Prevalence of blaZ gene and other virulence genes in penicillin-resistant Staphylococcus aureus isolated from bovine mastitis cases in Gansu, China

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Abstract: Staphylococcus aureus is a major etiological agent of bovine mastitis worldwide. In this study, 37 strains of S. aureus resistant to penicillin were isolated from bovine mastitis cases in Gansu Province for investigating blaZ and virulence-related genes, including tst, eta, etb, lukPV, lukED, lukM, hla, hlb, hld, and edin. Antibiotic resistance was based on disk diffusion method and blaZ and virulence-associated genes were studied by polymerase chain reaction. Penicillin resistance gene blaZ was detected in 35/37 (94.6%) of penicillin-resistant S. aureus isolates. tst, lukPV, lukED, hla, hlb, and hld were observed in 5.4%, 2.7%, 89.2%, 70.3%, 73.0%, and 70.3% of the penicillin-resistant isolates, respectively, while eta, etb, lukM, and edin were not detected in any isolates. blaZ carried by penicillin-resistant S. aureus isolates may be the main reason for phenotypic penicillin resistance. Virulence determinants encoded by lukED, hla, hlb, and hld genes may play important roles in bovine mastitis pathogenesis of penicillin-resistant S. aureus in Gansu Province.

Key words: Bovine mastitis, Staphylococcus aureus, antibiotic resistance, blaZ, virulence-related genes

Staphylococcus aureus is the primary contagious pathogen in bovine mastitis, causing severe economic losses to the dairy industry worldwide (1). Penicillin is the most commonly used drug in the treatment of mastitis (2), which has led to an increase in the number of resistant strains (3).

The pathogenic basis of S. aureus and its response to antibiotic therapy depend on various antibiotic resistance- and virulence-associated genes carried by the pathogen (4). Resistance gene blaZ is responsible for resistance to penicillin (5). Currently, over 40 virulence-associated genes have been reported among various S. aureus strains (6). Although some but not all of the virulence-related genes in S. aureus strains from bovine mastitis have been reported in South and East China (7,8), little is known yet about the virulence genes in penicillin-resistant S. aureus strains recovered from bovine mastitis in Northwest China. Therefore, the aims of this work were first to determine the genetic basis of penicillin resistance and second to investigate virulence-related genes in penicillin-resistant S. aureus strains from bovine mastitis cases in Gansu Province.

Thirty-seven bacterial isolates from bovine mastitis (26 isolates from clinical cases and 11 isolates from subclinical cases) were collected at 3 farms located in Gansu Province in China during 2014. Mastitis infection was confirmed by the California Mastitis Test. The isolates were identified as penicillin-resistant S. aureus strains by morphological characterization, biochemical testing, and disk diffusion method according to Clinical Laboratory Standards Institute standards (9). S. aureus ATCC 29213 was used as control isolate. The penicillin-resistant gene blaZ and several virulence-related genes encoding toxic shock syndrome (tst), exfoliatins (eta, etb), leukotoxins (lukPV, lukED, lukM), hemolysins (hla, hlb, hld), and EDIN (edin) were detected by simplex PCR according to Olsen et al. (10) and Jarraud et al. (11), respectively.

As shown in the Table, 94.6% of S. aureus isolates resistant to penicillin were shown to have the expected penicillin resistance gene blaZ. lukED was the most prevalent virulence gene (89.2%), followed by hlb (73.0%), hla (70.3%) hld (70.3%), tst (5.4%), and lukPV (2.7%). eta, etb, lukM, and edin were not detected in any strains. In the blaZ-positive isolates, lukED, hla, hlb, and hld were the most commonly occurring virulence-related genes,
Table. Distribution of blaZ and virulence-related genes in penicillin-resistant S. aureus from cows with mastitis in Gansu.

<table>
<thead>
<tr>
<th>Resistance genotype</th>
<th>No. (%) of strains</th>
<th>No. (%) of virulence-related genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tst</td>
<td>eta</td>
</tr>
<tr>
<td>blaZ*</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>blaZ</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>2</td>
</tr>
</tbody>
</table>

blaZ*: Strains positive for blaZ; blaZ : Strains negative for blaZ.

detected in 86.5%, 67.6%, 70.3%, and 67.6% of isolates, respectively. In contrast, tst and lukPV were found in only two isolates and one isolate, respectively. For the two blaZ-negative S. aureus isolates, the detection rates of lukED, hla, hlb, and hld were all 2.7%.

The activated blaZ could encode β-lactamase enzyme (penicillinase), which inactivates the antibiotic through hydrolysis of the peptide bond in the β-lactam ring (5). In this study, not all the penicillin-resistant S. aureus isolates exhibited genotypic resistance to penicillin, corresponding well to the findings of previous studies (12). This is in agreement with data from Gao et al. (13), showing that no resistance genes could be determined in some phenotypically resistant Streptococcus agalactiae isolates. In some isolates, phenotypic resistance may have been caused by point mutations rather than gene acquisition. Additionally, except for the general resistance mechanisms (14), other pathways such as biofilm formation may play a major role in the resistance mechanisms (15).

The penicillin-resistant S. aureus isolates in the present study showed a high frequency of lukED. The lukED gene could encode bicomponent leukotoxins with which S. aureus can target and kill innate immune cells critical for defense against bacterial infections (16). A high prevalence of hla, hlb, and hld was also observed in the S. aureus isolates, in agreement with findings reported in China and other countries (7,17–19). Hemolysins encoded by hla, hlb, or hld aid the S. aureus population in the invasion of the host cells and cause damage (20). The observation of frequent lukED, hla, hlb, and hld suggest that bicomponent leukotoxins and hemolysins encoded by these corresponding virulence-related genes may have crucial roles in pathogenesis of bovine mastitis caused by penicillin-resistant S. aureus in Gansu Province.

In conclusion, our results suggest that the blaZ carried by penicillin-resistant S. aureus may play a major role in the penicillin-resistant phenotype, but the resistance gene cannot be used alone as a diagnostic indicator for penicillin resistance. Further studies should be performed to develop accurate molecular indicators of antibiotic resistance. In addition, the detection of genes encoding virulence determinants suggests that lukED, hla, hlb, and hld are the most prevalent virulence-related genes in penicillin-resistant S. aureus from bovine mastitis cases in Gansu. These results emphasize the need for monitoring the genetic basis of antimicrobial resistance and virulence determinants in S. aureus.

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