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Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products

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ABSTRACT

The objectives of this study were to determine the prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk (cow and sheep) and dairy products (traditional cheese and kashk) in Mazandaran Province, Iran. A total of 2650 samples, including 1930 raw milk and 720 dairy products were purchased from retail stores. Out of 2650 samples, *S. aureus* was detected in 328 samples (12.4%) in which 53 (16.2%) were positive for methicillin-resistant *S. aureus*. The *S. aureus* isolates showed resistance to tetracycline (56.1%), followed by penicillin G (47.3%), oxacillin (16.2%), lincomycin (11.9%), clindamycin (11.3%), erythromycin (7.9%), streptomycin (5.8%), cefoxitin (5.5%), kanamycin (4%), chloramphenicol (3.7%), and gentamicin (2.1%). A high frequency of *blaZ* (46%) and *tetM* (34.8%) resistance genes was found in *S. aureus* isolates. The findings of this study revealed consumption of raw milk and dairy products as a potential risk of foodborne infection in this region.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive coccal bacterium belonging to the Staphylococcaceae family. This foodborne pathogen is considered as one of the world's leading causes of disease outbreaks related to food consumption, being responsible for a variety of manifestations and diseases. Several foodborne outbreaks of *S. aureus* intoxications have been documented to be associated with consumption of contaminated different types of food (Hyeon, Bing, Lee, Jeon, & Kim, 2013; Miwa, Kawamura, Masuda, & Akiyama, 2001; Nema, Agrawal, Kamboj, Goel, & Singh, 2007).

Farm fresh dairy products have maintained their popularity in many parts of the world and the small-scale producers of farm-dairies receive supports from the authorities involved in agricultural politics (Akineden, Hassan, Schneider, & Usleber, 2008; Jørgensen, Mørk, & Rørvik, 2005). Cheese production is normally processed using raw milk which drastically increases the contamination risks of dairy products with pathogenic bacteria. In addition, keeping the dairy animals near the dairy facilities might serve as another important factor to induce contamination problems (De Buyser, Dufour, Maire, & Lafarge, 2001; Oliver, Jayarao, & Almeida, 2005; Pak, Spahr, Jemmi, & Salman, 2002).

Staphylococcal enterotoxin outbreak has always been an indispensable threat to farm dairies (De Buyser, Janin, & Dilasser, 1985; Schønberg & Wåltorp, 2001) and the frequent reports of raw milk products contamination with different types of *S. aureus* clearly demonstrates the significance of this pathogen. In an investigation by Jørgensen, Mørk, Høgåsen, and Rørvik (2005), *S. aureus* was detected in 75% of bovine and 96% of caprine bulk milk samples collected from Norwegian farms. Detection of *S. aureus* in 38% of the cheeses made from raw milk goats' on-farm in another study in Sweden (Thaml, Hajdu, & Danielsson–Thaml, 1990) and in 61% (bovine) and 100% (caprine) of cheeses produced from raw milk in Italy (Cremonesi et al., 2007) reaffirms the fact that this pathogen have to be taken more into consideration. Although milk producing animals are the most important sources of *S. aureus* contamination, there are other factors that may affect the risks of contamination such as human handling, water, milking equipment and also the environment (Bergonier, De Crémoux, Rupp, Lagriffoul, & Berthelot, 2003; Jørgensen, Mørk, & Rørvik, 2005).

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Antimicrobial agents are administered to animals either to inhibit bacterial infections or to speed up their growth. Excessive application of these agents has led to the emergence of resistant strains as a growing problem in developed countries. *Penicillin has remained* the usual treatment for *S. aureus* infections in many countries. However, many strains of *S. aureus* are now resistant to penicillin, due to production of an enzyme called beta-lactamase or penicillinase. The treatment duration might vary based on severity or the area of infection (Chambers, 2001). High resistance level of *S. aureus* has also been reported against tetracycline, methicillin, kanamycin, gentamicin, streptomycin, *etc.* indicating the high potential of *S. aureus* for developing resistance against antimicrobial agents.

There are few studies on prevalence and antimicrobial susceptibility of *S. aureus* in raw milk and dairy products in Iran and further detailed investigations might provide more insights. Continuous monitoring of prevalence and profile of antimicrobial susceptibility could be helpful to treat infections more efficiently and also to reduce the development of antimicrobial resistant microorganisms. In the present study, we aim to investigate the prevalence and antimicrobial resistance level of *S. aureus* in unpasteurized milk and dairy products collected in Mazandaran Province, Iran.

2. Materials and method

2.1. Bacterial strains

A total of 2650 of raw cow milk ($n = 1035$), raw sheep milk ($n = 895$), traditional cheese ($n = 450$), and kashk (prepared by prolonged boiling yogurt) ($n = 270$) were purchased from randomly selected retail stores located in four cities of Mazandaran Province, North of Iran, since January 2006 to December 2013. Kashk and cheese may be produced from unpasteurized milk. Traditional dairy products in Iran are produced in small productive centers mostly located in urban areas and distributed unpacked. The samples were transferred into sterile plastic bags and transported immediately to the laboratory.

2.2. Isolation and detection

For isolation and detection of *S. aureus,* 10 ml (raw milk) or 10 g (kashk or cheese) of each sample was added to 90 ml sterile peptone water and homogenized. The samples were streaked onto Baird–Parker Agar (Oxoid, Basingstoke, UK) supplemented with 5% egg yolk and tellurite (Merck, Darmstadt, Germany). The plates were then incubated for 24–48 h at 37 °C. Colonies with typical black appearance and surrounded by clear zone were enumerated as *S. aureus* (Loncarevic et al., 2005). The presumptive colonies were confirmed using the API Staph identification system (bioMérieux, Marcy-l'Étoile, France).

2.3. Phenotypic detection of antimicrobial resistance

2.3.1. Antimicrobial susceptibility test

The isolates of *S. aureus* were subjected to antimicrobial susceptibility tests by the Kirby–Bauer disc diffusion method on Muller Hinton agar (Oxoid, Basingstoke, UK) (CLSI, 2006). Cefoxitin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (15 μg), oxacillin (1 μg), penicillin G (10 U), quinupristin-dalfopristin (15 μg), streptomycin (10 μg), tetracycline (30 μg), tobramycin (10 μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg) were used as antimicrobial agents (Oxoid, Basingstoke, UK).

2.3.2. D-test

Clindamycin-susceptible, erythromycin-resistant isolates were tested for inducible clindamycin resistance by disc diffusion test (D-test), and the positive isolates for the D-test, were considered resistant to clindamycin.

2.4. Antimicrobial resistance genes profiling

The PCR assays were performed as previously described for the cefoxitin (*cfxA*), chloramphenicol (*cat*::pC221, *cat*::pC194, *cat*::pC223, and *fexA*), ciprofloxacin (*qnrA*), erythromycin (*ermA, ermB, ermC, ermT, msrA, msrB*, and *mphC*), gentamicin (*aacA-aphD*), lincosamide (*lnuA,* and *lnuB*), oxacillin (*mecA)*, penicillin (*blaZ*), quinupristin-dalfopristin (*vgaA, vgaB,* and *vgaC*), streptomycin (*ant(6)-Ia*), tetracycline (*tetK, tetL,* and *tetM*), trimethoprim (*dfrG, dfrK,* and *dfrS1*), tobramycin and kanamycin (*ant(4′)-Ia*) resistance genes as well as the multidrug-resistance (*cfr*) (Argudin et al., 2011; Avelar et al., 2003; Bozdogan et al., 1999; Feβler et al., 2010; Gao et al., 2012; Kehrenberg & Schwarz, 2005, 2006; Lina et al., 1999; Mammeri, Van De Loo, Poirel, Martinez–Martinez, & Nordmann, 2005; Martineau, Picard, Grenier, et al., 2000; Martineau, Picard, Lansac, et al., 2000; Ounissi & Courvalin, 1987; Schmitz et al., 1999; Sutcliffe, Grebe, Tait-Kamradt, & Wondrack, 1996; Trzcinski, Cooper, Hryniewicz, & Dowson, 2000; Werner, Cuny, Schmitz, & Witte, 2001).

2.5. Statistical analysis

The relationship between contaminated samples and the different types of samples was analyzed using Chi-square analysis. All statistical analysis were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Out of 2650 raw milk and traditional dairy product samples, 328 (12.4%) samples including 162 (15.7%) raw cow milk, 86 (9.6%) raw sheep milk, 49 (10.9%) traditional cheese, and 31 (11.5%) kashk indicated *S. aureus* contamination. There is no significant difference between contaminated samples and different kinds of samples (raw milk and dairy products) ($P < 0.05$).

In this study, 119 (36.3%), 153 (46.6%), and 42 (12.8%) samples indicated resistance to one, two and more than two antimicrobial agents, respectively. Fifty three (16.2%) of the *S. aureus* isolates were identified as Methicillin-resistant *S. aureus* (MRSA) by antimicrobial susceptibility (oxacillin resistance). The resistance patterns of *S. aureus* to the tested antimicrobial agents are presented in Tables 1 and 2. Besides, 4.3% of the isolates were susceptible to all of the tested antimicrobial agents. The phenotypic resistance profiles of the *S. aureus* isolates are as follows: tetracycline, 56.1%; penicillin, 47.3%; oxacillin, 16.2%, lincomycin, 11.9%; clindamycin, 11.3%; erythromycin, 7.9%; streptomycin, 5.8%; cefoxitin, 5.5%; kanamycin, 4%; chloramphenicol, 3.7%; and gentamicin, 2.1%. Twenty five clindamycin-susceptible, erythromycin-resistant isolates were examined by D-test, where inducible clindamycin resistance was observed in 11 of 25 (44%) of these isolates and were reported as clindamycin resistant. Meanwhile, the tests demonstrated the susceptibility of all *S. aureus* isolates to ciprofloxacin, quinupristin-dalfopristin, tobramycin, and trimethoprim-sulfamethoxazole. In addition, no significant difference was noticed between resistant profiles and source of isolates ($P < 0.05$).

All of the resistant isolates of *S. aureus* to oxacillin and gentamicin carried *mecA* and *aacA-aphD* genes, respectively. All of the 53 MRSA isolates were confirmed by detection of *mecA* gene. The *blaZ, ant(6)-Ia, cfxA,* and *ant(4′)-Ia* genes were detected in 97.4%, 73.7%,