent a different step in cell wall synthesis are the glycopeptides, vancomycin and teicoplanin. Other agents have an antibacterial effect by inhibiting protein synthesis. Representatives of this group include the aminoglycosides, tetracyclines, macrolides and chloramphenicol which interfere with ribosome function. In addition, there are antibiotics that inhibit DNA synthesis, including quinolones, fluoroquinolones and sulfonamides.

Table 1. 2: Antibiotic types and mechanism of action and resistance

Antibiotics Types	Mechanism of action	Major resistance mechanisms
Beta-lactams	Inactivate PBPs (peptidoglycan synthesis	• Beta-lactamases • Low affinity PBPs • Decreased transport
Glycopeptides	Bind to precursor of peptidoglycan	• Modification of precursor
Aminoglycosides	Inhibit protein synthesis (bind to 30S subunit)	• Modifying enzymes (add adenyl or PO4)
Macrolides	Inhibit protein synthesis (bind to 50S subunit)	• Methylation of rRNA • Efflux pumps
Quinolones	Inhibit topoisomerases (DNA synthesis	• Altered target enzyme • Efflux pumps

1.7.2 Resistance Mechanisms

Mechanisms of antimicrobial resistance (Figures, 1.2 and 1.3) come in four general forms: (48)

1. Enzymes that destroy or modify the antimicrobial substrate.
2. Target site alteration like alteration of DNA gyrase, a target of fluoroquinolones.
3. Bypass pathways that substitute for a metabolic pathway.
4. Barrier to penetration or efflux pumps that exclude the agent.

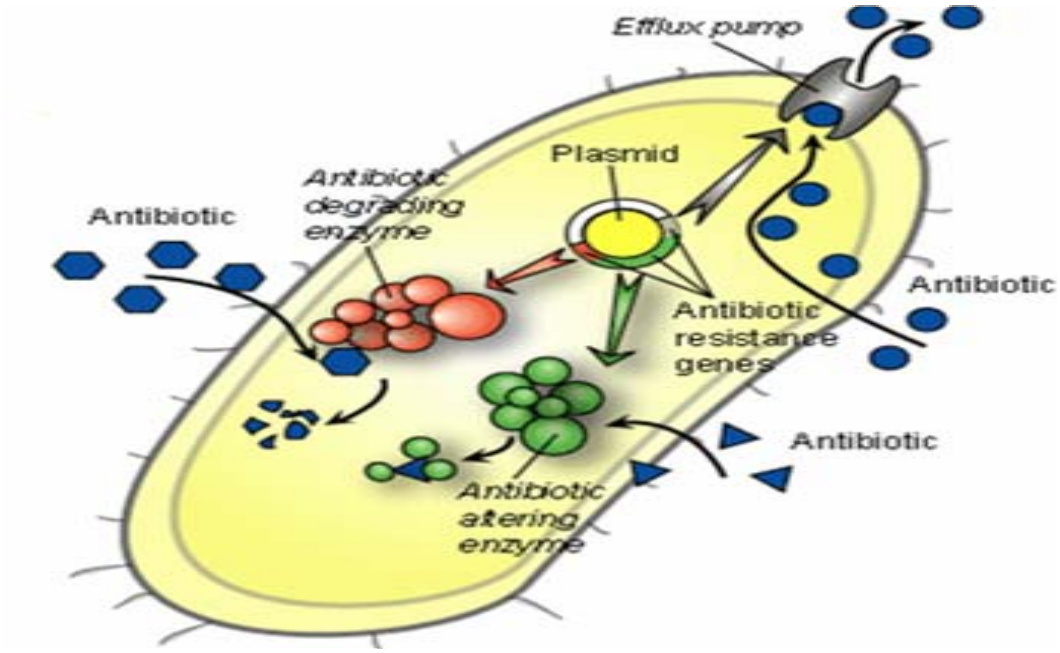


Figure 1.2: Mechanisms of antimicrobial resistance (136).

There are three hypotheses about the rapid spread of penicillin resistance: The first one emphasizes that the altered penicillin-binding protein (PBP) genes arise by interspecies recombinational events in which segments of native PBP genes are replaced with the corresponding segments from related species (49,50). The second one hypothesized that horizontal transfer, of altered PBP genes from resistant strains to susceptible strains, is the main cause of spreading the resistance to the susceptible strains (51). The last hypothesis stated that the importation and clonal spread of a small number of resistant clones are important factors in the global increase in the incidence of penicillin-resistant *pneumococci* (52, 53).

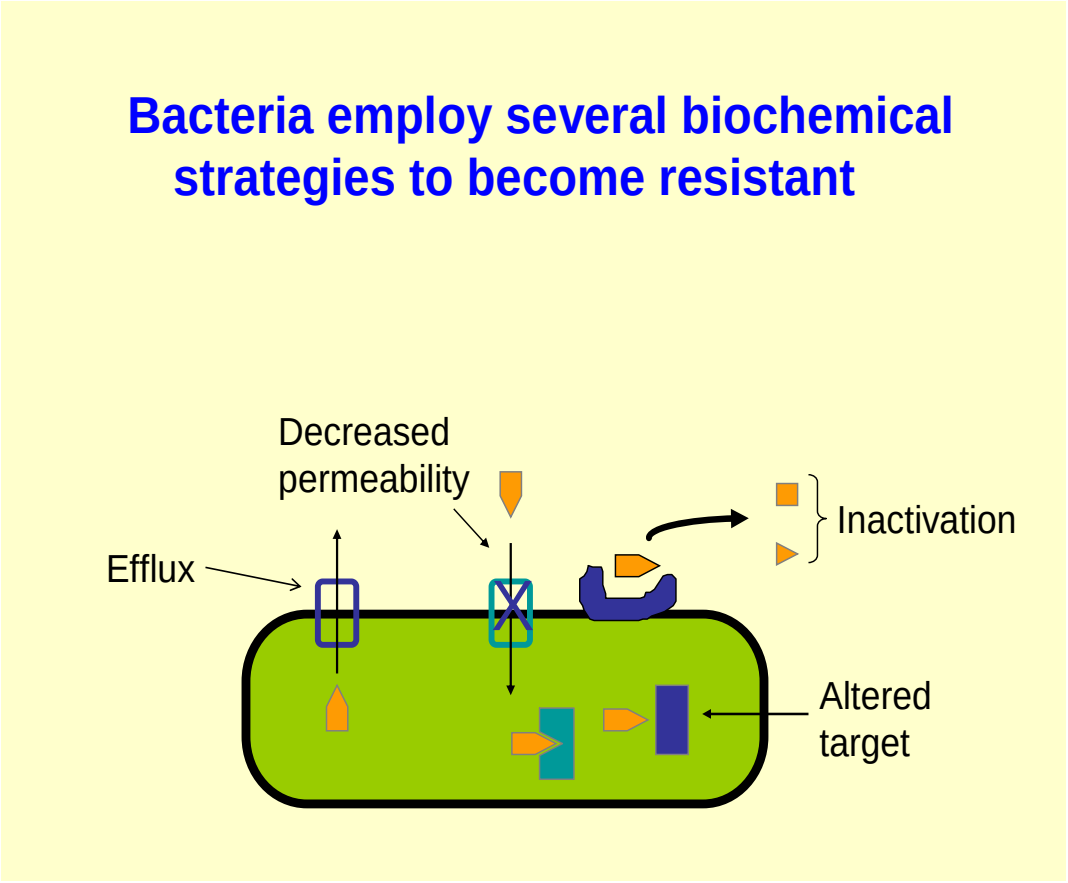


Figure 1. 3: Biochemical Mechanisms of antimicrobial resistance (137).

1.7.2.1 Beta-Lactam Drugs: Penicillins

1.7.2.1.1 Basic structure

Chambers *et al* (100) describes the chemical composition of penicillins. All penicillins have a "core" consisting of a thiazolidine ring that is attached to a β-lactam ring. The β-lactam ring carries a secondary amino group (RNH-). The core structure, 6-aminopenicillanic acid, is essential to the activity of these antimicrobial agents. The β-lactam ring is enzymatically cleaved by the bacterial enzyme β-lactamase. Penicilloic acid, the product of this enzymatic cleavage, does not show antibacterial activity.

1.7.2.1.2 Mode of action

Beta-lactam antibiotics produce a bactericidal effect by inhibiting the membrane-bound enzymes responsible for catalyzing vital stages in the biosynthesis of the cell wall. This is achieved by the antibiotic binding directly and covalently to one or more of the penicillin-sensitive enzymes called penicillin-binding proteins (PBPs). Specifically, bound penicillin prevents PBPs from catalyzing the transpeptidase reaction of peptidoglycan synthesis. The transpeptidase reaction involves the removal of the terminal alanine from the D-alanyl-D-alanine peptide chain of one murein subunit with the creation of a bond to the penultimate alanine of another murein subunit. As β-lactam drugs are structural analogues of the D-alanyl-D-alanine substrate, they become covalently bound to the PBPs, thereby inhibiting them.

1.7.2.1.3 Mechanism of resistance.

There are three main mechanism of resistance to the penicillins: (i) Cleavage of the β-lactam ring by β-lactamases/penicillinases, (ii) alterations in the target PBPs that reduce their affinity to the penicillins and (iii) a permeability barrier preventing penetration of the antibiotic into the cell (35). The first two mechanisms are especially important to β-lactam resistance in *S. aureus*. The last mechanism pertains particularly to gram-negative bacteria which have an intrinsic permeability barrier mediated by their outer cell membrane.

Inactivation of β-lactam drugs: β-lactamase production appears to be the most common mechanism of resistance, with the discovery and identification of more than 100 distinct β-lactamases (100). In terms of β-lactamase mediated resistance, the action of penicillin is prevented when the β-lactam ring of the antibiotic is hydrolyzed by β -lactamase. These molecules are extracellular enzymes which are divided into four types, A through D. In *S. aureus*, serotypes A and C have high activity. Genes for β-lactamase production, *blaZ oxpenP* are usually plasmid encoded, but these resistance genes may sometimes be found on the chromosome of the bacteria. blaZ is the gene that codes for the β -lactamase enzyme. In *S. aureus*, blaZ is carried by plasmids and is located on mobile genetic elements acquired from other bacteria. Three *S. aureus* transposons carry the blaZ gene: Tn4001, Tn4002 and Tn552. Tn552 encoded β-lactamase resistance is the most common in *S. aureus* plasmid

Methicillin resistance is another important β-lactam drug resistance mechanism in *S. aureus.* Methicillin is a semi-synthetic penicillin derivative. Resistance to this β-lactam drug in *S. aureus* is of great concern to medical and scientific personnel. The genes for methicillin resistance are located on the chromosome. The players in the signaling pathway for methicillin resistance are *mecA mecRl, mecR2* and *mecl. mecA* codes for a penicillin-binding protein, PBP2a (also called PBP2) which has a lower binding affinity for β-lactam drugs than regular PBPs. PBPs are transpeptidases involved in the construction of the bacterial cell wall.

The regulation of methicillin resistance resembles that of β-lactamase expression. The chromosomally located gene, *mecRl,* like the plasmid located *blaRl,* codes for a sensor-transducer that is part of a two-component signaling system. Mecl is a repressor of *mecA.* When Mecl is bound to DNA, PBP2a is not produced. When Mecl is unbound, then *mecA* is transcribed and PBP2a is produced. Lewis and Dyke (107) found that Mecl is also an effective regulator of *blaZ* and *blal.* MecR2, like BlaR2, is an accessory molecule involved in regulating PBP2a production.

There are a number of auxiliary genes involved in the expression of high-level methicillin resistance in *S. aureus.* The role of auxiliary genes was discovered through the observation that different levels of resistance to methicillin occurred in strains of staphylococci that had similar levels of expression of the *mecA* gene and produced comparable amounts of PBP2a protein (85). Researchers wanted to determine the reason(s) for the differing phenotype in cells producing the same amount of protein. It was postulated that one or more unknown cofactors were needed for high level resistance to methicillin. Studies revealed that four genes, *femA, femB, femC andfemD,* play an important role in the expression of methicillin resistance.

Alteration of the target: *S. aureus* has a chromosomally encoded resistance to β-lactam drugs via the *mec* region which confers resistance to methicillin, β-lactamase resistant penicillin, by encoding altered PBPs. There are two proposed mechanisms of PBP mediated resistance. The first is through mutations in the PBPs which provide immunity to all β-lactam antibiotics. The second mechanism is the result of the synthesis of a new PBP having reduced affinity for β-lactam antibiotics. It is believed that these altered or new PBPs are β-lactam-resistant transpeptidases which function in cell wall thickening and in septum formation (35).

1.7.2.2 Aminoglycosides

1.7.2.2.1 Basic structure

Aminoglycosides have a hexose ring, either streptidine as in streptomycin or 2-deoxystreptamine for all other aminoglycosides (106).

1.7.2.2.2 Mode of action.

Aminoglycosides are taken into the bacterial cell in three stages (35). During stage I, the antibiotic binds to outer surface components of the cell in an energy-independent manner. For gram-negative bacteria, the antibiotic passes across the outer membrane by passive diffusion via porin channels. In stage II, there is energy-dependent transport of the antibiotic across the inner cell membrane into the cytoplasm and driven by the transmembrane electrochemical gradient (106).Aminoglycosides are positively charged and the cell has an internal negative charge. Stage III consists of a second, more rapid uptake of the antibiotic into the cell which occurs after the first wave of antibiotic molecules bind to the ribosomes (35). Once inside the cell, the antibiotic binds to specific proteins of the 30S ribosomal subunit and inhibits protein synthesis. Inhibition of protein synthesis occurs by three different mechanisms (106).

1) They interfere with the initiation complex for peptide formation.
2) They induce misreading of mRNA which causes the incorporation of incorrect amino acids into the peptide. This results in non-functional or toxic proteins.
3) They cause the dissociation of polysomes into nonfunctional monosomes. It is believed that for the aminoglycoside streptomycin, the antibiotic binds directly to the 16S rRNA and distorts the aminoacyl site (101). This prevents the correct positioning of the tRNA for initiation of protein synthesis.

1.7.2.2.3 Mechanism of resistance

Three primary mechanisms of bacterial resistance to aminoglycosides have been discovered. The first method of resistance to aminoglycosides is via an alteration in the ribosomal target site. Mutations in the genes encoding ribosomal receptor proteins can result in changes in the structure of the ribosome such that it no longer binds the antibiotic or these receptor proteins may be absent. A second mechanism of resistance is impaired uptake of the antibiotic that diminishes the effective intracellular concentration of the antibiotic. This apparent impermeability may be caused by factors that affect the energetics of the cell membrane (35), however the precise mechanism is not known.

It has been proposed that membrane impermeability may be a result of genotypic changes such as mutations in or deletions of porin proteins or other proteins involved in the transport and maintenance of the electrochemical gradient. Another suggested reason for impermeability is a phenotypic change owing to growth conditions under which the oxygen-dependent transport process is not functional (106). The third mechanism of resistance is the most common and is due to the chemical inactivation of the aminoglycoside by specific enzymes. Aminoglycosides may be acetylated at secondary amino groups by aminoglycoside acetyltransferases (AAC), adenylated at hydroxyl groups by aminoglycoside adenyltransferases (AAD) or phosphorylated at hydroxyl groups by phosphotransferases (APH). Modified aminoglycoside antibiotics no longer bind to ribosomes and accordingly are unable to inhibit proteins synthesis.

1.7.2.3 Tetracyclines

1.7.2.3.1 Basic structure

All tetracyclines have a common structure. Free tetracyclines are crystalline, amphoteric substances of low solubility. They are available for use as hydrochlorides which are more soluble (103).

1.7.2.3.2 Mode of action

Tetracyclines inhibit protein synthesis. Tetracyclines enter bacterial cells by a dual mechanism involving passive diffusion and an energy-dependent process (active transport). Once inside the cell, tetracyclines bind reversibly to the 30S subunit of the bacterial ribosome. Binding to the ribosomal subunit sterically inhibits the binding of the aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex. Consequently, amino acids cannot be added to a growing peptide chain.

1.7.2.3.3 Mechanism of resistance

There are three main mechanisms of resistance to tetracyclines.

1) Decreased intracellular accumulation of the drug due to impaired influx or increased efflux via an active transport protein pump.
2) Ribosome protection due to the production of proteins which interfere with the tetracycline binding to the ribosome.
3) Enzymatic inactivation of tetracycline by chemical modification.

In *S. aureus*, resistance is due to active efflux of the antibiotic out of the cell. Tetracycline resistance determinants may be chromosomally-encoded or plasmid-encoded (35).

1.7.3 Plasmids and Antibiotic Resistance

Resistance to antibiotics and antimicrobial agents may be encoded by genes on the bacterial chromosome or genes located on extrachromosomal plasmids. Plasmids are usually the means by which most antibiotic resistance determinants are first acquired. Four plasmid classes, I - IV, have been defined for staphylococcal species (54), though additional plasmid classes may exist. Plasmid classes are based on size, copy number and resistance markers carried. For example, plasmids belonging to class I are small, approximately 1-5 kb. They have a high copy number, usually 15-20 copies per cell, and encode a single antibiotic resistance. Plasmids of class II are intermediate in size and copy number. They encode a combination of β-lactamase and inorganic ion resistances.Class III plasmids are large (40-60 kb) and encode multiple antibiotics resistances, usually including gentamicin resistance. In addition, many plasmids in this class are conjugative. Class IV consists of plasmids of intermediate size which encode a combination of resistances penicillin, heavy metals, aminoglycosides and fusidic acid.

Some *S. aureus* strains, especially those isolated from clinical infections contain more than one type of plasmid and can be resistant to a wide range of drugs (55). Accordingly, plasmids play a major role in the pathogenicity of this organism.

1.7.4 Factors Contributing to Increased Antibiotic Resistance

1.7.4.1 Misuse of Antibiotics

Of the factors contributing to the rise in antibiotic resistant bacteria, human misuse and abuse plays a critical role. Such misuse includes indiscriminate prescribing and dispensing of antibiotics and failure of patients to complete the full course of antibiotic treatment. The microenvironment created by the constant use of antibiotics generates 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.

Selection pressure refers to the many environmental conditions that act on populations of microorganisms. Some individuals in the population may survive and proliferate in a selective environment by virtue of novel alterations or mutations in their genetic material. Most discourses on antibiotic resistance and the effect of selection pressure examine selection in Darwinian terms. Accordingly, selection pressure allows for the differential proliferation of a sub-population. The degree of success an organism displays in its ability to proliferate in a selective environment is a measure of its fitness in that environment.

Treating bacterial infections with less than optimal doses of antibiotics exerts a selective pressure that is highly suited for selecting individuals in a population that are incrementally more fit than the rest of the population. At sub-therapeutic levels, only the most sensitive organisms are killed. Those organisms in the population with an inherent resistance to the antibiotic or with non-lethal mutations survive and propagate. The selected organisms are more antibiotic resistant than the original population and can be killed only with exposure to higher concentrations of antibiotics.

1.7.4.2 Use of Antibiotics in Livestock

The use of antibiotics in food animals is thought to contribute heavily to the emerging resistance patterns in human pathogens. Although it is disputed by farmers and veterinarians, there is mounting evidence to support this claim.

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 (109). Many of the broad and narrow spectrum antibiotics used for therapeutic treatment of infected animals are the same as those used in humans. Unfortunately, many of the antibiotics used for nutritive purposes to promote growth development are used at sub-therapeutic levels. This practice is believed to generate a reservoir of drug resistant microorganisms in the environment. At low antibiotic levels, the bacterial population is subjected to a selection pressure that effectively amplifies the few microorganisms that are adapted to the environment.

There are well documented cases that link antibiotic use in animal husbandry to antibiotic resistant bacterial infections in humans. In November 1999, a 62-year-old Danish woman died from Salmonella-induced food poisoning (110). The pathogen was *Salmonella enterica* serotype *typhimurium* DTI04. Scientific investigation revealed that she contracted the infection from contaminated pork products that were traced back to a single Danish herd. The herd had been treated with enrofloxacin, a quinolone compound similar to ciprofloxacin. Ciprofloxacin is used to treat human cases of salmonellosis. This particular *Salmonella* strain was resistant to seven other antibiotics.

In April 2000, a 12-year-old boy from a Nebraskan farm acquired a multi-drug resistant *Salmonella* infection from an infected cow (111). The cows had been given ceftiofur, a cephalosporin, to treat severe diarrhea in the herd. The infecting strain of *Salmonella enterica* serotype *typhimurium* was resistant to 13 antibiotics, including ceftriaxone, a drug that is used to treat salmonellosis in children. The boy lived, but this outcome had little to do with his course of antibiotic treatment. This report describes the first case of a *Salmonella* "super-bug" observed in the United States. Shortly thereafter, another case of ceftnaxone-resistant *Salmonella* infection was reported in a 4-year-old Ohio girl. She contracted the infection during a visit to her grandparents' farm.

A point of concern for investigators is the fact that ceftriaxone resistance is encoded by a plasmid. The plasmid-borne nature of ceftriaxone resistance means that it can be readily transmitted by horizontal transfer between bacteria.

The efficacy of ceftriaxone treatment in children should not be compromised as it is the main drug prescribed by physicians for salmonellosis. Quinolone antibiotics like ciprofloxacin cannot be used in children because they impede bone development. Another link between antibiotic use in animals and antibiotic resistant bacteria in humans is *Campylobacter jejuni*. Since 1994 when the FDA approved the use of quinolone drugs in poultry, the incidence of quinolone resistant bacteria in humans increased from 1% to 17% (112).

Of greater concern is the emergence of vancomycin-resistant strains of enterococcus (VRE). In Europe, enterococci resistant to vancomycin seem to coincide with avoparcin use in livestock in this region. In 1994, Germany banned the use of avoparcin as a growth promoter for livestock. By 1997, they had observed a decrease from 12% to 2.5% in the number of reported VRE.

Examples such as these are not surprising given the quantity of antibiotics administered to animals each year all over the world. It is important to note that many classes of antibiotics are shared between the agricultural community and the medical community. Teuber (109) provides concrete numbers for examination: In France in 1989, 50 tons of β-lactam antibiotics were used to treat farm animals, along with 57 tons of aminoglycosides, 99.6 tons of chloramphenicol, 116.8 tons of tetracylines, 37 tons of macrolides, 138.6 tons of sulfonamides and 126.2 tons of other assorted antibiotics; in the Netherlands for 1990, 300 tons of antibiotics was for veterinary use; and in the United Sates for 1978, 558 tons of antibiotics and antimicrobials were added to animal feed for growth promotion.

Industrial livestock production may be threatening the effectiveness of the mainstays of our health system: antibiotics. Animals living so closely together are at risk for spreading disease, so 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 (2004) are fed to animals that are not sick (141).

Many in the scientific community have expressed concern that the system will increase antibiotic resistance and put human health at risk. A 2002 study found antibiotics in 1/3 of ground and surface water samples taken near hog confined animal feeding operation (CAFO) and in 2/3 of samples near poultry CAFOs. Studies were carried out in the United States by An Emerging Public Health Crisis. Washington, DC: KAW, (2004) showed that manure lagoons can leach antibiotic resistant bacteria along with other contaminants (142). Antibiotic resistance should be a major concern for us all: doctors report (2007) a growing number of illnesses that no longer respond to antibiotic treatment, resulting in prolonged illness or death with resistant strains of an illness(142) The American Medical Association now opposes the use of antibiotics in farm animals that are not sick (143)

1.8 Methicillin-resistant *Staphylococcus aureus*

Methicillin resistant S. *aureus* (MRSA), which are often resistant to several classes of antibiotics, is the most common cause of nosocomial infections. The incidence of MRSA infections has dramatically increased in the last five years due to the worldwide emergence of community strains of MRSA among healthy people lacking traditional health care associated risk factors(78,79) The extent and transmission dynamics of MRSA in the community, however, remains poorly described. Several studies have demonstrated that community-associated MRSA (CA-MRSA) strains originating from patients with no antecedent hospital exposure were clonally distinct from hospital endemic MRSA strains (80, 81). The apparent phenotypic and genotypic differences between CA-MRSA and hospital-acquired MRSA (HA-MRSA) were noted in anecdotal reports, case series, and outbreak studies, which often compared few CA-MRSA strains to historical HA-MRSA control isolates from worldwide collections (82,83).

Such comparison of contemporary cases to historical controls is flawed because one cannot eliminate potential biases due to other factors that may have changed over time (e.g. clonal shifts). The need for concurrent control is clear. A better study design would compare a single series of contemporaneous patients with either CA-MRSA or HA-MRSA disease treated at the same health care location. The molecular genetic characterization of MRSA strains combined with better epidemiologic designs will enable the identification of the transmission dynamics of CA-MRSA and HA-MRSA.

1.8.1 Hospital- acquired MRSA infection

Staphylococcus aureus has emerged over the past several decades as a leading cause of hospital-associated infections (84). A significant component in the success of *S. aureus* has been its acquisition of antibiotic resistance determinants. As new antibiotics have come into use, *S. aureus* has responded soon after with resistant strains. This phenomenon has made therapy of staphylococcal diseases a global challenge. Penicillin-resistant strains, for example, appeared in hospitalized patients within a short time after the introduction of the antibiotic; over time, penicillin-resistant strains have spread into the community to the extent that penicillin is now only of very limited value as a treatment for *S. aureus* infections (78). *S. aureus* with broad resistance to the entire β-lactam class-termed methicillin resistant *S. aureus*, or MRSA-appeared in 1961, one year after methicillin was introduced into clinical use (86). Since then MRSA have spread in hospitals throughout the world (84).

1.8.2 Community-associated MRSA disease

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. Four features, in addition to a lack of nosocomial risk factors, appear to differentiate CA-MRSA from endemic hospital MRSA:

1) They are susceptible to most antibiotics other than β-lactams (87, 88).
2) They carry the type IV *SCCmec* element encoding resistance to the entire β-lactam class of antibiotics (89, 90).
3) They carry toxin genes such as Panton-Valentine leukocidin (PVL) and a variety of other enterotoxins (91, 92).
4) They are not related to genotypes endemic in the hospital (80, 91, 92).

1.9 Staphylococcal Chromosome Cassette *mec (SCCmec)*

Resistance to β-lactam antibiotics by HA-MRSA and CA-MRSA are mediated by production of β-lactamase and an altered target penicillin-binding protein (PBP), PBP2a. PBP2a, a bacterial cell wall synthetic PBP that putatively functions as a transpeptidase, is encoded by *mecA,* and confers broad resistance the entire β-lactam class (93). *mecA* is harbored on the staphylococcal chromosome cassette *mec (SCCmec),* which can be horizontally transferred between staphylococcal species.

Unlike hospital MRSA, which tend to be resistant to multiple antibiotics, CA-MRSA tended to be non-multidrug resistant (87, 88). It is thought that the fitness cost of multi-resistance reduce survival of HA-MRSA in the community. Indeed, CA-MRSA isolates multiplied in vitro much faster than HA-MRSA (98). HA-MRSA carried type I-III *SCCmec*; these genetic islands also harbor various resistance genes for non-β-lactam antibiotic classes (94, 95). CA-MRSA, however, almost always carry the smaller type IV *SCCmec* element (21 to 24 kb), which is devoid of non-β-lactam resistance determinants (89,90).

Recent DNA microarray analysis of worldwide hospital MRSA strains identified *mecA* in at least five divergent MRSA clonal lineages, suggesting that MRSA strains evolved a number of times by means of horizontal transfer of *mecA* (96), This multiple origin scenario is supported by phylogenetic analysis of *S. aureus* clonal lineages based on multilocus sequence typing (MLST) (99). Importantly, the majority of CA-MRSA isolates from diverse locations belong to the same five hospital MRSA clonal lineages. This suggests the horizontal transfer of type IV *SCCmec* into common MSSA progenitor lineages. Moreover, CA-MRSA from the US Midwest (97) and Australia (98), representing ST-1 and ST-298 (as defined by MLST), define two other CA-MRSA clonal lineages not associated with healthcare settings.

1.10 Antimicrobial resistance in Turkey

The results of antimicrobial resistance studies conducted in Turkey have shown a high prevalence of resistance to antibiotics in *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter species* as well as in Gram-positive cocci, e.g. *staphylococci and enterococci* (56, 57, 58, 59, and 60). The production of extended-spectrum beta-lactamases (ESBLs) appeared to be a major mechanism of resistance to beta-lactam antibiotics in enterobacteriaceae (57). The prevalence of ESBLs is as high as 15.5% in *E. coli* and 55.5% in *K. pneumoniae* (59). The prevalence of PER-1 type of beta-lactamases is 46% in *Acinetobacter species* and 11% in strains of *P. aeruginosa* (60). Extended-spectrum derivatives of OXA enzymes from *P. aeruginosa* were first found in a Turkish hospital in 1991-1992.

In a multicentre study high resistance rates to aminoglycosides among Gram-negative isolates were found (61).The resistance rates for gentamicin, tobramycin, netilmicin amitacin and isepamicin were 94.5%, 82.4%, 53.6%, 49.7% and 29.7%, respectively. The resistance rates were higher than those in most of the other countries surveyed in earlier studies. The most common aminoglycoside resistance mechanisms [AAC (3)-II (GTN), AAC (6')-I (TNA), and ANT (2")-I (GT)] in earlier studies were also found in the present isolates of *Klebsiella spp., Enterobacter spp. and E. coli*, with increased complexity. In addition to these older mechanisms, two new aminoglycoside resistance mechanisms, namely AAC(6')-III (TNAI) and AAC(6')-IV (GTNA), were also found at significant frequencies (11.9% and 26.9%, respectively) in these isolates of *Enterobacteriaceae* (AT=435)(61).

In Turkey, the first unexpected vancomycin-resistant isolate reported in 1999 was an *Enterococus faecium* strain with a Van A phenotype (62), then vancomycin-resistant *E. faecium* was isolated from a blood culture(63). Gram-negative microorganisms producing extended spectrum β-lactamase (ESBL) are a common cause of nosocomial infections In Turkey, (57). Nosocomial bloodstream infections in a Turkish university hospital: study of Gram-negative bacilli and their sensitivity patterns was inducted that the resistance rates for some antimicrobials in Turkey are higher than in European and USA surveys (58).

1.10.1 Methicillin-resistant *Staphylococcus aureus* (MRSA) in Turkey.

In Turkey, the ratio of MRSA causing hospital infections is not low. Topeli *et al.* (67) found that the rate of methicillin resistance among *S. aureus* strains causing bacteremia in Intensive care unit (ICU) patients was 37.7% in a Turkish hospital. Önciil et al. (68) reported that MRSA strains were isolated from 25% of the infections in a burn unit located in Turkey. In another study, the ratio of MRSA in hospital infections in ICU was reported as 22.2% (69). On the other hand, there is insufficient data on community-acquired MRSA infections and the ratio of colonization of MRSA in the community. Cesur and Cokca (70) determined the colonization ratio of MRSA as 2.6 and 6% in community members and in healthcare workers, respectively.

Investigation of colonization with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in an outpatient population in Izmir – Turkey was carried out by Erdenizmenli M. *et. al*. recommended that surveillance studies should be carried out in every geographical region to detect the prevalence of MRSA strains, and appropriate infection control measures should be taken to prevent infection with these strains (71).

The low rate of methicillin-resistant *Staphylococcus aureus* (MRSA) in Turkish children was reported by Soysal A. *et. al*. in 2006 (72). There are no other studies showing data from Turkey extent in the literature.

1.11 Antimicrobial resistance in Libya

The problem of antibiotic resistance is very serious in Libya, as it appears to be on the increase, particularly with the emergence of resistance to newer drugs that include the fluoroquinolones (e.g. ciprofloxacin) among the clinically important bacterial species (139).

The high prevalence of resistant bacteria in Libya seems to be related to antibiotic usage

1. Easy availability without prescription at drug stores,
2. Injudicious use in hospitals, and
3. Uncontrolled use in animal husbandry.

1.11.1 Methicillin-resistant *Staphylococcus aureus* (MRSA) in Libya.

Epidemiological dates on MRSA in Africa are scarce. The prevalence of MRSA was determined in eight African countries between 1996 and 1997 and was relatively high in Nigeria, Kenya, and Cameroon (21 to30) and below 10% in Tunisia and Algeria (113). In Algeria, the rate of MRSA increased to 14% in 2001 (114), and the prevalence of PVL-positive MRSA has increased in 2006, these strains were resistant to multiple antibiotics, including gentamicin and ofloxacin (115).

There are few studies showing data from Libya extent in the literature. Those studies indicate that the MRSA among *Staphylococcus aureus* isolated from different cities in Libya; Tripoli 25% 1996, Benghazi 24% 2000, and Misurata 26% 2001, (139,140).

1.12 AIMS OF THE STUDY

Over the past years, antimicrobial resistance is an increasing problem worldwide, impacting infection control efforts and costs of antimicrobial treatment. Numerous factors contribute to the problem, including unnecessary antimicrobial prescribing by trained and untrained health workers, uncontrolled dispensing by drug vendors, poor antibiotic prophylaxis in surgery, and poor infection control practices. (73)

In Turkey, there are two categories for the sale of drugs to the public; prescription-only drugs (e.g. antibiotics) and pharmacy drugs which can only be sold under the supervision a pharmacist in a pharmacy. However, practice, antibiotics can be given to patients without prescription. It is recognized that exposure to a given antibiotic will usually increase with non-prescription availability. (74) For the above reasons the aims of this thesis includes the followings:

The study including some clinical specimens collected from four different hospitals (two hospitals in Turkey and two hospitals in Libya).

1. To study the antibiotic resistant in *S. aureus* including MRSA strains in Turkish hospitals and Libyan hospitals.
2. To identify and category the MRSA strains in both countries
3. To compare the genetic differences between the MRSA strains in Turkish specimens and Libyan specimens.
4. To compare epidemiological data between Turkey and Libya.

CHAPTER 2

MATERIALS AND METHODS

This chapter includes details about the research methodology used in this thesis. The identification of *S. aureus* in microbiology laboratory was done by the following experiments:

1. The collection of samples:
    a. Turkish Hospitals.
    b. Libyan Hospitals.
2. Culture on media.
    a. On Blood agar.
    b. On Mueller Hinton agar.
    c. On Nutrient agar.
    d. In Nutrient broth.
3. Gram's stain.
4. Catalase test.
5. Coagulase test.
6. Antibiotic susceptibility test:
    a. Disk diffusion method.
    b. Minimum Inhibitory Concentration (MIC) Etest
7. Storage the Samples in Deep Freezing.
8. BD Phoenix Automated Microbiology System**.**
9. Genetic studies.

2.1 The collection of samples

Four hundred and fifty six samples were collected from both Turkish and Libyan Hospitals. In Turkey, clinical *S. aureus* strains were gathered from the Hacettepe Hospital (No., 200) and Ankara Hospital (No., 106), these clinical samples were collected between January and May 2007 and the total of specimens was 306. In Libya, strains of *S. aureus* were collected from Aljalla Hospital (No., 88) and Jamahyria Hospital (No., 62) in the period between June and August 2007, the total collected samples were 150. The collection of samples was registered in the questionnaires as represented in appendices (appendix A, appendix B and appendix C).

2.2 Culture on media.

All media were prepared in laboratory depending on the manufacturers' instruction and the media included blood agar (Oxoid), Mueller Hinton agar (BD), Nutrient agar (Oxoid), and nutrient broth (Oxoid).

2.2.1 Blood agar

Blood agar was prepared by weighing 37 grams of blood agar base powder and dissolving it in 1 liter of distilled water and allows it to sack for 10 minutes. Then, the solution was mixed and sterilized by autoclaving for 10 minutes at 121˚C. After that the solution was cooled to 47˚C and 5-7% sterile defibrinated blood was added. Finally, the solution was mixed well before pouring and was stored in a cool dry place (refrigerator 4-8 ˚C).

2.2.2 Mueller Hinton agar

Muller Hinton agar was prepared by suspending 35 grams in 1 liter of distilled water and further boiling it to dissolve completely. Then, the solution was sterilized by autoclaving at 121˚C for 15 minutes and kept tightly closed in a cool dry place (refrigerator 4-8 ˚C). It is worthy mentioning here that the pH of the solution is 7.3± 0.1.

2.2.3 Nutrient agar

Nutrient agar was prepared by weighing 37 grams of nutrient agar base powder and dissolving it in 1 liter of distilled water, allowing it to sack for 15 minutes. Then, we stirred the solution and sterilized it by autoclaving for 10 minutes at 121˚C. After that, the solution was mixed well and then poured in Petri dishes. Finally, the media was stored in a cool dry place (refrigerator 4-8 ˚C).

2.2.4 Nutrient broth

Nutrient broth was prepared by dissolving 15 grams of Nutrient broth powder in 1 liter of distilled water. After mixing well and distributing into final containers (test tubes), the solution was sterilized by autoclaving at 121˚C for 15 minutes and kept tightly closed away from bright light in a cool dry place (refrigerator 4-8 ˚C).

2.3 Gram's stain

Gram's stain was done in order to see the morphology of bacteria under microscope**.** The procedure is described below.

2.3.1 Reagents

1. Crystal violet.
2. Gram's iodine.
3. Acetone Alcohol.
4. Safranin counterstain.

2.3.2 Gram's Stain Procedure

Firstly, a thin smear from bacterial suspension was prepared on a clean slide and allowed it to air dry. In order not to wash off during the staining, the smear was fixed on the slide by passing the slide, right side up, through a flame three or four times. After fixation, the smear was flooded with crystal violet solution and let it stand for 1 minute and then the smear was washed gently with tap water. Then, the smear was flooded with Gram's iodine solution and let it stand for 1 minute. As a following step, the smear was washed with tap water until the excess iodine solution was removed and then the smear was decolorized by adding acetone alcohol. This usually takes 5 to 10 seconds. After that, the smear was washed gently with tap water and the slide was flooded with safranin counterstain for 30 to 60 seconds and again the smear was washed gently with tap water following by blot dry. Finally, the stained smear was examined under the 100 X (oil immersion) objective of the microscope.

2.3.3 Interpretation:

Upon examining the slides by microscope, the cells were differantiated by stain color. Gram-positive baceria have a dark blue to purple stain color, while Gram-negative bacteria have a pink to red color. In our case, we found that our bacteria which is *S. aureus* showed a dark blue to purple stain color meaning that it is a Gram-positive bacteria.

2.4 Catalase test

Catalase test was performed by slide method in order to differentiate between *Staphylococcus* and *Streptococcus.* This test was done because both *Staphylococcus* and *Streptococcus* are Gram-positive cocci bacteria. Catalase test gives a positive result with *Staphylococcus* and a negative result with *Streptococcus.*

2.4 1 Principle:

The catalase test is used primarily in differentiation between certain genera and species of bacteria. Catalase is an enzyme presents in most cytochrome containing aerobic and facultative anaerobic bacteria. An important exception is *Streptococcus* species. The test is performed by exposing the test organism to hydrogen peroxide and observing the immediate oxygen production.

2.4.2 Reagents and Equipment:

1. Hydrogen peroxide, 3% Store at 15-30° C
2. Slides
3. Sterile sticks or inoculating loop

2.4.3 Procedure:

By using a loop, an 18-24 hour old pure colony and placed it on a clean microscope slide. By using a Pasteur pipette or a dropper, a drop of 3% H_2O_2 was dropped over the bacteria on the slide and observed immediate bubbling.

2.4.4 Interpretation:

After exposing our slide sample to hydrogen peroxide immediate oxygen bubbling was observed. That means that our bacteria are *Staphylococcus* and not *Streptococcus* because *Streptococcus* bacteria don't interact with hydrogen peroxide to produce immediate oxygen bubbling.

2.4.5 Procedural Notes: (Interferences)

- In order to obtain a reliable result, nutrient agar was used instead of blood agar because red blood cells in blood agar contain catalase enzymes that give a false-positive result when reacted with hydrogen peroxide.
- Colonies older than 18-24 hours may lose their catalase activity, and may produce a false-negative result.
- Hydrogen peroxide is an unstable reagent that breaks down easily upon exposure to light. So, this reagent must be kept in dark bottles.
- Care must be taken while performing the test, since bacteria may be aerosolized as a result of bubbling.

1.5 Coagulase test

The coagulase test is commonly used in clinical microbiology laboratories to distinguish between strains of *Staphylococci.* When a negative coagulase test is obtained, it means that the tested strain is less invasive and non-pathogenic, but when a positive result is obtained, the tested strain is pathogenic.

2.5 1 Principle:

S. aureus produces coagulase, an enzyme that induces plasma coagulation by activating Prothrombin. In this study, coagulase test was done by two methods; slide method and tube method.

2.5.2 Coagulase test (Slide method)

There are two kinds of coagulase enzyme, bound coagulase enzyme and free coagulase enzyme. Slide test is used for the bound coagulase enzyme.

2.5.2.1 Procedure

Two drops of thawed rabbit plasma was placed inside a small circle on a clean glass slide, after that a single colony was added by using a wooden pick or sterile loop and emulsifying it in the plasma, fibrin threads form between the cells due to coagulase, causing them to agglutinate. By doing so, there will a visible clumping of cells within 10-15 seconds. This test was done for the bound coagulase enzyme.

2.5.2.2 Interpretation

In order to be sure that our sample is *S. aureus*, we did this test. As we said before, *S. aureus* bacteria have coagulase enzyme that reacts with rabbit plasma and fibrin is formed between the cells causing them to agglutinate. In our test, we got a positive results because we observed the clumping shape. It is worthy to mention that sometimes even when we got a negative result, it doesn't mean that our sample is not *S. aureus* because it may give a positive result when tube method is done depending on free coagulase enzyme.

1.5.3 Coagulase test (tube method)

Coagulase tube test was performed for all isolated strains in order to confirm the diagnosis of *S. aureus*. This test was used for the free coagulase.

1.5.3.1 procedure

After inoculating a tube with a 0.5 ml of plasma with the bacterial inoculum, the tube was incubated at 37˚C and checked every 30 minutes. Some strains will give positive reaction in few hours, other strains take longer time. If there was no clot after 4 hours, the tube would be incubated at 37˚C for 18-24 hours. In this test, any degree of coagulation is considered as a positive test for the free coagulase.

2.5.3.2 Interpretation

If a clot is observed due to the reaction of coagulase enzyme with the rabbit plasma within 4 hours at 37°C or after overnight incubation at 37°C, the result is considered as positive, but however, if no clot formation is observed after 24 hours, the result is considered as negative.

2.6 Antibiotic susceptibility test

In vitro susceptibility tests were performed on Mueller-Hinton agar by two methods:

1. The disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) standards.
2. The Minimum Inhibitory Concentration (MIC) as described by the Etest method in accordance with CLSI standards.

All MRSA strains were determined by disk diffusion method and confirmed by MIC method using the Oxacillin Etest (AB Biodisk, Solna, Sweden).

2.6.1 Disk diffusion method

In this study, the following antibiotic disks were tested: Oxacillin (Methicillin) (OX 1µg), Vancomycin (V 30µg), Ampicillin (AMP 10µg), Amoxicillin (AMX 10µg) Tetracyclin (TE 30µg), Erythromycin (E 15µg), Gentamycin (G 10µg), Ciprofloxacin (CIP 5µg), Clindamycin (DA 2µg), Chloramphenicol (C 30µg) Cefotetan (CTT 30µg) and Nitrofurantoin (F 300µg).

2.6.1.1 Procedure

After streaking the colony on Mueller-Hinton agar**,** the antibiotic disks was added after 15 minutes on the plates by sterile forceps. Then, the plates were incubated at 37˚C for overnight (18- 24 hours). The results were read according to NCCLS standards and all MRSA strains were confirmed by MIC method using the Oxacillin Etest (AB Biodisk, Solna, Sweden).

2.6.2 Minimum Inhibitory Concentration (MIC).

Before using the Etest gradient strips from an unopened package, the package was checked for any damage. If the strips were damaged, we wouldn't use it. In addition to that when the strips were removed from the freezer or refrigerator, they were allowed to reach room temperature (+4˚C /approx. 30 minutes, -20˚C / approx. 60 minutes). We ensured that moisture condensing on the outer surface of the package has evaporated completely before opening it. When packages' temperature reached room temperature, the strips were used immediately.

2.6.2.1 Etest method

The isolated viable colonies from overnight agar plate were suspended into broth tubes and a sterile swab was dipped into the suspension to press out excess fluid and then the entire surface of the agar plate was swabbed evenly in all directions by the same sterile swab. After that, the agar surface was left for 15 minutes on the bench or in an incubator to dry and an Oxacillin strip was applied onto the agar surface using sterile forceps. Once applied, the strips was attached firmly on the Mueller-Hinton agar and not allowed to move at all. Finally, the plates were incubated at 35˚C for a full 24 hours for MRSA and incubated for 48 hours if the result was negative after 24 hours.

2.6.2.2 Precautions

- Mueller Hinton + 2% NaCl were only used as hypertonicity which was optimal for *mecA* detection.
- An inoculum turbidity of at least 0.5 McFarland, was used. A heavier inoculum was necessary to detect low to moderate resistance mutations.

- MRSA and MRSE expression may be delayed. So, MRSA strain was confirmed after 48 hours of inoculation when necessary.
- Viable colonies were used for inoculum preparation, remembering that McFarland turbidity standard do not guarantee a correct colony count (cfu/ml).

2.6.2.3 Reading

The plates were read only when sufficient growth was seen after 24 hours for MRSA and 48 hours for MRSE. Besides, the MIC result was read where the ellipse intersects the MIC scale on the strip. Also, the point of complete inhibition of all growth including hazes and isolated colonies were read. Moreover, we used the illustrations in figures (2.1 to 2.9) to select the correct end points when different growth patterns were seen.

Figure 2.1 Susceptible strain. MIC 0.5 µg/ml.

Figure 2.2 Homogeneously resistant strain. MIC ≥256 µg/ml.

Figure 2.3 Heterogeneous resistance. MIC ≥256 µg/ml.

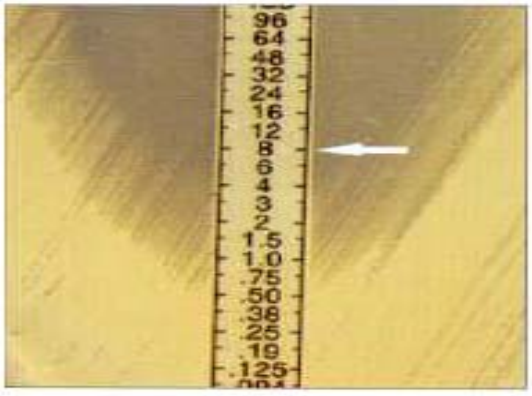


Figure 2.4 Diffuse Zone edge. MIC 8 µg/ml.

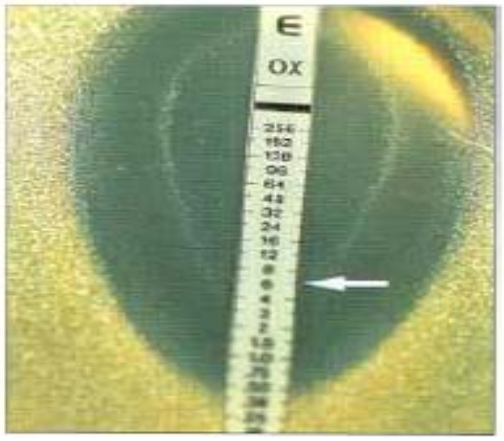


Figure 2.5 Translucent Film. The plate is tilted to read. MIC 6 µg/ml.

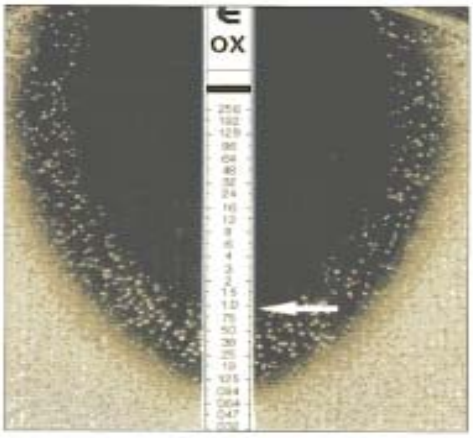


Figure 2.6 Subpopulation of macrocolonies. MIC 1 µg/ml.

Figure 2.7 Resistant subpopulation. MIC 256 µg/ml.

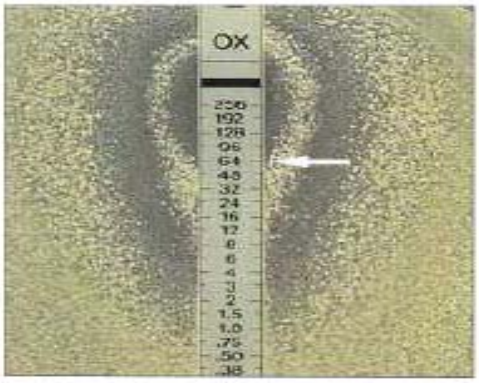


Figure 2.8 Induction of resistance at 8-64µg/ml. MIC 64 µg/ml.

Figure 2.9 Isolated colonies in ellipse. MIC ≥256 µg/ml.

2.6.2.4 Interpretation

In this test, NCCLS interpretive criteria for susceptibility categorization were used. Since Etest comprises a continuous gradient, MIC values in-between two-fold dilutions can be obtained. These values were rounded up to the next two-fold dilution before categorization. For example: when Oxacillin breakpoints are **S**: ≤2, **R**: ≥4 µg/ml, an Etest MIC of 3µg/ml was rounded up to 4 µg/ml and the category reported as resistant (**R**).

2.6.2.5 Storage of Etest

The packages were stored either at controlled room temperature (20-22˚C), or in refrigerator (4-8˚C), or in freezer (-20˚C), as stated on the product label. The Etest gradient strips were kept until dried when left over from an opened package. The opened package were either resealed as instructed or placed in an airtight storage container with desiccant and stored at the same temperature already labeled or at -20˚C. We ensured that the batch number and expiry date were marked on the storage container.

2.7 Storage the Samples in Deep Freezing.

Long term storage of microorganisms is a challenge in routine microbiology. The microorganisms were stored at low temperatures using a mechanical technique called microbank that offers the least possibility of the disturbance and permits ready access to stored material. Microbank is a sterile vial containing porous beads which serve as carriers to support microorganisms.

2.7.1 Microbank procedure (Preparation)

Under aseptic conditions, the screw cap cryovials were opened and inoculated the cryopreservative fluid with young colonial growth (18-24 hours) which were picked from a pure culture. Then, the vial was closed tightly and inverted for 4-5 times to emulsify the microorganisms. At this point, the microorganisms will be bound to the porous beads. The excess cryopresevative fluid was well aspirated leaving the inoculated beads as free of liquid as possible and then the vial finger tight was closed. After that each vial containing only one microorganism was labeled by using a permanent Marker. Finally, the inoculated cryovials were stored at -70˚C for long term results.

2.7.2 Microbank procedure (Recovery)

Under aseptic conditions, the cryovial was opened and a sterile needle or forceps was used to remove one colored bead. Then, the vial was closed and was returned as soon as possible to low temperature storage because excessive changes in temperature reduce the viability of the organisms. After that, the inoculated beads were sometimes directly streaked on solid medium and other times the inoculated beads were dropped into an appropriate liquid medium. When used effectively, each cryovial stores approximately 25 identical potential cultures.

2.7.3 Limitations

- Microbank is offered solely as a mean of providing extended storage possibilities for microorganisms.
- In use, we practiced aseptic technique to ensure continued integrity of the stored microorganism.
- Microbank were only used when we were sure that non of the following conditions were present before inoculation:
    a. The vial shows any evidence of leakage (loss of cryopreservative).
    b. Turbidity in cryopreservative suggesting contamination.
    c. The expiry date on the outer label has elapsed.
- After the beads were removed and used, we did not return it to the cryovial for any reason.

2.7.4 Safety precautions

- Microbiological safety cabinets were always used when making and manipulating a heavy suspension of a culture.
- Biohazard precautions were observed when discarding used or partly used cryovials.
- When storing Microbank in liquid nitrogen, the following precautions should be taken:
    a. Ensure that the cryovial screw cap is tightened normally: over-tightening may cause distortion of the silicone O-ring in the cap which may cause leakage.
    b. Ensure that the thread of the cryovial and screw cap is completely dry before closing: liquid drops impair the seal in liquid nitrogen.
    c. All Microbank vials should always be stored in gas phase, above liquid nitrogen. If immersed, they might develop leaks or even shatter when returned to room temperature.
    d. When removing vials from liquid nitrogen containers always use safety equipment such as gloves, hoods, face shields etc…

2.7.5 Storage of microbank

Before use, new Microbank was stored at 4˚C or at room temperature. Under these conditions, the Microbank can be used until the date of expiry which shown on the product label. After use, the inoculated Microbank was stored at -70˚C for long time.

2.8 BD Phoenix Automated Microbiology System.

Detection of glycopeptide intermediate or resistant *Staphylococcus aureus* strains by using BD phoenix Automated Microbiology system is a reliable method (64).
A significant number of *Staphylococcus aureus* isolates express resistance to methicillin, leaving vancomycin (VA) as the last line of defense in therapy. The increasing prevalence of VA intermediate strains suggests an alarming trend with respect to viable treatment options. The Phoenix Automated Microbiology System (BD Diagnostic Systems) was used for accuracy in identification and susceptibility testing of glycopeptide intermediate or resistant strains. The overall Phoenix identification results demonstrated a 98% agreement with expected identification. In addition to the results suggest that the Phoenix system can be reliably used to detect resistance of *S. aureus* to glycopeptides (64).

2.8.1 Identification

Identification systems were utilized to obtain the identification (genus and species) of an organism. These systems contain fluorogenic and chromogenic substrates. When the organism comes into contact with the substrates, the organism either reacts with the substrate (positive reaction) or there is no reaction (negative reaction). When the positive and negative reactions are combined, the identification of the organism is determined. This automated system was used at Jamahyria Hospital in Libya and also this instrument is present in Hacettepe Hospital (Figure, 2.10).

2.8.2 Susceptibility

Susceptibility systems were utilized to determine which antimicrobics will be the most effective in treating an organism. The organism is tested against various concentrations of antimicrobics, determining the organism's resistance

(ineffective) or susceptibility (effective) to the antimicrobics. The identification and susceptibility of an organism are the necessary information required by physicians from the laboratory in order to determine patient's treatment.

Figure 2.10 **BD Phoenix Automated Microbiology System**

2.9 Genetic Studies

The genetic studies was done according to QIAGEN protocols for multiplex detection and identification of *Staphylococcus* species and methicillin-resistance gene in a single reaction (StaphPlex Panel), in QIAGEN Laboratories in Turkey . The StaphPlex Panel enables detection of 18 different molecular targets corresponding to 13 different phenotypes Table (2.1). This includes five coagulase negative staphylococci and *S. aureus*. In addition to methicillin-resistance, other common antimicrobial-resistance mechanisms were also detected.

Table (2.1) The detectable phenotypes and the target genes.

Detectable phenotypes	Targeted genes	Target names
Coagulase negative staphylococcus	*tuf*	cons
S. epidermidis	*tuf*	epi
S. haemolyticus	*tuf*	haem
S. hominis	*tuf*	homi
S. lugdunensis	*tuf*	lug
S. simulans	*tuf*	sim
Staphylococcus aureus	*nuc*	nuc
Methicillinresistance	*mecA*	mecA
Community acquired MRSA	*mecA, ccrBIV, pvl*	mecA, ccrBIV, pvl
Hospital acquired MRSA	*mecA, ccrBI-III*	mecA, ccrBI-III
Aminoglycoside resistance	aacA	aacA
MLS resistance	*ermA, ermC*	ermA, ermC
Tetracycline resistance	*tetM, tetK*	tetM, tetK

2.9.1 StaphPlex Principle

The StaphPlex Panel is a product that is capable of amplifying and detecting 18 gene targets, which can identify Gram-positive *staphylococci* bacteria and drug-resistance genes. This provides a rapid and convenient method for differentiating between common potentially drug-resistant *staphylococci.* Table (2.2) lists each *staphylococcus*, its potential for drug resistance, and the designated target symbol.

Table (2.2) StaphPlex pathogen gene targets

Pathogen	Known drug resistance	Target genes	Target symbols
All *Staphylococcus* spp.	Aminoglycosides	*aacA*	aacA
All *Staphylococcus* spp.	β-lactams	*mecA*	mecA
Coagulase-negative staphylococci	–	*tuf*	cons
Staphylococcus aureus	–	*nuc*	nuc
Staphylococcus aureus	MRSA	*ccrBI* *ccrBII* *ccrBIII* *ccrBIV* *pvl*	ccrBI ccrBII ccrBIII ccrBIV pvl
Staphylococcus aureus	Macrolides, lincosamides, and streptogramins resistance	*ermA* *ermC*	ermA ermC
Staphylococcus aureus	All tetracyclines	*tetM*	tetM
Staphylococcus aureus	Tetracyclines, except minocycline	*tetK*	tetK
Staphylococcus epidermidis	–	*tuf*	epi
Staphylococcus haemolyticus	–	*tuf*	haem
Staphylococcus hominis	–	*tuf*	homi
Staphylococcus lugdunensis	–	*tuf*	lug
Staphylococcus simulans	–	*tuf*	sim

2.9.2 Panel Contents and Storage

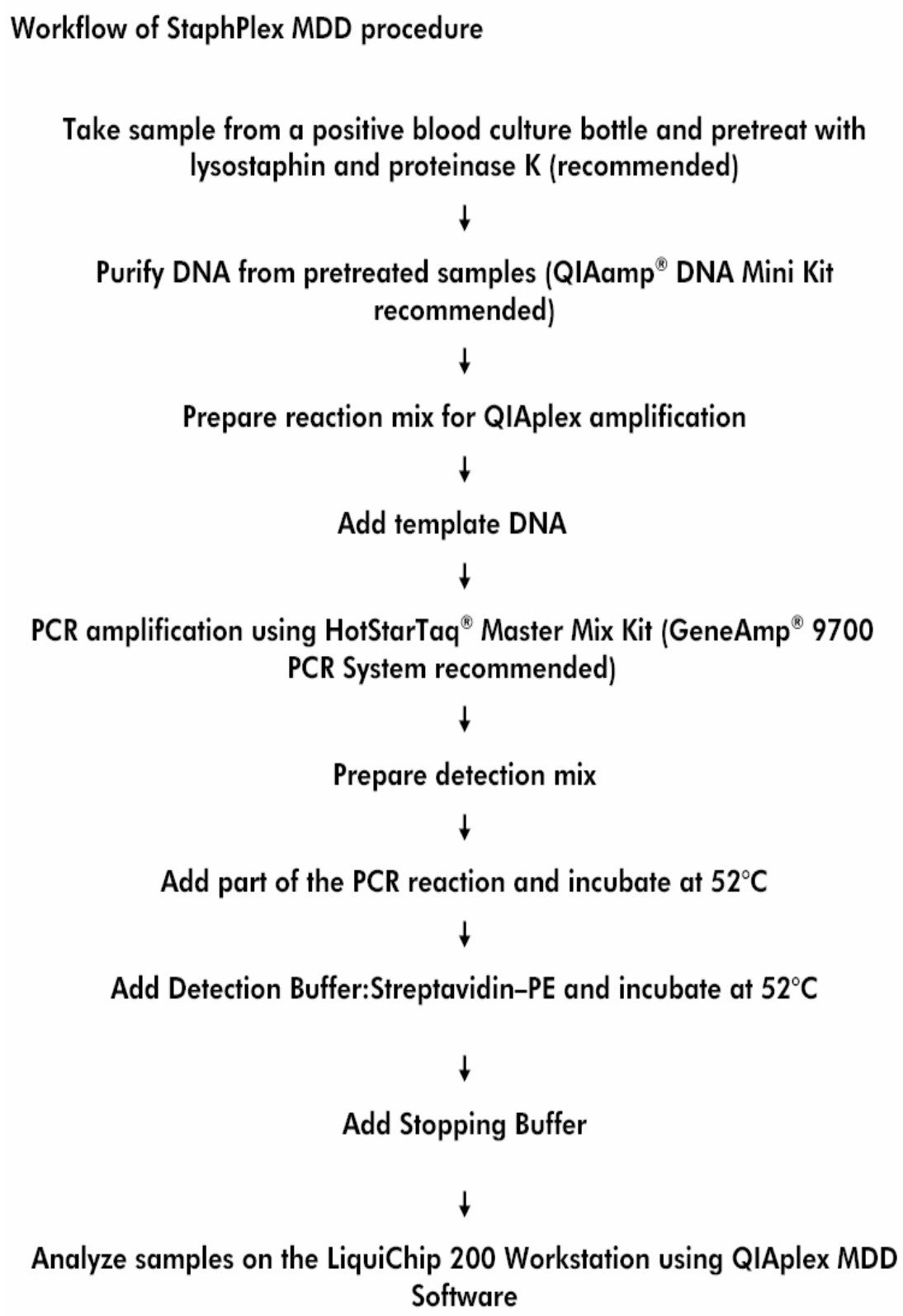
1. StaphPlex SuperPrimers.
2. AmpCheck.
3. StaphPlex Bead Mix.
4. Streptavidin-PE.
5. Detection Buffer.
6. Stopping Buffer.

StaphPlex SuperPrimers and AmpCheck were stored at 2-8°C upon arrival. Also, StaphPlex Bead Mix and Streptavidin-PE were stored in the dark at 2-8°C. Besides, the detection Buffer and the Stopping Buffer were stored at 2-8°C or at room temperature (15-25°C). If the buffers were stored at 2-8°C, they should be warmed to room temperature and mixed thoroughly before use.

2.9.3 Equipments and Reagents

1. HotStarTaq Master Mix Kit.
2. Pipettes and pipette tips.
3. Nuclease-free PCR tubes.
4. GeneAmp 9700 PCR System.
5. Nucleic acid decontaminating solutions.
6. RNase-free water.
7. Microcentrifuge tubes, racks and Vortexer.
8. Heating block or thermal cycler set at 52°C.
9. LiquiChip 200 Workstation (QIAGEN).
10. QIAplex MDD Software.

2.9.4 An overview of the StaphPlex procedure (138).



2.9.5 Protocol 1: PCR Amplification of Staphylococci DNA

This protocol is optimized for use with HotStarTaq Master Mix (QIAGEN) and the GeneAmp 9700 PCR System (Applied Biosystems) running in 9600 emulation mode. QIAGEN has not verified the performance of this protocol using other equipment and reagents.

2.9.5.1 Important points before starting

- A preincubation step with lysostaphin and proteinase K to lyse the rigid multilayered cell wall of Gram-positive bacteria prior to DNA purification is recommended.
- Commonly available DNA purification products should produce adequate DNA yield for this assay. Using the QIAamp DNA Mini Kit (cat. nos. 51304 or 51306) with an elution volume of 50µl is preferred.
- HotStarTaq DNA Polymerase requires an activation step of 15 min at 95°C.
- Set up all the reaction mixtures in an area separated from the one used for DNA preparation or PCR product analysis.
- Used disposable tips containing hydrophobic filters to minimize cross-contamination.

2.9.5.2 Things to do before starting

Before using AmpCheck for the first time, a 1/50 dilution was prepared by adding 245 µl RNase-free water to the tube containing the AmpCheck and then by mixing the solution well.

2.9.5.3 Protocol 1 procedure

HotStarTaq Master Mix, StaphPlex SuperPrimers, and RNase-free water were mixed by vortexing briefly. It was important to mix the solutions before used in order to avoid localized concentrations of salt. Then, the solution was prepared according to Table (2.3). It was not necessary to keep reaction vessels on ice since HotStarTaq DNA Polymerase was inactive at room temperature. The reaction mix typically contained all the components needed for PCR except the template DNA. The solution was prepared with a 10% volume greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included. An additional reaction for amplification of AmpCheck should always be included as an amplification check. HotStarTaq Master Mix was provided as a 2x concentrate (i.e., a 25 µl volume of the HotStarTaq Master Mix was required for amplification reactions with a final volume of 50 µl). After that, the solution was mixed thoroughly and dispensed appropriate volumes into PCR tube and then mixed gently (e.g., by pipetting the reaction mix up and down a few times). It was not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of HotStarTaq DNA Polymerase.

Table (2.3) Composition of reaction mix for QIAplex amplification reaction

Component	Volume/reaction	Final concentration
HotStarTaq Master Mix*	25 µl	2.5 units HotStarTaq DNA Polymerase 1x PCR Buffer† 200 µM of each dNTP
StaphPlex SuperPrimers	6 µl	–
RNase-free water*	14 µl	–
Template DNA (added at step 4)	5 µl	–
Total volume	**50 µl**	–

* Included in the HotStarTaq Master Mix Kit.

† Contains 15 mM $MgCl_2$.

Then, the template DNA was added into the individual tubes containing the reaction mix except the control tube in which RNase-free water was substituted for the DNA sample volume. Also, an amplification check was included with 5 µl AmpCheck (diluted 1/50) instead of template DNA. After that, the thermal cycler was programmed according to the manufacturer's instructions, using the conditions outlined in Table (2.4).

Note: Using the GeneAmp 9700 PCR System (Applied Biosystems) running in 9600 emulation mode is recommended. QIAGEN has not verified the performance of this protocol using other equipment.

Note: Each PCR program must start with an initial heat activation step at 95°C for 15 min.

Table (2.4) Optimized cycling protocol using the geneAmp 9700 PCR System running in 9600 emulation mode

Initial activation step:*	**15 min**	**95°C**
Enrichment cycling		
Denaturation:	30 s	94°C
Annealing:	2 min	55°C
Extension:	1 min	72°C
Number of cycles:	15	
2-step cycling		
Denaturation:	30 s	94°C
Annealing/extension:	1.5 min	70°C
Number of cycles:	6	
3-step cycling		
Denaturation:	20 s	94°C
Annealing:	20 s	55°C
Extension:	30 s	72°C
Number of cycles:	35	
Final extension:	3 min	72°C

* HotStarTaq DNA Polymerase is activated by this heating step.

Finally, the PCR tubes were placed in the thermal cycler and the cycling program was started. After amplification, detection of Amplified *Staphylococci* DNA was done by Protocol 2 (Figure, 2.11 and Figure 2.12).

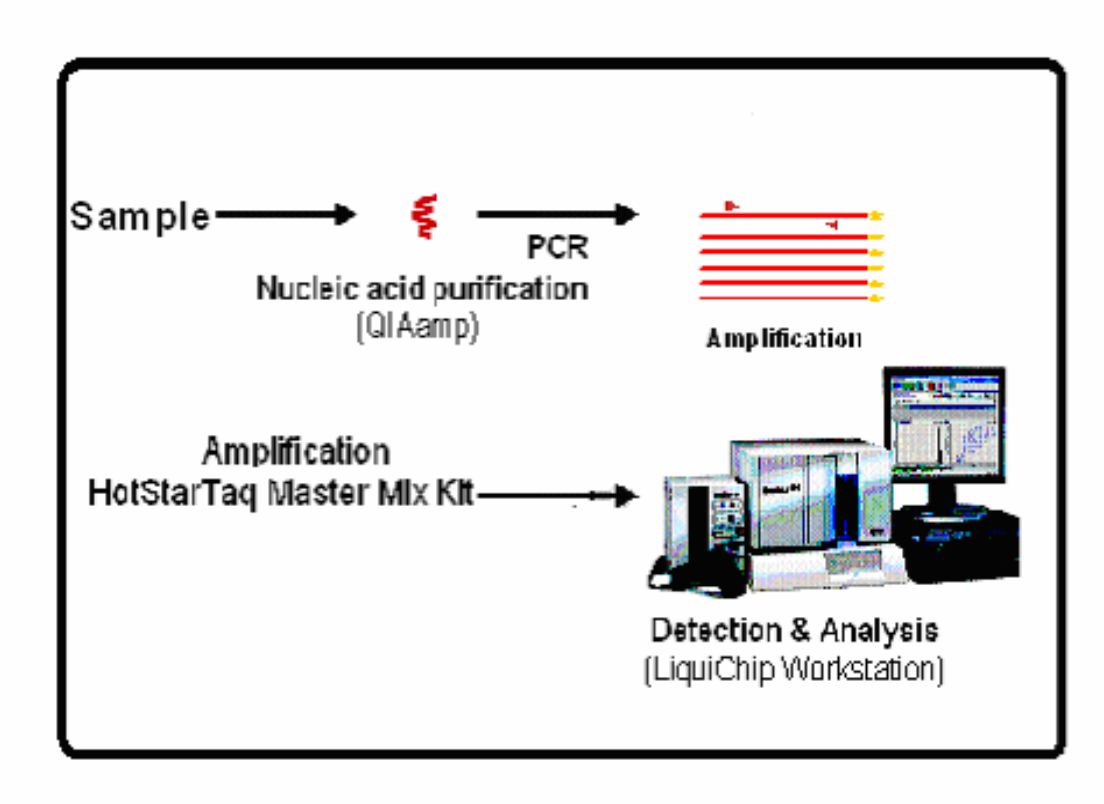


Figure 2.11: Steps from DNA purification until detection and analysis the results (138).

2.9.6 Protocol 2: Detection of Amplified Staphylococci DNA

This protocol is optimized for use with the LiquiChip 200 Workstation (QIAGEN) using QIAplex MDD Software.

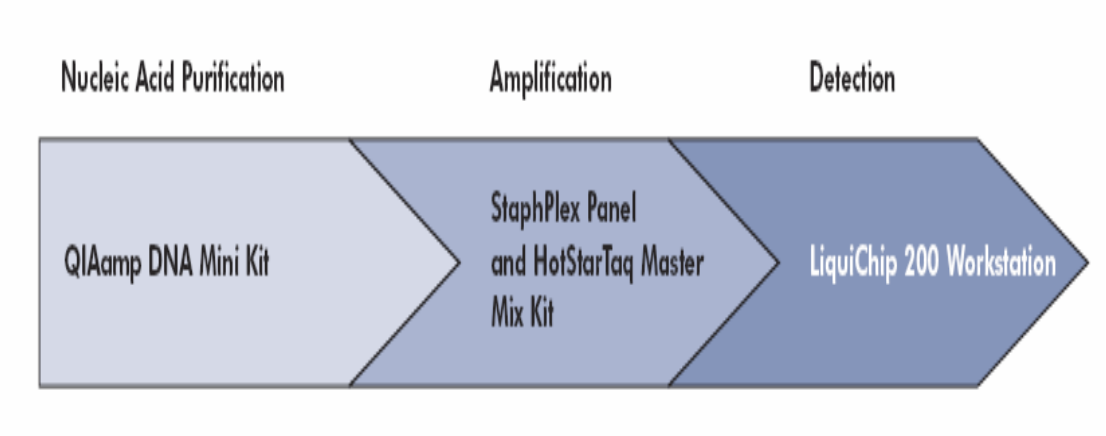


Figure 2.12 StaphPlex panel Workflow (138)

2.9.6.1 Important points before starting

- Set up all reaction mixtures in an area separated from the one used for DNA preparation or PCR setup.
- Used disposable tips containing hydrophobic filters to minimize cross-contamination.
- From the beginning of the hybridization procedure, the detection reaction must be kept at 52°C until analysis on the LiquiChip 200 Workstation.

2.9.6.2 Things to do before starting

Detection Buffer and Stopping Buffer should be brought to room temperature (15-25ºC) in order to use them. Also, heating block or thermal cycler should be heated to 52°C.

2.9.6.3 Protocol 2 procedure

Stopping Buffer were heated to 52°C, and kept at 52°C until use. Then, the StaphPlex Bead Mix was mixed by vortexing for at least 15 seconds without forgetting to protect the StaphPlex Bead Mix and reaction mix from light by foiling the tubes as well as the 96-well plate. Later, a detection mix was prepared according to Table (2.5).

Table (2.5) Composition of detection mix

Component	Volume/reaction
Detection Buffer	35 µl
ResPlex I Bead Mix	10 µl
Total volume	**45 µl**

The detection mix contains all the components needed for the reaction except the PCR products and the diluted Streptavidin-PE. Then a volume of detection mix was prepared 10% greater than that required for the total number of assays to be performed.

After vortexing the reaction thoroughly, the solution was dispensed (45µl) into each well of the 96-well flat-bottom plate and for each assay, the PCR reaction (5 µl from "Protocol 1: PCR Amplification of *Staphylococci* DNA") was added to a sample and mixed thoroughly by pipetting up and down. Afterwards, the samples were incubated at 52°C in the dark for 10 min. During this time, a fresh 1:1 mixture of detection buffer: Streptavidin-PE at room temperature was prepared. The detection buffer was prepared in an enough amounts in order to use 10 µl of the mixture per assay.

For example, 50µl Streptavidin-PE was added to 50µl Detection Buffer. The solution was prepared in a volume 10% greater than that required for the total number of detection assays. After incubation for 10 min in the previous steps, 10 µl Detection Buffer, Streptavidin-PE, was added to each sample and mixed gently by pipetting up and down and then the samples were incubated at 52°C in the dark for 5 min.

Keeping the samples at 52°C, Stopping Buffer (120 µl Prewarmed to 52 C˚) was added to each reaction. After incubation is complete, the samples were analyzed on the LiquiChip 200 Workstation using QIAplex MDD Software Figure (2.13). Alternatively, the Luminex 100 IS System or Luminex 200 System with QIAplex MDD Software can be used

Figure 2.13: LiquiChip 200 Workstation using QIAplex MDD Software (138).

CHAPTER 3

RESULTS

The following tests were carried out within the scope of the thesis:

1) The collection of clinical samples from Hospitals:
    - Turkish hospitals (Hacettepe Hospital and Ankara Hospital).
    - Libyan hospitals (Aljalla Hospital and Jamahyria Hospital).
2) Microbiological identification of *S. aureus* in microbiology laboratory:
    - Culture on media: (Blood agar, Mueller Hinton agar, Nutrient agar and in Nutrient broth).
    - Gram's stain, Catalase test and Coagulase test.
3) Antibiotic susceptibility test:
    - Disk diffusion method.
    - Minimum Inhibitory Concentration (MIC) Etest
4) BD Phoenix Automated Microbiology System
    - Identification
    - Susceptibility
5) Genetics investigations studies.

Genetic investigation studies were done for some isolates from both Turkish hospitals and Libyan hospitals.

3.1 The collection of *S. aureus* from clinical samples in Turkish hospitals

In Turkish hospitals, the clinical samples were collected from both Hacettepe and Ankara Hospitals. Patients were aged between 1 and 84 years; most of them were old in age (Appendix F).

Overall three hundred and six (306) clinical *S. aureus* samples were collected from patients in laboratory departments of both hospitals. Two hundred (200) samples were colleted from Hacettepe Hospital and one hundred and six (106) samples were collected from Ankara Hospital. During the collections of *Staphylococcus aureus* samples from the patients, it was found out that some of the patients were suffering from both *Staphylococcus aureus* and the Coagulase-negative *Staphylococcus* species (CoNS). Different strains of *Staphylococcus aureus* were isolated showing methicillin resistance (MRSA), methicillin susceptibility (MSSA), and multi-resistance.

In Turkish hospitals, *S. aureus* strains were isolated from different clinical samples' sources including pus, wounds, blood, urine, catheter, aspiration, burn and sputum (Table 3.1 below).

Table 3.1: The number of samples according to the sources of collection

Type of Specimens	No. Of Samples	Hacettepe Hospital	Ankara hospital
Pus	108	83	25
Wounds	75	32	43
Blood	51	33	18
Urine	20	15	5
Catheter	19	14	5
Aspiration	15	8	7
Burn	10	9	1
Sputum	8	6	2
Total	306	200	106

From the collected clinical samples from Hacettepe Hospital, 102 out of 200 were MRSA and the rest were MSSA, whereas the collected samples from Ankara Hospital showed that 68 out of 106 were MRSA and the rest were MSSA (Table 3.2).

Table 3.2: MRSA and MSSA results from both Turkish hospitals.

Hospitals	No. Of Samples	MRSA	MSSA
Hacettepe	200	102	98
Ankara	106	68	38

Most of the MRSA samples were isolated from blood, wounds, and pus. The percentage of MRSA in blood, wounds, pus and urine was 66%, 56% 47% and 30% respectively; whereas the percentage of MSSA in blood, wounds, pus and urine was 33%, 44% 53% and 70% respectively in both hospitals (Figure 3.1).

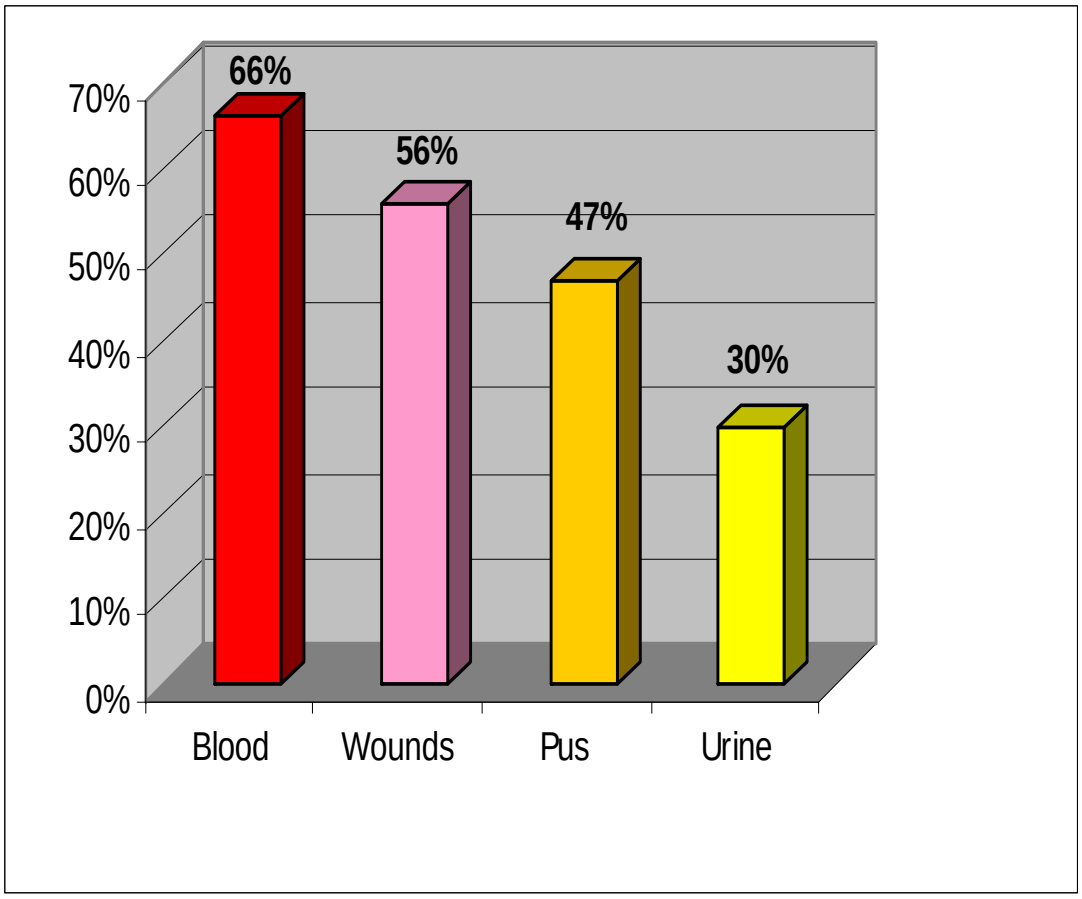


Figure 3. 1: Percentage of MRSA in Turkish hospitals according to clinical samples' sources.

The incidence of MRSA strain was higher in Ankara Hospital (64%) than in Hacettepe hospital (51%), (Figure 3.2).

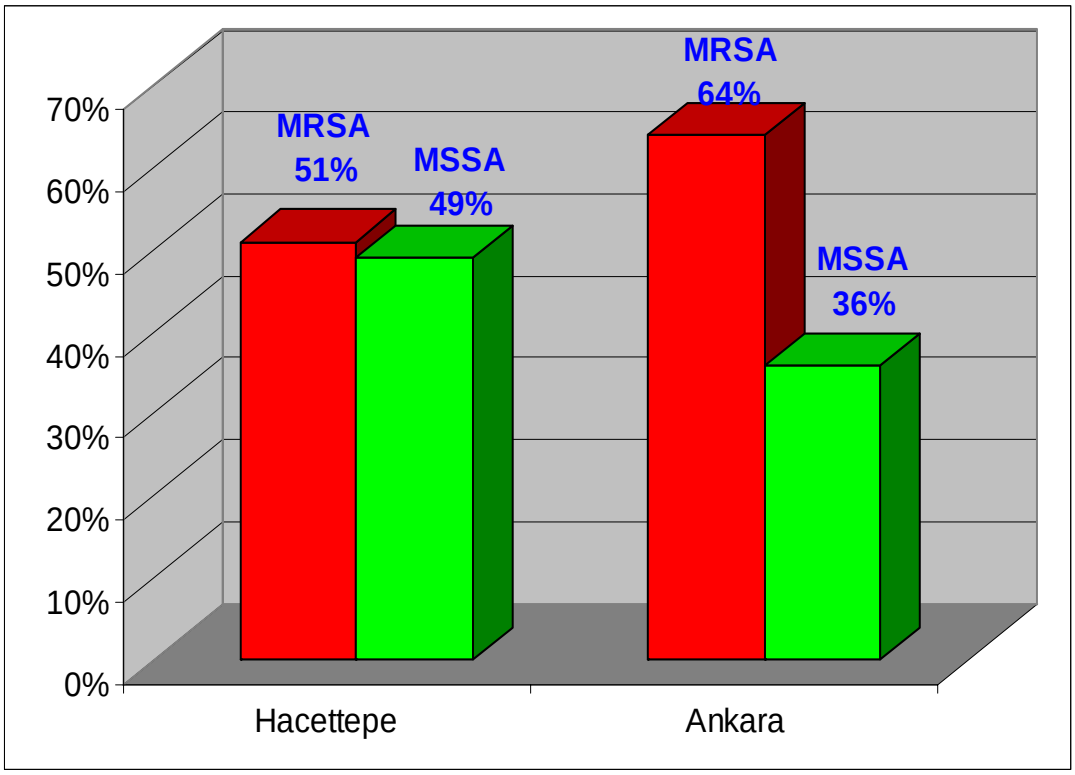


Figure 3.2 Percentages of MRSA and MSSA in both Hospitals

In Hacettepe hospital, the most of samples were colleted from pus 83 samples, blood 33 samples, wounds 32 samples and urine 33 samples, and fewer samples were collected from catheter (14), burn (9), aspiration (8) and sputum (6). The percentages of MRSA and MSSA, according to the kinds of clinical specimens in Hacettepe hospital, are shown in Table 3.3.

Table 3.3: Percentages of MRSA and MSSA according to the types of clinical specimens in Hacettepe hospital

Type of specimens	No. of samples	No. of MRSA	Percent. %	No. of MSSA	Percent. %
Pus	83	43	51%	40	49%
Blood	33	18	55%	15	45%
Wound	32	13	40%	19	60%
Urine	15	5	33%	10	67%
Catheter	14	6	43%	8	57%
Burn	9	4	45%	5	55%
Aspiration	8	6	75%	2	25%
Sputum	6	5	83%	1	17%

In Ankara hospital; most of the samples were collected from wounds 43 samples, pus 25 samples and blood 18 samples, and fewer samples were collected from Aspiration (7), urine (5), catheter (5), sputum (2) and burn (1). It was found that the incidence of MRSA strains were "between" 28 to 89 for the specimens sample size higher than 10 in Ankara Hospital. The specimen samples lower than 10 can be ignored since they may result in miss interpretation of the data due to low sample size (Table 3.4).

Table 3.4: Percentages of MRSA and MSSA according to the types of clinical specimens in Ankara hospital

Type of specimens	No. of samples	No. of MRSA	Percent. %	No. of MSSA	Percent. %
Wound	43	29	77%	14	23%
Pus	25	7	28%	18	72%
Blood	18	16	89%	2	11%
Aspiration	7	3	43%	4	57%
Urine	5	1	20%	4	80%
Catheter	5	5	100%	0	0%
Sputum	2	2	100%	0	0%
Burn	1	0	0%	1	100%

In Turkish hospitals, coagulase-negative *Staphylococcus* was isolated in addition to MRSA strain. Coagulase-negative *Staphylococcus* strain was collected from thirteen patients in both hospitals. In Hacettepe Hospital, 2 out of 4 were methicillin-resistant, while in Ankara Hospital, 5 out of 9 were methicillin-resistant. Unfortunately, seven out of thirteen patients were suffering from both *S. aureus* and coagulase-Negative *Staphylococcus* species. In these patients, we found two strains in one clinical sample and both of them were methicillin-resistant (Table 3.5).

Table 3.5: The number of Coagulase-Negative Staphylococcus samples in Turkish hospitals

Hospitals	No. Of Sample	Methicillin Resistant	Methicillin Susceptible
Hacettepe	4	2	2
Ankara	9	5	4
Total	13	7	6

3.2 Microbiological identification of *S. aureus* in Turkish hospitals

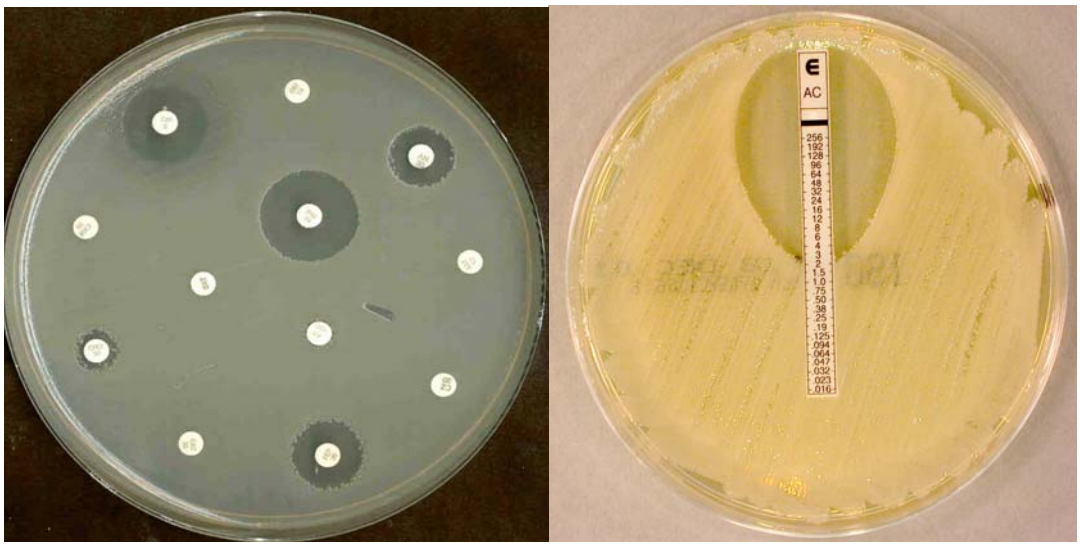
The identification of *S aureus* was performed by inoculating the clinical samples on blood agar media at 37C° for 24 hours for characterization of *S. aureus* on blood agar media such as; golden colonies and beta haemolysis (completely haemolysis of red blood cell on blood agar).

After the inoculation of the clinical samples on media, identification of *S aureus* was confirmed by Gram's stain, catalase test, and coagulase test. In this study, all samples were gram-positive cocci, catalase test positive and coagulase test positive. The catalase test was done in order to differentiate between *Staphylococcus* species and *streptococcus* species, whereas coagulase test was done to distinguish between *S. aureus* and coagulase-negative *Staphylococcus* species. The results of investigation of *S. aureus* on blood agar, Gram's stain, catalase test and coagulase test in Turkish Hospitals are shown in Appendix E.

3.3 Antibiotic susceptibility test in Turkish hospitals

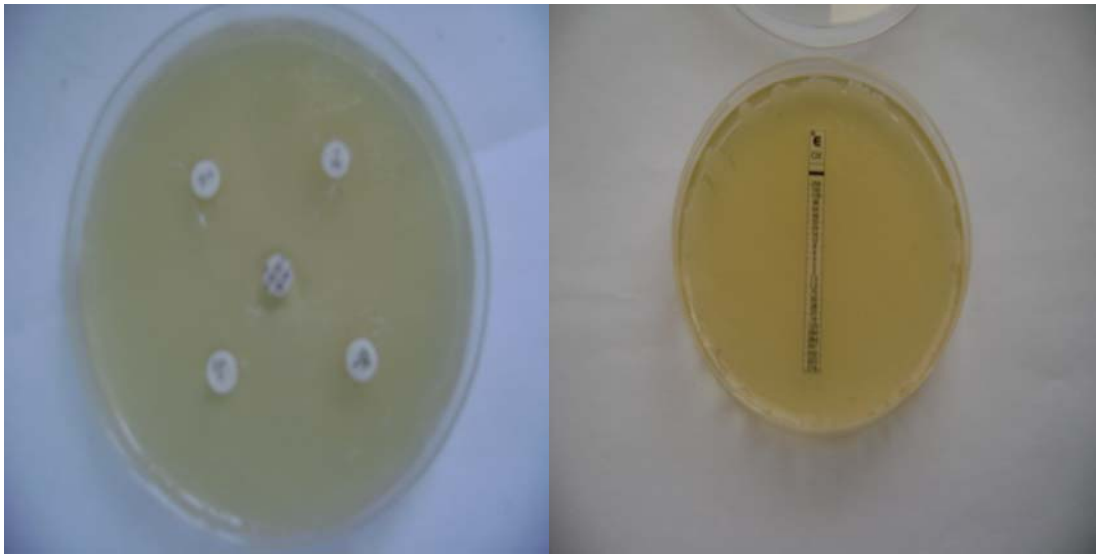
After the identification of *S. aureus,* susceptibility test was done by both Disk diffusion method and Minimum Inhibitory Concentration (MIC) Etest method. In this study, the following antibiotic disks were tested: Oxacillin (Methicillin) (OX 1μg), Vancomycin (V 30μg), Ampicillin (AMP 10μg), Amoxicillin (AMX 10μg) Tetracyclin (TE 30μg), Erythromycin (E 15μg), Gentamycin (G 10μg), Ciprofloxacin (CIP 5μg), Clindamycin (DA 2μg), Chloramphenicol (C 30μg), Cefotetan (CTT 30μg) and Nitrofuranton (F 300μg).

All clinical *S. aureus* samples were investigated by Disk diffusion method and only when MRSA strain was detected, the result were confirmed by Minimum Inhibitory Concentration (MIC) Etest. Moreover, the result of Oxacillin Etest was read after 24 hours and after 48 hours. After that, if the reading is negative, the sample was considered as MSSA strain. Examples of antibiotic susceptibility test results (on Mueller-Hinton agar) are shown in figure 3.3 and figure 3.4.



(a) **(b)**

Figure 3.3: Methicillin-susceptible Staphylococcus aureus (MSSA) strains (a) Disk diffusion method and (b) Etest method



(a) **(b)**

Figure 3.4: Methicillin-resistant Staphylococcus aureus (MRSA) strains (a) Disk diffusion method and (b) Etest method

In Turkish hospitals; the result of antibiotic susceptibility test indicated that all MRSA samples displayed an Oxacillin MIC was ≥256μg/ml and all MRSA samples were resistant to ampicillin and amoxicillin. In addition to that, the results illustrated that the majority of the isolates tested were resistant to ampicillin, amoxicillin, and tetracycline, while half of the *S. aureus* samples were resistant to erythromycin, ciprofloxacin, and gentamicin. The other isolates tested were resistant to clindamycin, cefotetan, chloramphenicol and vancomycin. All isolates were susceptible to nitrofurantoin.

Most MRSA samples were multiresistant to more than five classes of antibiotics including; Ampicillin, Amoxicillin, Tetracycline, Erythromycin and Ciprofloxacin. The results in this study indicated that the MRSA strains were (56%), while the MSSA strains were (44%). In addition to that the incidence of the Vancomycin-resistant *Staphylococcus aureus* strain (VRSA) and Vancomycin intermediately susceptible *Staphylococcus aureus* (VISA) was (2.2%) and (0%) respectively. The percentages of antibiotics resistant, susceptible and intermediate in Turkish hospitals are shown in Table 3.6.

Table 3.6: Percentages of antibiotics resistant, susceptible and intermediate in Turkish hospitals

Antibiotics types (Dosages)	Resistant (%)	Susceptible (%)	Intermediate (%)
Oxacillin, (OX: 1µg)	**56%**	**44%**	--
Ampicillin, (AMP: 10µg)	80.7%	19.3%	--
Amoxicillin,(AMX:10µg)	71.8%	28.2%	--
Tetracycline, (TE: 30µg)	53.2%	46.8%	--
Erythromycin, (E: 15µg)	49.3%	50.7%	--
Ciprofloxacin, (CIP: 5µg)	46.4%	53.6%	--
Gentamycin, (G: 10µg)	41.6%	58.4%	--
Clindamycin, (DA: 2µg)	30.7%	69.3%	--
Cefotetan, (CTT: 30µg)	14.7%	85.3%	--
Chloramphenicol, (C: 30µg)	13.7%	86.3%	--
Nitrofurantoin, (F: 300µg)	0.0%	100%	--
Vancomycin, (V: 30µg)	**2.2%**	**97.8%**	--

3.4 The collection of *S. aureus* from clinical samples in Libyan hospitals

In Libyan hospitals, the clinical samples were collected from both Aljalla and Jamahiriya Hospitals. Patients were aged between 3 days and 80 years most of them were in young age (Appendix F).

Overall one hundred and fifty (150) clinical *S. aureus* samples were collected from patients in laboratory departments in both hospitals. Eighty eight (88) samples were collected from Aljalla Hospital and sixty two (62) samples were collected from Jamahyria Hospital. During the collections of *Staphylococcus aureus* samples from the patients, it was found out that some of the patients were suffering from both methicillin-resistance *Staphylococcus aureus* (MRSA) strain and Vancomycin-resistance *Staphylococcus aureus* (VRSA) strain. Different strains of *Staphylococcus aureus* were isolated showing methicillin resistance (MRSA), methicillin susceptibility (MSSA), and multi-resistance.

In Libyan hospitals, *S. aureus* strains were isolated from different clinical samples' sources including pus, wounds, blood, urine, aspiration, sputum, burn, and semen (Table 3.7 below).

Table 3.7: The number of samples according to the sources of collection

Type of Specimens	No. Of Samples	Aljalla Hospital	Jamahiriya hospital
Pus	58	41	17
Wound	34	21	13
Blood	21	9	12
Urine	15	4	11
Aspiration	10	8	2
Sputum	6	2	4
Burn	5	3	2
Semen	1	0	1
Total	150	88	62

From the collected clinical samples from Aljalla Hospital, 52 out of 88 were MRSA and the rest were MSSA, whereas the collected samples from Jamahiriya Hospital showed that 38 out of 62 were MRSA and the rest were MSSA (Table 3.8 below.).

Table 3.8: The number of clinical samples in Libyan hospitals.

Hospitals	No. Of Samples	MRSA	MSSA
Aljalla Hospital	88	52	36
Jamahyria Hospital	62	38	24

Most of the MRSA samples were isolated from blood, wounds, and pus. The percentage of MRSA in blood, wounds, pus and urine was 71%, 62% 57% and 53% respectively; whereas the percentage of MSSA in blood, wounds, pus and urine was 29%, 38% 43% and 47% respectively in both hospitals (Figure 3.5).

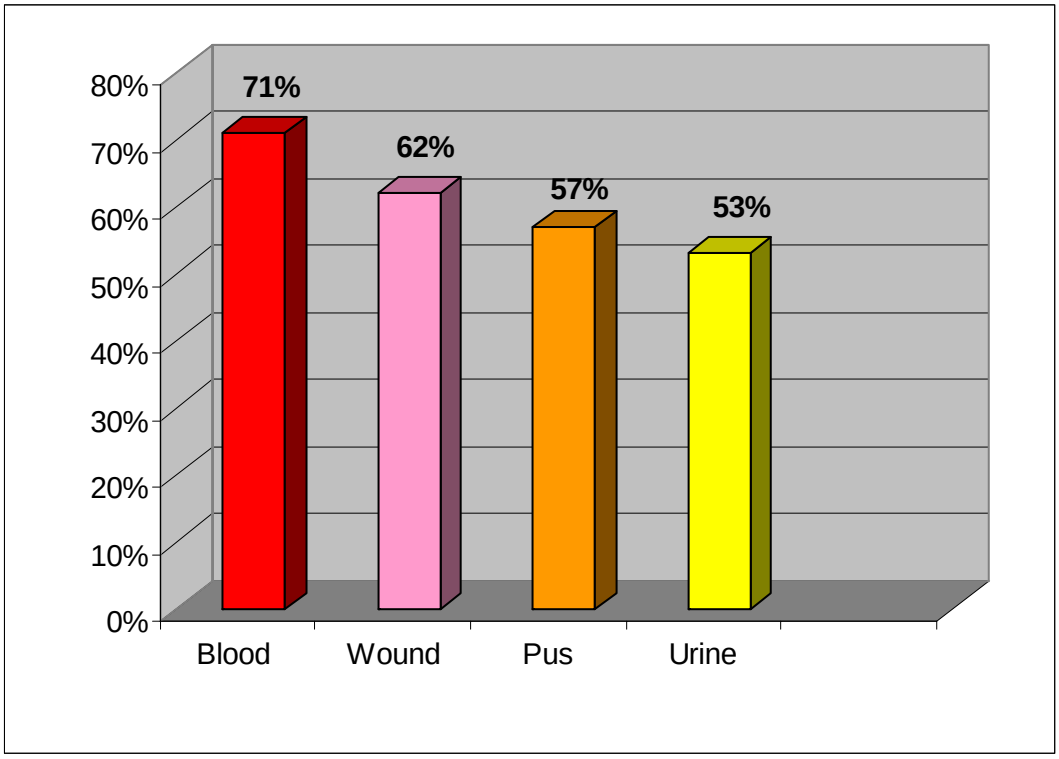


Figure 3. 5: The percentage of MRSA in Libyan hospitals according to clinical samples source.

The incidence of MRSA strain was high in both hospitals; Aljalla Hospital (56%) and Jamahiriya Hospital (62%), (Figure 3.6).

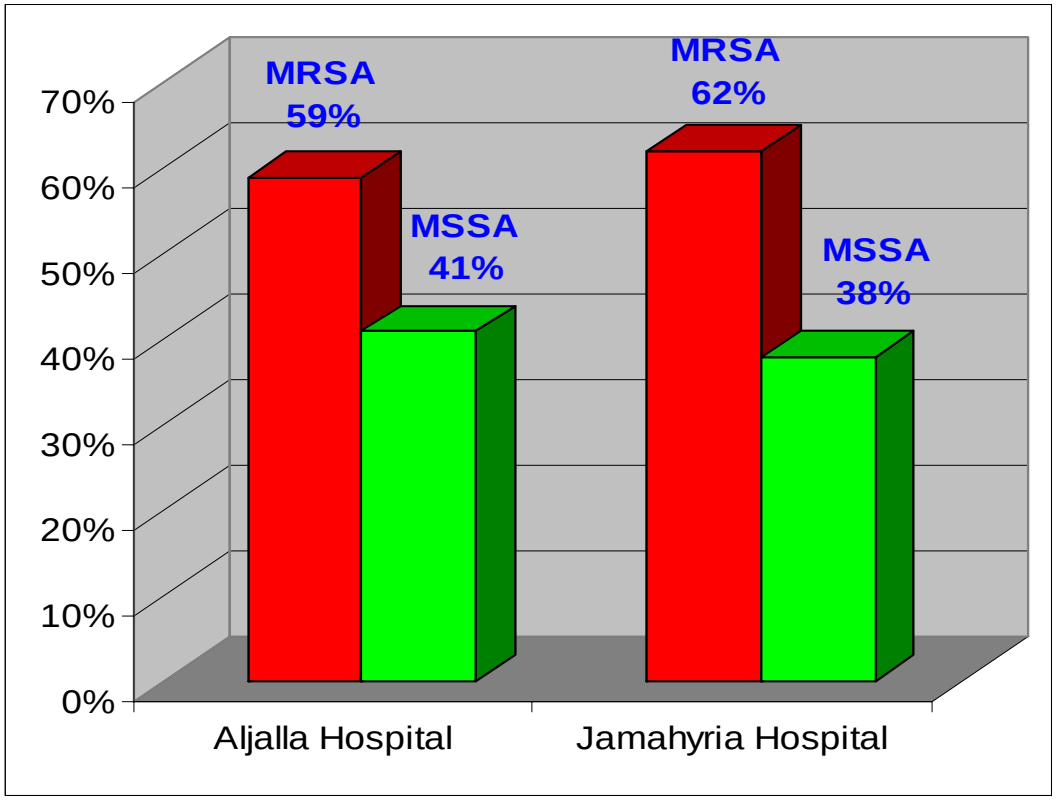


Figure 3.6: Percentages of MRSA and MSSA in both Hospitals

In Aljalla Hospital, most of the samples were collected from pus 41 samples, wounds 21 samples, blood 9 samples, aspiration 8 samples and fewer samples were collected from urine (4), burn (3), sputum (2) and semen (0). The percentages of MRSA and MSSA, according to the kinds of clinical specimens in Aljalla hospital, are shown in Table 3.9.

Table 3.9: The Percentages of MRSA and MSSA according to the types of clinical specimens in Aljalla hospital

Type of specimens	No. of samples	No. of MRSA	Percent. %	No. of MSSA	Percent. %
Pus	41	23	56 %	18	44 %
Wound	21	13	62 %	8	38 %
Blood	9	6	67%	3	33 %
Aspiration	8	5	63 %	3	37 %
Urine	4	2	50 %	2	50 %
Burn	3	1	33 %	2	67 %
Sputum	2	2	100 %	0	0 %
Semen	0	0	0 %	0	0 %

In Jamahiriya Hospital; most of the samples were collected from pus 17 samples, wounds 13 samples and blood 12 samples, and urine 11 samples and fewer samples were collected from sputum (4), burn (2), aspiration (2) and semen (1). It was found that the incidence of MRSA strains were "between" 56 to 67 for the specimens sample size higher than 8 in Jamahiriya Hospital. The specimen samples lower than 5 can be ignored since they may result in miss interpretation of the data due to low sample size (Table 3.10).

Table 3.10: The Percentages of MRSA and MSSA according to the types of clinical specimens in Jamahyria hospital

Type of specimens	No. of samples	No. of MRSA	Percent. %	No. of MSSA	Percent. %
Pus	17	10	59 %	7	41 %
Wound	13	8	61 %	5	39 %
Blood	12	9	75 %	3	25 %
Urine	11	6	55 %	5	45 %
Sputum	4	2	50 %	2	50 %
Burn	2	1	50 %	1	50 %
Aspiration	2	1	50 %	1	50 %
Semen	1	1	100 %	0	0 %

3.5 Microbiological identification of *S. aureus* in Libyan hospitals

The identification of *S aureus* was performed by inoculating the clinical samples on blood agar media at 37C˚ for 24 hours for characterization of *S. aureus* on blood agar media such as; golden colonies and beta haemolysis (completely haemolysis of red blood cell on blood agar). After the inoculation of the clinical samples on media, identification of *S aureus* was confirmed by Gram's stain, Catalase test, and Coagulase test.

In this study, all samples were gram-positive cocci, catalase test positive and coagulase test positive. The catalase test was done in order to differentiate between *Staphylococcus* species and *streptococcus* species, whereas coagulase test was done to distinguish between *S. aureus* and coagulase-negative *Staphylococcus* species. The results of investigation of *S. aureus* on blood agar, Gram's stain, catalase test and coagulase test in Libyan Hospitals are shown in Appendix E.

3.6 Antibiotic susceptibility test in Libyan hospitals

After the identification of *S. aureus,* susceptibility tests were done by both Disk diffusion method and Minimum Inhibitory Concentration (MIC) Etest method. In this study, the following antibiotic disks were tested: Oxacillin (Methicillin) (OX 1µg), Vancomycin (V 30µg), Ampicillin (AMP 10µg), Amoxicillin (AMX 10µg) Tetracyclin (TE 30µg), Erythromycin (E 15µg), Gentamycin (G 10µg), Ciprofloxacin (CIP 5µg), Clindamycin (DA 2µg), Chloramphenicol (C 30µg), Cefotetan (CTT 30µg) and Nitrofuranton (F 300µg).

All clinical *S. aureus* samples were investigated by Disk diffusion method and only when MRSA strain was detected, the result were confirmed by Minimum Inhibitory Concentration (MIC) Etest. Moreover, the result of Oxacillin Etest was read after 24 hours and after 48 hours. After that, if the reading is negative, the sample was considered as MSSA strain. Examples of antibiotic susceptibility test results (on Mueller-Hinton agar) are shown in figure 3.3 and figure 3.4.

In Libyan hospitals, the result of Antibiotic susceptibility test indicated that all MRSA samples displayed an Oxacillin MIC of ≥256μg/ml. The majority MRSA samples were multiresistant to more than one class of antibiotics. The results of antimicrobial susceptibility test indicate that the MRSA strains were high (59%) in both hospitals, while the MSSA strains were (41%). All MRSA strains were resistant to ampicillin and amoxicillin. Besides, the results showed that the majority of the isolated samples were resistant to Ampicillin, Amoxicillin. Moreover some of the strains were resistant to Ciprofloxacin and Gentamycin, Clindamycin, Cefotetan, Chloramphenicol and most of isolated strains were susceptible to Nitrofurantoin.

Most MRSA samples were multiresistant to more than five classes of antibiotics including; Ampicillin, Amoxicillin, Tetracycline, Erythromycin and Ciprofloxacin. In addition to that the incidence of the Vancomycin-resistant *Staphylococcus aureus* strain (VRSA) and Vancomycin intermediately susceptible *Staphylococcus aureus* (VISA) were quite high. The percentages of antibiotics resistant, susceptible and intermediate in Libyan hospitals are shown in Table 3.11.

Table 3.11: The percentage of antibiotics resistant susceptible and intermediate in Libyan hospitals

Antibiotics types (Dosages)	Resistant (%)	Susceptible (%)	Intermediate (%)
Oxacillin, (OX: 1µg)	**59%**	**41%**	--
Ampicillin, (AMP: 10µg)	90%	10%	--
Amoxicillin,(AMX:10µg)	86.7%	13.3%	--
Tetracycline, (TE: 30µg)	**16.7%**	**77.6%**	**5.7%**
Erythromycin, (E: 15µg)	**34.6%**	**58.1%**	**7.3%**
Ciprofloxacin, (CIP: 5µg)	36%	64%	--
Gentamycin, (G: 10µg)	27.3%	72.7%	--
Clindamycin, (DA: 2µg)	34.6%	65.4%	--
Cefotetan, (CTT: 30µg)	26.7%	73.3%	--
Chloramphenicol, (C: 30µg)	20%	80%	--
Nitrofurantoin, (F: 300µg)	1.3%	98.7%	--
Vancomycin, (V: 30µg)	**7%**	**67%**	**26%**

In Libyan hospitals, the percentages of the Vancomycin-resistance *Staphylococcus aureus* (VRSA) strain and Vancomycin-intermediately *Staphylococcus aureus* (VISA) in both hospitals were 7% and 26% respectively. In addition to that, the percentages of the Tetracyclin resistant strain, tetracycline Intermediate strain, Erythromycin resistant strain and erythromycin Intermediate strain in Libyan hospitals were 16.7%, 5.7%, 34.6% and 7.3% respectively. The percentages of the resistant, intermediate and susceptible for Vancomycin, Tetracyclin, and Erythromycin in Libyan patients are shown in Table 3.12.

Table 3.12: The percentage of the VISA strain in Libyan hospitals

Antibiotics types (Dosages)	**Resistant (%)**	**Intermediate (%)**	**Susceptible (%)**
Vancomycin, (V: 30µg)	7%	**26%**	67%
Tetracycline, (TE: 30µg).	16.7%	**5.7%**	77.6%
Erythromycin, (E: 15µg).	34.6%	**7.3%**	58.1%

In both countries, the majority MRSA strains were multiresistant to more than five classes of antibiotics including; Ampicillin, Amoxicillin, Tetracycline, Erythromycin and Ciprofloxacin. Moreover, most of the MRSA samples were isolated from blood, wounds, and pus with 68%, 57% and 50% respectively. The results of this study indicated that the incidence of MRSA strain was (56%), and the MSSA strain was (44%) in Turkish hospitals (Hacettepe Hospital and Ankara Hospital in Ankara city), whereas the prevalence of MRSA strain was (59%), and the MSSA strain was (41%) in Libyan hospitals (Aljalla Hospital and Jamahyria Hospital in Benghazi city). The percentages of MRSA and MSSA in Turkish hospitals and Libyan hospitals are shown in Figure 3.7.

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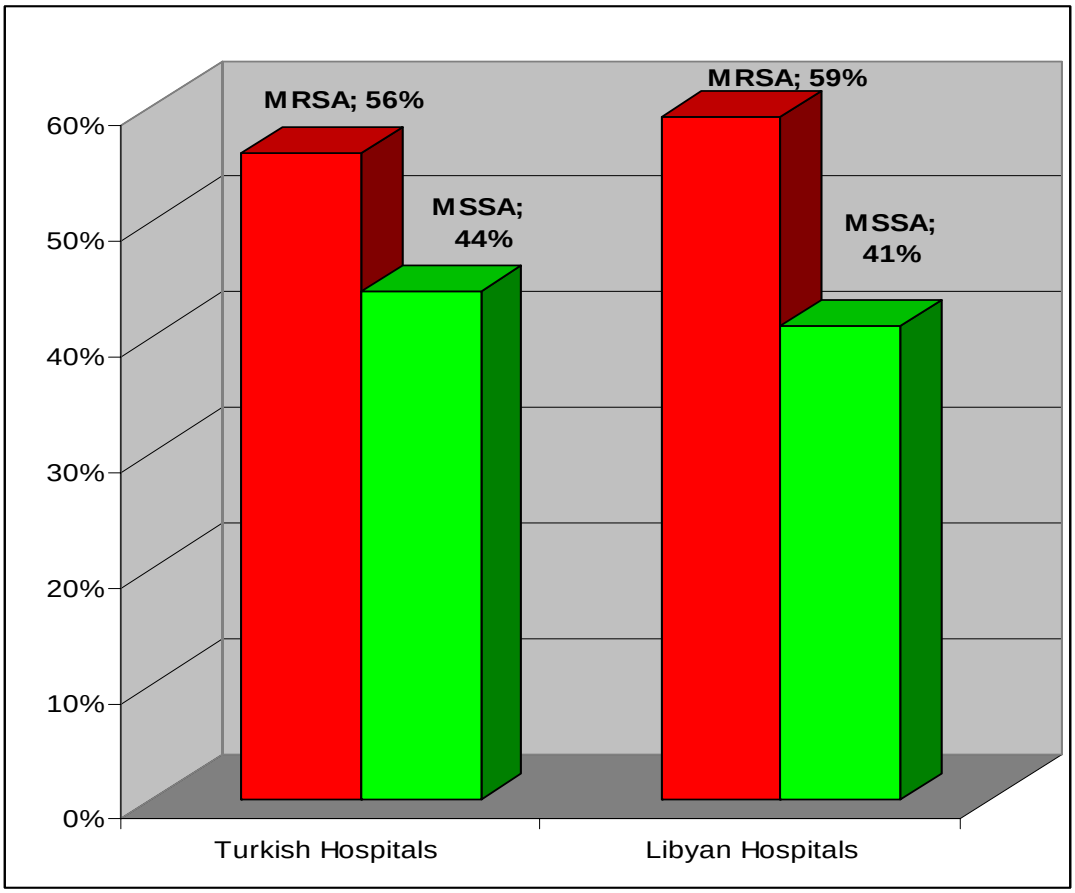

Figure 3.7: The Percentages of MRSA and MSSA In both countries

3.7 Genetics Studies

Genetic investigation studies were done for 30 samples from Turkish hospitals and 20 samples from Libyan hospitals. Because of the high cost of the genetic identification per sample, we had to decrease the number of the samples to be tested from 450 to only 50 (30 Turkish patients and 20 Libyan patients).

The StaphPlex system used for genetic investigation of isolates consists of three components: specimen processing and nucleic acid extraction, 18-pelex PCR amplification, and amplification product detection by analyzing the samples on the LiquiChip 200 Workstation using QIAplex MDD Software. In this computer system, the intensity of the median fluorescence is the major step to ensure the presence of the genes causing the resistance response. Whenever the value of the intensity is 250 or more for a certain gene, it means that this gene is responsible for the resistance response in the sample. The detectable phenotypes, target genes and target names are present in Table 3.13. The intensities of the resistance genes found in our samples are shown as green colour in Tables 3.14 and 3.15 and the results of positive and negative genes are given in Appendix D.

Table 3.13: The detectable phenotypes and Target genes

NO.	Detectable phenotypes	Target genes	Target names
1	**Methicillin resistance**	mecA	mecA
2	**Hospitals acquired MRSA**	ccrB-I	ccrB-I
3	**Hospitals acquired MRSA**	ccrB-II	ccrB-II
4	**Hospitals acquired MRSA**	ccrB-III	ccrB-III
5	**Community acquired MRSA**	ccrB-IV	ccrB-IV
6	**Panton-Valentine Leukocidin**	PVL	PVL
7	**Tetracycline resistance**	tetM	tetM
8	**Tetracycline resistance**	tetK	tetK
9	**Coagulase-negative *staphylococcus***	tuf	cons
10	***S. epidermidis***	tuf	epi
11	***S. haemolyticus***	tuf	haem
12	***S. haminis***	tuf	hami
13	***S. lugdunensis***	tuf	lug
14	***S. aureus***	tuf	nuc
15	***S. simulans***	tuf	sim
16	**MLS resistance**	ermA	ermA
17	**MLS resistance**	ermC	ermC
18	**Aminoglycoside resistance**	aacA	aacA

Table 3.14: The results of genetic investigations studies in Turkish hospitals

Sample No	nuc	mecA	ccr-BI	ccr-BII	homi	sim	ccr-BIII	ccr-BIV	aacA	ermA	ermC	lug	tetM	tetK	epi	PVL	CoNS	haem
1	317	281	102	128	103	154	208	167	130	191	158	126	266	108	125	151	156	104
2	331	303	115	130	149	139	229	144	148	200	171	142	293	119	118	169	150	143
3	151	277	115	124	149	132	193	116	144	147	156	142	305	152	172	151	259	179
4	327	314	131	121	149	150	191	144	134	156	202	158	232	153	139	160	215	151
5	333	326	143	140	171	202	238	132	148	190	182	180	289	142	144	159	166	138
6	299	295	105	148	131	131	231	121	119	161	212	140	233	100	112	132	148	101
7	298	142	97	118	129	134	141	116	109	123	132	144	295	97	128	252	133	99
8	315	277	104	113	115	158	214	108	124	210	135	120	290	125	102	137	166	106
9	327	123	112	106	128	162	142	124	111	111	152	137	154	105	134	140	138	117
10	290	250	91	113	121	129	187	107	93	133	205	110	183	93	114	160	237	120
11	279	224	97	110	107	120	169	99	90	114	122	94	260	81	84	131	119	97
12	280	273	65	87	98	116	138	106	114	128	132	102	185	95	95	130	209	164
13	283	260	66	110	103	94	166	100	80	125	113	92	224	88	108	103	86	62
14	259	122	82	104	124	135	139	137	104	96	120	110	93	129	90	109	123	104
15	252	120	67	69	70	81	106	74	75	72	92	79	102	77	75	77	121	82
Negative	32	41	40	41	43	49	47	47	44	40	41	46	42	47	41	44	40	44

Table 3.14: The results of genetic investigations studies in Turkish hospitals (continued)

Sample No	nuc	mecA	ccr-BI	ccr-BII	homi	sim	ccr-BIII	ccr-BIV	aacA	ermA	ermC	lug	tetM	tetK	epi	PVL	CoNS	haem
16	263	233	63	79	80	87	124	77	87	107	98	80	197	77	74	104	89	73
17	289	258	82	87	144	129	164	118	100	148	222	116	220	105	123	111	123	117
18	334	158	63	64	72	68	120	81	66	89	82	66	139	62	66	96	70	66
19	312	150	60	64	64	74	92	83	69	98	91	77	135	67	74	87	71	59
20	253	241	96	80	97	88	156	91	100	107	177	101	212	89	93	116	98	76
21	256	263	51	58	70	79	118	70	55	98	73	60	185	37	56	60	74	49
22	361	549	49	48	51	50	239	46	40	170	66	47	482	42	39	47	52	44
23	481	750	48	71	64	64	59	368	55	76	75	57	55	52	50	67	66	49
24	450	726	57	55	42	54	56	394	49	50	178	45	43	58	43	50	64	48
25	375	682	46	53	49	52	79	361	47	72	174	48	96	39	45	62	74	51
26	329	94	46	56	54	68	73	53	47	58	69	52	471	483	55	164	56	52
27	295	150	27	53	44	55	55	66	50	48	97	47	751	504	48	105	53	49
28	317	499	46	51	52	55	239	58	44	150	171	39	466	67	38	45	59	51
29	257	217	16	11	5	25	80	29	12	41	64	15	483	32	14	13	23	21
30	315	364	39	59	49	61	148	64	51	113	79	69	306	45	40	58	53	51
Negative	32	41	40	41	43	49	47	47	44	40	41	46	42	47	41	44	40	44

Table 3.15: The results of genetic investigations studies in Libyan hospitals

Sample No	nuc	mecA	ccr-BI	ccr-BII	homi	sim	ccr-BIII	ccr-BIV	aacA	ermA	ermC	lug	tetM	tetK	epi	PVL	CoNS	haem
1	325	171	88	87	94	88	81	123	74	79	101	81	71	78	79	89	86	76
2	287	223	65	70	78	81	133	88	70	140	100	85	172	82	75	95	86	63
3	327	207	74	79	80	92	105	134	71	83	91	69	64	83	80	86	142	76
4	287	262	111	97	119	79	124	156	87	59	118	108	86	97	84	363	111	93
5	296	277	73	92	76	100	92	185	70	77	120	103	73	108	83	348	98	87
6	312	324	26	42	19	33	25	165	24	27	26	28	42	59	35	624	35	29
7	253	488	45	52	49	51	50	223	40	48	57	56	45	48	44	754	50	65
8	305	316	41	54	48	51	44	155	39	34	47	44	47	65	45	727	53	42
9	284	265	47	48	35	46	102	50	43	119	50	47	241	44	36	57	47	47
10	304	37	43	33	39	31	43	50	35	33	58	39	36	50	49	42	41	38
11	386	241	41	38	39	42	44	128	39	41	42	38	39	56	44	713	37	36
12	323	343	31	43	29	48	42	177	36	44	43	38	48	64	43	669	47	37
13	380	47	31	40	33	59	43	51	30	42	38	31	41	46	40	64	60	38
14	270	414	44	47	27	54	39	240	29	40	38	37	40	46	44	734	51	39
15	398	616	45	61	50	70	48	298	51	48	48	43	62	56	53	1049	61	54
16	279	537	52	56	39	39	42	223	41	45	51	46	39	94	40	922	48	47
17	271	509	51	57	38	48	56	236	38	33	64	50	52	104	53	997	56	53
18	342	523	54	59	50	59	68	248	59	60	67	48	53	107	50	661	224	46
19	333	93	42	57	55	51	59	80	40	48	66	42	52	41	48	805	71	46
20	304	484	52	57	53	63	51	227	43	62	63	52	65	116	55	772	82	63
Negative	13	15	11	24	14	20	14	29	7	20	14	10	29	25	24	13	10	13

In Turkish hospitals, the result of the genetic investigation studies has shown that the mecA gene is present as one of the resistant genes in eighteen isolates (60%). Besides, three isolates (10%) carry the community acquired MRSA type IV (ccr-BIV) gene. It worth's mentioning that the hospital acquired MRSA types (ccr-BI, II, III) genes were not detected in any of isolated studied. The majority of the Turkish hospitals' samples carried Tetracycline resistance genes with (43%) for tetM gene and two (7%) for tetK gene, it was also observed that only one isolate carried PVL gene (3%) (Figure 3.8.). It is of great importance to mention that in some of the isolates the multiresistant effect was observed due to the presence of more than one positive gene in isolates. For example, in addition to the Methicillin resistance genes, the incidence of Tetracycline resistance gene was quite high (tetM and tetK 50%) in Turkish hospital isolates. So, these samples show resistance to both Methicillin and Tetracycline and in turn considered as multiresistant strains. Another example of multiresistant we came across in our study, is the existence of one sample (No. 3) carrying mecA gene, Tetracycline resistance gene (tetM), and coagulase-negative staphylococcus gene (CoNS), (Figure 3.9).

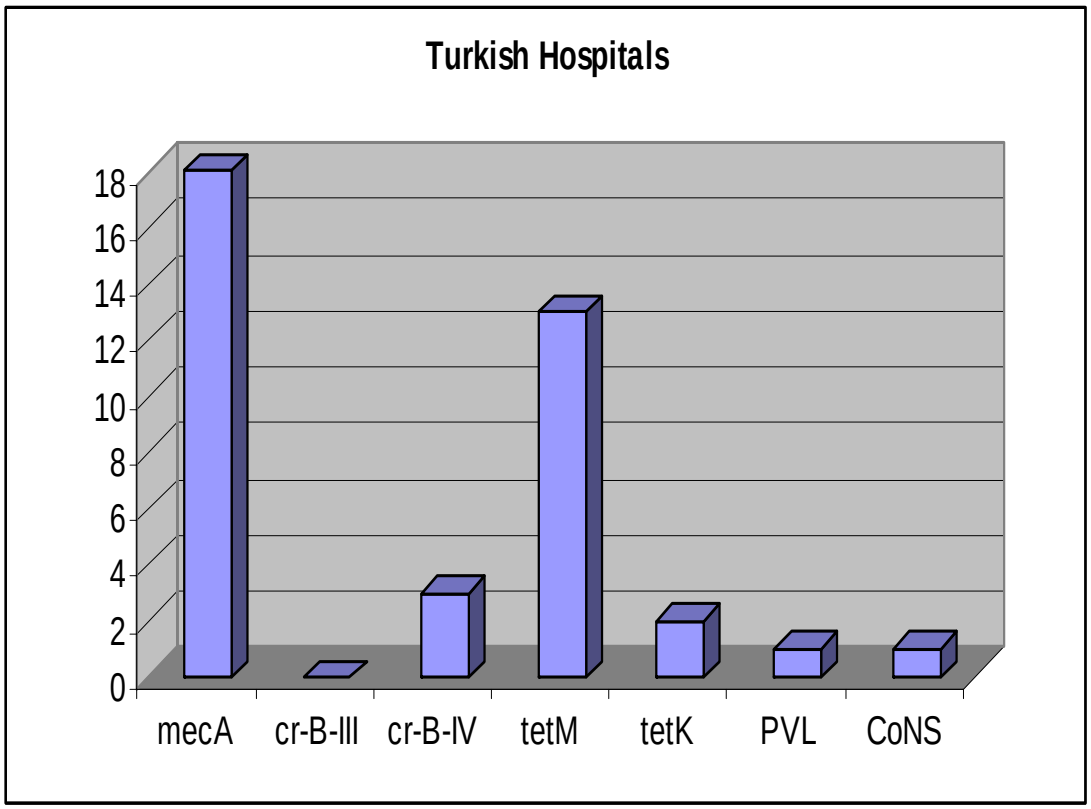

Figure 3.8: Resistance genes in Turkish hospitals samples

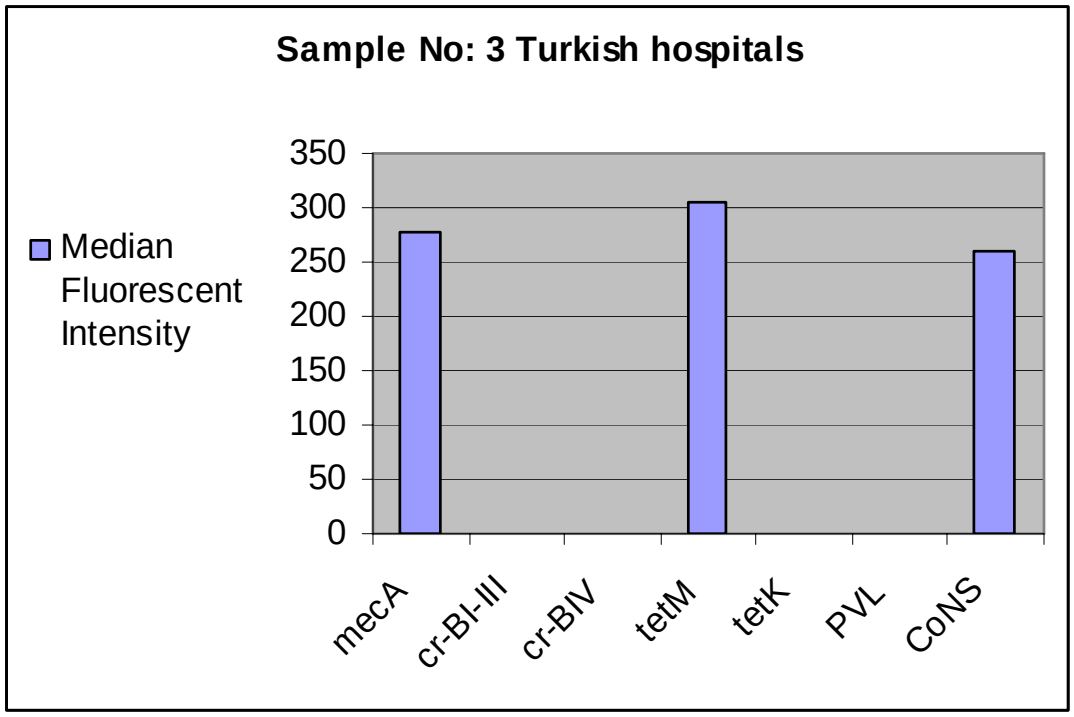

Figure 3.9: Result of genetic studies in sample No. 3 (Turkish Hospitals)

In Libyan hospitals, the result of the genetic investigation studies has also shown that the mecA gene is the primary resistance gene found in Libyan isolates (65%). Besides, there was one isolate out of twenty (5%) that carried the community acquired MRSA type IV (ccr-BIV) gene. It worth's mentioning that the hospital acquired MRSA types (ccr-BI, II, III) genes were not detected in any of the twenty isolates. The majority of the Libyan hospitals' isolates carried Panton-Valentine Leukocidin (PVL) genes with a result of fourteen out of twenty isolates (70%). Also, it was shown that not even one isolate carried the Tetracycline resistant genes (tetM and tetK) in both hospitals. The results of these genes are shown below in table 3.16 and figure 3.10. As we said before, some isolates may contain more than one resistance gene and show multiresistant effects. An example from the Libyan samples (No.15) is the presence of both resistance mecA gene and resistance ccr-BIV gene which is accompanied by the presence of Panton-Valentine Leukocidin (PVL) gene as shown in figure 3.11 below.

Table 3.16: The number of positive genes in Libyan hospitals

Results	mecA	ccr-B I,II,III	ccr-B IV	tet M	tet K	PVL	CoNS
Positive samples	**13**	0	**1**	0	0	**14**	0
Negative samples	7	20	19	0	0	6	20
Total	20	20	20	20	20	20	20

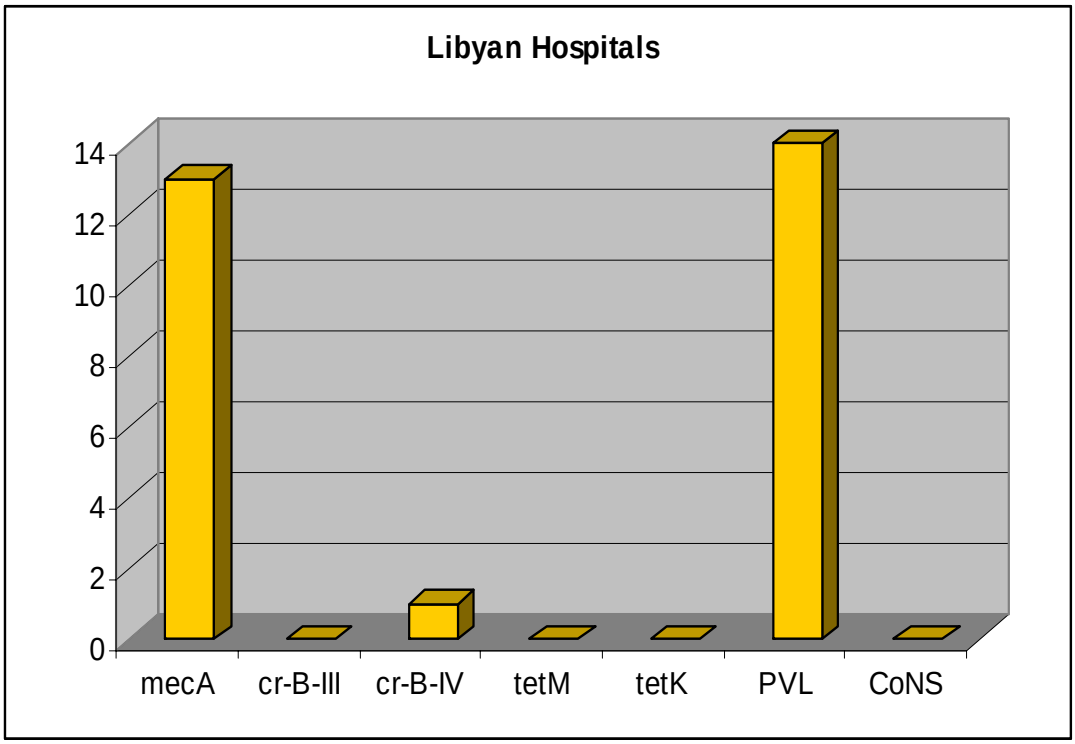

Figure 3.10: Resistance genes from Libyan hospitals' samples

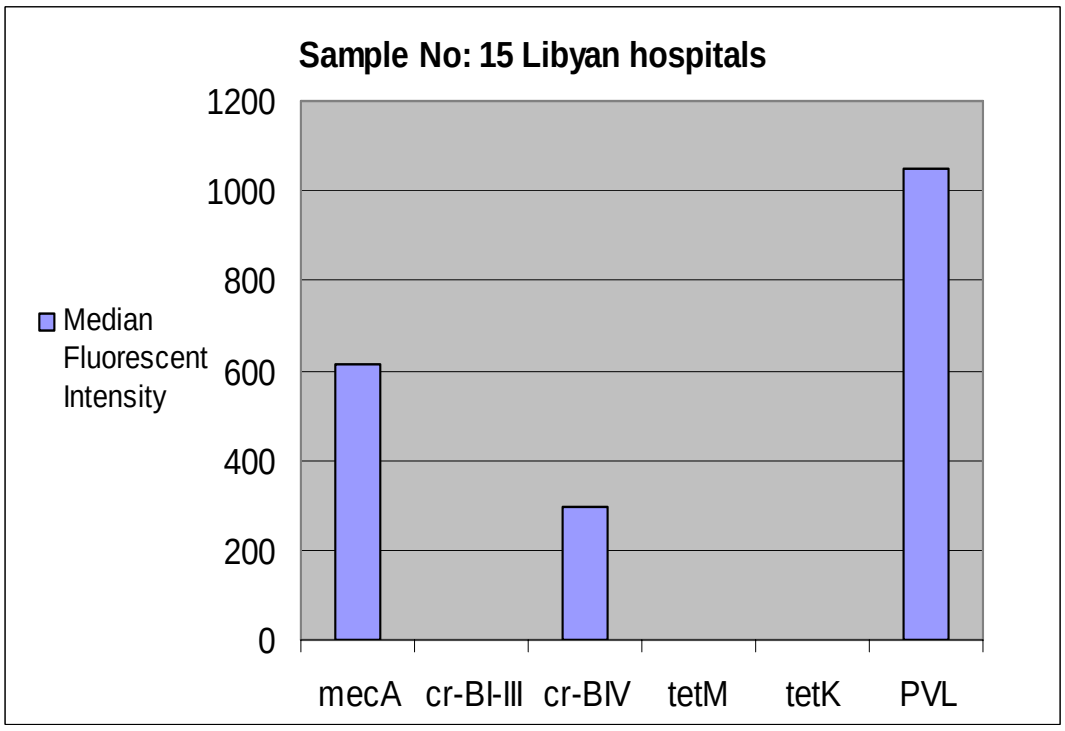

Figure 3. 11: Result of genetic studies in sample No.15 (Libyan Hospital)

CHAPTER 4

DISCUSSION

In this chapter; we examine the clinical and genetic results of the MRSA strains from the hospitals of both countries Turkey and Libya. (Turkish hospitals including; Hacettepe Hospital and Ankara Hospital in Ankara city and Libyan hospitals including; Aljalla Hospital and Jamahyria Hospital in Benghazi city). Three hundred and six clinical *Staphylococcus aureus* isolates were gathered in Turkish hospitals, whereas one hundred and fifty isolates of *S. aureus* were from Libyan Hospitals.

The clinical samples were collected from both gender, the males more than females in Turkish Hospitals (Males; 55% and females; 45%), whereas the females were slightly more than males in Libyan hospitals (Females; 52% and males; 48%). The results of the descriptive statistics in Turkish patients and Libyan patients are shown in Appendix F.

Methicillin resistant strains of *Staphylococcus aureus* (MRSA), which are often resistant to several classes of antibiotics, are the most common cause of nosocomial diseases. MRSA were first identified in the early sixties in those countries where methicillin was introduced. Subsequently MRSA travelled the world and was detected in hospitals on five out of six continents. Despite this major achievement, the picture is incomplete: still, there are numerous geographic regions for which it is currently unknown whether or not these major clones circulate there as well. It is important to fill these gaps in knowledge, because this may help to distinguish the major MRSA clones from the less important ones.

As a consequence, this characterization will identify those clones that are most epidemics (116). Current genomics approaches may be used to identify traits of the epidemic MRSA that determine facile dissemination. This, in the end, may be instrumental in the development of measures that limit the spread of even the epidemic MRSA.

Recent molecular phylogeny has substantiated that MRSA has emerged in six major clonal lineages (82,96). The prevalence of methicillin-resistant *Staphylococcus aureus* in many countries is increasing and, in hospitals in some areas, more than half of all *S. aureus* disease isolates are MRSA. MRSA strains are becoming increasingly multiresistant, and have recently developed resistance to vancomycin, used successfully to treat MRSA for more than 30 years. Nosocomial methicillin-resistant Staphylococcus aureus infections represent a major challenge to hospital microbiologists because of the emergence and spread of clones with decreased susceptibility to many antibiotic classes. Since the mid to late 1990s, hospital MRSA isolates have increased in prevalence in Europe, the USA and elsewhere (117, 118). In one European study of 25 university hospitals (119), one-quarter of 3051 *S. aureus* isolates collected were MRSA, with a geographical bias towards higher rates in southern countries such as Italy (50.5%) and Portugal (54%), and lower rates in northern European countries, including The Netherlands (2%), Austria (9%) and Switzerland (2%). MRSA infections are associated with increased morbidity; mortality and length of hospital stay, and represent a major financial burden on healthcare services (120, 121).

The first strain of MRSA was isolated in 1961 (86), 2 years after the introduction of methicillin; this strain rapidly spread to other countries throughout the 1960s, and became a problem in the USA in the 1970s. The antibiotic of choice for treating MRSA infections is the glycopeptide vancomycin, however vancomycin intermediately susceptible *S. aureus*

(VISA), was detected in Japan in 1997 (122,123), caused widespread alarm among physicians fearful of an era of untreatable MRSA infections. Reports of VISA isolates with an MIC ± 8 mg⁄L have so far been very rare, but two recent reports of fully vancomycin-resistant *S. aureus* (VRSA) from Michigan (124) and Pennsylvania (125) (MICs of 32 and 128 mg⁄L, respectively) in the USA have again caused alarm, and it is as yet unclear whether either VISA or VRSA isolates will become epidemic, leading to an exacerbation of the global MRSA problem.

In a climate of increasing *S. aureus* antibiotic resistance, the study of MRSA epidemiology has assumed new importance, because strategies to control the spread of MRSA at the local (hospital), national or international level require knowledge of how strains are spread and how MRSA epidemics occur. Epidemiological studies can be used to provide basic knowledge of the population biology of MRSA, and can help to answer fundamental questions such as: (1) how strains spread; (2) the number of major MRSA, VISA and VRSA clones circulating globally, and their relatedness to each other and to susceptible isolates; and (3) the ancestry of modern MRSA, VISA and VRSA strains. The answers to these questions have, until recently, been unclear, but several recent studies employing modern molecular typing technologies have now significantly increased our knowledge in these areas (126).

The widespread emergence of MRSA, especially in various types of nosocomial infections, is a serious clinical problem worldwide. The incidence of methicillin resistance among nosocomial isolates of *S. aureus* is higher than 70% in some Asian countries such as Taiwan, China, and Korea (127,128,129). Recently, MRSA has also emerged in the community setting in some countries, including Asian countries (130, 131). One of the cardinal features of the rapid emergence of MRSA in many parts of the world is the dissemination of specific clones; that has contributed to the accelerated increases in the incidence of MRSA.

Therefore, it is important to investigate the genotypic characteristics and evolutionary pathway of MRSA clones as well as the genetic relatedness of the strains isolated in different geographic regions. SCCmec typing analyzes a mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec), which contains the mecA gene encoding methicillin resistance (132) and is classified into four major types according to size and composition (133, 134).

The results of antimicrobial resistance studies conducted in Turkey have shown a high prevalence of resistance to antibiotics in both Gram positive and Gram negative bacteria. In Turkey, the ratio of MRSA causing hospital infections is not low. Topeli et al. 2000 (67) found that the rate of methicillin resistance among *S. aureus* strains causing bacteraemia in ICU patients was 37.7% in a Turkish hospital. Önciil et al. 2002 (68) reported that MRSA strains were isolated from 25% of the infections in a burn unit located in Turkey. In another study, the ratio of MRSA in hospital infections in ICU was reported as 22.2%. On the other hand, there is insufficient data on community-acquired MRSA infections and the ratio of colonization of MRSA in the community. Cesur and Cokca 2002 (70) determined the colonization ratio of MRSA as 2.6 and 6% in community members and in healthcare workers, respectively.

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 in Nigeria, Kenya, and Cameroon (21 to 30) and below 10% in Tunisia and Algeria (113). In Algeria, the rate of MRSA increased to 14% in 2001 (114), and the prevalence of PVL-positive MRSA was very high in 2006, these strains were resistant to multiple antibiotics, including gentamicin and ofloxacin (115). However, there are few studies showing data from Libya extent in the literature. Those studies indicate that the MRSA

among *Staphylococcus aureus* isolated from different cities in Libya; Tripoli 25% 1996, Benghazi 24% 2000, and Misurata 26% 2001, (139,140).

Our studies In Turkish hospitals, the MRSA strains were isolated from clinical samples including; pus (35%), wounds (25%), blood (17%), urine (7%), catheter (6%), Aspiration (5%), Burn (3%) and Sputum (2%) and most of the MRSA strains were isolated from blood, wounds, pus, and urine (66%, 56% 47% and 30% respectively).

The results in this study indicated that the MRSA strains were as high as 56% in Turkish hospitals and all MRSA strains displayed an Oxacillin MIC of ≥256µg/ml. and also they were resistant to both Ampicillin and Amoxicillin. In addition to that the majority of the isolates tested were highly resistant to Ampicillin (80.7%), Amoxicillin (71.8%), and Tetracyclin (53.2%), while about half of the strains were resistant to Erythromycin (49.3%) Ciprofloxacin (46.4%) and Gentamycin (41.6%), resistance to Clindamycin (30.7%), Cefotetan (14.7%), Chloramphencol (13.7%) and Vancomycin (2.2 %) were also detected for some isolates. All isolates were susceptible to Nitrofurantoin. Most MRSA isolates were multiresistant to more than five classes of antibiotics including; Ampicillin, Amoxicillin, Tetracycline, Erythromycin and Ciprofloxacin. In addition, the percentage of the Vancomycin-resistant isolates (VRSA) was 2.2% and Vancomycin intermediately susceptible *Staphylococcus aureus* (VISA) was not detected in Turkish hospital isolates

Besides those MRSA isolates, Coagulase Negative Staphylococcus was also isolated from clinical specimens in thirteen patients in Turkish hospitals. Seven of them were found to be Methicillin resistant and the other six were Methicillin susceptible. The explanation of these resistant coagulase negative isolates may be as the result of the horizontal transfer of mecA gene between MRSA and coagulase negative staphylococcal species.

In Libyan hospitals, the *Staphylococcus aureus* were isolated from clinical samples including; pus (39%), wounds (22%), blood (14%), urine (10%), Aspiration (6%), Burn (4%), Sputum (4%) and semen (1%). Incidences of the MRSA strains were high in isolates from blood, wounds, pus, and urine (71%, 62% 57% and 53%) respectively.

In Libyan hospitals, the majority MRSA isolates were multiresistant to more than three classes of antibiotics, the results of antimicrobial susceptibility test indicate that the MRSA isolates were high (59%) in both hospitals, and all MRSA strains were resistant to both Ampicillin and Amoxicillin like Turkish isolates. Some of the isolates were shown to be resistant to Ciprofloxacin (36%) and Gentamycin (27.3%), Clindamycin (34%), Cefotetan (26.7%), Chloramphencol (20%) and a large percentage (98.7%) of isolates were susceptible to Nitrofurantoin. The percentage of the VRSA and VISA in Libyan hospitals were 7% and 26% respectively, the intermediate resistance was also detected for Tetracyclin (5.7%) and Erythromycin (7.3%).

In both countries, the majority MRSA strains were multiresistant to more than five classes of antibiotics including; Ampicillin, Amoxicillin, Tetracycline, Erythromycin and Ciprofloxacin. Incidences of MRSA strains were high in blood, wounds and pus (68%, 57% and 50% respectively). Genetic investigation studies were done for some isolates (30 from Turkish hospitals and 20 from Libyan hospitals). The results of genetic investigation studies indicate that the mecA gene was present in the majority of isolates in both countries. Three samples out of thirty (10%) in Turkish hospital isolates and one case out of twenty isolates (5%) in Libyan hospitals specimens carry the community acquired MRSA type (ccrB-IV). Whereas no isolates out of fifty specimens carry the hospital acquired MRSA type (ccr-BI, II, III) in both countries.

The majority of Turkish hospital isolates carry Tetracycline resistance genes, thirteen out of thirty isolates (43%) for tetM gene and two out of thirty isolates (7%) for tetK gene. Whereas in Libyan isolates there was no sample out of twenty specimens carry the Tetracycline resistance genes. Many Libyan hospitals isolates carry Panton-Valentine Leukocidin gene (PVL), (70%). while one case only out of thirty isolates carries PVL gene (3%) in Turkish hospitals.

It is of great importance to mention that in some of the samples the multiresistant effect is due to the presence of more than one positive genes in isolates. For example, in addition to the Methicillin resistance genes, the incidence of Tetracycline resistance gene was quite high (tetM and tetK 50%) in Turkish hospitals isolates. So, these isolates show resistant to both Methicillin and Tetracycline and in turn considered as multiresistant strains. Another example of multiresistant is the existence of one isolate out of thirty that carried mecA gene, Tetracycline resistance gene (tetM), and coagulase-negative staphylococcus gene (CoNS).

The Panton-Valentine Leukocidin (PVL) is *S. aureus*-specific exotoxin often associated with severe skin infection and necrotizing pneumonia (135). PVL genes have been demonstrated primarily among CA-MRSA strains (135). The prevalence of Panton-Valentine Leukocidin gene was very high (PVL 70%) in Libyan hospital isolates. Four specimens out of fifty was shown to be CA-MRSA strains (ccr-BIV), however there was no isolate carrying HA-MRSA strains (ccr-BI, II, III) in both countries.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

1. The results of this thesis indicate that the incidence of MRSA strain was (56%), and MSSA strains was (44%) in Turkish hospitals (Hacettepe Hospital and Ankara Hospital, Ankara). Besides, the prevalence of MRSA strain was (59%), and MSSA strain was (41%) in Libyan hospitals (Aljalla Hospital and Jamahyria Hospital, Benghazi).

2. Although the percentages of VRSA strain and VISA strain in Libyan hospitals were (7%) and (26%) respectively, the percentages of VRSA and VISA were 2% and 0% in Turkish hospitals.

3. In both countries, the majority MRSA samples were multiresistant to more than five classes of antibiotics including; Ampicillin, Amoxicillin, Tetracycline, Erythromycin and Ciprofloxacin. Moreover, most of the MRSA samples were isolated from blood, wounds, and pus with (68%), (57%) and (50%) respectively.

4. Besides MRSA strain, Coagulase-Negative Staphylococcus was collected from clinical specimens from thirteen patients in Turkish hospitals. Unfortunately, seven of them were methicillin resistant and the other six were Methicillin Susceptible. The explanation of these isolated resistant strains may be as a result of the horizontal transfer of mecA gene between staphylococcal species.

5. The results of genetic investigations indicate the following:

a) The mecA gene presents in the majority of specimens in both countries.

b) The community acquired MRSA type IV gene (ccr-BIV) was found in three samples out of thirty (10%) in Turkish hospital specimens and one out of twenty isolates (5%) in Libyan hospital specimens. Conversely, the hospitals acquired MRSA types (ccr-BI, II, III) genes were not detected in any of the thirty isolates.

c) The majority of Turkish hospital isolates carried Tetracycline resistance genes, thirteen out of thirty specimens (43%) for tetM gene and two out of thirty isolates (7%) for tetK gene. On the other hand, it was shown that not even one sample carried the Tetracycline resistant genes (tetM and tetK) in Libyan hospitals.

d) The majority of Libyan hospital specimens carried Panton-Valentine Leukocidin (PVL) gene, (fourteen out of twenty isolates 70%). whereas only one out of thirty isolates (3%) in Turkish hospitals.

e) One isolate out of thirty from Turkish hospital samples carried mecA gene, Tetracycline resistance gene (tetM) and coagulase negative staphylococcus gene (CoNS). The existence of these genes together in one isolate is the cause of the multiresistant case.

6. Besides the Methicillin resistance gene, the incidence of Tetracycline resistance gene was quite high (tetM and tetK 50%) in Turkish hospital specimens, but in the Libyan hospitals the prevalence of Panton-Valentine Leukocidin gene was very high (PVL 70%).

5.2 Recommendations

1. The high prevalence of MRSA in Libya seems to be related to the high antibiotic usage;
   a) Easy availability of antibiotics at drug stores without a prescription.
   b) Injudicious use of antibiotics in hospitals.
   c) Uncontrolled use in animal husbandry.

2. The problem of antibiotic resistance is very serious in Libya, as it appears to be increasing, particularly with the emergence of resistance to newer drugs that include the fluoroquinolones (e.g. ciprofloxacin) among the clinically important bacterial species such as MRSA strains.

   It is urgently required to:

   a) Ban the sale of antibiotics without prescription,
   b) Use antibiotics more judiciously in hospitals by intensive teaching of the principles of the use of antibiotics, and
   c) Establish better control measures of nosocomial infections.
   d) Regulate the usage of antimicrobials for both humans and animals.

These issues are not easy to address and require the collective action of health authorities, the pharmaceutical community, health care providers, and consumers.

3. The obtained results indicate a high incidence of the VRSA and VISA in Libyan hospitals with 7% and 26% respectively. For that reason, I recommend more research to be carried about this topic in the future and especially in Libyan hospitals.
4. It is recommended that this study is extended in the future in a way that not only covers two hospitals in Ankara city and Benghazi city, but also covers more hospitals in other regions of Turkey and Libya.

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APPENDIX A: QUESTIONNAIRS

Table A. 1: Specimens' collection questionnaire

No	Sex	Age	Clinical Sample	Strain	Hospital

Table A. 2: Microbiological investigations questionnaire

No	Gram's Stain	Catalase Test	Coagulase Test

Table A. 3: Antibiotic Susceptibility Test questionnaire

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G

OX: Oxacillin 1µg (Methicillin), **V:** Vancomycin 30µg, **AMP:** Ampicillin 10µg, **AMX:** Amoxicillin 10µg, **TE:** Tetracyclin 30µg, **E:** Erythromycin 15µg, **G:** Gentamycin 10µg, **CIP:** Ciprofloxacin 5µg, **DA:** Clindamycin 2µg, **C:** Chloramphencol 30µg, **CTT:** Cefotetan 30µg, and **F:** Nitrofurantoin 300µg.
S: Susceptible, **R:** Resistant, **I:** Intermediate and **–** Not use for sometime.

APPENDIX B.

RESULTS OF INVESTIGATIONS IN TURKISH HOSPITALS

Table B. 1: Results of Specimens' collection in Turkish Hospitals

No	Sex	Age	Clinical Sample	Strain	Hospital
1	F	24	Wound	**MRSA**	Ankara hospital
2	M	64	Blood	**MRSA**	Ankara hospital
3	F	80	Catheter	**MRSA**	Ankara hospital
4	M	51	Blood	**MRSA**	Ankara hospital
5	F	50	Blood	**MRSA**	Ankara hospital
6	M	57	Wound	**MSSA**	Ankara hospital
7	M	18	Wound	**MSSA**	Ankara hospital
8	M	54	Wound	**MSSA**	Ankara hospital
9	M	57	Blood	**MSSA**	Ankara hospital
10	F	79	Urine	**MSSA**	Hacettepe hospital
11	M	43	Pus	**MRSA**	Hacettepe hospital
12	M	77	Pus	**MRSA**	Hacettepe hospital
13	M	51	Pus	**MRSA**	Hacettepe hospital
14	F	27	Pus	**MRSA**	Hacettepe hospital
15	M	26	Pus	**MRSA**	Hacettepe hospital
16	F	20	Pus	**MSSA**	Hacettepe hospital
17	M	51	Blood	**MRSA**	Ankara hospital
18	M	1	Blood	**CoNS**	Ankara hospital
19	M	46	Blood	**MRSA**	Ankara hospital
20	M	55	Blood	**CoNS**	Ankara hospital
21	M	66	Blood	**CoNS**	Ankara hospital
22	F	28	Wounds	**MRSA**	Ankara hospital

23	M	30	Wounds	**MRSA**	Ankara hospital
24	F	65	Blood	**MRSA**	Ankara hospital
25	M	75	Sputum	**MSSA**	Ankara hospital

Table B. 1: Results of Specimens' collection in Turkish Hospitals

(continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
26	M	72	Wound	**CoNS**	Ankara hospital
27	M	57	Pus	**MSSA**	Hacettepe hospital
28	F	27	Pus	**MRSA**	Hacettepe hospital
29	F	83	Catheter	**CoNS**	Hacettepe hospital
30	M	42	Blood	**CoNS**	Hacettepe hospital
31	F	60	Urine	**CoNS**	Hacettepe hospital
32	F	62	Pus	**CoNS**	Hacettepe hospital
33	F	47	Aspiration	**CoNS**	Ankara hospital
34	M	51	Aspiration	**CoNS**	Ankara hospital
35	M	9	Aspiration	**CoNS**	Ankara hospital
36	M	60	Aspiration	**CoNS**	Ankara hospital
37	M	61	Aspiration	**MRSA**	Ankara hospital
38	M	49	Catheter	**MRSA**	Ankara hospital
39	F	6	Pus	**MRSA**	Ankara hospital
40	M	44	Pus	**MSSA**	Ankara hospital
41	M	27	Pus	**MSSA**	Ankara hospital
42	M	63	Urine	**CoNS**	Ankara hospital
43	F	73	Blood	**MRSA**	Hacettepe hospital
44	M	47	Blood	**MRSA**	Hacettepe hospital
45	M	36	Wound	**MRSA**	Hacettepe hospital
46	F	42	Blood	**MRSA**	Hacettepe hospital
47	M	64	Blood	**MRSA**	Hacettepe hospital
48	M	64	Catheter	**MSSA**	Hacettepe hospital
49	M	74	Blood	**MSSA**	Hacettepe hospital
50	M	72	wound	**MSSA**	Hacettepe hospital
51	F	77	Aspiration	**MSSA**	Hacettepe hospital
52	M	64	Blood	**MSSA**	Hacettepe hospital
53	M	61	Blood	**MSSA**	Hacettepe hospital
54	M	67	Blood	**MSSA**	Hacettepe hospital
55	F	13	Burn	**MSSA**	Hacettepe hospital
56	M	56	Pus	**MRSA**	Hacettepe hospital
57	F	72	Urine	**MRSA**	Hacettepe hospital
58	F	22	Burn	**MRSA**	Hacettepe hospital
59	F	34	Blood	**MSSA**	Hacettepe hospital
60	M	71	Pus	**MSSA**	Hacettepe hospital

Table B. 1: Results of Specimens' collection in Turkish Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
61	M	61	Pus	**MRSA**	Hacettepe hospital
62	M	63	Pus	**MRSA**	Hacettepe hospital
63	M	31	Pus	**MSSA**	Hacettepe hospital
64	M	30	Pus	**MSSA**	Hacettepe hospital
65	F	81	Blood	**MRSA**	Ankara hospital
66	M	62	Wounds	**MRSA**	Ankara hospital
67	F	40	Wounds	**MRSA**	Ankara hospital
68	M	53	Catheter	**MRSA**	Ankara hospital
69	M	44	Wound	**MSSA**	Ankara hospital
70	M	55	Wound	**MRSA**	Ankara hospital
71	F	51	Wound	**MRSA**	Ankara hospital
72	F	59	Urine	**MRSA**	Ankara hospital
73	M	80	Blood	**MRSA**	Ankara hospital
74	F	75	Wound	**MSSA**	Ankara hospital
75	M	61	Sputum	**MRSA**	Hacettepe hospital
76	F	74	Catheter	**MRSA**	Hacettepe hospital
77	F	70	Blood	**MRSA**	Hacettepe hospital
78	M	55	Wound	**MSSA**	Hacettepe hospital
79	M	61	Blood	**MRSA**	Hacettepe hospital
80	M	24	Burn	**MSSA**	Hacettepe hospital
81	M	21	Burn	**MRSA**	Hacettepe hospital
82	M	65	Sputum	**MRSA**	Hacettepe hospital
83	M	71	Pus	**MRSA**	Hacettepe hospital
84	M	46	Blood	**MRSA**	Hacettepe hospital
85	M	24	Wound	**MRSA**	Ankara hospital
86	M	46	Blood	**MRSA**	Ankara hospital
87	F	58	Blood	**MRSA**	Ankara hospital
88	F	23	Pus	**MRSA**	Ankara hospital
89	M	1	Catheter	**MRSA**	Ankara hospital
90	M	28	Wound	**MRSA**	Ankara hospital
91	F	75	Wound	**MRSA**	Ankara hospital
92	F	45	Wound	**MSSA**	Ankara hospital
93	F	73	Pus	**MSSA**	Ankara hospital
94	M	46	Aspiration	**MSSA**	Ankara hospital
95	F	64	Pus	**MSSA**	Hacettepe hospital

96	M	31	Pus	**MRSA**	Hacettepe hospital
97	F	25	Pus	**MRSA**	Hacettepe hospital
98	M	17	Pus	**MRSA**	Hacettepe hospital

Table B. 1: Results of Specimens' collection in Turkish Hospitals

(continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
99	M	36	Pus	**MRSA**	Hacettepe hospital
100	M	52	Pus	**MRSA**	Hacettepe hospital
101	F	26	Wound	**MSSA**	Hacettepe hospital
102	M	19	Blood	**MRSA**	Hacettepe hospital
103	M	39	Wound	**MSSA**	Hacettepe hospital
104	F	45	Blood	**MSSA**	Hacettepe hospital
105	M	71	Pus	**MSSA**	Hacettepe hospital
106	M	51	Pus	**MRSA**	Hacettepe hospital
107	F	38	Pus	**MSSA**	Hacettepe hospital
108	F	46	Urine	**MSSA**	Hacettepe hospital
109	M	42	Pus	**MSSA**	Hacettepe hospital
110	F	78	Aspiration	**MRSA**	Hacettepe hospital
111	F	53	Wound	**MRSA**	Hacettepe hospital
112	F	38	Wound	**MRSA**	Ankara hospital
113	F	27	Aspiration	**MSSA**	Ankara hospital
114	M	1	Urine	**MSSA**	Ankara hospital
115	F	61	Wound	**MSSA**	Ankara hospital
116	M	25	Wound	**MSSA**	Ankara hospital
117	M	46	Wound	**MSSA**	Ankara hospital
118	F	42	Wound	**MRSA**	Ankara hospital
119	F	19	Pus	**MRSA**	Ankara hospital
120	F	42	Pus	**MSSA**	Ankara hospital
121	M	62	Wound	**MRSA**	Ankara hospital
122	M	16	Pus	**MSSA**	Hacettepe hospital
123	M	39	Pus	**MRSA**	Hacettepe hospital
124	M	52	Pus	**MRSA**	Hacettepe hospital
125	M	58	Aspiration	**MRSA**	Hacettepe hospital
126	F	16	Burn	**MRSA**	Hacettepe hospital
127	M	39	Pus	**MRSA**	Hacettepe hospital
128	M	19	Blood	**MSSA**	Hacettepe hospital
129	F	69	Blood	**MRSA**	Hacettepe hospital
130	M	17	Pus	**MRSA**	Hacettepe hospital
131	M	39	Pus	**MRSA**	Hacettepe hospital
132	M	72	Pus	**MSSA**	Hacettepe hospital
133	M	71	Pus	**MRSA**	Hacettepe hospital

134	M	14	Pus	**MRSA**	Hacettepe hospital
135	M	14	Pus	**MRSA**	Hacettepe hospital

Table B. 1: Results of Specimens' collection in Turkish Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
136	F	48	Wound	**MRSA**	Ankara hospital
137	F	20	Wound	**MSSA**	Ankara hospital
138	F	48	Wound	**MSSA**	Ankara hospital
139	M	1	Wound	**MRSA**	Ankara hospital
140	F	35	Wound	**MRSA**	Hacettepe hospital
141	F	72	Aspiration	**MRSA**	Hacettepe hospital
142	M	18	Pus	**MRSA**	Hacettepe hospital
143	M	42	Pus	**MSSA**	Hacettepe hospital
144	M	47	Blood	**MSSA**	Hacettepe hospital
145	F	57	Wound	**MSSA**	Hacettepe hospital
146	F	55	Wound	**MRSA**	Hacettepe hospital
147	F	72	Aspiration	**MRSA**	Hacettepe hospital
148	M	72	Aspiration	**MRSA**	Hacettepe hospital
149	M	45	Urine	**MSSA**	Hacettepe hospital
150	M	46	Urine	**MRSA**	Hacettepe hospital
151	M	52	Blood	**MSSA**	Hacettepe hospital
152	M	37	Wound	**MRSA**	Ankara hospital
153	M	12	Wound	**MSSA**	Ankara hospital
154	F	68	Wound	**MSSA**	Ankara hospital
155	M	51	Wound	**MRSA**	Ankara hospital
156	M	49	Wound	**MSSA**	Ankara hospital
157	F	1	Catheter	**MRSA**	Ankara hospital
158	M	1	Pus	**MSSA**	Ankara hospital
159	M	72	Pus	**MSSA**	Ankara hospital
160	M	1	Wound	**MRSA**	Ankara hospital
161	F	45	Wound	**MRSA**	Ankara hospital
162	F	72	Sputum	**MRSA**	Hacettepe hospital
163	M	12	Pus	**MRSA**	Hacettepe hospital
164	M	16	Burn	**MSSA**	Hacettepe hospital
165	M	41	Urine	**MSSA**	Hacettepe hospital
166	M	47	Wound	**MSSA**	Hacettepe hospital
167	M	40	Wound	**MSSA**	Hacettepe hospital
168	M	47	Pus	**MRSA**	Hacettepe hospital
169	M	39	Blood	**MSSA**	Hacettepe hospital
170	M	73	Blood	**MSSA**	Hacettepe hospital

Table B. 1: Results of Specimens' collection in Turkish Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
171	F	47	Wound	**MRSA**	Hacettepe hospital
172	M	64	Pus	**MSSA**	Hacettepe hospital
173	M	60	Pus	**MRSA**	Hacettepe hospital
174	M	5	Sputum	**MRSA**	Hacettepe hospital
175	M	69	Pus	**MRSA**	Hacettepe hospital
176	M	14	Pus	**MSSA**	Hacettepe hospital
177	M	48	Blood	**MRSA**	Ankara hospital
178	M	62	Wound	**MRSA**	Ankara hospital
179	F	63	Blood	**MRSA**	Ankara hospital
180	M	61	Catheter	**MRSA**	Ankara hospital
181	F	33	Wound	**MRSA**	Ankara hospital
182	F	72	Blood	**MRSA**	Hacettepe hospital
183	M	38	Blood	**MSSA**	Hacettepe hospital
184	F	66	Urine	**MSSA**	Hacettepe hospital
185	M	56	Wound	**MSSA**	Hacettepe hospital
186	M	31	Burn	**MSSA**	Hacettepe hospital
187	F	69	Pus	**MSSA**	Hacettepe hospital
188	M	76	Catheter	**MRSA**	Hacettepe hospital
189	M	52	Aspiration	**MRSA**	Hacettepe hospital
190	M	47	Blood	**MRSA**	Hacettepe hospital
191	M	38	Blood	**MRSA**	Hacettepe hospital
192	F	56	Burn	**MSSA**	Hacettepe hospital
193	M	70	Catheter	**MSSA**	Hacettepe hospital
194	F	72	Catheter	**MRSA**	Hacettepe hospital
195	M	30	Burn	**MRSA**	Hacettepe hospital
196	F	69	Pus	**MSSA**	Hacettepe hospital
197	F	84	Wound	**MSSA**	Hacettepe hospital
198	M	62	Wound	**MSSA**	Ankara hospital
199	F	60	Pus	**MSSA**	Ankara hospital
200	F	62	Blood	**MRSA**	Ankara hospital
201	F	52	Wound	**MSSA**	Ankara hospital
202	F	26	Pus	**MSSA**	Ankara hospital
203	F	25	Blood	**MRSA**	Hacettepe hospital
204	F	74	Wound	**MRSA**	Hacettepe hospital
205	F	77	Pus	**MSSA**	Hacettepe hospital

Table B. 1: Results of Specimens' collection in Turkish Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
206	F	46	Pus	**MSSA**	Hacettepe hospital
207	M	72	Blood	**MRSA**	Hacettepe hospital
208	M	50	Pus	**MRSA**	Hacettepe hospital
209	M	56	Blood	**MSSA**	Hacettepe hospital
210	F	21	Urine	**MSSA**	Hacettepe hospital
211	F	84	Catheter	**MSSA**	Hacettepe hospital
212	F	52	Catheter	**MSSA**	Hacettepe hospital
213	M	44	Pus	**MSSA**	Hacettepe hospital
214	F	74	Wound	**MRSA**	Hacettepe hospital
215	M	50	Wound	**MSSA**	Hacettepe hospital
216	M	48	Blood	**MRSA**	Ankara hospital
217	F	26	Wound	**MRSA**	Ankara hospital
218	M	38	Pus	**MSSA**	Hacettepe hospital
219	F	66	Wound	**MRSA**	Hacettepe hospital
220	M	56	Blood	**MRSA**	Hacettepe hospital
221	M	31	Wound	**MSSA**	Hacettepe hospital
222	M	25	Pus	**MRSA**	Hacettepe hospital
223	F	74	Wound	**MRSA**	Hacettepe hospital
224	M	73	Blood	**MRSA**	Hacettepe hospital
225	M	50	Pus	**MRSA**	Hacettepe hospital
226	M	30	Wound	**MSSA**	Hacettepe hospital
227	M	50	Wound	**MRSA**	Hacettepe hospital
228	F	23	Wound	**MSSA**	Hacettepe hospital
229	M	45	Wound	**MRSA**	Hacettepe hospital
230	M	51	Pus	**MSSA**	Hacettepe hospital
231	F	61	Pus	**MSSA**	Hacettepe hospital
232	M	45	Urine	**MRSA**	Hacettepe hospital
233	M	50	Wound	**MRSA**	Ankara hospital
234	F	30	Pus	**MRSA**	Ankara hospital
235	F	42	Pus	**MRSA**	Ankara hospital
236	M	75	Sputum	**MRSA**	Hacettepe hospital
237	F	38	Pus	**MRSA**	Hacettepe hospital
238	F	26	Pus	**MRSA**	Hacettepe hospital
239	F	48	Pus	**MRSA**	Ankara hospital
240	M	51	Pus	**MRSA**	Ankara hospital

Table B. 1: Results of Specimens' collection in Turkish Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
241	M	50	Wound	**MSSA**	Hacettepe hospital
242	F	69	Pus	**MSSA**	Hacettepe hospital
243	M	79	Pus	**MRSA**	Hacettepe hospital
244	M	63	Urine	**MRSA**	Hacettepe hospital
245	M	31	Wound	**MRSA**	Hacettepe hospital
246	M	76	Catheter	**MSSA**	Hacettepe hospital
247	M	51	Sputum	**MRSA**	Ankara hospital
248	M	56	Wound	**MRSA**	Ankara hospital
249	M	31	Burn	**MSSA**	Ankara hospital
250	F	69	Pus	**MSSA**	Ankara hospital
251	F	52	Pus	**MSSA**	Hacettepe hospital
252	F	50	Wound	**MSSA**	Hacettepe hospital
253	M	36	Urine	**MSSA**	Hacettepe hospital
254	M	65	Pus	**MSSA**	Hacettepe hospital
255	M	30	Pus	**MRSA**	Hacettepe hospital
256	F	30	Urine	**MSSA**	Hacettepe hospital
257	M	79	Pus	**MSSA**	Hacettepe hospital
258	M	47	Pus	**MRSA**	Hacettepe hospital
259	M	50	Wound	**MRSA**	Ankara hospital
260	F	23	Pus	**MRSA**	Ankara hospital
261	M	45	Sputum	**MRSA**	Ankara hospital
262	M	51	Pus	**MSSA**	Ankara hospital
263	F	61	Pus	**MRSA**	Ankara hospital
264	M	60	Pus	**MRSA**	Hacettepe hospital
265	M	50	Pus	**MSSA**	Hacettepe hospital
266	F	22	Wound	**MSSA**	Hacettepe hospital
267	M	50	Wound	**MRSA**	Hacettepe hospital
268	F	59	Pus	**MSSA**	Hacettepe hospital
269	F	66	Pus	**MRSA**	Hacettepe hospital
270	F	80	Pus	**MSSA**	Hacettepe hospital
271	M	38	Pus	**MRSA**	Ankara hospital
272	F	48	Wound	**MRSA**	Ankara hospital
273	M	37	Blood	**MRSA**	Ankara hospital
274	F	34	Urine	**MSSA**	Ankara hospital
275	F	29	Pus	**MSSA**	Ankara hospital

Table B. 1: Results of Specimens' collection in Turkish Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
276	M	84	Aspiration	**MSSA**	Hacettepe hospital
277	M	74	Catheter	**MSSA**	Hacettepe hospital
278	F	35	Blood	**MSSA**	Hacettepe hospital
279	M	42	Pus	**MSSA**	Hacettepe hospital
280	M	17	Pus	**MRSA**	Hacettepe hospital
281	M	25	Pus	**MSSA**	Hacettepe hospital
282	F	33	Pus	**MSSA**	Hacettepe hospital
283	M	83	Wound	**MSSA**	Hacettepe hospital
284	M	42	Pus	**MRSA**	Hacettepe hospital
285	M	52	Sputum	**MSSA**	Hacettepe hospital
286	M	74	Catheter	**MSSA**	Hacettepe hospital
287	M	13	Catheter	**MSSA**	Hacettepe hospital
288	F	45	Pus	**MRSA**	Hacettepe hospital
289	F	61	Blood	**MSSA**	Hacettepe hospital
290	M	50	Pus	**MRSA**	Hacettepe hospital
291	M	30	Burn	**MSSA**	Hacettepe hospital
292	F	69	Pus	**MSSA**	Hacettepe hospital
293	M	66	Pus	**MSSA**	Hacettepe hospital
294	F	48	Urine	**MSSA**	Hacettepe hospital
295	M	50	Pus	**MSSA**	Hacettepe hospital
296	F	38	Wound	**MSSA**	Hacettepe hospital
297	F	27	Aspiration	**MRSA**	Ankara hospital
298	M	1	Urine	**MSSA**	Ankara hospital
299	F	61	Wound	**MRSA**	Ankara hospital
300	F	39	Catheter	**MRSA**	Hacettepe hospital
301	F	30	Blood	**MRSA**	Hacettepe hospital
302	F	70	Urine	**MRSA**	Hacettepe hospital
303	M	80	Blood	**MSSA**	Hacettepe hospital
304	M	72	Wound	**MRSA**	Hacettepe hospital
305	M	40	Urine	**MSSA**	Hacettepe hospital
306	F	70	Wound	**MSSA**	Hacettepe hospital

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
1	**R**	S	S	R	S	R	R	S	R	S	R	-
2	**R**	S	R	R	R	R	R	S	R	R	S	-
3	**R**	S	S	R	S	R	S	S	R	R	R	-
4	**R**	S	S	S	R	R	S	S	R	S	S	-
5	**R**	S	S	R	R	R	S	S	R	R	R	-
6	**S**	S	S	R	R	R	R	S	S	R	R	-
7	**S**	S	R	S	R	R	S	S	S	R	R	-
8	**S**	S	S	S	R	S	S	S	S	S	R	-
9	**S**	S	S	S	S	R	S	S	S	S	S	-
10	**S**	S	S	R	S	S	R	S	S	R	R	-
11	**R**	S	S	S	S	R	S	S	S	S	R	-
12	**S**	S	R	R	R	R	S	S	S	S	R	-
13	**R**	S	S	R	S	R	R	S	R	R	R	-
14	**R**	S	S	R	R	R	S	S	R	R	R	-
15	**R**	S	S	S	R	R	R	S	S	S	R	-
16	**S**	S	S	S	R	S	S	S	S	S	S	-
17	**R**	S	S	S	R	R	S	S	R	S	R	-
18	**R**	S	S	R	R	R	R	S	S	S	R	-
19	**R**	S	S	R	R	R	R	S	R	R	R	-
20	**S**	R	S	R	S	S	S	S	S	R	R	-
21	**R**	R	S	S	R	R	R	S	R	R	R	-
22	**R**	S	S	R	R	S	R	S	S	S	S	-
23	**R**	S	S	R	R	R	R	S	R	R	R	-
24	**R**	S	S	R	R	R	S	S	R	R	R	-
25	**S**	S	R	R	S	R	S	S	R	R	R	-
26	**R**	S	S	R	R	R	S	S	S	R	R	-
27	**S**	S	S	S	S	R	S	S	S	S	S	-
28	**R**	S	R	R	R	R	R	S	R	R	R	-
29	**R**	S	R	R	R	R	R	S	R	R	R	-
30	**R**	S	S	S	S	R	S	S	S	S	S	-
31	**S**	S	R	R	R	R	R	S	S	S	S	-
32	**S**	S	S	S	R	R	S	S	R	S	S	-
33	**S**	S	S	S	S	S	S	S	S	S	S	-
34	**S**	S	S	R	S	S	S	S	R	R	R	-
35	**R**	S	R	R	S	R	R	S	S	R	R	-
36	**R**	S	R	R	S	R	S	S	R	R	S	-
37	**R**	S	S	R	R	R	R	S	R	S	R	-
38	**R**	S	S	R	R	R	R	S	R	R	R	-
39	**R**	S	S	R	R	R	R	S	R	S	R	-

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
40	**S**	S	R	R	R	S	S	S	S	S	S	-
41	**S**	S	R	S	R	R	S	S	R	R	R	-
42	**S**	S	S	S	S	R	S	S	R	S	R	-
43	**R**	S	S	S	S	R	R	S	R	S	R	-
44	**R**	S	S	R	R	R	R	S	R	S	R	-
45	**R**	S	S	R	R	R	R	S	R	S	S	-
46	**R**	S	S	R	R	S	S	S	R	S	S	-
47	**R**	S	S	R	R	R	R	S	S	S	S	-
48	**S**	S	S	R	R	R	R	S	S	S	S	-
49	**S**	S	S	S	S	S	S	S	S	S	S	-
50	**S**	S	S	R	R	S	R	S	S	S	S	-
51	**S**	S	S	S	S	S	S	S	S	S	S	-
52	**S**	S	S	R	R	R	S	S	S	S	S	-
53	**S**	S	S	R	R	R	R	S	S	S	S	-
54	**S**	S	R	R	R	R	R	S	S	R	S	-
55	**S**	S	S	R	S	S	S	S	S	S	R	-
56	**R**	S	S	R	R	R	R	S	R	S	S	-
57	**R**	-	S	S	S	R	R	S	S	S	S	-
58	**R**	-	R	R	R	R	R	S	S	S	S	-
59	**S**	-	R	R	R	R	S	S	S	S	R	-
60	**S**	-	S	R	R	R	R	S	S	S	R	-
61	**R**	-	R	R	R	R	R	S	R	S	R	-
62	**R**	-	S	R	R	R	R	S	S	R	R	-
63	**S**	-	S	S	S	S	R	S	S	S	S	-
64	**S**	-	S	S	S	S	R	S	S	S	S	-
65	**R**	-	S	R	S	S	S	S	S	S	R	-
66	**R**	-	S	R	R	S	R	S	S	S	S	-
67	**R**	-	R	R	S	S	S	S	S	S	S	-
68	**R**	-	S	R	R	R	S	S	S	S	R	-
69	**S**	-	R	R	S	R	S	S	S	S	R	-
70	**R**	-	S	R	R	S	S	S	S	S	S	-
71	**R**	-	S	R	R	S	S	S	S	S	S	-
72	**R**	-	S	R	R	S	S	S	S	S	S	-
73	**R**	-	S	R	S	S	S	S	S	S	S	-
74	**S**	-	S	S	S	S	S	S	S	R	R	-
75	**R**	-	S	S	S	S	S	S	R	R	S	-
76	**R**	-	S	S	S	S	R	S	S	S	S	-

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
77	**R**	-	S	S	S	R	S	S	S	S	R	-
78	**S**	-	S	S	S	S	S	S	S	S	S	-
79	**R**	-	R	R	R	R	R	S	R	S	R	-
80	**S**	-	S	R	S	S	S	S	S	S	R	-
81	**R**	-	R	R	R	R	R	S	R	S	R	-
82	**R**	-	R	S	R	R	R	S	S	S	R	-
83	**R**	-	R	R	R	S	S	S	S	R	R	-
84	**R**	-	S	S	R	R	R	S	R	S	R	-
85	**R**	-	R	R	R	R	R	S	R	R	R	-
86	**R**	-	R	R	R	R	R	S	R	R	R	-
87	**R**	-	S	S	R	R	R	S	R	S	R	-
88	**R**	-	R	R	R	R	S	S	R	R	R	-
89	**R**	-	R	S	R	R	R	S	R	R	R	-
90	**R**	-	R	R	R	R	R	S	R	R	R	-
91	**R**	-	R	R	R	R	R	S	S	R	R	-
92	**S**	-	R	R	R	R	S	S	R	R	R	-
93	**S**	-	R	R	R	R	R	S	S	R	R	-
94	**S**	-	S	S	R	R	R	S	S	S	R	-
95	**S**	-	S	S	R	S	S	S	S	S	S	-
96	**R**	-	R	S	S	R	S	S	S	S	S	-
97	**R**	-	R	S	S	R	S	S	S	R	S	-
98	**R**	-	S	S	R	S	S	S	S	R	S	-
99	**R**	-	S	R	R	R	S	S	R	S	R	-
100	**R**	S	S	S	R	R	S	S	R	S	S	R
101	**S**	S	S	R	R	R	R	S	R	S	S	R
102	**R**	S	S	S	S	R	R	S	R	R	R	S
103	**S**	S	S	S	S	R	R	S	S	R	R	S
104	**S**	S	S	S	S	R	R	S	S	S	S	S
105	**R**	S	S	S	S	R	R	S	S	S	S	S
106	**S**	S	S	S	S	S	S	S	S	S	S	S
107	**S**	S	S	R	S	S	S	S	S	S	S	S
108	**S**	R	S	S	S	R	R	S	S	S	S	S
109	**S**	S	S	S	S	R	R	S	S	S	S	S
110	**R**	S	S	S	R	R	S	S	R	S	S	R
111	**R**	S	S	R	R	R	S	S	R	S	S	R
112	**R**	S	R	S	S	R	R	S	-	S	S	S

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
113	**S**	S	R	S	S	R	R	S	-	S	R	S
114	**S**	S	S	S	S	R	R	S	-	R	R	S
115	**S**	S	S	S	R	R	S	S	-	S	S	S
116	**S**	S	R	S	S	S	S	S	-	R	S	R
117	**S**	S	S	S	S	R	R	S	-	S	S	S
118	**R**	S	S	R	R	R	R	S	-	S	R	S
119	**R**	S	R	R	R	R	R	S	-	R	R	R
120	**S**	S	S	R	R	R	R	S	-	S	R	R
121	**R**	S	R	R	R	R	R	S	-	R	R	S
122	**S**	S	S	R	S	R	S	S	-	S	S	S
123	**R**	S	S	S	S	R	R	S	-	S	R	R
124	**R**	S	S	R	R	R	R	S	-	R	R	R
125	**R**	S	S	R	R	R	R	S	-	S	R	R
126	**R**	R	S	R	R	R	R	S	-	R	S	S
127	**R**	S	S	R	R	R	R	S	-	S	R	R
128	**S**	S	S	S	S	S	S	S	-	S	S	S
129	**R**	S	S	R	S	R	R	S	-	R	S	S
130	**R**	S	S	R	R	R	R	S	-	S	R	R
131	**R**	S	S	R	R	R	R	S	-	R	R	R
132	**S**	S	S	S	S	S	S	S	-	S	S	S
133	**R**	S	S	S	S	R	R	S	-	S	R	S
134	**R**	S	R	R	R	R	R	S	-	R	S	S
135	**R**	S	R	R	R	R	R	S	-	R	S	R
136	**R**	S	S	R	S	R	R	S	-	R	S	S
137	**S**	S	S	S	S	R	R	S	-	S	S	S
138	**S**	S	S	S	S	R	R	S	-	R	S	S
139	**R**	S	S	R	R	R	R	S	-	R	R	R
140	**R**	S	S	S	S	R	R	S	-	S	R	R
141	**R**	S	S	R	S	R	R	S	-	S	R	R
142	**R**	S	S	R	S	R	R	S	-	S	R	R
143	**S**	S	S	S	S	R	R	S	-	S	S	S
144	**S**	S	S	S	S	S	S	S	-	S	S	S
145	**S**	S	S	S	S	R	R	S	-	S	S	S
146	**R**	S	S	S	R	R	R	S	-	S	R	R
147	**R**	S	R	R	R	R	R	S	-	S	R	R
148	**R**	S	S	R	R	R	R	S	-	R	R	R

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
149	**S**	S	S	S	S	R	R	S	-	S	S	S
150	**R**	S	S	R	R	R	R	S	-	R	R	R
151	**S**	S	S	S	S	R	R	S	-	S	S	S
152	**R**	S	S	R	R	R	R	S	-	R	R	R
153	**S**	S	S	S	S	S	S	S	-	S	S	S
154	**S**	S	S	R	S	R	R	S	-	S	S	S
155	**R**	S	S	R	R	R	R	S	-	R	R	R
156	**S**	S	S	S	R	R	R	S	-	S	S	S
157	**R**	S	S	R	R	R	R	S	-	R	R	R
158	**S**	S	S	S	S	R	R	S	-	R	R	R
159	**S**	S	S	S	S	R	R	S	-	S	S	S
161	**R**	S	S	R	R	R	R	S	-	R	R	R
162	**R**	S	S	R	R	R	R	S	-	S	S	R
163	**R**	S	S	R	R	R	R	S	-	S	R	R
164	**S**	S	S	S	S	R	R	S	-	S	S	S
165	**S**	S	S	S	S	S	S	S	-	S	S	S
166	**S**	S	S	S	R	R	R	S	-	S	R	S
167	**S**	S	S	S	R	R	R	S	-	S	R	S
168	**R**	S	S	R	R	R	R	S	-	S	S	S
169	**S**	S	S	S	S	S	S	S	-	S	S	S
170	**S**	S	S	S	S	S	R	S	-	S	R	S
171	**R**	S	S	S	R	R	R	S	-	S	R	S
172	**S**	S	S	S	S	R	S	S	-	S	R	S
173	**R**	S	S	R	R	R	R	S	-	R	R	R
174	**R**	S	S	R	R	R	R	S	-	R	R	S
175	**R**	S	S	R	R	R	R	S	-	S	S	S
176	**S**	S	S	S	S	R	R	S	-	R	S	R
177	**R**	S	S	R	R	R	R	S	-	R	R	R
178	**R**	S	S	R	R	R	R	S	-	R	R	S
179	**R**	S	S	R	R	R	R	S	-	R	R	S
180	**R**	S	S	R	R	R	R	S	-	R	R	S
181	**R**	S	S	R	R	R	R	S	-	R	R	S
182	**R**	S	S	R	R	R	R	S	-	S	R	R
183	**S**	S	S	S	S	R	R	S	-	S	S	S
184	**S**	S	S	S	S	R	R	S	-	S	S	S
185	**S**	S	S	S	S	S	S	S	-	S	S	S
186	**S**	S	S	S	S	R	R	S	-	S	S	S

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
187	**S**	S	S	S	S	R	R	S	-	S	R	S
188	**R**	S	S	R	R	R	S	S	-	R	S	R
189	**R**	S	S	R	R	R	R	S	-	S	R	R
190	**R**	S	S	S	S	R	R	S	-	S	S	S
191	**R**	S	S	R	R	R	R	S	-	S	R	R
192	**S**	S	S	S	S	R	R	S	-	S	S	S
193	**S**	S	S	S	R	S	S	S	-	S	S	S
194	**R**	R	S	R	R	R	R	S	-	R	R	R
195	**R**	R	S	R	R	R	R	S	-	R	R	R
196	**S**	S	S	S	S	R	R	S	-	S	S	S
197	**S**	S	S	S	R	R	R	S	-	S	S	S
198	**S**	S	S	S	S	R	R	S	-	S	S	S
199	**S**	S	S	S	S	S	S	S	-	S	S	S
200	**R**	R	S	R	R	R	R	S	-	R	R	R
201	**S**	S	S	S	S	R	R	S	-	S	S	S
202	**S**	S	S	S	S	S	S	S	-	S	S	S
203	**R**	S	S	R	R	R	R	S	-	S	R	R
204	**R**	S	S	R	R	R	R	-	-	S	R	R
205	**S**	S	S	S	R	R	R	-	-	S	S	S
206	**S**	S	S	S	S	S	S	-	-	S	S	S
207	**R**	S	S	R	R	R	R	-	-	S	R	R
208	**R**	S	S	R	R	R	R	-	-	R	R	R
209	**S**	S	S	S	S	R	R	-	-	S	S	S
210	**S**	S	S	S	S	R	R	-	-	S	S	S
211	**S**	S	S	S	S	R	R	-	-	S	S	S
212	**S**	S	S	S	S	R	R	-	-	S	S	S
213	**S**	S	S	S	S	R	R	-	-	S	S	S
214	**R**	S	S	R	R	R	R	-	-	R	R	R
215	**S**	S	S	S	S	R	R	-	-	S	S	S
216	**R**	S	S	R	R	R	R	-	-	R	R	R
217	**R**	S	S	R	R	R	R	-	-	R	R	R
218	**S**	S	S	S	S	S	S	-	-	S	S	S
219	**R**	S	S	R	R	R	R	-	-	S	R	R
220	**R**	S	S	R	R	R	R	-	-	S	R	R
221	**S**	S	S	S	S	S	S	-	-	S	S	S
222	**R**	S	S	R	R	R	R	-	-	R	R	R
223	**R**	S	S	R	R	R	R	-	-	R	R	R

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
224	**R**	S	S	R	R	R	R	-	-	S	R	R
225	**R**	S	S	R	R	R	R	-	-	R	R	R
226	**S**	S	S	R	R	R	R	-	-	S	R	S
227	**R**	S	S	R	R	R	R	-	-	R	R	R
228	**S**	S	S	S	S	R	R	-	-	S	S	S
229	**R**	S	S	R	R	R	R	-	-	S	R	R
230	**S**	S	S	S	S	S	S	-	-	S	S	S
231	**S**	S	S	S	S	R	R	-	-	S	S	S
232	**R**	S	S	R	R	R	R	-	-	S	R	R
233	**R**	S	R	R	R	R	R	-	-	R	R	R
234	**R**	S	S	R	R	R	R	-	-	S	R	R
235	**R**	S	S	R	R	R	R	-	-	S	R	R
236	**R**	S	S	R	R	R	R	-	-	R	R	R
237	**R**	S	S	R	R	R	R	-	-	S	R	R
238	**R**	S	S	R	R	R	R	-	-	S	R	R
239	**R**	S	S	S	R	R	R	-	-	S	R	S
240	**R**	S	S	S	R	R	R	-	-	R	R	R
241	**S**	S	S	S	S	S	S	-	-	S	S	S
242	**S**	S	S	S	S	R	R	-	-	S	S	S
243	**R**	S	S	R	R	R	R	-	-	R	R	R
244	**R**	S	S	S	R	R	R	-	-	S	R	R
245	**R**	S	S	R	R	R	R	-	-	R	R	R
246	**S**	S	S	S	S	S	S	-	-	S	S	S
247	**R**	S	S	R	R	R	R	-	-	S	R	R
248	**R**	S	S	R	R	R	R	-	-	R	R	R
249	**S**	S	S	S	S	S	S	-	-	S	S	S
250	**S**	S	S	S	S	R	R	-	-	S	S	S
251	**S**	S	S	S	S	R	R	-	-	S	S	S
252	**S**	S	S	S	S	S	S	-	-	S	S	S
253	**S**	S	S	R	S	R	R	-	-	S	S	S
254	**S**	S	S	R	S	R	R	-	-	S	R	S
255	**R**	S	S	S	R	R	R	-	-	S	S	S
256	**S**	S	S	S	S	S	S	-	-	S	S	S
257	**S**	S	S	R	S	S	S	-	-	R	R	R
258	**R**	S	S	R	S	R	R	-	-	S	S	S
259	**R**	S	R	R	R	R	R	-	-	R	R	R

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
260	**R**	S	S	R	R	R	R	-	-	R	R	R
261	**R**	S	S	R	R	R	R	-	-	R	R	R
262	**S**	S	S	S	S	R	R	-	-	S	S	S
263	**R**	S	S	R	R	R	R	-	-	R	R	R
264	**R**	S	S	R	R	R	R	-	-	S	R	R
265	**S**	S	S	S	S	R	R	-	-	S	S	S
266	**S**	S	S	S	S	R	R	-	-	S	S	S
267	**R**	S	S	R	R	R	R	-	-	S	R	R
268	**S**	S	S	S	S	R	R	-	-	S	S	S
269	**R**	S	S	S	S	R	R	-	-	S	S	S
270	**S**	S	S	S	S	R	R	-	-	S	S	S
271	**R**	S	S	R	R	R	R	-	-	R	R	R
272	**R**	S	S	R	R	R	R	-	-	S	R	R
273	**R**	S	S	R	R	R	R	-	-	R	R	R
274	**S**	S	S	S	S	R	R	-	-	S	S	S
275	**S**	S	S	S	S	R	R	-	-	S	S	S
276	**S**	S	S	S	S	R	R	-	-	S	S	S
277	**S**	S	S	S	S	R	R	-	-	S	S	S
278	**S**	S	S	S	S	S	S	-	-	S	S	S
279	**S**	S	S	S	S	R	R	-	-	S	S	S
280	**R**	S	R	R	R	R	R	-	-	R	R	R
281	**S**	S	S	S	S	S	S	-	-	S	S	S
282	**S**	S	S	S	S	R	R	-	-	S	S	S
283	**S**	S	S	S	S	R	R	-	-	S	S	S
284	**R**	S	S	R	R	R	R	-	-	R	R	R
285	**S**	S	S	S	S	S	S	-	-	S	S	S
286	**S**	S	S	S	S	R	R	-	-	S	S	S
287	**S**	S	S	S	S	R	R	-	-	S	S	S
288	**R**	S	R	R	R	R	R	-	-	R	R	R
289	**S**	S	S	S	S	S	S	-	-	S	S	S
290	**R**	S	S	R	R	R	R	-	-	S	R	R
291	**S**	S	S	S	S	R	R	-	-	S	S	S
292	**S**	S	S	S	S	R	R	-	-	S	S	S
293	**S**	S	S	S	S	S	S	-	-	S	S	S
294	**S**	S	S	S	S	R	R	-	-	S	S	S
295	**S**	S	S	S	S	R	R	-	-	S	S	S

Table B. 2: Results of Antibiotic Susceptibility Test in Turkish Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
296	**S**	S	S	S	S	S	S	-	-	S	S	S
297	**R**	S	S	R	R	R	R	-	-	S	R	R
298	**S**	S	S	S	S	R	R	-	-	S	S	S
299	**S**	S	S	S	S	R	R	-	-	S	S	S
300	**R**	S	S	R	R	R	R	-	-	S	R	R
301	**R**	R	S	R	R	R	R	-	-	S	R	R
302	**R**	S	S	R	R	R	R	-	-	S	R	R
303	**S**	S	S	S	S	R	R	-	-	S	S	S
304	**R**	S	S	R	R	R	R	-	-	S	R	R
305	**S**	S	S	S	S	S	S	-	-	S	S	S
306	**S**	S	S	S	S	R	R	-	-	S	S	S

APPENDIX C.

RESULTS OF INVESTIGATIONS IN LIBYAN HOSPITALS

Table C. 1: Results of Specimens' collection in Libyan hospitals

No	Sex	Age	Clinical Sample	Strain	Hospital
1	M	45	Pus	**MRSA**	Aljalla Hospital
2	F	18	Wounds	**MSSA**	Aljalla Hospital
3	M	20	Pus	**MRSA**	Aljalla Hospital
4	M	23	Wounds	**MRSA**	Aljalla Hospital
5	M	49	Aspiration	**MSSA**	Aljalla Hospital
6	F	16	Wounds	**MRSA**	Aljalla Hospital
7	M	52	Urine	**MRSA**	Jamahyria Hospital
8	M	37	Pus	**MSSA**	Jamahyria Hospital
9	M	12	Blood	**MRSA**	Jamahyria Hospital
10	F	68	Wounds	**MSSA**	Jamahyria Hospital
11	M	51	Urine	**MRSA**	Jamahyria Hospital
12	M	49	Blood	**MRSA**	Jamahyria Hospital
13	M	1	Sputum	**MRSA**	Jamahyria Hospital
14	F	13	Wounds	**MSSA**	Jamahyria Hospital
15	M	72	Pus	**MRSA**	Jamahyria Hospital
16	F	18	Wounds	**MSSA**	Aljalla Hospital
17	M	45	Aspiration	**MRSA**	Aljalla Hospital
18	M	72	Urine	**MRSA**	Aljalla Hospital
19	M	12	Blood	**MRSA**	Aljalla Hospital
20	M	16	Wounds	**MRSA**	Aljalla Hospital
21	M	41	Urine	**MRSA**	Jamahyria Hospital
22	F	47	Blood	**MRSA**	Jamahyria Hospital
23	M	40	Pus	**MRSA**	Jamahyria Hospital
24	F	47	Wounds	**MRSA**	Jamahyria Hospital
25	F	39	Pus	**MSSA**	Aljalla Hospital

Table C. 1: Results of Specimens' collection in Libyan Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
26	M	73	Pus	**MSSA**	Aljalla Hospital
27	M	47	Pus	**MSSA**	Aljalla Hospital
28	F	64	Wounds	**MRSA**	Aljalla Hospital
29	F	17	Pus	**MSSA**	Aljalla Hospital
30	M	5	Aspiration	**MRSA**	Aljalla Hospital
31	F	21	Wounds	**MRSA**	Aljalla Hospital
32	F	14	Burn	**MSSA**	Aljalla Hospital
33	F	48	Pus	**MRSA**	Aljalla Hospital
34	M	62	Pus	**MRSA**	Aljalla Hospital
35	F	63	Wounds	**MRSA**	Aljalla Hospital
36	M	61	Pus	**MRSA**	Aljalla Hospital
37	M	33	Pus	**MSSA**	Jamahyria Hospital
38	M	72	Burn	**MRSA**	Jamahyria Hospital
39	F	38	Wounds	**MSSA**	Jamahyria Hospital
40	M	66	Aspiration	**MRSA**	Jamahyria Hospital
41	F	56	Wounds	**MSSA**	Aljalla Hospital
42	M	31	pus	**MRSA**	Aljalla Hospital
43	F	23	Blood	**MRSA**	Jamahyria Hospital
44	M	76	Blood	**MRSA**	Jamahyria Hospital
45	F	52	Urine	**MRSA**	Jamahyria Hospital
46	F	47	Pus	**MSSA**	Jamahyria Hospital
47	M	38	Sputum	**MSSA**	Jamahyria Hospital
48	M	56	Wounds	**MRSA**	Jamahyria Hospital
49	M	70	Pus	**MRSA**	Aljalla Hospital
50	M	72	Blood	**MRSA**	Aljalla Hospital
51	F	30	Burn	**MRSA**	Aljalla Hospital
52	M	69	Pus	**MRSA**	Aljalla Hospital
53	F	80	Wounds	**MRSA**	Aljalla Hospital
54	M	62	Pus	**MSSA**	Aljalla Hospital
55	F	19	Pus	**MRSA**	Aljalla Hospital
56	M	26	Wounds	**MRSA**	Aljalla Hospital
57	F	52	Pus	**MRSA**	Aljalla Hospital
58	F	26	Blood	**MRSA**	Aljalla Hospital
59	F	25	Blood	**MRSA**	Jamahyria Hospital
60	F	74	Urine	**MSSA**	Jamahyria Hospital
61	M	27	Pus	**MRSA**	Jamahyria Hospital

Table C. 1: Results of Specimens' collection in Libyan Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
62	M	46	Pus	**MSSA**	Jamahyria Hospital
63	F	72	Wounds	**MRSA**	Jamahyria Hospital
64	M	50	Wounds	**MRSA**	Jamahyria Hospital
65	F	56	Urine	**MSSA**	Jamahyria Hospital
66	M	21	Blood	**MSSA**	Jamahyria Hospital
67	F	80	Pus	**MSSA**	Aljalla Hospital
68	M	52	Pus	**MRSA**	Aljalla Hospital
69	F	44	Pus	**MRSA**	Aljalla Hospital
70	M	74	Wounds	**MSSA**	Aljalla Hospital
71	F	50	Pus	**MSSA**	Aljalla Hospital
72	F	48	Pus	**MSSA**	Aljalla Hospital
73	F	26	Aspiration	**MRSA**	Aljalla Hospital
74	F	38	Pus	**MSSA**	Aljalla Hospital
75	M	26	Wounds	**MRSA**	Aljalla Hospital
76	F	15	Pus	**MSSA**	Aljalla Hospital
77	F	31	Pus	**MRSA**	Aljalla Hospital
78	M	25	Pus	**MRSA**	Jamahyria Hospital
79	M	14	Urine	**MSSA**	Jamahyria Hospital
80	M	73	Blood	**MSSA**	Jamahyria Hospital
81	M	50	Blood	**MSSA**	Jamahyria Hospital
82	F	30	Pus	**MRSA**	Aljalla Hospital
83	M	50	Sputum	**MRSA**	Aljalla Hospital
84	M	23	Pus	**MRSA**	Aljalla Hospital
85	F	45	Blood	**MSSA**	Aljalla Hospital
86	M	51	Aspiration	**MSSA**	Aljalla Hospital
87	F	61	Wounds	**MSSA**	Aljalla Hospital
88	F	45	Urine	**MSSA**	Aljalla Hospital
89	F	27	Pus	**MSSA**	Aljalla Hospital
90	M	30	Wounds	**MSSA**	Aljalla Hospital
91	F	42	Pus	**MSSA**	Jamahyria Hospital
92	F	21	Wounds	**MSSA**	Jamahyria Hospital
93	F	38	Pus	**MSSA**	Jamahyria Hospital
94	M	26	Aspiration	**MRSA**	Aljalla Hospital
95	F	48	Pus	**MSSA**	Aljalla Hospital
96	M	51	Pus	**MRSA**	Aljalla Hospital
97	F	50	Blood	**MRSA**	Aljalla Hospital
98	M	69	Wounds	**MSSA**	Aljalla Hospital

Table C 1: Results of Specimens' collection in Libyan Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
99	F	79	Blood	**MRSA**	Aljalla Hospital
100	M	3day	Blood	**MRSA**	Jamahyria Hospital
101	F	31	Sputum	**MRSA**	Aljalla Hospital
102	M	76	Pus	**MSSA**	Aljalla Hospital
103	M	51	Pus	**MRSA**	Aljalla Hospital
104	F	56	Blood	**MRSA**	Jamahyria Hospital
105	F	31	Urine	**MSSA**	Jamahyria Hospital
106	M	69	Blood	**MRSA**	Jamahyria Hospital
107	F	52	Pus	**MSSA**	Jamahyria Hospital
108	F	22	Burn	**MSSA**	Jamahyria Hospital
109	M	36	Urine	**MRSA**	Jamahyria Hospital
110	F	41	Pus	**MRSA**	Jamahyria Hospital
111	F	30	Pus	**MRSA**	Jamahyria Hospital
112	F	30	Wounds	**MRSA**	Jamahyria Hospital
113	F	79	Pus	**MRSA**	Aljalla Hospital
114	M	47	Pus	**MRSA**	Aljalla Hospital
115	F	18	Pus	**MSSA**	Aljalla Hospital
116	M	23	Wounds	**MRSA**	Aljalla Hospital
117	M	45	Blood	**MSSA**	Aljalla Hospital
118	F	51	Urine	**MRSA**	Aljalla Hospital
119	F	24	Pus	**MRSA**	Jamahyria Hospital
120	F	25	Semen	**MSSA**	Jamahyria Hospital
121	M	33	Aspiration	**MSSA**	Jamahyria Hospital
122	F	83	Pus	**MRSA**	Jamahyria Hospital
123	M	42	Wounds	**MRSA**	Jamahyria Hospital
124	M	52	Urine	**MRSA**	Jamahyria Hospital
125	M	74	blood	**MSSA**	Aljalla Hospital
126	F	13	Pus	**MSSA**	Aljalla Hospital
127	F	34	Burn	**MSSA**	Aljalla Hospital
128	M	61	Pus	**MRSA**	Aljalla Hospital
129	F	50	Sputum	**MRSA**	Jamahyria Hospital
130	M	48	Pus	**MRSA**	Jamahyria Hospital
131	F	69	Blood	**MRSA**	Jamahyria Hospital
132	M	27	Aspiration	**MRSA**	Aljalla Hospital
133	M	48	Wound	**MRSA**	Aljalla Hospital
134	F	50	Urine	**MSSA**	Jamahyria Hospital
135	M	38	Pus	**MRSA**	Jamahyria Hospital
136	F	27	Wounds	**MRSA**	Jamahyria Hospital

Table C. 1: Results of Specimens' collection in Libyan Hospitals (continued)

No	Sex	Age	Clinical Sample	Strain	Hospital
137	F	1	Sputum	**MSSA**	Jamahyria Hospital
138	F	30	Semen	**MRSA**	Jamahyria Hospital
139	M	39	Pus	**MRSA**	Aljalla Hospital
140	F	30	Pus	**MSSA**	Aljalla Hospital
141	F	20	Wounds	**MRSA**	Aljalla Hospital
142	M	80	Pus	**MSSA**	Aljalla Hospital
143	F	19	Wounds	**MRSA**	Aljalla Hospital
144	M	40	Wounds	**MRSA**	Aljalla Hospital
145	F	70	Aspiration	**MSSA**	Aljalla Hospital
146	F	33	Pus	**MSSA**	Aljalla Hospital
147	F	22	Pus	**MRSA**	Aljalla Hospital
148	M	16	Wounds	**MSSA**	Aljalla Hospital
149	M	40	Urine	**MRSA**	Aljalla Hospital
150	M	25	Pus	**MRSA**	Aljalla Hospital

Table C. 2: Results of Antibiotic Susceptibility Test in Libyan Hospitals

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
1	**R**	I	S	S	S	R	R	S	R	S	R	S
2	**S**	S	S	S	S	R	R	S	S	S	S	S
3	**R**	I	R	R	R	R	R	S	R	R	R	R
4	**R**	R	R	S	S	R	R	S	S	S	S	S
5	**S**	S	S	S	S	R	R	S	S	S	S	S
6	**R**	I	R	S	S	R	R	S	S	S	S	S
7	**R**	I	R	R	R	R	R	S	R	R	R	R
8	**S**	S	S	S	S	R	R	S	S	S	S	S
9	**R**	S	S	I	S	R	R	S	S	S	S	S
10	**S**	S	S	S	S	R	R	S	S	S	S	S
11	**R**	S	S	I	S	R	R	S	S	S	S	S
12	**R**	R	R	R	R	R	R	S	R	R	R	R
13	**R**	R	R	R	R	R	R	S	R	R	R	R
14	**S**	S	S	S	S	R	R	S	S	S	S	S
15	**R**	R	R	R	R	R	R	S	R	R	R	R
16	**S**	S	S	S	S	R	R	S	S	S	S	S
17	**R**	I	R	R	R	R	R	S	R	R	R	R
18	**R**	R	R	R	S	R	R	S	S	S	R	S
19	**R**	I	S	S	S	R	R	S	S	S	S	S
20	**R**	R	R	R	R	R	R	S	R	R	R	R
21	**R**	S	S	S	S	R	R	S	S	S	S	S
22	**R**	R	R	R	R	R	R	S	R	R	R	R
23	**R**	I	R	R	R	R	R	S	R	R	R	R
24	**R**	S	S	S	S	R	R	S	S	S	S	S
25	**S**	S	S	S	S	R	R	S	S	S	S	S
26	**S**	S	S	S	S	S	S	S	S	S	S	S
27	**S**	S	S	S	S	R	R	S	S	S	S	S
28	**R**	S	S	I	S	R	R	S	S	S	S	S
29	**S**	S	S	S	S	R	R	S	S	S	S	S
30	**R**	I	S	R	S	R	R	S	S	R	R	S
31	**R**	R	R	R	R	R	R	S	R	R	R	R
32	**S**	S	S	S	S	R	R	S	S	S	S	S
33	**R**	S	S	S	S	R	R	S	S	S	S	S
34	**R**	I	S	S	S	R	R	S	S	S	S	S
35	**R**	I	R	R	R	R	R	S	S	S	R	R
36	**R**	I	R	R	S	R	R	S	S	R	S	R
37	**S**	S	S	S	S	R	R	S	S	S	S	S

Table C 2: Results of Antibiotic Susceptibility Test in Libyan Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
38	**R**	I	S	I	S	R	R	S	S	S	S	S
39	**S**	S	S	S	S	R	R	S	S	S	S	S
40	**R**	S	S	R	S	R	R	S	S	R	R	R
41	**S**	S	S	S	S	R	R	S	S	S	S	S
42	**R**	S	S	R	S	R	R	S	S	R	R	R
43	**R**	S	S	S	S	R	R	S	S	S	S	S
44	**R**	I	R	R	R	R	R	S	S	S	R	R
45	**R**	S	R	S	R	R	S	S	R	R	S	S
46	**S**	S	S	S	S	R	R	S	S	S	S	S
47	**S**	S	S	S	S	R	R	S	S	S	S	S
48	**R**	S	S	R	S	R	R	S	S	R	R	R
49	**R**	I	S	R	S	R	R	S	S	R	S	R
50	**R**	S	S	S	S	R	R	S	S	S	S	S
51	**R**	S	S	R	S	R	R	S	S	S	R	R
52	**R**	S	S	R	S	R	R	S	S	R	R	S
53	**R**	I	S	R	S	R	R	S	S	R	R	S
54	**S**	S	S	S	S	R	R	S	S	S	S	S
55	**R**	S	S	R	S	R	R	S	S	R	R	S
56	**R**	S	S	R	S	R	R	S	S	R	R	R
57	**R**	S	S	S	S	S	R	R	S	S	S	S
58	**R**	S	S	R	S	R	R	S	S	R	S	R
59	**R**	S	S	R	S	R	R	S	S	S	R	R
60	**S**	S	S	S	S	R	R	S	S	S	S	S
61	**R**	I	R	R	R	R	R	S	S	S	R	R
62	**S**	S	S	S	S	R	R	S	S	S	S	S
63	**R**	I	S	R	S	R	R	S	S	R	R	R
64	**R**	S	S	R	S	R	R	S	S	R	R	R
65	**S**	S	S	S	S	R	R	S	S	S	S	S
66	**S**	S	S	S	S	S	R	S	S	S	S	S
67	**S**	S	S	S	S	R	R	S	S	S	S	S
68	**R**	R	S	I	S	R	R	S	S	S	S	S
69	**R**	S	S	R	S	R	R	S	S	R	R	R
70	**S**	S	S	S	S	R	R	S	S	S	S	S
71	**S**	S	S	S	S	R	R	S	S	S	S	S
72	**S**	S	S	S	S	S	S	S	S	S	S	S
73	**R**	R	S	R	S	R	R	S	S	R	R	S
74	**S**	S	S	S	S	R	R	S	S	S	S	S

Table C. 2: Results of Antibiotic Susceptibility Test in Libyan Hospitals (continued)

No.	OX	V	C	E	TE	AMP	AMX	F	CTT	DA	CIP	G
75	**R**	S	S	R	S	R	R	S	S	R	R	S
76	**S**	S	S	S	S	R	R	S	S	S	S	S
77	**R**	I	S	R	S	R	R	S	S	R	R	S
78	**R**	I	S	I	S	R	R	S	R	S	R	S
79	**S**	S	S	S	S	R	R	S	S	S	S	S
80	**S**	S	S	S	S	R	R	S	S	S	S	S
81	**S**	S	S	S	S	R	R	S	S	S	S	S
82	**R**	I	S	I	I	R	R	S	S	S	S	S
83	**R**	I	R	R	R	R	R	S	S	S	R	R
84	**R**	S	S	R	S	R	R	S	S	R	R	S
85	**S**	S	S	S	S	R	R	S	S	S	S	S
86	**S**	S	S	S	S	R	R	S	S	S	S	S
87	**S**	S	S	S	S	R	R	S	S	S	S	S
88	**S**	S	S	S	S	R	R	S	S	S	S	S
89	**S**	S	S	S	S	R	R	S	S	S	S	S
90	**S**	S	S	S	S	R	R	S	S	S	S	S
91	**S**	S	S	S	S	S	S	S	S	S	S	S
92	**S**	S	S	S	S	S	S	S	S	S	S	S
93	**S**	S	S	S	S	R	R	S	S	S	S	S
94	**R**	I	S	S	I	R	R	S	S	S	S	S
95	**S**	S	S	S	S	S	R	S	S	S	S	S
96	**R**	S	S	R	S	R	R	S	S	R	R	S
97	**R**	I	S	R	S	R	R	S	S	R	R	S
98	**S**	S	S	S	S	R	R	S	S	S	S	S
99	**R**	S	S	S	S	R	R	S	S	S	S	S
100	**R**	R	R	R	S	R	R	R	R	R	R	S
101	**R**	R	S	R	S	R	R	S	S	R	R	R
102	**S**	S	S	S	S	R	R	S	S	S	S	S
103	**R**	I	S	R	S	R	R	S	S	R	R	R
104	**R**	S	S	S	S	R	R	S	S	S	S	S
105	**S**	S	S	S	S	R	R	S	S	S	S	S
106	**R**	I	S	S	S	R	R	S	R	S	R	S
107	**S**	S	S	S	S	R	R	S	S	S	S	S
108	**S**	S	S	S	S	S	S	S	S	S	S	S
109	**R**	I	S	S	S	R	R	S	S	S	S	S
110	**R**	S	S	I	I	R	R	S	S	S	S	S

Table C. 2: Results of Antibiotic Susceptibility Test in Libyan Hospitals (continued)

111	**R**	S	S	S	I	R	R	S	S	S	S	S
112	**R**	S	R	R	R	R	R	S	R	R	R	R
113	**R**	S	S	R	S	R	R	S	S	R	R	R
114	**R**	I	S	I	I	R	R	S	S	S	S	S
115	**S**	S	S	S	S	R	R	S	S	S	S	S
116	**R**	S	R	R	R	R	R	S	R	R	R	R
117	**S**	S	S	S	S	R	S	S	S	S	S	S
118	**R**	I	S	R	S	R	R	S	S	R	R	S
119	**R**	I	R	R	R	R	R	S	S	S	R	R
120	**S**	S	S	S	S	S	R	S	S	S	S	S
121	**S**	S	S	S	S	R	R	S	S	S	S	S
122	**R**	S	S	R	S	R	R	S	S	S	S	R
123	**R**	S	S	S	S	R	R	S	S	S	S	S
124	**R**	I	S	S	S	R	R	S	R	S	R	S
125	**S**	S	S	S	S	R	R	S	S	S	S	S
126	**S**	S	S	S	S	R	R	S	S	S	S	S
127	**S**	S	S	S	S	S	S	S	S	S	S	S
128	**R**	I	S	S	S	R	R	S	S	S	S	R
129	**R**	S	R	R	R	R	R	S	R	S	R	R
130	**R**	R	R	S	S	R	R	S	S	R	R	S
131	**S**	S	S	S	S	S	S	S	S	S	S	S
132	**R**	I	R	R	R	R	R	S	S	S	R	R
133	**R**	S	R	S	R	R	S	S	R	R	S	R
134	**S**	S	S	S	S	R	R	S	S	S	S	S
135	**R**	S	S	S	S	R	R	S	S	S	S	S
136	**R**	I	S	I	I	R	R	S	S	S	S	S
137	**S**	S	S	S	S	S	S	S	S	S	S	S
138	**R**	I	R	R	R	R	R	S	S	S	R	R
139	**R**	I	R	R	R	R	R	S	S	S	R	R
140	**S**	S	S	S	S	R	R	S	S	S	S	S
141	**R**	I	R	R	R	R	R	S	S	S	R	R
142	**S**	S	S	S	S	S	S	S	S	S	S	S
143	**R**	S	S	I	I	R	R	S	S	S	S	S
144	**R**	I	R	R	R	R	R	S	R	R	R	R
145	**S**	S	S	S	S	R	R	S	S	S	S	S
146	**S**	S	S	S	S	S	R	S	S	S	S	S
147	**R**	I	S	S	S	R	R	S	R	S	R	S
148	**S**	S	S	S	S	S	S	S	S	S	S	S
149	**R**	I	S	S	S	R	R	S	S	S	S	S
150	**R**	S	S	S	S	R	R	S	S	S	S	S

APPENDIX D.

THE RESULTS OF GENETIC INVESTIGATIONS STUDIES IN BOTH COUNTRIES (50 ISOLATES).

Table D. 1: Types of 30 samples from Turkish hospital and 20 Samples from Libyan hospitals

No. of Sample	Type of Samples	Strains	Hospitals
1	Blood	MRSA	Ankara Hospital
2	Wounds	MRSA	Hacettepe Hospital
3	Blood	CoNS	Ankara Hospital
4	Catheter	MRSA	Hacettepe Hospital
5	Wounds	MRSA	Ankara Hospital
6	Urine	MRSA	Hacettepe Hospital
7	Blood	MRSA	Ankara Hospital
8	Aspiration	MRSA	Hacettepe Hospital
9	Wound	MRSA	Ankara Hospital
10	Blood	MRSA	Hacettepe Hospital
11	Blood	MRSA	Hacettepe Hospital
12	Blood	MRSA	Hacettepe Hospital
13	Blood	MRSA	Hacettepe Hospital
14	Catheter	MRSA	Hacettepe Hospital
15	Aspiration	MRSA	Ankara Hospital
16	Blood	MRSA	Ankara Hospital
17	Pus	MRSA	Ankara Hospital
18	Aspiration	MRSA	Ankara Hospital
19	Blood	MRSA	Hacettepe Hospital
20	Aspiration	MRSA	Hacettepe Hospital
21	Pus	MRSA	Jamahyria Hospital
22	Wounds	MRSA	Jamahyria Hospital
23	Pus	MRSA	Aljalla Hospital
24	Blood	MRSA	Aljalla Hospital
25	Pus	MRSA	Jamahyria Hospital
26	Wounds	MRSA	Jamahyria Hospital
27	Blood	MRSA	Jamahyria Hospital

Table D. 1: Types of 30 samples from Turkish hospital and 20 Samples from Libyan hospitals (continued)

No. of Sample	Type of Samples	Strains	Hospitals
28	Blood	MRSA	Jamahyria
29	Sputum	MRSA	Jamahyria
30	Pus	MRSA	Aljalla
31	Urine	MRSA	Jamahyria
32	Wounds	MRSA	Jamahyria
33	Pus	MRSA	Jamahyria
34	Pus	MRSA	Jamahyria
35	Pus	MRSA	Aljalla
36	Wounds	MRSA	Aljalla
37	Urine	MRSA	Aljalla
38	Aspiration	MRSA	Aljalla
39	Blood	MRSA	Aljalla
40	Pus	MRSA	Aljalla
41	Blood	MRSA	Hacettepe
42	Blood	MRSA	Hacettepe
43	Blood	MRSA	Hacettepe
44	Pus	MRSA	Hacettepe
45	Aspiration	MRSA	Ankara
46	Blood	MRSA	Ankara
47	Blood	MRSA	Hacettepe
48	Blood	MRSA	Ankara
49	Blood	MRSA	Ankara
50	Blood	MRSA	Ankara

APPENDIX D. The results of genetic investigations studies in both Countries

Table D. 2: The results of genetic investigations studies in Turkish hospitals

Sample No	nuc	mecA	ccr-BI	ccr-BII	homi	sim	ccr-BIII	ccr-BIV	aacA	ermA	ermC	lug	tetM	tetK	epi	PVL	CoNS	haem
1	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
2	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
3	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
8	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
9	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
12	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table D. 2: The results of genetic investigations studies in Turkish hospitals (continued)

Sample No	nuc	mecA	ccr-BI	ccr-BII	homi	sim	ccr-BIII	ccr-BIV	aacA	ermA	ermC	lug	tetM	tetK	epi	PVL	CoNS	haem
16	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
23	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
24	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
25	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
26	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
27	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
28	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
29	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
30	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table D. 3: The results of genetic investigations studies in Libyan hospitals

Sample No	nuc	mecA	ccr-BI	ccr-BII	homi	sim	ccr-BIII	ccr-BIV	aacA	ermA	ermC	lug	tetM	tetK	epi	PVL	CoNS	haem
1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
12	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
13	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
15	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
16	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
17	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
18	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
19	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
20	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX E: Microbiological identification of *S. aureus* in both countries

Figure E. 1: Beta haemolysis *S. aureus* on blood agar collected from Turkish Hospitals

Figure E. 2: Beta haemolysis S. aureus on blood agar collected from Libyan Hospitals.

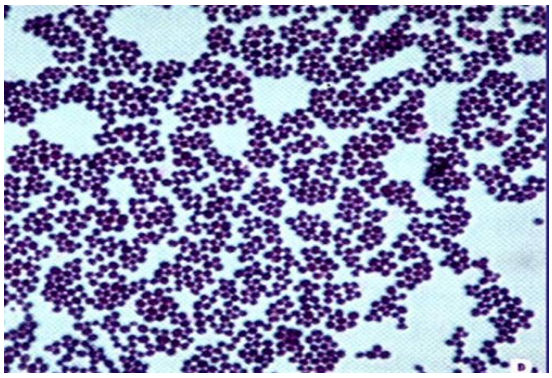

Figure E. 3: Microscopic slide, Gram positive cocci (*Staphylococci*)

Figure E. 4: Catalse test (Positive and Negative result)

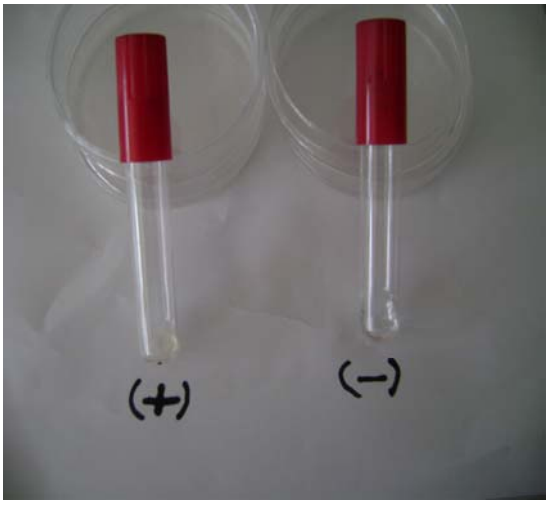

Figure E. 5: Coagulase test (Positive and Negative result)

APPEDIX F. RESULTS OF DESCRIPTIVE STATISTICS FOR AGES OF TURKISH PATIENTS AND LIBYAN PATIENTS

A. Descriptive Statistics: Ages of Turkish patients

Variable	N	Mean	Median	TrMean	StDev	SE Mean
Ages of	306	48.17	50.00	48.74	19.99	1.14

Variable	Minimum	Maximum	Q1	Q3
Ages of	1.00	84.00	33.75	64.00

B. Descriptive Statistics: Ages of Libyan patients

Variable	N	Mean	Median	TrMean	StDev	SE Mean
Ages of	150	42.23	42.00	42.06	20.12	1.64

Variable	Minimum	Maximum	Q1	Q3
Ages of	3day	80.00	26.00	53.00

CURRICULUM VITAE

Personal Information

Name: Annour M. Alalem
Nationality: Libyan
Date of Birth: December 7, 1967
Place of Birth: Tessawa, Libya
Marital Status: Married
e-mail: Annour_alalem@yahoo.com

Education

Degree	Institution	Graduation Year	GPA
M.S.	Laboratory medicine Department, Al-arab University, Benghazi, Libya	1999	84%
ADLM.	Laboratory medicine Department, Al-arab University, Benghazi, Libya	1996	78%
B.S.	Institute of Medical Technology Al-arab University, Benghazi, Libya	1992	82%
Health Diploma	Medical Laboratory Department, Institute of Health Benghazi, Libya	1986	93%

Professional Experience

He worked as a Laboratory medicine Technologist in General Morzok hospital from 1986 to 1992. In spring 1999, he received his M.Sc. from faculty of medicine, since then he has been a Laboratory medicine Doctor in General Morzok hospital.

He received a scholarship from his country to study for PhD at METU in Turkey; he has joined his PhD. program in Biochemistry Department of the Middle East Technical University in spring 2003-2004.

He published five articles in local conferences in the field of laboratory medicine and two articles in international conferences in the field of Hematology. His main areas of research interest are clinical biochemistry, clinical microbiology, and clinical hematology.