

Antimicrobial Resistance of *Staphylococcus aureus* Isolated from Bovine, Sheep and Goat Raw Milk

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Abstract: Objective of this study was to determine the prevalence rate of antimicrobial resistance of *S. aureus* isolated from dairy cow, sheep and goat milk in Iran. From September 2010 to September 2011, a total of 348 raw milk samples from cow, sheep and goat were collected from randomly selected herds in Fars, Chahar Mahal va Bakhtiari and Ghom, provinces, Iran. Overall, 46 raw milk samples (13.2%) were found to be contaminated with *S. aureus*. Susceptibilities of the isolates were determined for 11 antimicrobial drugs using the disk diffusion assay. Most of the isolates (82.6%) were resistant to one or more antimicrobial agent. Six isolates (13.0%) were resistant to single antibiotic and 16 isolates (34.8%) showed resistance to 2 antimicrobial agents. Multiresistance was found in 34.8% of *S. aureus* isolates. Resistance (resistance and intermediate resistance) to ampicillin was the most common finding (54.3%), followed by resistance to oxacillin (28.3%), tetracycline (26.1%), penicillin G (23.9%), erythromycin (23.9%), trimethoprim-sulfamethoxazole (17.4%) and cephalotin (2.2%). All isolates tested for antibiotic sensitivity were susceptible to methicillin, vancomycin, chloramphenicol and ciprofloxacin. Furthermore, impacts and dynamics of genetic antibiotic determinants should also be investigated using molecular methods.

Key words: Antimicrobial Resistance • Cow • Sheep • Goat • Milk • *Staphylococcus aureus*

INTRODUCTION

Milk is an excellent bacteriological medium for a large number of microorganisms. When the milk is drawn from the udder of a healthy animal, it contains organisms that have entered the teat canal through its opening. They are mechanically flushed out during milking. The number is ranged during milking between several hundreds to several thousand per milliliter [1]. *Staphylococcus aureus* isolates are normal inhabitants of skin and mucus membranes. The coagulase-positive staphylococci constitute the well known pathogenic species *S. aureus*. *S. aureus* mastitis is a serious problem in dairy production and infected animals may contaminate bulk milk. Additionally, human handlers, milking equipment, the environment and udder and teat skin of dairy animals may

be other possible sources of bulk milk contamination. *S. aureus* is still an important cause of foodborne intoxications worldwide [2-4]. The ability of *S. aureus* to grow and produce staphylococcal enterotoxins (SEs) under a wide range of conditions is evident from the variety of foods implicated in staphylococcal food poisoning (SFP) [2]. SFP is suspected when the symptoms including nausea, violent vomiting, abdominal cramps and diarrhea affect the patients between 1 and 8 h after food consumption [5].

Antibiotics are used to treat diseases of cattle, sheep, goat, water buffalo and other animals and as well as used as preservatives for milk [6]. The indiscriminate use of antibiotics has led to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective. Resistant bacteria occur in soil,

water, plants and animals. The resistant bacteria present in environments are in contact with human beings and animals. It has been estimated that nearly equal tonnage of antimicrobial agents are used in man and in agriculture worldwide [1]. Antimicrobial resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food [7]. *S. aureus* has been reported to frequently show multiple antimicrobial resistance patterns [8].

Determination of levels of *S. aureus* and an evaluation of the antibiotic-resistant phenotypes of the isolates could serve as a tool for determining the hygiene standards implemented during milking. Data on antibiotic resistance could also be used to characterize these opportunistic pathogens, which may further limit the risks associated with the consumption of contaminated milk and its products. The aims of this study were to determine the prevalence rate and antimicrobial resistance of *S. aureus* in cow, sheep and goat, raw milk in Fars, Chahar Mahal va Bakhtyari and Ghom, Iran.

MATERIALS AND METHODS

Sample Collection: From September 2010 to September 2011 a total of 348 cow (190), sheep (78) and goat (80), raw milk samples was collected from Fars, Chahar Mahal va Bakhtyari and Ghom provinces, Iran. The samples were immediately transported to the laboratory in a cooler with ice packs and were processed within an hour of collection.

Detection of *S. Aureus*: The samples were processed immediately upon arrival using aseptic techniques. To detect *S. aureus*, 1mL of each milk sample was inoculated on Baird - Parker agar (Difco, Detroit, Michigan, USA). After 24 - 48 h of incubation at 37°C, suspected colonies were sub-cultured on blood agar plate (Difco, Detroit, Michigan, USA) and incubated for 24 h at 37°C. To identify *S. aureus*, Gram stain, catalase, coagulase and Voges-Proskaver (VP) tests were conducted on suspected colonies [4, 9].

Antimicrobial Susceptibility Testing: One strain from each *S. aureus*-positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood, according to the Clinical Laboratory Standards Institute [10]. The following antimicrobial impregnated

disks (HiMedia Laboratories, Mumbai, India) were used: penicillin G (10 IU), cephalotin (30 µg), chloramphenicol (30 µg), ciprofloxacin (30 µg), erythromycin (15 µg), tetracycline (30 µg), oxacillin (15 µg), gentamycin (10 µg), trimethoprim-sulfamethoxazole (25 µg), methicillin (5µg) and vancomycin (30 µg). After incubation at 37°C for 48 h, the susceptibility of the *S. aureus* isolates to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI [10].

RESULTS AND DISCUSSION

In this study we described the isolation and antibiotic susceptibility characterization of *S. aureus* from cow, sheep and goat milk obtained from two provinces of Iran. Forty six of 348 samples (13.2%) were positive for *S. aureus*. Thirty four raw cow milk (17.9%), 7 raw sheep milk (9.0%) and 5 raw goat milk (6.3%), samples were contaminated with *S. aureus*. This contamination rate is similar that observed in the surveys previously conducted in other countries on several kinds of raw milk [3, 7, 11-13]. The resistance pattern of *S. aureus* isolates to 11 antimicrobial agents tested in this study is shown in Table 1. Most of the isolates (82.6%; n=38) were resistant to one or more antimicrobial agent. Six isolates (13.0%) were resistant/ intermediate resistance to single antibiotic and 16 isolates (34.8%) showed resistance/ intermediate resistance to 2 antimicrobial agents. Multiresistance which is defined as resistance to 3 or more antimicrobial agents was found in 34.8% of *S. aureus* isolates. Resistance (resistance and intermediate resistance) to ampicillin was the most common finding (54.3%), followed by oxacillin (28.3%), tetracycline (26.1%), penicillin G (23.9%), erythromycin (23.9%), trimethoprim-sulfamethoxazole (17.4%) and cephalotin (2.2%). All isolates tested for antibiotic sensitivity were susceptible to methicillin, vancomycin, chloramphenicol and ciprofloxacin.

The antimicrobial resistance profile of the tested *S. aureus* strains to different antibacterial agents revealed that 82.6% (n=38) of the strains were resistant to at least one antibiotic. As reported by other investigators, the resistance of *S. aureus* isolates to β -lactams such as ampicillin, penicillin, tetracycline and oxacillin was evident [14-17]. The finding that a large number of *S. aureus* were resistant to ampicillin, penicillin, tetracycline and oxacillin are, however, a cause for concern and should be further investigated. These drugs are used in veterinary medicine in Iran.

Table 1: Antimicrobial resistance profiles of *Staphylococcus aureus* isolated from dairy cow, sheep and goat raw milk samples in Iran

Antimicrobial agent	<i>S. aureus</i> (N = 46)		
	Resistance	Intermediate resistance	Susceptible
Ampicillin	18 (39.1%)	7 (15.2%)	21 (45.6%)
Cephalotin	1 (2.2%)	0 (0.0%)	45 (97.8%)
Chloramphenicol	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ciprofloxacin	0 (0.0%)	0 (0.0%)	0 (0.0%)
Erythromycin	5 (10.9%)	6 (13.0%)	35 (76.1%)
Methicillin	0 (0.0%)	0 (0.0%)	0 (0.0%)
Oxacillin	7 (15.2%)	6 (13.0%)	33 (71.7%)
Penicillin G	8 (17.4%)	3 (6.5%)	35 (76.1%)
Tetracycline	7 (15.2%)	5 (10.9%)	34 (73.9%)
Trimethoprim-sulfamethoxazole	4 (8.7%)	4 (8.7%)	38 (82.6%)
Vancomycin	0 (0.0%)	0 (0.0%)	0 (0.0%)

Erythromycin resistance was almost non-existent in the cow milk samples. Despite the fact that a large number of *S. aureus* isolated from both sheep and goat milk samples was resistant to erythromycin, it was evident from our results that this antibiotic was not frequently used in animals by large scale farmers. About 14% of the isolates from cow milk samples were resistant to erythromycin.

Trimethoprim-sulfamethoxazole was the drug to which almost small proportion of the isolates were resistant (17.4%). Similarly, a previous study reported that only a small percentage (15.7% to 23.8%) of *S. aureus* isolated from meat and dairy products was resistant to sulfamethoxazole [7, 18]. This drug is not used in dairy cattle farms in the sampled area of Iran.

The results presented herein are similar to previous studies in which Gram-positive bacteria were generally susceptible to vancomycin, methicillin, chloramphenicol, cephalotin and ciprofloxacin [15, 17, 19-21]. These drugs are no longer used in veterinary medicine in many countries [22, 23] including Iran, which may account for the results reported here; and there are only a few reports on MRSA Associated with mastitis [20]. However, High levels of methicillin resistance *S. aureus* (MRSA) have been identified in patients in the United States and some European countries [24]. In these countries, 44.4%, 34.7%, 41.8% and 32.4% of isolates from patients in the United States, France, Italy and Spain, respectively, were resistant to methicillin.

Since antibiotic-resistant isolates might be transmitted to humans by the consumption of food products containing such resistant bacteria, the use of antibiotics as growth promoters in animal husbandry, especially of those commonly used for both human and animal care should be avoided [25]. The present study demonstrated that the resistant strains may have been transferred to cow, sheep and goat then to milk, which can

be the reason of infection in human beings if they take raw milk. These can be treated by improving hygienic conditions and careful handling of cow during milking. Furthermore, impacts and dynamics of genetic antibiotic determinants should also be investigated using molecular methods.

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