# Antibiotic Susceptibility and Plasmid Profiles of *Staphylococcus aureus* Strains Isolated from Bovine Subclinical Mastitis in Tabriz, Iran

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## ABSTRACT

**Background and Objectives**: Mastitis caused by *Staphylococcus aureus* is a widely distributed disease in cattle, goats and sheep. The infection is often subclinical in cattle, leading to reduced milk production and quality, but acute catarrhal or even gangrenous inflammation may also occur. The aim of this study was to investigate resistance of *S. aureus* isolates from bovine subclinical mastitis to a number of antibiotics.

**Methods**: Milk samples were collected from 120 cows in different regions of Tabriz, Iran. Milk samples were cultured and bacteriological identification was performed. Antimicrobial susceptibility of the isolates was assessed by determining minimum inhibitory concentration. Plasmid DNA was extracted by an alkaline lysis method.

**Results:** The highest frequency of resistance was observed against gentamicin (100%) and β- lactam antibiotics including amoxicillin (96%), ampicillin (40%) and penicillin (96%). The isolates mostly contained large plasmids, which might harbor acquired antibiotic resistance.

**Conclusion:** The results confirm the high frequency of antibiotic resistance among staphylococci isolated from bovine subclinical mastitis.

**Keywords:** Anti-infective Agents, Cattle, *Staphylococcus Aureus*, Mastitis, Plasmids.

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## INTRODUCTION

Mastitis is one of the most costly diseases in dairy cattle (1). The disease is usually caused by staphylococci that are shed in milk, and serves as a source of infection for the healthy cattle (2). Staphylococci contain plasmids that have been proven to be related with antibiotic resistance in some cases (3). From the epidemiological point of view, it is important to determine the origin of the organisms involved in the etiology of the disease. Therefore, this study aimed to investigate the presence of plasmids and their relationship with antibiotic resistance in strains isolated from dairy herds with subclinical mastitis.

## MATERIAL AND METHODS

In this study, 120 apparently healthy Holstein dairy cows (at different calving and lactation stages) were selected from 15 herds in different regions of Tabriz, Iran. Milk samples were cultured and bacteriological identification was performed for each sample. The colonies suspected of being staphylococci were sub-cultured on blood agar plates, along with a *Streptococcus agalactiae* culture for the CAMP test (4).

Antimicrobial susceptibility of the isolates was assessed by determining minimum inhibitory concentration (MIC) using microdilution method in accordance with instructions of the clinical laboratory standard institute. MIC was defined as the lowest concentration that inhibited the visible growth of bacteria. The plates were incubated for 24 hours at 37 ºC. Plasmid DNA was extracted by an alkalin lysis method described by Anderson and McKay (3). After electrophoresis on gels stained with ethidium bromide, the results were observed and photographed under UV illumination.

## RESULTS

Overall, *S. aureus* was isolated from 50 samples (41.66%). Results of the antimicrobial susceptibility testing for *S. aureus* isolates are summarized in Table 1. All isolates were resistant against gentamicin. In addition, most isolates were resistant against penicillin (96%) and amoxicillin (96%). Enrofloxacin, erythromycin and oxytetracycline were the most effective antibiotics against *S. aureus* isolates.

Plasmids were detected in 44 isolates (88%) with four different molecular weights. Most isolates showed three plasmid bands (21.2, 13.7 and 8.3 Kb). Twelve isolates contained a single plasmid, while 29 isolates contained multiple plasmids (Table 1). Table 2 represents the five different plasmid profiles detected for the *S. aureus* isolates.

**Table 1- Antibiotic resistance and frequency distribution of MICs for *S. aureus* (n=50) isolates from subclinical mastitis in dairy cows**

| Antibiotic | Resistance (%) | ≤0.06 | Distribution (%) of MICs (mg/L) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | ≤128 |
| Ampicillin | 40 | - | 4 | 4 | 8 | - | 8 | 12 | 12 | 12 | 18 | 10 | 12 |
| Penicillin | 96 | 2 | 2 | 6 | 10 | 2 | 6 | 14 | 10 | 18 | 20 | 10 | - |
| Amoxicillin | 96 | - | 4 | 4 | 10 | 8 | 16 | 16 | 18 | 24 | - | - | - |
| Cloxacillin | 40 | 6 | 4 | 4 | 8 | 10 | 28 | 22 | 10 | 4 | - | 4 | - |
| Erythromycin | 8 | 36 | 12 | 16 | 16 | 12 | 6 | - | 2 | - | - | - | - |
| Enrofloxacin | 2 | 54 | 16 | 14 | 12 | 2 | 2 | - | - | - | - | - | - |
| Oxytetracycline | 14 | 36 | 20 | 6 | 12 | 12 | 4 | 4 | 6 | - | - | - | - |
| Gentamicin | 100 | - | - | - | - | 8 | 28 | 14 | 16 | 18 | 6 | 10 | - |

**Table 2- Antibiotic resistance patterns and plasmid profiles of *S. aureus* isolates**

| Isolates | Number of plasmids | Molecular weight (Kb) | Antibiotic-resistance patterns | Profile |
|---|---|---|---|---|
| 1 | 1 | 21.2 | AM, Pen G, Amox, GE | 1 |
| 2 | 1 | 21.2 | Clox,OXT, GE | 1 |
| 3 | 1 | 21.2 | Pen G,GE | 1 |
| 4 | - | - | Pen G, Amox, GE | |
| 5 | 3 | 21.2, 13.7, 8.3 | Pen G, GE | 2 |
| 6 | - | - | AM, Pen G, Amox, GE | |
| 7 | 3 | 21.2, 13.7, 8.3 | AM, Clox, GE | 2 |
| 8 | 3 | 21.2, 13.7, 8.3 | Clox, GE | 2 |
| 9 | 3 | 21.2, 13.7, 8.3 | AM, Pen G, Amox, GE | 2 |
| 10 | 3 | 21.2, 13.7, 8.3 | AM, Pen, Amox, GE | 2 |
| 11 | 3 | 21.2, 13.7, 8.3 | AM, Pen, GE | 2 |
| 12 | 3 | 21.2, 13.7, 8.3 | Pen, Clox, GE | 2 |
| 13 | 3 | 21.2, 13.7, 8.3 | Pen G, Amox, Clox, GE | 2 |
| 14 | 3 | 21.2, 13.7, 8.3 | Pen G, Amox, GE | 2 |
| 15 | 1 | 21.2 | AM, Pen G, GE | 1 |
| 16 | 1 | 21.2 | GE | 1 |
| 17 | - | - | AM, Amox, GE | |
| 18 | - | - | AM, Amox, Clox, GE | |
| 19 | - | - | AM,, Pen G, Amox, GE | |
| 20 | - | - | Pen, Amox, Gen | |
| 21 | 3 | 21.2, 13.7, 8.3 | Clox, Ery, GE | 2 |
| 22 | 3 | 21.2, 13.7, 8.3 | Pen G, Clox, OXT, GE | 2 |
| 23 | 3 | 21.2, 13.7, 8.3 | GE | 2 |
| 24 | 1 | 21.2 | Pen G, Amp, Amox, GE | 1 |
| 25 | 3 | 21.2, 13.7, 8.3 | Pen G, Clox, OXT, GE | 2 |
| 26 | 3 | 21.2, 13.7, 8.3 | AM, Pen G, Clox, GE | 2 |
| 27 | 3 | 21.2, 13.7, 8.3 | AM, GE | 2 |
| 28 | 3 | 21.2, 13.7, 8.3 | AM, GE | 2 |
| 29 | 3 | 21.2, 13.7, 8.3 | Amp, Pen G, Amox, GE | 2 |
| 30 | 3 | 21.2, 13.7, 8.3 | Pen G, Amox, GE | 2 |
| 31 | 3 | 21.2, 13.7, 8.3 | Pen G, Amox, GE | 2 |
| 32 | 3 | 21.2, 13.7, 8.3 | AM, Amox, GE | 2 |
| 33 | 3 | 21.2, 13.7, 8.3 | Pen G, Amox, Clox, GE | 2 |
| 34 | 3 | 21.2, 13.7, 8.3 | GE | 2 |
| 35 | 3 | 21.2, 13.7, 8.3 | AM, Pen G, Amox, GE | 2 |
| 36 | 3 | 21.2, 13.7, 8.3 | Pen G, Amox, GE | 2 |
| 37 | 2 | 21.2, 51.4 | Pen G, Amox, GE | 3 |
| 38 | 1 | 21.2 | Pen G, Clox, OXT, GE | 1 |
| 39 | 1 | 21.2 | OXT, GE | 1 |
| 40 | 1 | 21.2 | Amox, Clox, GE | 1 |
| 41 | 1 | 21.2 | AM, Pen G, Amox, GE | 1 |
| 42 | 1 | 21.2 | AM, Pen G, Amox, Clox,GE | 1 |
| 43 | 1 | 21.2 | AM, Pen G, Amox, GE | 1 |
| 44 | 3 | 21.2, 13.7, 9.4 | AM, Pen G, Amox, GE | 4 |
| 45 | 3 | 21.2, 13.7, 9.4 | Clox, GE | 4 |
| 46 | 2 | 21.2, 9.4 | Pen G, GE | 5 |
| 47 | 2 | 21.2, 9.4 | AM, Pen G, Amox, Clox, GE | 5 |
| 48 | 1 | 21.2 | AM, PenG, Amox, Clox, GE | 1 |
| 49 | 1 | 21.2 | PenG, Amox, Clox, GE | 1 |
| 50 | 1 | 21.2 | AM, Pen G, Amox, Clox, GE | 1 |

**AM= ampicillin, GE= gentamicin, OXT= oxytetracycline, PenG= penicillin G, Amox= amoxicillin, Clox=cloxacillin, Ery=erythromycin**

## DISCUSSION

*S. aureus* was isolated from milk samples collected under strict inclusion criteria by systematic sampling from different regions of Tabriz. According to the recommendations by the European Food Safety Authority, information on acquisition of decreased susceptibility is most relevant for monitoring purposes. However, one should not always deduce that reduced susceptibility implies clinical resistance. In Iran, several studies have been performed on mastitis. Gooraninejad et al. found 209 *S. aureus* isolates among 365 samples collected from Ahvaz, Southeast of Iran (6). We found that all *S. aureus* isolates were resistant to gentamicin. However, other studies reported the prevalence of gentamicin-resistance to be 0.5%, 39.9% and 51.2% among *S. aureus* isolates (7-9). The high resistance to penicillin (96%) detected in our study is consistent with the results reported by Yonis et al. (4). The increasing rate of penicillin resistance in *S. aureus* strains involved in mastitis may be related to the extensive use of this antibiotic in mastitis treatment. In earlier studies conducted in Iran, the rates of antibiotic resistance among *S. aureus* strains isolated from mastitis are higher than that reported in our study. For instance, a study has reported that 60% of the *S. aureus* isolates were resistant to ampicillin. High frequency of antibiotic resistance was also observed in other regions. In Sweden, Bengtsson et al. found that 1.9 % of isolates from acute clinical mastitis are resistant to erythromycin (8). Another study in Iran has reported that all isolates were resistant to cloxacillin, and sensitive to cefalotin and vancomycin (10). Among 66 isolates, Adwan detected ampicillin and amoxicillin resistance in 35 and 50 isolates, respectively (11). The most common mechanism of aminoglycosides resistance is based on production of an enzyme that closely resembles aminoglycoside 6´-N-acetyltransferase (1). Efficient treatment can be achieved only if the treatment is preceded by an antimicrobial susceptibility test and selection of the most accurate agent.

The high prevalence of aminoglycoside resistance could be because they are still one of the most widely used antibiotics for treatment of bovine mastitis. Frequent contact of bacteria with an antibiotic can increase the chance of resistance and decrease treatment efficiency. In our study, the MIC values for erythromycin ranged between 0.06 and 2 µg/ml (except for one strain with MIC of 8 µg/ml). However, since the observed MIC values for most strains were lower than the susceptibility breakpoint for this agent, we recommend using this peptide for treatment of infections caused by staphylococci including bovine mastitis. Furthermore, identification of highly resistant strains does not disqualify this antibiotic as a potential agent for treatment of bovine mastitis. Study of Parulraj et al. has reported the MIC of erythromycin to be between 0.305 and 2.44 µg/ml (12). Our results clearly indicate the potential of erythromycin and especially enrofloxacin for treatment of bovine mastitis. The positive therapeutic effects of oxytetracycline in treatment of bovine mastitis have been demonstrated by several studies. Preez revealed that oxytetracycline and erythromycin therapy has favorable clinical effects (13). Plasmid profiling has been proven useful for differentiation of *S. epidermidis* strains (14), but not carrier and invasive *S. epidermidis*. Aslantas et al. reported that 94% of *S. aureus* strains have different plasmid profiles and molecular weights (14). Since resistance genes could exist in plasmids of *S. aureus* strains, there seems to be some correlation between the presence of plasmids and resistance to antibiotics. It is well-established that plasmids of *S. aureus* are not very stable (15). A small variation of plasmid patterns could be responsible for altered antibiotic resistance.

## CONCLUSION

The results of the present study indicate that enrofloxacin and erythromycin are promising effective agents for treatment of staphylococcal infections. On the other hand, differentiation of the strains using antibiotic susceptibility testing and determination of plasmid profiles can provide additional information that could be of use in characterization of bacteria.

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## CONFLICT OF INTEREST

There is no conflict of interest.

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