Antimicrobial susceptibility pattern and **SCC**<i>mec</i> types of methicillin-resistant coagulase-negative staphylococci from subclinical bovine mastitis in Hatay, Turkey

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Abstract

Eighty-nine isolates of coagulase-negative staphylococci (CoNS) of eight species from subclinical bovine mastitis were screened for the phenotypic and genotypic methicillin-resistance. In addition, all methicillin-resistant (MR) isolates indicating the **mecA** gene were examined by PCR for the antimicrobial susceptibility patterns, and staphylococcal cassette chromosome **mec** (SCC**mec**) types were also determined by multiplex PCR. A total of 21 (23.6%) CoNS isolates were found to be resistant to oxacillin in broth microdilution assay. All isolates phenotypically resistant to oxacillin did not have the **mecA** gene, which was only found in 14.6% (13) of the isolates. Most MR-CoNS isolates were highly resistant to erythromycin (92.3%), fusidic acid (84.6%), penicillin (76.9%), and rifampycin (61.5%), and susceptible to mupirocin (100%), tetracycline (100%), vancomycin (100%), clindamycin (92.3%), and sulfamethoxazole-trimethoprim (69.2%). In conclusion, a high rate of antimicrobial resistance among MR-CoNS isolated from food producing animals emphasises the need for periodic surveillance of their resistance.

**Keywords:** bovine mastitis, staphylococci, antibiotic resistance.

Introduction

Coagulase-negative staphylococci (CoNS) are among the most frequently isolated bacteria from the subclinical mastitis in cows and sheep (17), and recently they have been recognised as emerging pathogens (16). These bacteria are present in the teat skin of healthy cattle as a normal microbiota. CoNS are also a well-recognised nosocomial pathogens causing a range of human diseases including bacteraemia, endocarditis, catheter related infections, urinary tract infections, and endophthalmitis (8).

Many isolates of CoNS are becoming resistant to most antibiotics, particularly to β-lactam antibiotics due to the **mecA** gene carried by the mobile genetic element called staphylococcal cassette chromosome **mec** (SCC**mec**) (5, 20). Methicillin-resistant coagulase-negative staphylococci (MR-CoNS) have been also found to be resistant to the antibiotics commonly used to treat health care-associated infections and community-acquired diseases in humans (9, 12). Therefore, the prevalence and antimicrobial sensitivity patterns of MR-CoNS need to be established for the selection of appropriate treatment for both human and animal diseases associated with these organisms.

It is well-known that food of animal origin contaminated with MR-CoNS is of a great concern of public health and also has a role in the dissemination of resistant bacteria to humans (7, 11). However, to date, there are not many studies performed on MR-CoNS among subclinical bovine mastitis in Turkey (10, 22). Thus, the objectives of the study covered phenotypic (broth microdilution and disc diffusion) and genotypic (methicillin resistance gene (**mecA**)) characterisation of methicillin-resistance in CoNS strains from subclinical bovine mastitis, and determination of SCC**mec** types by PCR, as well as susceptibility patterns of a panel of antimicrobials commonly used against staphylococci.
Material and Methods

A total of 89 CoNS isolates (23 S. haemolyticus, 19 S. epidermidis, 13 S. lentus, 12 S. chromogenes, 8 S. xylosus, 7 S. sciuri, 4 S. warneri, 3 S. simulans) from cases of subclinical bovine mastitis were included in this study. The strains were isolated and identified between 2008 and 2010 in Hatay province, Turkey. All isolates were biochemically identified by standard identification system (API® Staph, BioMerieux, USA). All strains were screened for their oxacillin susceptibility by broth microdilution method and disc diffusion assay (Oxoid, U.K.). Oxacillin was tested at the concentration of 0.03-128 µg/mL. The minimal inhibitory concentrations (MICs) of 12 antimicrobial agents (amoxicillin/clavulanic acid, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, mupirocin, penicillin, rifampin, vancomycin, tetracycline, and trimethoprim-sulfamethoxazole) were determined for mecA-positive isolates using VITEK®2 Compact System (BioMerieux, France). Because no Clinical and Laboratory Standards Institute (CLSI) breakpoints were available for mupirocin and fusidic acid, the breakpoints were adapted from Fuchs et al. (4) and Toma and Barriault (21) respectively. For all other agents, the breakpoints obtained from the CLSI (2) were applied. S. aureus strain (ATCC 25923) provided by Dr. Kılıç (the Refik Saydam Hygiene Center, Ankara) was used for the quality control.

Bacterial DNA was extracted by the method described by Hesselbarth and Schwarz (6). All CoNS were also screened by PCR for the presence of the mecA gene, as previously reported (1). The primers used to detect mecA gene were: 5′-CCTAG TAAAGCTCCGGAA-3′ and 5′-CTAGTCCATTCC GTTCCA-3′, and the PCR reaction was as follows: initial denaturation at 95°C for 2 min, denaturation at 94°C for 2 min (30 cycles), annealing at 58°C for 30 sec and extension at 72°C for 30 sec, with a final extension step at 72°C for 7 min. SCCmec typing was performed according to Kondo et al. (13).

Results

Out of 89 CoNS strains isolated from bovine mastitis, six (10.5%) isolates were found to be resistant to oxacillin by disc diffusion method while 21 (23.6%) isolates demonstrated resistance to oxacillin by broth microdilution method. However, among the all CoNS isolates, 13 (14.6%) were mecA positive by PCR: seven isolates of S. haemolyticus, four S. lentus, one S. warneri, and one S. epidermidis were mecA positive (Table 1).

MR-CoNS also showed resistance to other tested antibiotics, i.e.: 92.3% to erythromycin, 84.6% to fusidic acid, 76.9% to penicillin, and 61.5% to rifampicin. Out of 13 MR-CoNS isolates, 92.3% were susceptible to gentamicin and clindamycin, 69.2% to sulfamethoxazole-trimethoprim, and 61.5% to amoxicillin/clavulanic acid. In addition, all MR-CoNS isolates were found to be susceptible to tetracycline (Table 1). Gentamicin resistance was encountered in only one S. epidermidis strain, while fusidic acid also did not reveal activity against S. epidermidis and S. warneri in the resistance range (MIC, ≥2 µg/mL). All MR-CoNS isolates presented only SCCmec type V (Table 1).

Discussion

In the past, CoNS had long been considered to be non-pathogenic, but the fact is that the role of these organisms in a variety of clinical symptoms in animals and humans is significant. The prevalence and antimicrobial susceptibility of MR-CoNS, one of the most important causes of mastitis in animals responsible for a number of community-acquired and hospital-acquired infections in humans, was reported in this study.

β-lactam antibiotics have been widely used in veterinary medicine mainly for prevention and treatment of mastitis (23). However, the emerging antimicrobial resistance to these agents limits the use of this antimicrobials, and causes overuse of other agents such as glycopeptides. Thus, several studies have been conducted to examine the presence of mecA gene encoding PBP2a among CoNS isolates from the mastitis (10, 19, 24).

In this study, even though 21 (23.6%) isolates demonstrated resistance to oxacillin by broth microdilution method, only 13 (14.6%) were considered as methicillin-resistant because detection of the mecA gene is the best tool for identification of methicillin-resistant isolates (24). The study conducted in Germany by Feßler and Billerbeck (3), in which genotypic and phenotypic tests were applied in order to investigate methicillin-resistance in CoNS isolates from bovine mastitis, also reported that some CoNS isolates exhibiting oxacillin resistance (MICs, ≥0.5 µg/mL) did not harbour the mecA gene. Misestimation of methicillin-resistance in CoNS by phenotypic tests has been also reported by other investigators (19, 24). The mecA positive CoNS from a variety of animal species have been isolated so far, and the prevalence of mecA positive CoNS obtained from subclinical mastitis in this study was found to be similar to the results of previous studies conducted in European countries (3, 19), but higher than that reported by Ünal and Çınar (22) in Turkey. In addition, the frequency of the mecA gene in S. chromogenes was reported to be very rare (19). Similarly, in the study, there were no S. chromogenes isolates, which displayed phenotypic and genotypic (mecA positive) resistance to oxacillin. However, our results are contradictory to observations of Sampiljon et al. (19) and Vanderhaeghen et al. (23), who noted that determination of mecA among S. epidermidis was
the most common, whereas only a single *S. epidermidis* strain being positive for this gene, was found.

The glycopeptide vancomycin is reported to be the drug of choice for effective treatment of methicillin-resistant staphylococci infections in humans (15). No mupirocin and vancomycin resistant strains in MR-CoNS were recovered during this study. It is not surprising considering the fact that these agents are not the drug of choice for mastitis in dairy cattle. The rate of erythromycin resistance was found to be higher than in the results observed in Germany (14). This is likely due to the fact that macrolides agents are the main group of antibiotic used for either controlling or preventing from bacterial infections of dairy cattle in Turkey. With regard to the zoonotic potential of staphylococci for humans (11) and also capacity of transferring their resistance to other bacteria (5), infrequency of vancomycin and mupirocin resistance emphasises the need for suitable antibiotic treatment in order to control the emergence of resistance against these last-resort antibiotics.

The presence of SCCmec elements in MR-CoNS was also investigated in this study. We only identified SCCmec types V on MR-CoNS, although different SCCmec typing on MR-CoNS was reported so far (18). Four different SCCmec types on MR-CoNS were found in study conducted by Inegöl and Türcüylilmaz (10). This was the first detected SCCmec element on MR-CoNS from the animal sources in Turkey. It was previously shown that MR-CoNS isolates from different geographical areas were found to carry different types of SCCmec elements (18).

Overall, the relatively high prevalence of MR-CoNS was observed in this study, revealing the wide spread use of this agent to cure or to prevent bacterial diseases in animals. The emergence of resistant bacteria, particularly multidrug-resistance, has been considered as a public health problem worldwide. In dairy farms and processing plants, CoNS contamination of milk and the final products, such as cheese could occur due to improper cleaning and disinfecting the equipment and mishandling the products. Another problem may occur is the dissemination of antimicrobial resistant bacteria in the environment. The results obtained showed a high rate of antimicrobial resistance among MR-CoNS isolated from food producing animals, emphasising the need of periodic surveillance to prevent from the dissemination of antimicrobial resistance genes and resistant pathogens to humans in Turkey.

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<table>
<thead>
<tr>
<th>Table 1. Characteristics of the 13 mecA positive CoNS</th>
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<tr>
<td><strong>Isolate ID</strong></td>
<td><strong>Species</strong></td>
</tr>
<tr>
<td></td>
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<tr>
<td>B-1</td>
<td><em>S. haemolyticus</em> 0</td>
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<tr>
<td>B-133</td>
<td><em>S. haemolyticus</em> 12</td>
</tr>
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<td>B-10</td>
<td><em>S. haemolyticus</em> 10</td>
</tr>
<tr>
<td>B-181</td>
<td><em>S. lentus</em> 10</td>
</tr>
<tr>
<td>B-163</td>
<td><em>S. lentus</em> 10</td>
</tr>
<tr>
<td>B-151</td>
<td><em>S. lentus</em> 10</td>
</tr>
<tr>
<td>B-147</td>
<td><em>S. lentus</em> 10</td>
</tr>
<tr>
<td>B-202</td>
<td><em>S. epidermidis</em> 10</td>
</tr>
<tr>
<td>B-106</td>
<td><em>S. warneri</em> 15</td>
</tr>
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| Resistant isolates (%) | 76.9 | 100 | 38.5 | 7.7 | 46.1 | 92.3 | 7.7 | 0 | 0 | 84.6 | 0 | 61.5 | 30.8 |

aAntimicrobial abbreviations (P, penicillin; OXA, oxacillin; AMC, amoxicillin-clavulanic acid; CN, gentamicin; CIP, ciprofloxacin; E, erythromycin; DA, clindamycin; VA, vancomycin; TE, tetracycline; FA, fusidic acid; MUP, mupirocin; SXT, trimethoprim-sulfamethoxazole; RA, rifampycin)
bThe MIC values of amoxicillin/clavulanic acid (2/1) and sulfamethoxazole/trimethoprim (19:1) are given as amoxicillin and trimethoprim MIC values, respectively
References