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## ORIGINAL ARTICLE

# Antimicrobial Susceptibility Pattern in the Bacteria Isolated from Surgical Site Infection: Emphasis on *Staphylococcus Aureus*; Yasuj City, Southwest Iran

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### SUMMARY

***Background:*** **Surgical site infections (SSIs) in surgical wards remains the most common cause of postoperative complications and realistically is the third most common origin of healthcare-related conditions. *Staphylococcus aureus* is undoubtedly the most common bacteria causing SSIs. The current study aimed at investigating the antimicrobial susceptibility pattern in bacteria isolated from SSIs, evaluation of tetracycline resistance genes, and SCC*mec* typing in *S. aureus* isolates isolated from patients with SSIs from 2018 to 2019 in Yasuj, Kohgiluyeh, and Boyer-Ahmad Province, Iran.**

***Methods:*** **This study diligently investigated 240 potential patients. Antimicrobial susceptibility testing was performed properly by the disk diffusion method. For the final confirmation of isolated bacteria, PCR was used. The presence of tet genes and SCC*mec* typing was carried out by multiplex PCR.**

***Results:*** **The results showed that the most common isolated pathogens included *S. aureus, E. coli, P. aeruginosa, Coagulase-negative Staphylococci,* and *K. pneumonia* in 58.8%, 19.8%, 9.2%, 6.8% and 5.4% of cases, respectively. The majority of the Gram positive isolates were resistant against penicillin (86%) and Gram negative were resistant against ciprofloxacin (75.6%). In isolates of Staphylococcus aureus, the mecA gene was detected in 63.6% of isolates. The predominant SCC*mec* types were type III (59.1%) and type I (18.4%). The tetK and tetM genes were detected in 80.7% and 71.9% of the *S. aureus* isolates, respectively. There was a statistically significant difference between tet genes (tetK and tetM) from the viewpoint of resistance to tetracycline (p = 0.024).**

***Conclusions:*** **According to the results of the current study, it is recommended to administer vancomycin, amikacin, and imipenem in Yasuj to treat SSIs.**

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**KEY WORDS**

surgical site infections, antibiotic resistance, *Staphylococcus aureus*, SCC*mec* typing, tet resistance genes

## INTRODUCTION

Surgical site infections (SSIs) are infections consequent to the surgery that present within 30 days of the operative procedure, or within 1 year after surgery, if an implant is placed at the surgical site [1,2]. According to

CDC criteria for wound classification (2015), wounds are classified as clean, clean-contaminated, contaminated, and dirty or infected [3]. SSIs can be classified into three categories: 1) Superficial incisional infections 2) Deep incisional infection 3) Organ/space infections [4]. There are ordinarily two groups of established risk factors for SSIs. The first group is patient-related factors including (age, weakened immune system, alcoholism, diabetes, obesity, smoking, history of chemotherapy, etc.). The second group is exogenous factors which consist of foreign material in the surgical site, duration of surgery, excessive movement of staff in operating room, staff with skin infections, surgical technique, type of surgery, etc. [5]. SSIs are the most common cause of postoperative complications in surgical wards (i.e., 38% of nosocomial infections in surgical patients) and is the third most common cause of healthcare-related infections [6]. Some other studies suggest they account for 14 - 16% of all nosocomial infections, especially in low income and developing countries [7,8]. SSIs increase morbidity and mortality, prolong hospitalization on average by 7 to 13 days [9], raise the cost of care, and increase morbidity and mortality [10]. Identifying microorganisms plays an important role in selecting appropriate antibiotics to treat of SSIs. SSIs are caused by various bacteria (including: *S. aureus*, *P. aeruginosa*, *E. coli*, *Coagulase neg. Staphylococci*, *Enterococci*), the most common microorganism isolated from SSIs is *S. aureus* [11,12]. *S. aureus* infections can be challenging to treat in the SSIs due to increased resistance to antimicrobial drugs [13]. Treatment of such a controversial condition has always been problematic because of drug resistance. One of the effective antibiotics used for skin infections caused by *S. aureus* is tetracycline, aberrant use appropriate for antibiotics, and increasing antimicrobial resistance cause the condition of SSIs. Since the onset of treatments in SSIs are based on physician's experience, providing up-to-date information about common infecting microorganisms. In addition, antimicrobial susceptibility patterns based on the geographic regions will enable physicians to more efficiently treat the suffering patients. The purpose of this study: 1) Frequency of microorganisms isolated from SSIs. 2) Antimicrobial susceptibility pattern in bacteria isolated. 3) SCC*mec* typing *S. aureus* isolates. 4) Evaluate tetracycline resistance genes in *S. aureus* isolates.

## MATERIALS AND METHODS

### Sampling method

This descriptive cross-sectional study was conducted in Imam Sajjad and Shahid Beheshti hospitals in Yasuj, Kohgiluyeh and Boyer-Ahmad Province located in southwestern Iran from January to December 2019. It included 240 patients who underwent surgery in the afore-mentioned hospitals during the period of the study. Patients with the following criteria entered the study: above 10 years, purulent drainage with or without laboratory confirmation, having at least one of the symptoms of infection including pain or allergy, local swelling, redness or heat. Patients unwilling to voluntarily enter the study and those suffering from immunodeficiency were excluded. The samples were collected with two sterile swabs: one swab for Gram stain and the other one was inoculated on McConkey agar and blood agar (5% sheep blood) plates. Next, they were incubated at 37°C for 24 hours. Bacteria were detected based on colony morphology and Gram staining and the following tests: MR-VP, citrate utilization, TSIA, urease, oxidase, oxidative/fermentative for Gram-negative, and catalase test for Gram-positive cocci and coagulase test, culture on Mannitol Salt Agar for *S. aureus* [14]. Finally, PCR was used to confirm the isolated bacteria. The specific primers are shown in Table 1.

### Antimicrobial susceptibility testing

Antibiotic susceptibility pattern using by Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) [15] was used as a quality control *(E. coli* ATCC 25922 and *S. aureus* ATCC 25923). The isolates were tested against, ampicillin (10 µg), gentamicin (10 µg), vancomycin (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), penicillin (10 IU), imipenem (10 µg), amikacin (30 µg), clarithromycin (15 µg), clindamycin (2 µg), ceftazidime (30 µg) [15,16].

### DNA extraction

DNA extraction was accomplished through a boiling method described (with some minor modifications) [17]. Several colonies freshly (24 hours) obtained from Mueller Hinton Agar or Nutrient Agar were dissolved in 300 µL sterile distilled water and placed in boiling water (at 100°C) for 10 minutes. To obtain the template DNA for the polymerase chain reaction, the bacterial solution was centrifuged (Sigma, Germany) for 10 minutes at 12,000 rpm. Afterward, 100 µL of the supernatant was centrifuged (DNA template) and stored at -20°C for PCR.

### PCR assay

The polymerase chain reaction (PCR) was performed by the specific primers for target genes (Table 1). The mixture was prepared separately for each gene in a total volume of 25 µL including 12.5 µL of Master Mix (Amplicon, Denmark), 2 µL of each primer, 5 µL of bacterial DNA and 3.5 µL of sterile distilled water. The PCR program was as follows: initial denaturation at 94°C for 4 minutes, followed by 34 cycles (denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes for nucA gene, and initial denaturation at 94°C for 4 minutes, followed by 30 cycles (denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 5 minutes for oprL gene, initial denaturation at 95°C for 4 minutes followed by 28

cycles of amplification at 95°C for 1 minute, annealing at 57°C for 1 minute, extension at 72°C for 1 minute, final extension step was performed at 72°C for 5 minutes, for Ure-D gene with AroE gene. Multiplex-PCR mixtures for detection of the (tet genes and SCC*mec* typing) were prepared separately. The reaction mixture for tet genes was prepared in a total volume of 50 µL including 25 µL of Master Mix (Amplicon, Denmark), 2 µL of each primer, 9 µL of bacterial DNA for tet genes. DNA amplification was performed in a thermocycler machine (Bio-Rad T100, Singapore): initial denaturation at 95°C for 5 minutes followed by 35 cycles of amplification at 94°C for 45 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 45 seconds. A final extension step was performed at 72°C for 7 minutes, for tet genes, and SCC*mec* typing using the specific primers and amplification conditions as was described by Zhang et al. [18]. After amplification, PCR products were electrophoresed (Major Science MP300, Taiwan) on 1.2% agarose gel (Pishgam, Iran) at 90 V for 45 minutes. The PCR products were stained with Gel Stain (Pishgam, Iran). They were then visualized by Gel Documentation (Major Science, Taiwan).

**Statistical analysis**

Data collected from the survey was analyzed using SPSS, version 20. Comparison was performed by chi-square test or Fisher's exact test and "p-values of less than 0.05 were regarded as statistically significant".

## RESULTS

A total of 240 patients participated in the study. Bacterial growth was seen in 89 cases (37.1%). Of the patients whose culture was positive, 44 (49.4%) were males and 45 (50.6%) were females. The mean age of the patients was 40.88 years. The hospitalization locations included general surgery 41 (46.1%), orthopedic 27 (30.3%), gynecological surgery 17 (19.1%), and neurosurgery 4 (4.5%). The highest isolates were from the clean-contaminated (74.8%), followed by clean (17.6%) and then contaminated (7.6%) (Figure 1). Among the positive cultures, 131 isolates were isolated and among them, gram-positive cocci in 86 (65.6%) cases and gram-negative bacilli in 45 (34.4%) cases. *S. aureus* was the predominant isolated organism (58.8%), followed by *E. coli* (19.8%), *P. aeruginosa* (9.2%), *Coagulase-negative Staphylococci* (6.8%), and *K. pneumonia* (5.4%) (Figure 1). According to the results of the study, in the gram-positive isolates 86% were resistant to penicillin, 84.9% to ampicillin, 72.1% to tetracycline, 72.1% to clarithromycin, 67.4% to clindamycin, 66.3% to amikacin, 62.8 % to gentamicin, 55.8% to vancomycin, and in the gram-negative isolates: 75.6% to ciprofloxacin, 71.1% to levofloxacin, 64.4% to ceftriaxone, 62.2% to ceftazidime, 60% to gentamicin, 55.6% to tetracycline and imipenem, and 48.9% to amikacin. Table 2 presents the antibiotic resistance patterns of the Gram-positive and Gram-negative bacterial isolates. Of the seventy-seven *S. aureus* isolates, according to mecA gene, 63.6% and 36.4% isolates were MRSA and MSSA, respectively. The most predominant MRSA genotypes were SCC*mec* type III (59.1%, 29/49), followed by type I (18.4%, 9/49), types IVb and V accounted for 8.2% (4/49) and 6.1% (3/49), respectively. Examining tetracycline susceptibility of the isolates revealed that there was resistance in 57 cases (74%) of *S. aureus* isolates. The tetK and tetM genes were detected in 46 cases (80.7%) and 41 cases (71.9%) of 57 tetracycline-resistant isolates. None of the tetracycline-resistant isolates had tetL or tetO gene. There was a statistically significant difference between tet genes (tetK and tetM) and tetracycline resistance ($p = 0.024$). Moreover, the results of the simultaneous presence of tetracycline resistance genes revealed that tetK and tetM were present in 30 isolates (52.6%) simultaneously. Out of 57 tetracycline resistant isolates, 37 isolates (64.9%) were MRSA. The tetK and tetM genes were detected in 32 cases (86.5%), and 24 cases (64.9%) of 37 tetracycline-resistant MRSA isolates and 19 isolates had at least two genes simultaneously. Twenty isolates (35.1%) were *methicillin-susceptible Staphylococcus aureus* (MSSA). The tetK, tetM genes were detected in 14 (70%) and 17 (85%) of 20 tetracycline-resistant MRSA isolates Table 3. There was a statistically significant difference between the resistance genes in MRSA and MSSA ($p = 0.0001$).

## DISCUSSION

Surgical site infections are the third most common cause of nosocomial infection. SSIs cause prolongation of hospital stay, increased treatment cost, and higher morbidity and mortality rates. The prevalence of organisms varies in different studies. Almost like Chaudhary R et al. [19], the present study revealed Gram-positive cocci and Gram-negative bacilli bacteria constituted 58.4% and 41.6% of the isolates, respectively. The primitive organisms isolated in this study like some other studies [11,20-23] included *S. aureus*, *E. coli, P. aeruginosa*, *Coagulase-negative Staphylococci*, and, *K. pneumonia*. The differences observed in various studies may be due to principal differences in health plans, infection control processes, and appropriate treatments. In the current study, *S. aureus* - similar to other prior investigations [6,11,24] - was the most primitive organism causing SSIs. Like Ahmed et al. [25], 58.8% of the isolates were *S. aureus*. The possible reason for such a notable occurrence could be the well-established fact that *S. aureus* represents the normal flora of skin and nose. In the present investigation, the antibiotic susceptibility pattern of gram-positive isolates revealed the most significant resistance to penicillin (86%), whereas gram-negative isolates showed the highest resistance to ciprofloxacin (75.6%). Gram-positive isolates revealed the highest sensitivity to vancomycin which confirms

**Table 1. The primers used in this study.**

| Target gene | Primer sequence (5' → 3') | Amplicon length, bp | Reference |
|---|---|---|---|
| **nucA** | **F - CTG GCA TAT GTA TGG CAA TTG TT<br>R - TAT TGA CCT GAA TCA GCG TTG TCT** | **670** | **[34]** |
| **aroE** | **F - AAGGTGCGAATGTGACGGTG<br>R - AACTGGTTCTACGTCAGGCA** | **620** | **[35]** |
| **Ure-D** | **F - CCC GTT TTA CCC GGA AGA AG<br>R - GGA AAG AAG ATG GCA TCC TGC** | **243** | **[36]** |
| **OprL** | **F - ATGGAAATGCTGAAATTCGGC<br>R - CTTCTTCAGCTCGACGCGACG** | **504** | **[37]** |
| **mecA** | **F - GTG AAG ATA TAC CAA GTG ATT<br>R - ATG CGC TAT AGA TTG AAA GGA T** | **147** | **[18]** |
| **tetK** | **F - GTAGCGACAATAGGTAATAGT<br>R - GTAGTGACAATAAACCTCCTA** | **360** | **[32]** |
| **tetM** | **F - AGTGGAGCGATTACAGAA<br>R - CATATGTCCTGGCGTGTCTA** | **158** | **[32]** |
| **tetL** | **F - ATAAATTGTTTCGGGTCGGTAAT<br>R - AACCAGCCAACTAATGACAATGAT** | **1077** | **[32]** |
| **tetO** | **F - AACTTAGGCATTCTGGCTCAC<br>R - TCCCACTGTTCCATATCGTCA** | **514** | **[32]** |
| **SCC*mec* I** | **F - GCTTTAAAGAGTGTCGTTACAGG<br>R - GTTCTCTCATAGTATGACGTCC** | **613** | **[18]** |
| **SCC*mec* II** | **F - CGTTGAAGATGATGAAGCG<br>R - CGAAATCAATGGTTAATGGACC** | **398** | **[18]** |
| **SCC*mec* III** | **F - CCATATTGTGTACGATGCG<br>R - CCTTAGTTGTCGTAACAGATCG** | **280** | **[18]** |
| **SCC*mec* IVa** | **F - GCCTTATTCGAAGAAACCG<br>R - CTACTCTTCTGAAAAGCGTCG** | **776** | **[18]** |
| **SCC*mec* IVb** | **F - TCTGGAATTACTTCAGCTGC<br>R - AAACAATATTGCTCTCCCTC** | **493** | **[18]** |
| **SCC*mec* IVc** | **F - ACAATATTTGTATTATCGGAGAGC<br>R - TTGGTATGAGGTATTGCTGG** | **200** | **[18]** |
| **SCC*mec* IVd** | **F - CTCAAAATACGGACCCCAATACA<br>R - TGCTCCAGTAATTGCTAAAG** | **881** | **[18]** |
| **SCC*mec* V** | **F - GAACATTGTTACTTAAATGAGCG<br>R - TGAAAGTTGTACCCTTGACACC** | **325** | **[18]** |

**Table 2. The antibiotic resistance patterns of the Gram-positive and Gram-negative bacterial isolates.**

| Antibiotics | *S. aureus* | *Cons* | *E. coli* | *K. pneumonia* | *P. aeruginosa* |
|---|---|---|---|---|---|
| **Penicillin** | **88.3%** | **66.7%** | **-** | **-** | **-** |
| **Ampicillin** | **87%** | **66.7%** | **-** | **-** | **-** |
| **Clarithromycin** | **72.7%** | **66.7%** | **-** | **-** | **-** |
| **Amikacin** | **68.8%** | **44.4%** | **57.7%** | **0** | **58.3%** |
| **Clindamycin** | **68.8%** | **55.6%** | **-** | **-** | **-** |
| **Tetracycline** | **74%** | **55.6%** | **57.7%** | **14.3%** | **75%** |
| **Gentamycin** | **64.9%** | **44.4%** | **57.7%** | **28.6%** | **83.3%** |
| **Vancomycin** | **58.4%** | **33.3%** | **-** | **-** | **-** |
| **Ciprofloxacin** | **-** | **-** | **76.9%** | **71.4%** | **75%** |
| **Ceftriaxone** | **-** | **-** | **61.5%** | **28.6%** | **91.7%** |
| **Ceftazidime** | **-** | **-** | **61.5%** | **57.1%** | **66.7%** |
| **Imipenem** | **-** | **-** | **53.8%** | **71.4%** | **50%** |

**Table 3. Distribution of tet genes in MRSA and MSSA strains.**

| Resistance genes | MRSA (n = 37) | MSSA (n = 20) | p-value |
|---|---|---|---|
| **tetM** | **24 (64.9%)** | **17 (85%)** | **0.0001** |
| **TetK** | **32 (86.5%)** | **14 (70%)** | **0.0001** |
| **tetK and tetM** | **19** | **11** | **0.0001** |

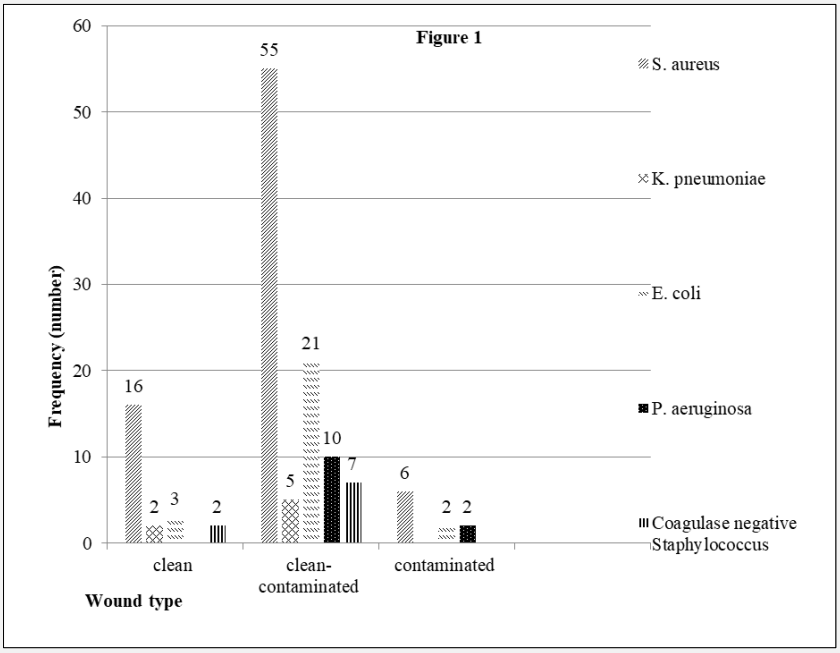


**Figure 1. Isolation of bacteria based on the type of wound.**

another research by Jnaneshwara et al. [21]. On the other hand, gram-negative isolates showed the most susceptibility to amikacin and imipenem just as stated by Chaudhary et al. [19]. Moreover, the highest sensitivity to imipenem observed for *E. coli* and *P. aeruginosa* in our study positively confirmed an earlier study by Charles Obinna et al. [26]. *S. aureus* remained the most important pathogen following surgical procedures. In the present study, 70.03% of *S. aureus* isolates were resistant to tetracycline. The resistance level was higher than the investigation of Shrief et al. [27]. In comparison with Jafari et al. [28], our study indicated a lower rate of tetracycline-resistant isolates to be genuinely MRSA (64.9%). There are two main mechanisms leading *S. aureus* resistant to tetracycline. The first one is "active efflux" mediated by plasmid-encoded genes, tetK and tetL. The second mechanism is "ribosomal protection" mediated by transposon or chromosomal tetM and tetO determinants [29]. In our study, the tetK and tetM genes were respectively detected in 80.7% and 71.9% of tetracycline-resistant isolates. The frequency of the tetK gene in the present investigation was similar to Khorramrooz et al. [30]. Like more prior studies [27, 30,31], tetL and tetO were observed in none of the isolates. Very likely, these genes retained no role in causing tetracycline resistance in our study. In MRSA isolates obtained from our study, the frequency of tetK and tetM genes were 86.5% and 64.9%, respectively. The frequencies were higher than previous studies [32,33]. Different resistance of bacteria to antibiotics reported in different studies might be because of different health plans in different countries, the difference in antibiotics prescribed by physicians, arbitrary use of antibiotics by patients, and geographical climates.

## CONCLUSION

Since treatment begins empirically, identification of the bacteria that cause surgical site infection in each area and antibiotic susceptibility in them can play an important role in the management of these infections and potentially leading to decreased mortality and morbidity of patients. The results of the current study indicated that the most commonly isolated pathogens were *S. aureus*, *E. coli*, *P. aeruginosa*, *Coagulase negative Staphylococci* and *K. pneumonia*. According to the results of the current study, it is recommended to administer vancomycin, amikacin and imipenem in Yasuj to treat SSIs. In *S. aureus* isolates: the predominant SCC*mec* types were type III and type I. Examination of tetracycline resistance genes in isolated *S. aureus* showed that tetK gene frequency was higher.

**Ethical Approval**:
The study was approved by the Ethical Committee of Yasuj University of Medical Sciences (YUMS). Ethical code number: IR.YUMS.REC.1397.124.

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**Disclosure:**
This study was the result of Farzad Mazloomirad's research project.

**Declaration of Interest:**
The authors declare that they have no conflicts of interest.

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