

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Antibiotic Susceptibilities and SDS-PAGE Protein Profiles of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) Strains Obtained from Denizli Hospital

---

Göksel Doğan, Gülümser Acar Doğanlı,  
Yasemin Gürsoy and Nazime Mercan Doğan

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55457>

---

## 1. Introduction

Soon after two years of introducing methicillin, *S. aureus* strains developed resistance to methicillin by through the gain of the *mecA* gene (MRSA). At first *S. aureus* strains were exclusively related to hospital acquired (HA) MRSA, but as from 1990s, community acquired (CA) MRSA came into view [1].

In both HA-MRSAs and CA- MRSAs are referred to as a significant factor of serious infections in high morbidity and mortality including bacteremia, pneumonia, endocarditis, osteomyelitis and toxic shock syndrome [2,3,4,5,6]. The factors that increase the prevalence of nosocomial bacteremia are the increase in older age groups in society, life period prolongation of people with chronic diseases, widespread use of immunosuppressive drugs, increase in interventional procedures for the purposes of diagnostic and therapeutic. Generally, some of staphylococ infections are nosocomial, other infections have occurred by depending on MRSAs. The colonization rate with MRSA has increased in parallel duration of hospitalization. These strains have been found resistant against penicillins, combinations of betalactam/betalactamase inhibitory, cephalosporins, combinations of monobactams and carbapenems. To identify the resistance of staphylococcus's against methicillin antibiotic, those methods as of disc diffusion, tube dilution or microdilution, agar scanning, agar dilution, automate susceptibility tests, DNA hybridisation techniques and polymerase chain reaction have been used [7,8,9]. The aim of this study is to identify antibiotic susceptibility and specificity of MRSAs isolated from various clinic samples with various methods including disc diffusion, SDS-PAGE and DNase test.

## 2. Methicillin resistant *Staphylococcus aureus*

### 2.1. General properties

*S. aureus* (including MRSA strains) are clusterforming, facultative aerobic, Gram-positive cocci (at 0.5-1.7  $\mu$  of diameters) with intrinsic ability to ferment carbohydrates, producing white to deep yellow pigmentation on solid culture media [2].

50% of cell wall of Staphylococci has composed from peptidoglycan. Peptidoglycan chains consist of alternative polysaccharide subunits included N-acetyl glucosamines and N-acetyl muramik acid. These chains are cross linkage by pentaglycine bridges that are specific for *S. aureus* and tetra peptide chains binded with N- acetyl muramik acid. Peptidoglycan can show endotoxin properties and structural differences among strains can lead to extensive intravenous coagulation [10,11].

Most researchers demonstrated that MRSA caused various diseases ranging from soft, superficial dermatological diseases to acute and potentially fatal systemic enervations [2,12,13]. Some MRSA live as a normal flora member in human mucose membrans and skins, others lead to ichor, abscess formation, various piogen infections and fatal septicemia. MRSA strains have also been detected in domestic animals and birds such as horses, cattle, chickens and dogs as well as associated individuals [2].

*Staphylococcus aureus* is coagulase positive and major patogen for humans. In general pathologic Staphylococci hemolysis the blood, coagulates the plasma, and also produces various extracellular enzymes and toxins. They have developed quickly against antimicrobial drugs [14].

### 2.2. Culture properties

Staphylococci grows easily aerobic or microaerofilic conditions. Optimal temperature is 37°C for these bacteria. Colonies of them are orbicular, sleek, bouffant and brilliant in solid media. In general, *S. aureus* has colonie colours that change from white to golden yellow. However, in many colonies, pigment occurs by those bacteria after long incubation time. It does not occur under anaerobic conditions or in broth [14].

### 2.3. Growing properties

Staphylococci have produced catalase variously then Streptococci. Staphylococci fermentate carbohydrates slowly by constituting acid, but they do not produce gases. On the other hand, pathogenic Staphylococci have generated so many extracellular substances. Even though, Staphylococci are durable against conditions of dry air, temperature (50°C, 30 m) and 9% of NaCl, but they can be inactivated easily with powerful chemicals such as 3% of hexaclorofen. Other one theme, these bacteria are sensitive against many of antimicrobial drugs. Resistant separates several of categories;

Mechanism of methicillin resistant: The resistant against beta-lactame antibiotics (methicillin, oxacillin, nafcillin, kloxacillin and dikloxacillin) not having been hydrolysed with beta-lacta-

mase enzyme has denominated as methicillin resistant. Thus, it is clear that the resistant is chromosomal and is not with beta-lactamase enzyme inactivated the antibiotic [15,16].

PBP 2a (PBP 2'): Although methicillin sensitive *S. Aureus* (MSSA) has five types of penicillin binding proteins (PBP), in addition these proteins, methicillin resistant *S. Aureus* (MRSA) has also a different penicillin binding protein named as PBP 2' or PBP 2a. This protein has 78 kDa of molecular weight [17]. PBP 2a shows lower affinity against beta-lactam antibiotics than other PBPs. Hence, the enzyme is single transpeptidase that has the ability to continue of peptidoglycan synthesis by showing high affinity in presence of beta-lactam antibiotics [13,18]. The gene encoded PBP 2a is 2.1 kb and named *mecA*. Though, all of MRSAs have this gene, but there is no this gene at MSSA strains. The emergence of methicillin resistant phenotypically has been able to show variability among bacteria. The phenotypically expression of methicillin resistant is possible in two ways; homogen and heterogen [17]. In homogen resistant, all of cells show high levels of resistance in presence of high methicillin concentrations by growing [19,11]. In heterogen resistance, even though all cells have *mecA* gene had information that needs for methicillin resistance, only some cells show resistance.

- a. ***mecR1-mecI* sistemi:** The *mecA* have been controlled with two regulator genes. Those genes are *mecR1* and *mecI*. Also these genes are similar to *blaR1* and *blaI*, which are regulatory genes of beta-lactamase, in terms of structure, function and mechanism of regulation. *MecI* and *mecR1* have the same regulator role for *mecA*. *MecR1* encodes a signal stimulant protein, while *mecI* encodes a protein suppressed the *mecA* [15,16]
- b. Other factors affected resistant phenotype:
  - Beta-lactamase plasmid: Production of beta-lactamase enzyme is encoded by *blaZ* gene and is controlled by genes of *blaR1* and *blaI* which are antireceptor and receptor, respectively. *BlaR1* which is a transmembrane protein binds to beta-lactam in presence of it and leads to start the synthesis of beta-lactamase enzyme by providing signal transmission from out of cell to inside of cell [20]. At the same time, it has been thought that genes of *blaR1* and *blaI* have had a role for occurring of methicillin resistant phenotypically [17].
  - Fem Factors: The obtaining sensitive strains from methicillin resistance strains by transposons via inactivation had led to identification of genes beyond of *mec*. These genes placed out of *mec* gene region had defined as "auxiliary" or "factors essential for the expression of methicillin resistance" or shortly "fem" genes [21]. Both MRSA and MSSA strains have fem factors variously from *mecA* gene. Also, it indicated that some conditions increased the occurring of methicillin resistant were correlated with modifications at cell autolysis.

## 2.4. Enzymes and toxins

Staphylococci can lead to diseases by large diffusion at tissue and producing many extracellular substances. Some of these extracellular substances are enzymes, others are toxins. Most toxins are under genetic control of plasmids. Some of them can be under both chromosomal and extrachromosomal control [22,23,24,25].

## 2.5. Coagulase and clumping factor

*S.aureus* produces coagulase being an enzyme providing coagulation of the plasma. Coagulase that can be dropped out freely binds the prothrombin and initiates polymerization of fibrin. It can also accumulate the fibrin at surface of Staphylococci. It thinks that products of coagulase are similar to invasive pathogenic power.

Clumping Factor which is responsible for binding of organism to fibrin and fibrinogen-is a surface component of *S.aureus*. When clumping factor comes up with plasma, *S.aureus* forms clumps. Clumping Factor is discrete from coagulase [22,25].

## 2.6. Enzymes

Other enzymes produced by Staphylococcus's are staphylokinase, proteinase, DNase and enzymes having different properties such as  $\beta$ -lactamase.

## 2.7. Exotoxins

Alfa toxin is a heterogen protein acting by depending on large spectrum of eucariotic cell membranes.  $\alpha$  toxin is a power hemolysin (a substance that causes the fragmentation of erythrocytes).  $\beta$  toxin have reduced the sphingomyelin, therefore it is toxic for many cells including human red blood cells. Additionally, another toxin,  $\delta$  is heterogen. It fractionates the biological membranes and can have a role at diarrheal patients because of *S.aureus*.

## 2.8. Leukocidin

This toxin of *S.aureus* has two components. It affects white blood cells in humans and rabbits. These two components have moved synergistic like  $\gamma$  toxin at the membrane of white blood cells. The toxin is an important virulence factor in community acquired MRSA (CA-MRSA) strains.

## 2.9. Toxic shock syndrome toxin

Many *S.aureus* strains isolated from patients with toxic shock syndrome produce the toxic shock syndrome toxin-1 (TSST-1) also namely enterotoxin F. TSST-1 is the super antigen and binds the MHC-II molecules that leads to T cell stimulation TSST-1. This toxin is related to febrile, shock and multisystem involvement which are scope skin disease.

## 2.10. Diagnostic laboratory tests

### 2.10.1. Gram staining

*S. Aureus* bacteria appears violet colour and looks like bunch of grapes by gram staining. It is impossible to separate pathogenic organism (*S.aureus*) from saprophytic organisms (*S.epidermidis*) by gram staining.

### 2.10.2. Catalase test

MRSAs produce catalase, which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci from the streptococci [14].

### 2.10.3. Susceptibility tests

At choosing of advisable antibiotic drug associated with therapy of infection, a number of factors such as potential infectious agents, antibiotic susceptibility, host factors that may affect the activity of the drug in vivo, the location of the infection, pharmacokinetic and pharmacodynamic properties of the drug should be evaluate [26]. Generally, *in vitro* procedures applied for determination of antimicrobial activity of an antibiotic is named as susceptibility tests. Susceptibility tests apply in the cases of not foreseeable susceptibility against antibacterial agent which will be applied at treatment of aerob and facultative anaerob bacteria which are clinically important. Susceptibility against antimicrobial drugs can be detected with a lot of methods. In most, inhibitor activity of drugs (bacteriostatic) evaluates in applied method. The applied methods with this purpose can consider of; 1. dilution methods in liquid and solid media; 2. disc diffusion method; 3. gradient diffusion (E-test) method; 4. the detection of enzymes which inactivate antimicrobial agents [26].

At disc diffusion method, paper discs is absorbed a specific amount of antibiotic place onto plate inoculated with the test microorganism. Thus, antibiotic absorbed by disc diffuses into agar and inhibits the growing of bacteria at effect levels of antibiotic. At the end of this situation, a circular inhibition zone where does not grow bacteria occurs at ambient of the disc. The categories of susceptibility as of sensitive, medium and resistance identify by measuring diameter of this zone. The limit values related to these categories detect for every antimicrobial agent by regarding accessible serum levels [26,27]. For example, Staphylococci are *mecA* positive and are resistant against methicillin at Müller-Hinton agar contented 6 of µg/mL of oxacillin and 4% of NaCl.

## 2.11. Community and hospital acquired MRSA infections

Molecular epidemiology of community acquired MRSA (CA-MRSA) is tolerably different from hospital acquired MRSA (HA-MRSA). CA-infections commonly cause of skin and soft tissue infections, bacteremia and endocarditis [2].

Panton-Valentine leukocidin (PVL) gene encoded a toxin which is responsible from virulence of bacteria and type 4 *SCCmec* genetic component frequently are presence at CA-MRSA isolates [22,16]. HA-MRSA isolates generally have type I, II or III of staphylococcal cassette chromosome (*SCCmec*) genetic component. This gene domain is responsible from showing resistant of bacteria against antibiotics made from beta-lactam and even other drugs such as clindamycin, gentamicin and florocinolone. Characterisation of the staphylococcal cassette chromosome (SCC) *mec* type has led to better discrimination of hospital acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA) [28]. SCCs are mobile elements characterized by association of a *mec complex* and *ccr genes* coding for integration into or excision from the chromosome. Three types of SCC (types I, II and III) were originally described in hospital-



acquired MRSA strains (HA-MRSA), most of them isolated before 1990. A fourth type (type IV) was recently described, first in community-acquired MRSA isolates (CA-MRSA) and then in several MRSA backgrounds, including hospital isolates [29].

To compare HA-MRSA strains with CA-MRSA, It needs to examine several aspects. Initially, chromosomal elements for meticillin resistance in community-associated strains are chromosome cassette mec (SCCmec) types IV or V, being smaller and more active than SCCmec types I–III found in hospital-acquired MRSA. In HA-MRSA, the larger gene elements are correlated with reduced bacterial ability as well as decreased toxin generation. Also, the PVL toxin is more common in CA-MRSA than in MSSA. Another thing, increased expression of certain virulence determinants which can cause more acute disease (e.g. phenol-soluble modulins-PSMs-) is available in CA MRSA. The last one,, while all *S. aureus* strains have an aptness to generate biofilms, it suggest that variations in biofilm matrix in CA-MRSA compared to other strains. But, there is no sufficient evidence in the literature that any of MRSA strain sample has a larger capability to cause invasive infection than MSSA strains [7].

## 2.12. Clinic Infections

### 2.12.1. Skin and soft tissue infections

CA-MRSA strains can be infected skin and soft tissue infections, which characteristically occur in healthy people without preconditions. For instance, the US had one of the biggest CA-MRSA epidemic, with one strain named USA300, being liable for most of infections. In 2005, 13.7% of all invasive MRSA infections in the US were community associated. [30]. Skin, soft tissue and bone infections seems at most. These infections can be confined from a localized infektion to more general infections such as cellulite, impedingo, folliculit, boll, carboncul and surgical wound infection. Staphylococcal bone marrow dermatitis (osteomyelitis) occurs typically at young children because of bacteremia. More severe manifestations can include necrotizing pneumonia, pyomyositis, sepsis, osteomyelitis and necrotizing fasciitis [7]. Even though, some of *S.aureus* infections corellate with development of TSS, nowadays most of patients have skin and soft tissue infections with this disease. For instance, Staphylococcal scalded skin syndrome seems at most in young children. It is less common in adults and older children. The reason is that some of toxins (exfoliative toxins; ETA and ETB) bind with GM4-like glycolipids in newborns sensitive epidermis. GM4-like glycolipids are not available in adult and children [31,32] Figure 1 shows a sample of skin and soft tissue infection of MRSA strains.

## 2.13. Systemic infections

### 2.13.1. Pneumonia

At 1918 in registries, obtained from influence pandemia of young individuals, most of deaths related with bacterial super infections that *S.aureus* strains lead to those infections. Recently, *S.aureus* isolates produced PVL toxin has relevanted to infections of skin and soft tissue such as pnömonia at healthy young people. PVL is a bi-component exotoxin transmitted by bacteriophages that is encoded by two genes, *luk* FPV and *luk* S-PV. PVL genes are carried by



**Figure 1.** Skin and Soft tissue Infection of MRSA [33]

nearly every CA-MRSA strain as well as a small proportion of clinical MSSA strains. This suggests that PVL has an important role in fitness, transmissibility and virulence, but the role of PVL in the pathogenesis of CA-MRSA infections is controversial [7]. PVL is a toxin fragmented speedy the white blood cells. Also, these strains can be contained other virulence factors and toxins. Consequently, this kind of infections have high rates of death.

#### 2.13.2. Treatment

It is necessary the using of a penicillin which is resistant against  $\beta$ -lactamase in bacteremia, endocarditis, pneumonia and other infections which occur by *S.aureus* strains for long times. Vancomycin has been preferred at methicillin resistant strains. If the infections are because of *S.aureus* which not produce  $\beta$ -lactamase, penicillin G has to choose for those infections. *S.aureus* strains isolated from clinic infections which are resistant against penicillin G always produce penicillinases. Those strains are generally sensitive against penicillins which are resistant  $\beta$ -lactamases, cephalosporins or vancomycin. Resistant of methicillin is independent from producing of  $\beta$ -lactamase.

Resistant of drugs such as penicillin, tetracyclin, aminoglycosid and, erythromycin which has detected by plasmids can be transferred to other staphylococci by transduction and conjugation. Antibiotics such as linezolid, daptomycin and dalbapristin can be used at serious staphylococcal or enterococcal infections of patients which have common drug resistance.



3. Material and method

3.1. MRSA isolates

Twenty-six isolates, which were isolated from intensive care unit (ICU) between September 2009-February 2010, were clinical isolates from ICU patients in Denizli Hospital. The isolates were identified as MRSA in hospital before obtaining. These isolates were obtained from tracheal aspirates, catheter tip, wounds, urine, blood, and effluxion, in a total of 26 strains were taken into account. Colonies were also checked by Gram-staining. Isolates were also identified by growth on Dnase test agar with methyl gren agar by DNase test. Table 1 shows the isolates and their origins.

Code of Strains	Source	Code of Strains	Source
MRSA 1	tracheal aspirates	MRSA 14	tracheal aspirates
MRSA 2	effluxion	MRSA 15	tracheal aspirates
MRSA 3	tracheal aspirates	MRSA 16	tracheal aspirates
MRSA 4	blood	MRSA 17	tracheal aspirates
MRSA 5	blood	MRSA 18	urine
MRSA 6	catheter tip	MRSA 19	tracheal aspirates
MRSA 7	blood	MRSA 20	blood
MRSA 8	blood	MRSA 21	tracheal aspirates
MRSA 9	tracheal aspirates	MRSA 22	catheter tip
MRSA 10	blood	MRSA 23	blood
MRSA 11	urine	MRSA 24	wound
MRSA 12	tracheal aspirates	MRSA 25	blood
MRSA 13	tracheal aspirates	MRSA 26	wound

Table shows MRSA samples obtained from 26 of patients, Denizli hospital

Table 1. MRSA strains and isolation resources

3.2. Media

Tryptic soy broth (TSB) medium (g/l:pepton from casein 17, pepton from soy meal 3, D(+)-glucose 2.5, NACL Dipotassium hydrogen phosphate) was used for cultering MRSA

strains at the study of antibiotic susceptibility. Tryptic soy agar (TSA) solid medium (g/l: pepton from casein 15.0; pepton from soymeal 5.0; Sodium chloride 5.0; agar-agar 15.0) was used for antibiogram tests. DNAase test agar with methyl green medium was used to determinate the DNAase activity. All of media were autoclaved at 121 °C for 15 min. and stored at 4 °C until using.

### 3.3. Antimicrobial susceptibilities

To determinate multiple antibiotic resistance of MRSA strains, antibiotic susceptibilities were investigated by disc diffusion method. Susceptibility to methicillin by disc diffusion had already determined by cefoxitin (30 µg) discs before samples taking from hospital. The zone of inhibition was interpreted after 24 h of incubation at 35 °C. Plates were read at 24 and 48 h of incubation at 37 °C. In addition to cefoxitin (FOX, 30 µg), the following antibiotics were tested: vancomycin (VA, 30 µg), tetracycline (TE, 30 µg), erythromycin (E, 15 µg), clindamycin (DA, 2 µg), rifampicin (RA, 5 mcg), linezolid (LZD, 30 µg), sulfamethoxazole / trimethoprim (SXT, 25 mcg), penicillin-G (P, 10 unit), amikacin (AK, 30 mcg), seforoksimsodium (CXM, 30 mcg), Novobiosin (NV, 5 µg), ampicillin (AM, 10 mcg) and gentamicin (CN, 10 mcg) antibiotics.

### 3.4. Extraction of Whole Cell Proteins (WCPs)

The method of Laemmli [34] was used by a little modification for electrophoresis. After overnight incubation at 37 °C for 24 h. in 5 ml of TSB (Merc) media, samples were centrifuged for 20 min at 6000 rpm. The pellets were washed three times with sterile distilled water and stirred after adding 25 µl SDS sample buffer (0.06 M Tris, 2.5 % glycerol, 0.5 % SDS, 1.25 % β-mercaptoethanol and 0.001 % bromophenol blue). The proteins were denatured in boiling water for 10 min. Samples stayed in eppendorf tubes were uploaded to electrophoresis apparatus.

## 4. SDS-PAGE

Denatured whole cell proteins were analyzed by SDS-PAGE according to Laemmli. This method used 4 % acrylamide stacking gel and 10 % acrylamide separating gel. MBI Fermentas SM0661 kit was used as molecular weight standard in SDS-PAGE. Electrophoresis was performed with buffer system in a Biolab gel apparatus model V20-CDC. The gel was run at 150 V for 2 h at stacking gel, 200 V for 4 h at separating gel until the bromophenol blue had reached the bottom. Gels were then stained with Coomassie Brilliant Blue R 250 (Sigma).

### 4.1. Determination of DNase activity

Following overnight incubation at 37 °C for 24 h. in TSB (Merc) media, 26 of MRSA strains were inoculated a full loop of cells to petridishes where place DNase test agar with methyl

green media and were applied the spot test. Inoculated cells were incubated for overnight at 37 °C for 24 h. After incubation, 1 N of HCl was dumped onto plates which grow MRSA colonies. It was checked whether achromatic color zone occur or not. The colonies occurring achromatic color zone were accepted as DNase positive strains, others were accepted DNase negative strains.

## 5. Results

### 5.1. Antimicrobial susceptibilities

Antimicrobial susceptibility testing was performed as recommended by the National Committee on Clinical Laboratory Standards [35,36]. MRSA samples that we used in our study were activated by culturing freshly at 37 °C for 24 h. two times. 100 µl from these activated fresh culture samples were done smear cultivation to petridishes of TSA medium. Antibiotic discs were fixed up to surface of the media by using Disc diffusion method under steril conditions. The samples were incubated at 37 °C for 24 h. and the diameters of zone were measured.

In general, It was determinated that all of strains showed resistance against all of antibiotics. No zone of inhibition has been seen only against ceftiofur (FOX, 30 µg) antibiotic. It was observed that the zone did not occur except a few strains and strains were resistant against penicillin, amikacin (AK, 30 mcg), sulfamethoxazole / trimethoprim (SXT, 25 mcg), ampicillin (AM, 10 mcg), (CN, 10 mcg), erythromycin (E, 15 µg) and rifampicin (RA, 5 mcg) antibiotics. Beyond linezolid (LZD, 30 µg) which have started clinic using also in our country recently and used alternatively as an antimicrobial agent against glykopeptits in M-R strains, it was determinated that MRSA strains used in this study were resistant against this antibiotic. Even though it was detected that there was no resistance against vancomycin (VA, 30 µg) for MRSA infection in many studies, the sensitivity of vancomycin (VA, 30 µg) decreased significantly against MRSA strains in our study. Table 2 shows the results of antibiotic susceptibility.

### 5.2. Determination of DNase activity

According to the DNase tests, three of strains are DNase negative, the rest of 26 strains are DNase positive. Strains were 11.54% DNase negative and 88.46% DNase positive. Table 2 shows the results of DNase activity (Also, see figure 3).

### 5.3. SDS-PAGE

With regard to SDS PAGE analyzes whole cell proteins (WCPs) of MRSA strains showed the similar protein profile bands. Figure 4 and 5 show the band profiles obtained from WCPs (Also, see figure 4).

Code of Strains	Antibiotics														Dnaz Activity
	VA 30	TE 30	E 15	DA 2	RA 5	LZD 30	FOX 30	SXT 25	P 10	AK 30	CXM 30	NV 5	AM 10	CN 10	
MRSA1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA6	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA7	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA9	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA11	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Negative
MRSA12	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA13	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA14	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Negative
MRSA16	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA17	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA18	R	R	R	R		R	R	MS	R	R	R	R	R	R	Negative
MRSA19	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA20	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA21	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA22	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA23	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA24	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA25	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive
MRSA26	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Positive

R; Resistant, MS; Medium Sensitive, S; Sensitive (No sensitive strains in our study)

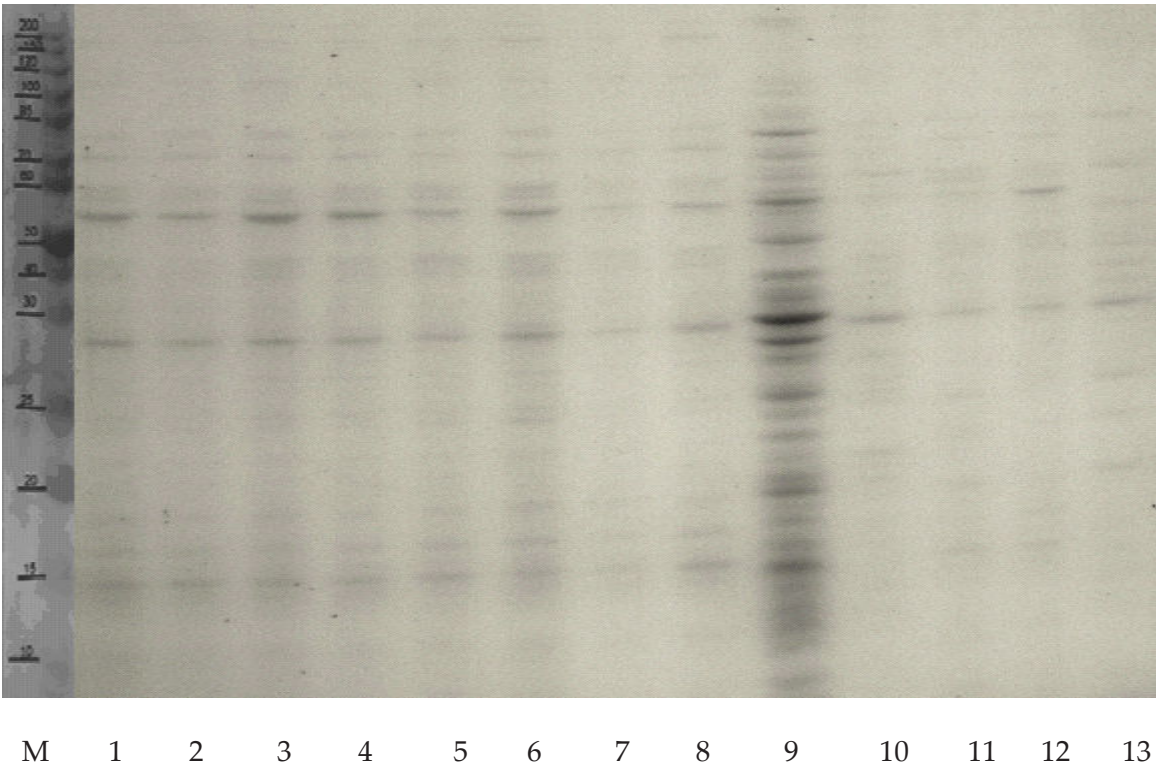
Table 2. Antibiotics susceptibility and DNase activity of MRSA strains



Figure 2. MRSA-24 antibiotic inhibition zones

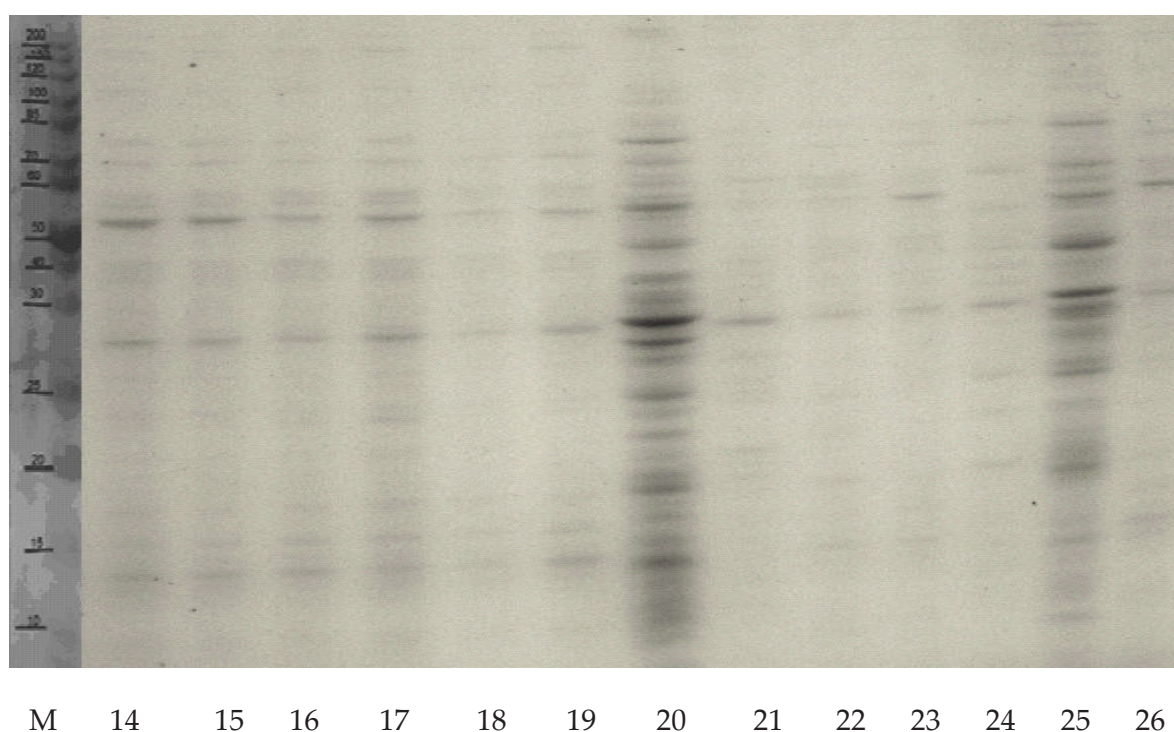


**Figure 3.** DNase activity test



**Figure 4.** Whole cell protein profiles of MRSA strains by SDS-PAGE. Line 1-13: MRSA strains from ICU; Line M: Molecular weight standard in kD.





**Figure 5.** Whole cell protein profiles of MRSA strains by SDS-PAGE. Line 14-26: MRSA strains from ICU; Line M: Molecular weight standard in kD.

## 6. Discussion

Most of methods have been applied to determinate the MRSA strains. In the present study, SDS- PAGE was used for typing MRSA strains obtained from the various wards of patients in ICU. By SDS-PAGE (WCPs), all of the 26 MRSA strains, of which 11 from tracheal aspirat, 8 from blood, 2 from urine, 2 from wounds, 2 from catheter tip and the other one from effluxion were compared proteins profiles each other. Results of this study in MRSA strains by SDS-PAGE and previous studies obviously indicate that valuable epidemiological informations can be demonstrated with electrophoretic methods. It is reported that WCPs can not be used because of the high similarities between their band patterns examined in the differentiation of MRSA strains [37]. In order to overcome this problem, it is necessary to make SDS-PAGE with the use of FPs and by N-PAGE using WCPs. Also this problem can dissolve when 5-7.5 % gel concentration uses in SDS-PAGE as opposed to 10% gel concentration [37]. In this study we applied only SDS-PAGE with use WCPs because of inadequate time of study term.

By using various antibiotic discs, in study of antibiotic susceptibility, generally all of the MRSA strains showed multiple drug resistance. The high level morbidity and mortality of MRSA infections has caused to trace the prevalence of multiple antibiotic resistant staphylococci mainly on the brink of MRSA. In Turkey between 1996-1999, the average of resistant against methicillin was detected 47,5 %. Vancomycin being glycopeptit antibiotic



is very important because of methicillin resistant in staphylococcus's. Until now the resistance of glycopeptit was no informed in many studies which have done in our country [9,38]. In our study, we investigated that none of MRSA strains were resistance against Vancomycin. Susceptibilities of antibiotic groups such as fucidic acid, sulfamethoxazole / trimethoprim, clindamycin, erithromycin, and quinolone have come into prominence because of alternativ treatment options of those antibiotics without glycopeptit antibiotics in patients having light and middle infections of MRSA and remediable on erect pozition. SXT is an other antibiotic not to be  $\beta$ -lactam which uses in resistant staphilococcus's. Even though, susceptibility of SXT for MRSA strains was 81% in our study, it was 91% in study of Sengoz [19]. Also we found that rate of resistant strains for eritromycin antibiotic was 73%. In one study done in laboratory of clinic microbiology, Haydar Paşa Hospital, this rate for eritromycin was found 71%. In general it was considered that eritromycin antibiotic is not to be alternativ antibiotic option for treatment in MRSA's. Clindamycin is an alternativ antibiotic which can use in infections of staphylococcus. We found that resistant of clindamycin to MRSA strain was 23% in present study. A few studies which had been done in Turkey ; in study of Gonluugur resistant of clindamycin was 39%, in study of Dogan it was 54% [10,39]. Linezolid is the first member of antibiotics which are from oksazolidinon group. Linezolid do not show the cross resistant with other antibiotics due to different effect mechanism of those antibiotics. Also, to resistant evolve is power against in vitro linezolid. Resistant evolving occurs typically with single nucleotid chancing in genes which encode the 23 S rRNA. In present study, susceptibility of linezolid was found 100%. It was found that linezolid antibiotic was effectiv and safe in 70% of facts with *S.aureus* infection which is not respondet or tolerant [40]. Unfortunately in our country, there is limeted number of study associated with Linezolid. This rate of resistant was 92% against gentamicin in our study.

In this study, we investigated antibiotic susceptibilities, DNase activity and protein profiles by SDS-PAGE of MRSA strains. It was determinate that MRSA strains were resistant against all of antibiotics. A significant decrease in Vancomycin susceptibility is particularly notable. Moreover, the strains were 11.54% DNase negative and 88.46% DNase positive. Also strains showed similarities of band pattern for protein profiles by SDS-PAGE. Studies of Sacilik et al. (1999) and Van Belkum et al. (1997) [41] supported that dissemination of MRSA strains in Turkish hospitals probably originated from the same clone. In order to demonstrate such informations and to understand clearly, it is nessesary to make more molecular genetic analyzes and studies in many zones of Turkey.

## Acknowledgements

We acknowledge Nilüfer AYDINLIK for her valuable assistance. We are also grateful personel of Denizli Hospital for MRSA samples.

## Author details

Göksel Doğan\*, Gülümser Acar Doğanlı, Yasemin Gürsoy and Nazime Mercan Doğan

\*Address all correspondence to: [gksldogan@hotmail.com](mailto:gksldogan@hotmail.com)

Pamukkale University, Faculty of Arts and Sciences , Department of Biology, Denizli, Turkey

## References

- [1] Goss, C.H., Muhlebach, M.S., 2011. *Staphylococcus aureus* and MRSA in cystic fibrosis. *Journal of Cystic Fibrosis* 10; 298–306
- [2] Azaez Akande, O., 2010. Global trend of methicillin-resistant *Staphylococcus aureus* and emerging challenges for control. *Afr. J. Clin. Exper. Microbiol* 11(3): 150-158.
- [3] Rello, J., Diaz, E. 2003. Pneumonia in the intensive care unit. *Crt. Care Med.*; 31: 2544 - 2551.
- [4] Graffunder. E.M., Venezia, R.A., 2002. Risk factors associated with nosocomial methicillin resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J. Antimicrob. Chemother.*; 49:999 - 1005.
- [5] Gottlieb, G.S., Fowler, V.G. Jr., Kong, L.K. et al. 2000. *Staphylococcus aureus* bacteremia in the surgical patients: a prospective analysis of 73 postoperative patients who developed *Staphylococcus aureus* bacteremia at a tertiary care facility. *J. Am. Coll. Surg.*; 190:50 - 57.
- [6] Mylotte, J.M., Tayara, A. 2000. *Staphylococcus aureus* bacteremia: predictors of 30 - day mortality in a large cohort. *Clin. Infect. Dis.*; 31: 1170 - 1174.
- [7] Watkins, R.R., David, M.Z., Salata, R.A., 2012. Current concepts on the virulence mechanisms of methicillin-resistant *Staphylococcus aureus*. *Journal of Medical Microbiology Papers in Press*. Published June 28, 2012 as doi:10.1099/jmm.0.043513-0.
- [8] Chang, S., Sievert, D.M., Hageman, J.C., 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. *N Engl J Med.*; 348:1342–1347
- [9] Chang, F.Y., MacDonald, B.B., Peacock, J.E., Jr, 2003. A prospective multicenter study of *Staphylococcus aureus* bacteremia: incidence of endocarditis, risk factors for mortality, and clinical impact of methicillin resistance. *Medicine* ; 82:322–332.

- [10] Cosgrove, S.E., Qi Y, Kaye K.S., 2005. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. *Infect Control Hosp Epidemiol*; 26:166–174.
- [11] Chambers, H.F., 1988. Methicillin-resistant staphylococci. *Clin Microbiol Rev*; 1:173-86.
- [12] Moran, G.J., Amii, R.N., Abrahamian, F.M., Talan, D.A 2005. Methicillin resistant *Staphylococcus aureus* in community - acquired skin infections. *Emerg. Infect. Dis*; 11 (11): 928 - 930.
- [13] Chambers, H.F., 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *American society for clinical microbiology*. 4; 781-91.
- [14] <http://sci.kufauniv.com/teaching/hazim/bacteria/strept.doc>.
- [15] Koneman, E.W., Allen, S.D., William, M.J., Schereckenberger, P.C., Winn, W.C., 2006. Gram-positive cocci, Part I: Staphylococci and related gram-positive cocci. Winn WC Jr et al (editors). *Color atlas and textbook of diagnostic microbiology*, 6th ed. Lippincott Williams and Wilkins; 623–71.
- [16] Jorgensen, J.H., 1997. Laboratory issues in the detection and reporting of antibacterial resistance. *Infect Dis Clin North Am*; 11:785-802.
- [17] Hartman, B.J., Tomasz, A., 1986. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus*. *Antimicrob Agents and Chemother*; 29:85-92.
- [18] Kuwahara-Arai, K., Kondo, N., Hori, S., Tateda-suzuki, E., Hiramatsu, K., 1996. Suppression of methicillin resistance in a *mecA* containing pre-methicillin-resistant *Staphylococcus aureus* strain is caused by the *mecI* mediated repression of PBP 2' production. *Antimicrob Agents Chemother*; 40:2680-5.
- [19] Gulay, Z., 1999. Gülay Z. Antimikrobiyal ilaçlara direnç. Mutlu G, İmir T, Cengiz T, Ustaçelebi Ş, Tümbay E, Mete Ö (eds). *Temel ve Klinik Mikrobiyoloji*. Ankara: Güneş Kitabevi, 1999:91-108.
- [20] Maranan, M.C., Moreira, B., Boyle-Vavra, S., Daum, R.S., 1997. Antimicrobial resistance in staphylococci. *Infect Dis Clin North Am*; 11:813-49.
- [21] Hackbarth, C.J., Chambers, H.F., 1993. *blaI* and *blaR1* regulate  $\beta$ -lactamase and PBP 2a production in methicillin resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*; 37:1144-9.
- [22] Brooks, G.F., Carroll, K.C., Butel, J.S., Morse, S., 2007. The Staphylococci. In: Jawetz, Melnick and Adelberg's medical microbiology. 24th ed. New York; McGraw-Hill.
- [23] Bronner, S., Monteil, H., Prévost. G., 2004. Regulation of virulence determinants in *Staphylococcus aureus*: Complexity and applications. *FEMS Microbiol Rev*; 28:183.

- [24] Mulligan, M.E., Murray-Leisure, K.A., Standiford, H.C., 1993. Methicillin- resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis and epidemiology with implications for prevention and management. *Am J Med*; 94:313-28.
- [25] Thorsherry, C., 1984. Methicillin-resistant (heteroresistant) *Staphylococci* Antimicrobial Newsletter 1; 6.
- [26] Franklin, D.L., 1998. *Staphylococcus aureus* infections. *N Engl J Med*; 339:520-32.
- [27] Novick RP, Schelievert P, Ruzin A. 2001. Pathogenicity and resistance islands of staphylococci. *Microbes and Infect*;3:585.
- [28] Boye, K., Bartels, M.D., Andersen, I.S., Møller, J.A., Westh, H., 2007. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I–V. *Clinical Microbiology and Infection* Volume 13, Issue 7, pages 725–727.
- [29] Donnio, P.Y., Preney, L., Gautier-Lerestif, A.L., Avril, J.L., Lafforgue, N., 2004. Changes in staphylococcal cassette chromosome type and antibiotic resistance profile in methicillin-resistant *Staphylococcus aureus* isolates from a French hospital over an 11 year period. *Journal of Antimicrobial Chemotherapy* (2004) 53, 808–813.
- [30] Klevens, R.M. et al., 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States, *J. Am. Med. Assoc.* 298, 1763–1771.
- [31] Kirca, Catar, F., 2008. *Staphylococcus aureus* suslarında metisilin direnci tanısında kullanılan bazı fenotipik yöntemlerin karsılaştırılması. *Uzmanlık Tezi*, Gazi Üniversitesi, Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Ankara, Türkiye.
- [32] Murray, P.R., Rosenthal, K.S., Kobayashi, G.S., Pfaller M.A., 2002. *Staphylococcus* and related organisms. Mosby Inc. St. Louis: 202-216.
- [33] [www.havasteril.com/images/xx/uygulamalar.htm](http://www.havasteril.com/images/xx/uygulamalar.htm)
- [34] Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature (London)*, 227: 680-85.
- [35] National Committee for Clinical Laboratory Standards, 2000a. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 5th ed. Approved standard M7–A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- [36] National Committee for Clinical Laboratory Standards, 2000b. Performance standard for antimicrobial susceptibility testing. Document M100–S10. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- [37] Sacilik, S.C., Osmanoglu, O., Palbiyikoglu, U., Bengisun, J.S., Cokmus, C., 2000. Analysis of Methicillin Resistant *Staphylococcus aureus* Isolates by Polyacrylamide Gel Electrophoresis in an Intensive Care Unit of Ibni-Sina Hospital. *Turk J Med Sci.* 30 367-371.

- [38] Pa, W., 1997. National Committee for Clinical Laboratory Standarts. Performance standarts for antimicrobial disc susceptibility tests. Approved standart M2-A5, National Committee for Clinical Laboratory Standarts, 1997.
- [39] Ridenour GA, Wong ES, Call MA, 2006. Duration of colonization with methicillin resistant *Staphylococcus aureus* among patients in the intensive care unit: implications for intervention. *Infect Control Hosp Epidemiol*,; 27:271–278.
- [40] Doganay M., 1998. Nozokomiyal sepsis: önemi ve tanımlar, *Hastane Enfeksiyon Dergisi*; 2(4):179-81.
- [41] Van Belkum, A., Van Leeuwen, W., Werkooyen R., Sacilik, S.C., Cokmus, C., Verbrugh H., 1997. Dissemination of a single clone of methicillin-resistant *Staphylococcus aureus* among Turkish hospitals. *J Clin Microbiol*, 35: 978-81,