Beta lactamase production rate and antimicrobial susceptibility of Staphylococcus aureus isolated from clinical and subclinical mastitis cases in Turkey

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SUMMARY

A total of 183 strains of Staphylococcus aureus were investigated for their ß-lactamase production and susceptibility to several antimicrobial agents. Strains were isolated from bovine clinical and subclinical mastitis cases in the following geographic locations: Ankara, Central Anatolia, and Balikesir, Western Anatolia. ß-lactamase production rate of all isolates was determined by using nitrocefin-containing identification sticks (OXOID BR66A). Sixty-two (33.8 %) of isolates tested were positive for ß-lactamase production. Antimicrobial susceptibility was examined by mean of agar disk diffusion and micro dilution susceptibility tests. In the agar disk diffusion test, the following disks were included: penicillin G (Oxoid 10 IU), ampicillin (Oxoid 10 µg), cloxacillin (Oxoid 5 µg) and amoxicillin plus clavulanic acid (Oxoid 30 µg). The percentage of resistance against penicillin G, ampicillin, amoxicillin plus clavulanic acid and cloxacillin was 91.9 %, 90.3 %, 4.8 %, 4.8 % in ß-lactamase positive isolates and 7%, 5.7 % and 0 % in ß-lactamase negative isolates, respectively. The average minimum inhibitory concentrations that inhibit 50 % and 90 % of ß-lactamase positive and negative isolates for penicillin G, ampicillin, amoxicillin plus clavulanic acid and cloxacillin were ≤ 0.06, ≤ 0.06, ≤ 0.06, ≤ 0.06, 2, 0.125, 0.5 µg/ml in ß-lactamase positive isolates and ≤ 0.06, ≤ 0.06, ≤ 0.06, ≤ 0.06, 0.5, 0.5, 0.125, 0.5 µg/ml in ß-lactamase negative isolates, respectively. Three out of the 183 isolates tested were resistant to all antibiotics tested.

It was concluded that there was good agreement between resistance to penicillin G and ampicillin and ß-lactamase production of S. aureus. Therefore, the analysis of ß-lactamase production status of S. aureus strains from mastitis cases at regular intervals would provide valuable information to practitioners.

KEY-WORDS : Mastitis, Beta-lactamase, Staphylococcus aureus, antibiotic susceptibility.

Introduction

Bovine mastitis is generally considered to be the most costly disease in worldwide dairy industry. The high cost is associated with a drop in milk production, discharged milk, high culling rate and treatment cost [22]. Recent studies conducted in Turkey revealed that the majority of mastitis cases are caused by staphylococci, streptococci and Gram-negative bacilli with S. aureus being the most frequently isolated species [1, 10, 20].

ß-lactam antimicrobial agents such as penicillin’s and cephalosporins are commonly used to treat mastitis. Widespread use of these agents has resulted in the emergence of resistant organisms [4, 5, 14, 21]. The way bacteria develop resistance to ß-lactam antibiotics is mainly through ß-lactamase enzymes. ß-lactamases render bacteria resistant to ß-lactam antibiotics by hydrolyzing the ß-lactam ring of penicillin’s and cephalosporins [16]. Both Gram-positive and Gram-negative bacteria can become resistant to ß-lactam antibiotics via production of ß-lactamase [2]. There is wide correlation between ß-lactamase production of bacteria and resistance against penicillin. Therefore, measuring of the ß-lactamase production rate of bacteria is the most useful parameter for determining potential penicillin resistance [2, 3, 4, 9, 14]. A few studies on ß-lactamase production of S. aureus strains isolated from mastitis cases in Turkey are available. HADIMLI et al. [7], ESKIIZMIRLIER and ÖNCEL [6] and SEZEN et al. [19] determined 46 %, 64 % and 100 % ß-lactamase production rate among S. aureus tested respectively.
The purpose of this study was to investigate the β-lactamase production rate and antibacterial susceptibility of S. aureus isolated mastitis cases in two different geographic locations of Turkey.

Materials and methods

SOURCE AND IDENTIFICATION OF BACTERIAL ISOLATES

A total of 183 isolates of S. aureus were evaluated in the study. Tested strains were isolated from clinical and subclinical mastitis cases in the following locations (102 strains): Ankara, Central Anatolia, and (81 strains) Balikesir, Western Anatolia.

For isolation, lacteal secretions from infected quarters were aseptically collected in sterile test tubes. A 0.01 ml portion of lacteal secretions were streaked on to blood agar and McConkey agar plates and incubated at 37 °C for 48 hours. Isolates were presumptively characterized as staphylococci based on colony morphology. All strains were identified by standard procedures [8]. The coagulase test was performed by using rabbit plasma in tubes. Staphylococcus spp. tested positive for free coagulase was classified as S. aureus.

β-LACTAMASE PRODUCTION

All isolates were tested for β-lactamase production by using nitrocefin-containing identification sticks (OXOID BR66A). Briefly, sticks were touched to colony material from a 24-h culture of test bacteria and kept up to 24 hours in an incubator at 37°C with moisture. A change in the color of the sticks indicated β-lactamase production.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility tests were performed on Mueller-Hinton agar plates following the Disk diffusion susceptibility test procedures as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [13]. The following disks included: penicillin G (Oxoid 10 IU), ampicillin (Oxoid 10 μg), cloxacillin (Oxoid 5 μg) and amoxicillin plus clavulanic acid (Oxoid 30 μg).

Minimum inhibitory concentrations (MIC) of penicillin, ampicillin, amoxicillin plus clavulanic acid and cloxacillin against the S. aureus strains tested were determined using the broth microdilution method as recommended by NCCLS [13]. Standard antibiotic powders were provided by Pfizer Inc. All the compounds were tested in the dilution range of 0.06 - 64 μg/ml. S. aureus ATCC 29213 was included as control. The MIC is defined as the lowest concentration of antimicrobial agent that inhibits growth of the organism.

Results

The MIC values for the quality control organism were within the recommended ranges for all the antimicrobials tested. Of the tested S. aureus strains, 33.8% (62 strains) were positive for β-lactamase production (Table I). In disk diffusion tests, β-lactamase positive strains showed high resistance against Penicillin G and ampicillin. In contrast, the activity of both penicillin G and ampicillin was higher against β-lactamase negative strains in comparison to β-lactamase positive strains. Amoxicillin plus clavulanic acid and cloxacillin were highly active against both β-lactamase positive and negative strains. Three out of the 183 strains evaluated were resistant to all antimicrobials tested. The MIC values were consistent with the results of the disk diffusion tests. The MICs required to inhibit 50% and 90% of the isolates tested and the percentage of resistance detected in the agar diffusion test are presented in Table I.

Discussion

The role of β-lactamases in the resistance development of S. aureus against β-lactam antibiotics has been described [3, 5, 9, 12, 21]. Similarly, recent studies from Turkey have revealed that the β-lactamase production of S. aureus strains isolated from mastitis cases is as high as those observed throughout the world [6, 7, 19]. The β-lactamase production rate given in the present study (33.8%) is less than those reported in previous studies in Turkey. This could be the result of variation in susceptibility patterns between different geographical locations, or even in different herds in the same location. Our findings together with the results of previously conducted studies in Turkey suggest that the existence of β-lactamase positive S. aureus strains could pose a problem to practitioners in the treatment of mastitis. These findings highlight the importance of local data on susceptibility patterns of antimicrobials, as most of the veterinarians in the field can not benefit from laboratory services in course of mastitis therapy.

Some researchers stated that the exposure of isolated bacteria to penicillin before testing for β-lactamase production resulted in higher β-lactamase rate [5]. This indicates induction of β-lactamase by penicillin. As penicillin induction was not performed before β-lactamase testing in this study, the possible effect of these phenomena on the β-lactamase production of isolates is not yet known.

In antimicrobial susceptibility tests, complete correlation was observed between positive β-lactamase results and the resistance to penicillin G and ampicillin. Average MIC50 values for penicillin G and ampicillin for β-lactamase negative isolates were low (both were 0.5 μg/ml) compared to those for β-lactamase positive isolates (both were 2 μg/ml).

In contrast, amoxicillin plus clavulanic acid and cloxacillin demonstrated high activity against both β-lactamase positive and negative isolates. Our results agree in general with those reported in previous studies demonstrating that β-lactamase production is a good indicator of penicillin resistance [3, 4, 11, 12, 14, 21].

High activity of amoxicillin plus clavulanic acid and cloxacillin is dependent on their resistance to β-lactamase enzymes. Cloxacillin is anti-staphylococcal penicillin that is naturally resistant to β-lactamase produced by gram-positive bacteria. [16]. Cloxacillin has been used in treatment of mas-
titis caused by gram-positive bacteria for more than 30 years without any resistance problem. High activity of cloxacillin in this study is consistent with previous studies [15, 17, 18, 23] in which cloxacillin were found to be highly active against both ß-lactamase positive and negative S. aureus isolates. Three ß-lactamase positive isolates were found to be resistant to cloxacillin. Three of these isolates were also resistant to other antibiotics tested including amoxicillin plus clavulanic acid. NCCLS recommends oxacillin in place of cloxacillin in antimicrobial susceptibility tests as it is more stable and gives more reliable results. Resistance to oxacillin demonstrates the existence of methicillin-resistant S. aureus strains (MRSA). Isolates of this study which are both resistant to cloxacillin and amoxicillin plus clavulanic acid were suspected of MRSA. MRSA has not been reported in the veterinary field in Turkey. As MRSA shows multiple resistances to different antibiotics including oxacillin, isolates of this study should be tested for antibiotics of other classes and presence of MecA gene to prove MRSA. On the other hand, it can be explained that the isolates that were resistant to cloxacillin, could be due to hyper production of ß-lactamase rather than to the altered penicillin-binding protein found in MRSA positive strains as suggested by DE OLIVEIRA et al. [5].

Clavulanic acid that is part of amoxicillin plus clavulanic acid combination is a ß-lactamase inhibitor. Although it has weak antibacterial effect, when used in combination with penicillins and cephalosporins it shows a synergistic effect by inhibiting ß-lactamase [16]. There are several studies demonstrating the activity of amoxicillin plus clavulanic acid against bacteria that cause mastitis [5, 14]. Owens and Watts [14] demonstrated 100 % activity of amoxicillin plus clavulanic acid against 722 isolates of S. aureus and Coagulase negative staphylococci including ß-lactamase positive strains by using agar gel diffusion test. DE OLIVEIRA et al. [5] determined MIC of several antibiotics for 811 strains of S. aureus with 35.6 % ß-lactamase activity and reported MIC90 value for amoxicillin plus clavulanic acid as ranged between < 0.06 - 0.125, demonstrating high activity of combination. This result is in agreement with MIC90 value range of < 0.06- 0.125 reported in this study. Three out of the sixty-two ß-lactamase positive isolates tested in this study was resistant to amoxicillin plus clavulanic acid. Resistance to this antimicrobial could be interpreted as being the result of different resistance mechanisms that were not investigated in this study [16].

From the results presented here it appears that ß-lactamase production is worth consideration for S. aureus strains isolated from mastitis cases in Turkey. Commercially available sticks used in this study were found to be useful and labor-saving in the detection of ß-lactamase activity of S. aureus. Since a strong correlation was observed between ß-lactamase production and resistance to ampicillin, and penicillin G, ß-lactamase production of S. aureus strains from mastitis cases should be tested regularly. The results of studies concerning the activity of available antibiotics are beneficial to practitioners and provide them with useful data on the resistance profiles of available antibiotics and assist them choosing the best alternative. Broadening the study by examining the activity of antimicrobials in other groups would be most beneficial.

References