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Short communication: Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from bovine clinical mastitis

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ABSTRACT

The aims of this study were to determine the prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from bovine clinical mastitis in Varamin, Tehran Province, Iran. All of the isolated *Staph. aureus* were identified by morphology and culture and confirmed using the API Staph identification system (bioMérieux, Marcy-l'Étoile, France). Antibiotic resistance genes were detected by PCR with oligonucleotide primers specific for each gene. *Staphylococcus aureus* was recovered from 43 of 207 (20.1%) bovine clinical milk samples. Using disk diffusion, methicillin-resistant *Staph. aureus* was detected in 5 of 43 (11.6%) samples. The pathogen showed high resistance against penicillin G (86%) and tetracycline (76.7%). The *blaZ* (penicillin) (86%), *tetM* (tetracycline), and *ermC* (erythromycin) genes (39.5% each) were the most prevalent antibiotic resistance genes. The findings of this study are useful for designing specific control programs for bovine clinical mastitis caused by *Staph. aureus* in this region of Iran.

Key words: *Staphylococcus aureus*, clinical mastitis, antibiotic susceptibility, resistance gene

Short Communication

Staphylococcus aureus is a major cause of clinical and subclinical mastitis in dairy herds. The pathogen can be transmitted from cows, the environment, and equipment into the udder via the milking machine and other fomites (e.g., milkers' hands). In Iran, bovine mastitis is often treated with tetracycline, penicillin, clindamycin, erythromycin, oxacillin, or ciprofloxacin. However, the practice of incorporating antibiotics into feeds to control and treat diseases in cows on dairy farms has increased, and antibiotic administration in animals could be one of the main causes of antibiotic resistance in pathogens (Schwartz et al., 2003; Jamali et al., 2013).

The antibiotic resistance genes *mecA* (oxacillin); *tetM, tetK, tetL* (tetracycline); *ermA*, *ermB*, *ermC*, *ermT*, *msrA*, *msrB*, *mphC* (erythromycin); *blaZ* (penicillin); *aacA-aphD* (gentamicin); *ant(4')-Ia* (kanamycin and tobramycin); *qnrA* (fluoroquinolone); *lnuA* (lincomycin); *fexA* (chloramphenicol); *cfr* (multidrug resistance; phenicols-lincosamides-oxazolidinones-pleuromutilin-streptogramins A); and *dfrG*, *dfrK*, and *dfrS1* (trimethoprim) have been reported among *Staph. aureus* isolates in previous studies (Lina et al., 1999; Martineau et al., 2000a,b; Mammeri et al., 2005; Kehrenberg and Schwarz, 2006; Lüthje and Schwarz, 2006; Fessler et al., 2010; Argudín et al., 2011; Gao et al., 2012). Methicillin-resistant *Staph. aureus* (**MRSA**) is a significant cause of human nosocomial infections in many parts of the world. The aim of this study was to investigate the prevalence, antibiotic resistance profiles, and related resistance genes among these isolates of *Staph. aureus* isolated from bovine clinical mastitis cases in Varamin, Tehran Province, Iran.

Two hundred and seven mastitic milk samples were collected from one infected teat of cows suffering from clinical mastitis by field veterinarians distributed throughout Varamin, Iran, from November 2008 to July 2010. Samples were transported on ice to the laboratory within 3 h of sampling.

Isolation of *Staph. aureus* in this study was carried out according to National Mastitis Council (1990) methods. Briefly, 15 μL of each mastitic milk sample was plated on blood agar base (Oxoid, Basingstoke, UK) with 5% sheep blood and was incubated for 24 h at 37°C under anaerobic condition. Presumptive colonies of *Staph. aureus* (based on colony morphology) were confirmed using the API Staph identification system (bioMérieux, Marcy-l'Étoile, France).

Antibiotic susceptibility was determined by the disk diffusion method after culture on Mueller Hinton agar (Oxoid; CLSI, 2006). The following panel of antibiotic agents (Oxoid) was applied: tetracycline, penicillin, clindamycin, erythromycin, oxacillin, ciprofloxacin, chloramphenicol, gentamicin, cefoxitin, trimethoprim-

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sulphametoxazol, kanamycin, lincomycin, tobramycin, streptomycin, and quinupristin-dalfopristin. Clindamycin-susceptible, erythromycin-resistant isolates were tested for inducible clindamycin resistance by disk diffusion induction test (**D-test**), and isolates positive for the D-test were considered resistant to clindamycin (Jorgensen et al., 2004).

The PCR assays were performed as described previously for the antibiotic resistance genes *mecA* (oxacillin resistance); *tetM*, *tetK*, *tetL* (tetracycline resistance); *ermA*, *ermB*, *ermC*, *ermT*, *msrA*, *msrB*, *mphC* (erythromycin resistance); *blaZ* (penicillin resistance); *aacA-aphD* (gentamicin resistance); *qnrA* (fluoroquinolone resistance); *lnuA*, *lnuB* (lincomycin resistance); *vgaA*, *vgaB*, *vgaC* (quinupristin-dalfopristin resistance); *cat*::pC221, *cat*::pC194, *cat*::pC223; *fexA* (chloramphenicol resistance); *cfr* (multidrug resistance); *dfrG*, *dfrK*, *dfrS1* (trimethoprim resistance); *cfxA* (cefoxitin resistance); *ant(4')-Ia* (kanamycin and tobramycin resistance); and *ant(6)-Ia* (streptomycin resistance) (Ounissi and Courvalin, 1987; Sutcliffe et al., 1996; Bozdogan et al., 1999; Lina et al., 1999; Schmitz et al., 1999; Martineau et al., 2000a,b; Trzcinski et al., 2000; Werner et al., 2001; Avelar et al., 2003; Kehrenberg and Schwarz, 2005; Mammeri et al., 2005; Kehrenberg and Schwarz, 2006; Lüthje and Schwarz, 2006; Fessler et al., 2010; Argudín et al., 2011; Gao et al., 2012).

Staphylococcus aureus isolates were detected in 43 of 207 (20.1%) bovine clinical mastitis samples. Five (11.6%) isolates were identified as MRSA by antibiotic susceptibility (resistant to oxacillin) and confirmed by *mecA* gene detection (Table 1). The *Staph. aureus* isolates showed resistance to penicillin G (86%), tetracycline (76.7%), erythromycin (39.5%), clindamycin (34.9%), cefoxitin (16.3%), oxacillin, chloramphenicol, trimethoprim-sulfamethoxazole (11.6% each), lincomycin (9.3% each), gentamicin (7%), quinupristin-dalfopristin and streptomycin (2.3% each). Fifteen clindamycin-susceptible, erythromycin-resistant isolates were tested by D-test. Inducible clindamycin resistance was observed in 9 of 15 (60%) of these isolates and were reported as clindamycin resistant (Table 1). All *Staph. aureus* isolates were susceptible to ciprofloxacin, kanamycin, and tobramycin.

The correlation between phenotypic antibiotic resistance and PCR results is shown in Table 1. All *Staph. aureus* isolates resistant to penicillin G, gentamicin, and oxacillin contained the *blaZ*, *aacA-aphD*, and *mecA* genes, respectively. For cefoxitin resistance, the *cfxA* gene was present in 6 of 7 (85.7%) of the cefoxitin-resistant isolates. Out of 33 tetracycline resistant isolates, 81.8, 51.5, and 12.1% isolates contained the *tetM*, *tetK*, and *tetL* genes, respectively, with each gene found alone or in combination in the following isolate percentages: *tetK* (15.2%), *tetM* (45.5%), *tetK tetL* (3%), *tetL tetM* (3%), *tetK tetM* (27.3%), and *tetK tetL tetM* (6.1%). For erythromycin resistance, the *ermC* gene was present in all erythromycin-resistant isolates, alone (41.2%) or together with *ermB* (23.5%), *ermA* (5.9%), both *ermB* and *ermA* genes (17.6%), or *ermT* (11.8%). The *msrA* and *msrB* genes were detected in 29.4 and 52.9% of erythromycin-resistant isolates, respectively. Furthermore, the *mphC* gene was found in 10 of 17 (58.8%) of the erythromycin-resistant isolates. Four and 1 of the trimethoprim-sulphametoxazol-resistant isolates harbored the *dfrG* and *dfrK* genes, respectively. No isolate was positive for the trimethoprim-resistance gene *dfrS1.* Moreover, no isolate was positive for the chloramphenicol-resistance *cat*::pC221, *cat*::pC194, *cat*::pC223 genes, whereas the *fexA* gene was detected in 1 isolate. This isolate also carried lincomycin resistance gene *lnuA* and the multidrug resistance gene, *cfr.* The quinupristin-dalfopristin resistant isolate was negative for the *vgaA*, *vgaB*, and *vgaC* genes. No *qnrA* (fluoroquinolone resistance gene), *ant(4')-Ia* (kanamycin and tobramycin resistance gene), or *ant(6)-Ia* (streptomycin resistance gene) were detected in the tested isolates.

Although the prevalence of *Staph. aureus* from bovine mastitis has frequently been reported in other countries, few published studies have addressed the problem in Iran. In the current study, *Staph. aureus* was detected in 20.1% of the 207 mastitic milk samples. However, in prior studies, Dastmalchi Saei et al. (2009) and Momtaz et al. (2011) detected *Staph. aureus* in 15.7 and 23.9% of combined clinical and subclinical mastitis, respectively, in 2 different provinces of Iran. Moreover, Boynukara et al. (2008) and Oliveira et al. (2012) reported that 22.1 and 15.2% of bovine mastitic milk samples in Turkey and Brazil were contaminated with *Staph. aureus,* respectively. Methicillin-resistant *Staph. aureus* is an important bacterial pathogen in humans and animals worldwide, but to the best of our knowledge, there is no published information regarding MRSA from bovine clinical mastitis in Iran. Although the prevalence of MRSA in bovine clinical samples was 11.6% (5/43) in this study, Vanderhaeghen et al. (2010) detected MRSA in 9.3% of clinical and subclinical mastitis cases in Belgian cows. Variations in findings may be attributable to different sample sizes and study locations.

Over the last decade, penicillin and tetracycline have been administered to animals through food to treat infectious diseases, particularly on dairy farms in Iran (Jamali et al., 2013). The high resistance rate of *Staph. aureus* isolated from bovine clinical mastitis to penicillin G and tetracycline could be due to widespread use of these antibiotics on dairy farms. Our findings are in agreement with earlier results: a high resistance