

## ORIGINAL ARTICLE

# Antimicrobial Resistance of *Staphylococcus Aureus* Isolated From Bovine Mastitis in China

D. Shi, Y. Hao, A. Zhang, B. Wulan and X. Fan

College of Veterinary, Inner Mongolia agricultural University, Inner Mongolia, China

**Keywords:**antibiotic sensitivity testing; bovine mastitis;  
*Staphylococcus aureus***Correspondence:**Y. Hao. College of Veterinary, Inner Mongolia  
Agricultural University, Huhhot, Inner  
Mongolia, 010018 China.  
Tel.: +864714308677; Fax: +864714309175;  
E-mail: yongqinghao1960@yahoo.com.cn

Received for publication February 8, 2010

doi:10.1111/j.1865-1682.2010.01139.x

**Summary**

Mastitis is one of the largest production concerns in the dairy industry worldwide. Mastitis caused by *Staphylococcus aureus* is a major concern to the dairy industry because of its resistance to antibiotic treatment. In this report, the results of antibiotic susceptibility test, carried out on 236 *Staphylococcus aureus* isolated from milk samples which were collected from cases of mastitis in cow herds of China, are presented. The regions and number of isolates include Inner Mongolia (112), Hebei (58) and Heilongjiang (66). Susceptibility to ampicillin, penicillin G, amoxicillin, piperacillin, cephalexin, cephazolin, cefotaxime, ceftazidime, cefoxitin, SMZ-TMP, gentamycin, kanamycin, norfloxacin, ciprofloxacin, ofloxacin, furaxone, torlamycin, roxithromycin, clindamycin and vancomycin was determined by the disc diffusion method. Antibiotic susceptibility testing showed 87.30% (206 of 236) isolates were resistant to penicillin G. This result compares with the reports from other countries; the overall level of resistance was generally high for all antimicrobial agents tested.

**Introduction**

Bovine mastitis is an inflammation of the udder caused by microbial infection and imposes a serious economic burden in the dairy industry (Miles et al., 1992). Among the mastitis pathogens, *Staphylococcus aureus* (*S. aureus*) is considered an agent of major concern because of the low response to treatment and its propensity to recur chronically. Targeted antimicrobial therapy plays an important role in mastitis control by reducing the levels of herd infection and by preventing new infections. *S. aureus* isolates resistant to antibiotics have been reported in studies in many countries. (Owens et al., 1997; Aarestrup et al., 1998; Lange et al., 1999; De Oliveira et al., 2000). Hence, antimicrobial resistance monitoring of *S. aureus* isolates from herds will be important for the establishment of a more effective targeting of therapy for staphylococcal udder infections.

The province surveyed in this study contains over 45% of the dairy cows currently in China and produces 56% of the milk (source: National Bureau statistics,

China statistical yearbook). Eight hundred thirty-five (835) quarter milk samples were collected from 288 dairy cows at 18 farms during 2005–2006. A cow with a SCC  $\geq 500\,000$  cells/ml was considered to have mastitis. Cultures resulted in the isolation of 285 *S. aureus* strains. The regions and number of isolates included inner Mongolia (112), Hebei province (58) and Heilongjiang (66). Susceptibility to ampicillin, penicillin G, amoxicillin, piperacillin, cephalexin, cephazolin, cefotaxime, ceftazidime, cefoxitin, SMZ-TMP, gentamycin, kanamycin, norfloxacin, ciprofloxacin, ofloxacin, furaxone, torlamycin, roxithromycin, clindamycin and vancomycin was determined by the disc diffusion method.

**Materials and Methods****Sample collection and microbiological analysis**

Eight hundred thirty-five quarter milk samples were collected from 288 dairy cows at 18 farms during 2005–2006. A cow with a SCC  $\geq 500\,000$  cells/ml was considered to

have mastitis. A total of 236 strains of *S. aureus* were used in the study.

Before sampling, the teat ends were cleaned with warm water and alcohol swabs and allowed to dry. The first few streams were discarded, and then 5 ml of secretion was collected in 10-ml sterile tubes. Samples were cooled and immediately transported to the Veterinary microbiological laboratory (Imau, Huhhot, China). From each milk sample, 0.1 ml was plated on blood agar plate, Chapman's agar plate and Baird-Parker agar and incubated at 37°C for 48 h. Suspect isolates were identified by conventional methods, including Gram staining, colony morphology, coagulase, haemolysis, DNase, tests for catalase, acetoin and anaerobic fermentation of mannitol. The confirmed isolates of *S. aureus* were preserved at -70°C in LB medium containing 15% of glycerol.

### Antibiotic susceptibility testing

Antibiotic susceptibility test was performed using disc diffusion method on Mueller-Hinton Agar (Oxoid) according to the National Committee of Clinical Laboratory Standards (NCCLS). Five colonies from the blood agar medium, incubated at 37°C for 18 h, were suspended in 2 ml of sterile saline. The bacterial suspension was adjusted to a density approximately equal to  $1.5 \times 10^8$  CFU/ml according to the McFarland standard. A dry sterile cotton wool swab was placed in the bacterial suspension, and

excess liquid was expressed against the inside of the tube. The bacterial suspension was inoculated onto Mueller-Hinton agar with the swab to ensure that the whole surface of the agar was covered. Susceptibility test discs were obtained commercially (Beijing Tiantan Microorganism Reagent Co., Ltd., Beijing, China). The antibiotic discs, containing the following antibiotics: ampicillin (10 µg), penicillin G (10 µg), amoxicillin (20 µg), piperacillin (100 µg), cephalexin (30 µg), Cephazolin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftiofur (30 µg), SMZ-TMP (23.75/1.25 µg), gentamicin (10 µg), kanamycin (30 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), Ofloxacin (5 µg), furaxone; (300 µg), erythromycin (15 µg), ROX (15 µg), clindamycin (2 µg) and vancomycin (30 µg), were dispensed on the surface of the medium with a sterile forceps and incubated aerobically at 37°C for 18 h. The results were recorded as resistant or susceptible based on the measurement of the inhibition zone diameter using a sliding calliper according to the interpretive standards of NCCLS. The reference strain used for antibiotic susceptibility assays was *S. aureus* ATCC 25923.

## Results

### Antimicrobial susceptibility

The antibiotic resistance rates of *S. aureus* isolated from bovine mastitis were detailed in Table 1. As can be seen, *S. aureus* isolates showed the highest *in vitro* resistance rate

**Table 1.** Antibiotic susceptibility of *S. aureus* isolated from bovine mastitis

			<i>S. aureus</i> (n = 236 isolates)		
	Antibiotic	Abbreviation	Resistant	Moderate sensitive	Hypersensitive
			n (%)	n (%)	n (%)
Penicillins	Ampicillin	AM	113 (47.88)	34 (14.41)	89 (37.71)
	Penicillin G	P	206 (87.30)		30 (12.70)
	Amoxicillin	AMX	75 (31.78)	64 (27.12)	97 (41.10)
	Piperacillin	PIP	82 (34.75)	49 (20.76)	105 (44.49)
Cephalosporins	Cephalexin	CX	60 (25.42)	37 (15.68)	139 (58.90)
	Cephazolin	CZ	109 (46.19)	34 (14.47)	93 (39.44)
	Cefotaxime	CTX	67 (28.39)	90 (38.14)	79 (33.47)
	Ceftazidime	CAZ	97 (41.10)	52 (22.04)	87 (36.86)
	Cefoxitin	FOX	52 (22.03)	45 (19.07)	139 (58.90)
Sulfamido	Smz-tmp		135 (57.20)	22 (9.33)	79 (33.47)
Aminoglycosides	Gentamicin	GM	41 (17.37)	19 (8.05)	176 (74.58)
	Kanamycin	K	41 (17.37)	30 (12.71)	165 (69.92)
Quinolones	Norfloxacin	NOR	52 (22.05)	30 (12.70)	154 (65.25)
	Ciprofloxacin	CIP	48 (20.34)	52 (22.03)	136 (57.63)
	Ofloxacin	OFL	45 (19.06)	34 (14.41)	157 (66.53)
Nitro furfuran	Furaxone	FR	138 (58.47)	26 (11.02)	72 (30.51)
Macrolides	Torlamycin	E	108 (45.76)	56 (23.73)	72 (30.51)
	Roxithromycin	ROX	139 (58.90)	41 (17.37)	56 (23.73)
Lincosamide	Clindamycin	CM	112 (47.45)	34 (14.41)	90 (38.14)
Glycopeptides	Vancomycin	VA	127 (53.81)		109 (46.19)

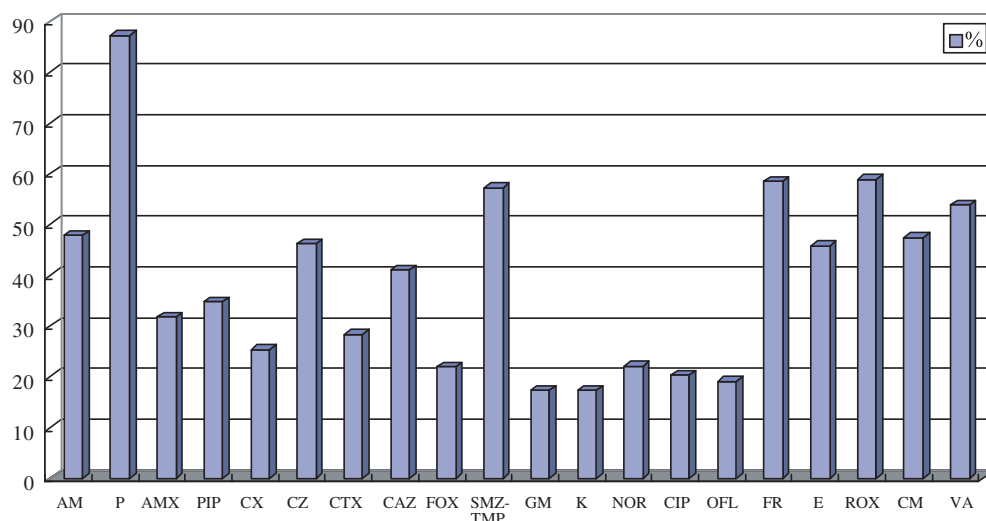


Fig. 1. Antibiotic resistance of *S. aureus* isolated from bovine mastitis.

(87.30%) to penicillin G, of the total province surveyed, Inner Mongolia (91.1%), Hebei (85.4%) and Heilongjiang (81.8%). Only four of the isolated strains were susceptible to all antibiotics tested, while the remaining 232 isolates were resistant at least to one of the antibiotics. Antibiotic resistance determination revealed that 135 isolates (57.2%) were resistant to seven or more antibiotics. Also, resistance to SMZ-TMP (57.20%), furaxone (58.47%), roxithromycin (58.90%), vancomycin (53.81%) and ampicillin (47.88%) was observed among *S. aureus* strains. In conclusion, the resistance demonstrated by the isolates tested was generally high for all antimicrobial agents tested (Fig. 1).

## Discussion

*S. aureus* has been the main subject of studies on antibiotic resistance because of its importance for mastitis in dairy cows. The occurrence of bovine mastitis has serious consequences for animal and public health. Antimicrobial susceptibility of *S. aureus* isolated from bovine mastitis varies widely by region (Makovec and Ruegg, 2003; Gill et al., 2006). In this study, of the 236 isolates evaluated for antimicrobial resistance, isolates resistant to multiple antibiotics were most common. The widespread of resistance to penicillin G could be a consequence of the frequently used in intramammary infections in China. In cases of mastitis choosing the wrong antibiotic or applying an incomplete treatment of animals also contributes significantly to the development of bacterial resistance. Countries with a policy of prudent use of antibiotics in veterinary practices have shown lower levels of resistance compared with other countries (Rabello et al., 2005; Aarestrup et al., 1998; Grave et al., 1999; De Oliveira et al., 2000).

A large number of the isolates were found to be resistant to long established antibiotics (penicillin G, ampicillin, roxithromycin etc.). Among the 20 antibiotics tested against the isolated *S. aureus* strains, penicillin G was the least effective. This result is similar to or higher than the results reported from other countries' studies (Gentilini et al., 2000; Malinowski et al., 2002).

Resistance to more than one kind of antibiotics was observed in approximately 98% of the *S. aureus* isolates (232 out of 236) in this study. The high multiple resistance levels must be of concern because antibiotic resistance is carried on plasmids that can pass from one staphylococcal species to another (Werckenthin et al., 2001).

Overall, the antimicrobial susceptibility results of the 236 strains of *S. aureus* tested in our study demonstrated high resistance patterns. This might be attributed to misuse of antibacterial agents in China, where these are often dispensed without a prescription. A high rate of resistance to these antibiotics was perhaps inevitable. To date, *S. aureus* mastitis has been particularly difficult to treat, and antibiotic-resistant strains have become common. Growing concerns surrounding antibiotic resistance have spurred studies on treatment methods. Although resistance level may provide important information towards the development of effective prevention and treatment strategies for this disease, eradication of *S. aureus* mastitis has not been possible. A vaccine to protect against this infection would be beneficial in the future.

## Conclusion

In this article, the result observed in antibiotic susceptibility testing shows the high levels of resistance of *S. aureus*

isolated from cases of bovine mastitis in China. In the treatment of the infected bovines, it is important to avoid the selection of ineffective antibiotic. This study will be helpful to the correlative science research.

### Acknowledgements

The authors gratefully acknowledge the participation of the owners and managers of the herds with which this research was conducted; without their participation this study would not have been possible. This study was supported by National Natural Science Foundation of China (30160183).

### References

- Aarestrup, F. M., F. Bager, N. E. Jensen, M. Madsen, A. Meyling, and H. C. Wegener, 1998: Resistance to antimicrobial agents used for animal therapy in pathogenic, zoonotic and indicator bacteria isolated from different food animals in Denmark: a baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). *APMIS* 106, 745–770.
- De Oliveira, A. P., J. L. Watts, S. A. Salmon, and F. M. Aarestrup, 2000: Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and United States. *J. Dairy Sci.* 83, 855–862.
- Gentilini, E., G. Denamiel, P. Llorente, S. Godaly, M. Rebuelto, and O. de Gregorio, 2000: Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Argentina. *J. Dairy Sci.* 83, 1224–1227.
- Gill, J. J., J. C. Pacan, M. E. Carson, K. E. Leslie, M. W. Griffiths, and P. M. Sabour, 2006: Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical *Staphylococcus aureus* mastitis in lactating dairy cattle. *Antimicrob. Agents Chemother.* 50, 2912–2918.
- Grave, K., C. Greko, L. Nilsson, K. Odensvik, T. Mork, and M. Ronning, 1999: The usage of veterinary antibacterial drugs for mastitis in cattle in Norway and Sweden during 1990–1997. *Prev. Vet. Med.* 42, 25–55.
- Lange, C., M. Cardoso, D. Senczek, and S. Schwarz, 1999: Molecular subtyping of *Staphylococcus aureus* isolates from cases of bovine mastitis in Brazil. *Vet. Microbiol.* 67, 127–141.
- Makovec, J. A., and P. L. Ruegg, 2003: Antimicrobial resistance of bacteria isolated from dairy cow milk samples submitted for bacterial culture: 8,905 samples (1994–2001). *J. Am. Vet. Med. Assoc.* 222, 1582–1589.
- Malinowski, E., A. Klossowska, M. Kaczmarowski, H. Lassa, and K. Kuzma, 2002: Antimicrobial susceptibility of staphylococci isolated from affected with mastitis cows. *Bull. Vet. Inst. Pulawy.* 46, 289–294.
- Miles, H., W. Lesser, and P. Sears, 1992: The economic implications of bioengineered mastitis control. *J. Dairy Sci.* 75, 596–605.
- Owens, W. E., C. H. Ray, J. L. Watts, and R. J. Yancey, 1997: Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. *J. Dairy Sci.* 80, 313–317.
- Rabello, R. F., C. R. V. M. Souza, R. S. Duarte, R. M. M. Lopes, L. M. Teixeira, and A. C. D. Castro, 2005: Characterization of *Staphylococcus aureus* Isolates recovered from Bovine Mastitis in Rio de Janeiro, Brazil. *J. Dairy Sci.* 88, 3211–3219.
- Werckenthin, C., M. Cardoso, J. L. Martel, and S. Schwarz, 2001: Antimicrobial resistance in staphylococci from animals with particular reference to bovine *Staphylococcus aureus*, porcine *Staphylococcus hyicus*, and canine *Staphylococcus intermedius*. *Vet. Res.* 32, 341–362.