

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*, *hnb*, *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*, *hnb*, 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*, *hnb*, 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*, *hnb*, *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 *hnb* (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*, *hnb*, and *hld* were the most commonly occurring virulence-related genes,

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Table. Distribution of *blaZ* and virulence-related genes in penicillin-resistant *S. aureus* from cows with mastitis in Gansu.

Resistance genotype	No. (%) of strains	No. (%) of virulence-related genes									
		<i>tst</i>	<i>eta</i>	<i>etb</i>	<i>lukPV</i>	<i>lukED</i>	<i>lukM</i>	<i>hla</i>	<i>hlb</i>	<i>hld</i>	<i>edin</i>
<i>blaZ</i> ⁺	35 (94.6)	2 (5.4)	0 (0)	0 (0)	1 (2.7)	32 (86.5)	0 (0)	25 (67.6)	26 (70.3)	25 (67.6)	0 (0)
<i>blaZ</i> ⁻	2 (5.4)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	0 (0)	1 (2.7)	1 (2.7)	1 (2.7)	0 (0)
Total	37 (100)	2 (5.4)	0 (0)	0 (0)	1 (2.7)	33 (89.2)	0 (0)	26 (70.3)	27 (73.0)	26 (70.3)	0 (0)

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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