INTRODUCTION

*Staphylococcus aureus* is one of the most common community and hospital-acquired pathogens, responsible for a huge array of infections. Antibiotic resistance in *S. aureus* has become a serious problem in many parts of the world, emphasizing the need to better understand mechanisms involved in the emergence and spread of resistant strains. Low-level resistance is often under estimated. It is not only a gateway to high-level clinical resistance, but also gateway to often unsuspected phenomena such as resistance, to unrelated compounds, increased virulence or bacterial adaptation to adverse in vivo conditions. Low level resistance usually emerges under antibiotic pressure but can also be selected by antiseptics and a variety of non-antimicrobial compounds [1]. Hospital–acquired infections due to methicillin resistant *S. aureus* (MRSA) continue to be a major problem in many countries. There is a wide range in the prevalence of MRSA strains between different countries and even between hospitals in the same country. The extent of the spread of these organisms from hospital to hospital also shows variation [2]. Biofilm formation, which increases antibiotic resistance capabilities and is considered to be a virulence factor, also causes treatment failure and recurrent staphylococcal infections in burn patients [3]. Udobi et al. (2013) reported that their study has become very expedient because of the significant epidemic potential of these organisms and the high morbidity and mortality rates in Orthopaedic hospital they cause in humans with rapid development of resistance which has made it into a major clinical problem worldwide [4]. The report of methicillin-resistant *Staphylococcus aureus* (MRSA)...
encoding a divergent mecA gene in 2011 was highly significant. This homologue, designated mecC, poses diagnostic problems with the potential to be misdiagnosed as methicillin-sensitive S. aureus, with important potential consequences for individual patients and for the surveillance of MRSA [5].

We aimed in the present study to determine the status of antimicrobial resistance, underlying conditions, and determination of methicillin resistance S.aureus isolates with beta-lactamase from different hospitals in Kahramanmaraş, Turkey.

MATERIALS AND METHOD

Isolation of bacterial strains and identification:

29 isolates were collected from different hospitals in Kahramanmaraş between 2006-2007 and recorded at specimens. Isolates were considered to be presumptive Staphylococcus spp. if they were gram-positive coccus that Staphylococcus spp., was isolated by conventional methods. Reproductive characteristics and Gram staining, catalase and coagulase tests were conducted in blood medium [6]. Detection of Meticillin resistance, 1 µg oxacillin disk was used Mueller Hinton agar containing with 4% NaCl [7]. Oxacillin susceptibility was used to determine the percentage of S. aureus that were MRSA [8].

Multiple Antibiotic Resistance Index:

For all isolates, we calculated the MAR index values (a/b, where a represents the number of antibiotics the isolate was resistant to, b represents the total number of antibiotics the isolate was tested against). A MAR index value ≥ 0.2 is observed when isolates are exposed to high risk sources of human or animal contamination, where antibiotics use is common; in contrast a MAR index value < or = 0.2 observed when antibiotics are seldom or never used [9,10].

Test for antibiotic resistance:

Antibiotic resistance was determined by Kirby-Bauer disc diffusion method [11]. Using Mueller-Hinton agar (Difco) according to Clinical and Laboratory Standards Institute (CLSI (2005) recommendations. Twelve different antibiotics (belonging to 7 classes) were used in this study. For antibiotic resistance determination, the isolates were grown in Luria- Bertani (LB) broth until the turbidity equal to the 0.5 Mc Farland standart. Cultures were swabbed on to the Mueller–Hinton agar and all isolates were tested against Meropenem (MER, 10µg/ml), Amoxicillin (AMO, 20µg/ml), Penicillin (PEN, 10µg/ml), Nitrofrantoin (NIT, 300µg/ml), Cefazolin (CEF, 30µg/ml), Cefoxitin (CEFX, 30µg/ml), Ceftriaxone (CEFT, 30µg/ml), Gentamycin (GEN, 10µg/ml), Tetracycline (TET, 30µg/ml), Streptomycine (STR, 10µg/ml), Chlorampenicol (CHL, 30µg/ml), Oflaxain (OFL, 5µg/ml). The isolates those grown in inoculation were evaluated as resistant and the others were evaluated as susceptible [12]. The antibiotic discs were dispensed sufficiently separated from each other so as to avoid overlapping of inhibition zones. The plates were incubated at 37°C, and the diameters of the inhibition zones were measured after 18 hr. All susceptibility tests were carried out in duplicate and were repeated twice if discordant results had been obtained.

Iodometric Slide Method:

The production of β-lactamase was detected with the iodometric slide test [13,14]. Previously, iodine solution was added to penicillin solution. Later, emulsify organism to be tested in a drop of freshly prepared the penicillin-iodine solution on flamed side of a glass slide; made a heavy suspension. Then starch solution was added. Initially, solution of all samples will turn purple. An indication of β-lactamase production is clearing of purple color to white within 5 min. But the entire mixture does not have to clear; clearing of definite clumps or areas is sufficient to denote a positive result. Starch and iodine react in solution to produce a purple color. β-lactamase causes the β-lactam ring to open with production of penicilloic acid, which reacts with iodine, making it unavailable to react with starch. The presence of β-lactamase is indicated by decolorization of (or failure to form) the starch-iodine complex. Intact (active) penicillin does not bind iodine, whereas penicilloic acid does. Penicilloic acid acts as a reducing agent to reduce iodine in the complex. All the bacterial isolates were tested for the production of β-lactamases.

RESULTS AND DISCUSSIONS

A total of 29 isolates of S.aureus were tested. Majorities of S.aureus strains were isoated from blood and urine but minorities of S.aureus strains were isolated from Sperm, Urethra, Catheter type, Urine, Blood, Probe type, Wound, Abscess, Pustule, Derma, Cerebral Spinal Fluid. It was given prevalence of the antibiotic resistance among 29 clinical Methicillin resistant Staphylococcus aureus isolates in Table 1.

Penicillin resistance rate was the highest (79%). The resistance rate for Cef and Amo were 69 %, followed by Cefx and Cef 51 %, Gen and Tet 48 %, Str and Ofl 44%, Chl 34%, Nit 17%, Mer 10%.
Some researchers used similar our antibiotics. For example Tahnkivole et al (2002) found that maximum strains were resistant to penicillin (100%) [15]. Some researchers found that penicillin resistance rate was the highest in clinical samples like ours [16,21]. The reason for this, penicillins are bactericidal, inhibiting bacterial cell wall synthesis [22]. These bacteria offer resistance to penicillins by production of beta lactamases and by permeability barrier of the cell surface [23].

Cephalosporins are widely used and therapeutically important antibiotics. They inhibit bacterial cell wall synthesis in a manner similar to that of penicillin. The explosive growth of cephalosporins during the past decade has made a well accepted system of classification by ‘’generation’’ based on general feature of antimicrobial activity. The first generation cephalosporins have good activity against gram positive bacteria and relatively modest activity against gram negative bacteria. Cefazolin resistance rate was 51%. Several studies have reported increased resistance of S.aureus isolates to Cefazolin like us [24], Tanaka et al. in 1987 and Otsuki et al in1986 reported good antibacterial activity of cefazolin against S.aureus [25,26].

The 69% resistance of amoxicillin to MR S.aureus isolates as recorded in this work is in conformity with the findings of Ikekewu et al, in which 63% amoxicillin resistant strains of S.aureus was reported [27]. 100% resistance of S.aureus isolates to amoxicillin has also been reported [28]. Amoxicillin resistance rate was reported from 22.2% to 91.9% in a different studies [29-32].

Among the aminoglycosides, gentamycine resistance rate was 48%. Our results were similar to Durmaz et al (1997) in which 52% gentamycine resistance rate was reported [33]. Different studies were reported that gentamycine resistance rate was from 13.7% to 95% [20,21,32,34-38]. The effectiveness of gentamycine confirms the findings of Barber and Waterworth(1966), who found it to be four times more active than kanamycin against S.aureus [39]. In view of its effectiveness, gentamycine will likely prove itself useful in the treatment of certain types of Staphylococcal infections [16].

Among the tetracyclins antibiotics is a tetracycline. Resistance to tetracycline was showed in 48%. Tetracycline is a bacteriostatic antibiotic and used to select mutants of multidrug resistance [40]. Different studies showed that tetracycline resistance rate was from 2.6% to 87.5% [17,20,32,40-43].

As for the streptomycin resistance rate, it was reported that 44%. Different studies showed that streptomycin resistance rate was from 0% and 69.5% [17,20,44]. Our results were in accordance with previous works.

Among the fluoroquinolones antibiotics, when it comes to, oflaxacin resistance rate, it was found 44%. Some researchers showed that oflaxacin resistance rate was 8% to 100% [45-47]. The results changes to researchers. For instance, 100 % sensitivity of S.aureus isolates to oflaxacin has been reported [48]. In addition, high sensitivity of oflaxacin against S.aureus isolates was also highlighted by Chalita et al (2004) [49].

When it comes to, chloramfenicol resistance rate, it was found 34%. Chloramfenicol resistance rate was reported from 11% to 48.6% in a different studies [17,20]. This findings is consistent with previous report.

As for the nitrofurantoin resistance rate, it was found 17%. Different studies were reported that nitrofurantoin resistance rate was between 0.8% and 31.11% [34,44]. Ekşi et al reported that uriner system infection caused by S.aureus, nitrofurantoin may be treatment [34].

Meropenem is β lactam antibiotics of the carbapenem class. Meropenem resistance rate was showed in 10% in our study. Different studies were showed that meropenem resistance rate was from 0 % to 30% [50-52]. The reason for the variation could most likely be attributable to strain differentiation.

When it comes to ceftriaxone resistance rate, it was found 51%. Different studies were reported that ceftriaxone resistance rate was between 23.07% and 87.5% [53-56].

β – lactamase production by far the most important and most widespread mechanism of resistance, this type of resistance has spread from country to country in both hospitals and community acquired infection. It has been passed between bacterial species an even genera. Moreover, it has extended to cover more and more β lactam agent including third generation cephalosporins. β-lactam antibiotics, such as penicillins, cephalosporins and cephemycins, all have a similiar core structure consisting of a β lactam agents including third generation cephalosporins [57-59]. For most bacterial species the incidence of β lactamase production in throughout the world are remarkably similar. Indience of β-lactamase production in S.aureus has consistently been reported to be from 74% to 90% in different studies pertentage rate [60-63]. Torimiro et al.,(2013) reported that β-

### Table 1: Antibiotic resistance of 29 Staphylococcus aureus strains according to the disc diffusion.

<table>
<thead>
<tr>
<th>Antibiotic group</th>
<th>Antibiotics</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Beta-Lactams:</td>
<td>Penicillin (PEN), Amoxicillin (AMO), Meropenem (MER)</td>
<td>79%, 69%, 10%</td>
</tr>
<tr>
<td>2. Nitrofurantoin:</td>
<td>Nitrofurantoin (NIT)</td>
<td>17%</td>
</tr>
<tr>
<td>3. Cephalosporins:</td>
<td>Cefazolin (CEF), Cefotaxim (CEF), Ceftriaxone (CEFT)</td>
<td>69%, 51%, 51%</td>
</tr>
<tr>
<td>4. Aminoglycosides:</td>
<td>Gentamycine (GEN), Streptomycine (STR)</td>
<td>48%, 44%</td>
</tr>
<tr>
<td>5. Tetracyclines:</td>
<td>Tetracycline (TET)</td>
<td>48%</td>
</tr>
<tr>
<td>6. Macrolides:</td>
<td>Chloramphenicol (CHL)</td>
<td>34%</td>
</tr>
<tr>
<td>7. Quinolones:</td>
<td>Ofloxacin (OFL)</td>
<td>44%</td>
</tr>
</tbody>
</table>
lactamase production and resistance to amoxicillin, amoxicillin/clavulanic acid (augmentin) and ceftriaxone resistance was significant. The study suggests strict infection control measures and encouragement of prudent antibiotic use [64].

Among the 29 isolates of MR S. aureus were showed 15 (51%) beta lactamase activity especially isolated from blood. 14 isolates of MR S. aureus were showed no beta lactamase activity, especially isolated from urine. It was given in table 2.

### Table 2: Multiple Antibiotic Resistance Index and beta lactamase activity of 29 Staphylococcus aureus strains.

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Total Isolates</th>
<th>Multiple Antibiotic Resistance Index (MAR)</th>
<th>Beta lactamase (+) or (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm</td>
<td>1</td>
<td>0.3</td>
<td>1(+)</td>
</tr>
<tr>
<td>Urethra</td>
<td>1</td>
<td>0.42</td>
<td>1(-)</td>
</tr>
<tr>
<td>Catheter tip</td>
<td>1</td>
<td>0.75</td>
<td>1(-)</td>
</tr>
<tr>
<td>Urine</td>
<td>3</td>
<td>0.08 (2isl), 0.5</td>
<td>2(-), 1(+)</td>
</tr>
<tr>
<td>Blood</td>
<td>13</td>
<td>0.17, 0.42, 0.5 (2isl), 0.67(4isl), 0.75 (4isl), 0.83</td>
<td>9(+), 4(-)</td>
</tr>
<tr>
<td>Probe tip</td>
<td>1</td>
<td>0.5</td>
<td>1(-)</td>
</tr>
<tr>
<td>Wound</td>
<td>2</td>
<td>0.33,05</td>
<td>2(-)</td>
</tr>
<tr>
<td>Abscess</td>
<td>3</td>
<td>0.42, 0.5, 0.58</td>
<td>2(+),1(-)</td>
</tr>
<tr>
<td>Pustule</td>
<td>2</td>
<td>0.17 (2isl)</td>
<td>1(+), 1(-)</td>
</tr>
<tr>
<td>Derma</td>
<td>1</td>
<td>0.33</td>
<td>1(-)</td>
</tr>
<tr>
<td>Cerebral Spinal Fluid</td>
<td>1</td>
<td>0.3</td>
<td>1(+)</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td>15(+), 14(-)</td>
</tr>
</tbody>
</table>

Isl: Isolates

According to these results, out of 29 isolates, 24 (83%) isolates showed Multiple Antibiotic Resistance four to twelve antibiotics. It was given in table 2 Staphylococcus aureus strains of Multiple Antibiotic Resistance Index. Our results were similar to Amaechi and Ugbo (2006) in which 79,3% multiple antibiotic resistance index was reported [32]. Most common reason for multi drug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing which may be due to due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or patients poor economic status. There is a difference between antibiogram of MRSA and MSSA isolates and routine testing of methicillin resistance should be done using cefoxitin disc which is at present the most sensitive method [65].

In conclusion, this research work has shown that there are rather high prevalence of multiple-antibiotic resistant S. aureus among the clinical samples and this should be concern. This finding is in agreement with most of the international studies showing the shifting towards Methicillin resistant Staphylococcus aureus septicemia. The high prevalence rates of antibiotic resistance among clinical isolates from septisemic cases achieved in our study compared with previous studies performed in international studies obviously indicating the misuse of antibiotic among our society. Regions surveillance studies in Turkey will be most useful to the clinicians in deciding out the right empirical treatment and will help to control and prevent infections caused by Methicillin resistant Staphylococcus aureus. Furthermore our data suggest that the most antibiotic resistance rates were Penicillin like our previous research [66], later amoxicillin and cefazolin.

Our study has several limitations. Firstly, it is likely that the effectiveness of hospital surveillance programmes to enrol patients on admission may be heavily influenced by institutional and local factors. Therefore, the generalizability of our findings may be limited. Secondly, some of our data was collected retrospectively from medical records and is therefore subject to the inaccuracies inherent to data collected in this way.

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**REFERENCES**


