



Full Length Article

# Staphylococcal species associated with bovine mastitis in the North West of Iran: Emerging of coagulase-negative staphylococci



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## KEYWORDS

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Staphylococcal species

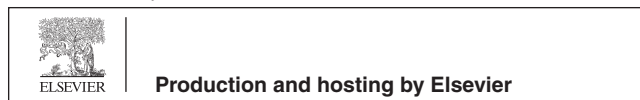
**Abstract** Staphylococcal mastitis is a major and costly problem of dairy cattle all over the world. The objective of this study was to isolate and identify the main staphylococcal species causing bovine mastitis in 7 dairy herds located in 4 different areas of East and West Azerbaijan provinces, Iran. Of the 158 mastitic milk samples collected, 113 staphylococcal isolates were identified (71.5%) on the basis of cultural and biochemical features as well as by genus specific PCR. Then, species level identification of staphylococcal isolates was carried out using restriction fragment length polymorphism (RFLP) analysis of the *gap* gene (933 bp). On the basis of polymerase chain reaction-RFLP, 10 different patterns were identified. Of 113 isolates, 5 (4.4%) were *Staphylococcus aureus* and 108 (95.6%) were coagulase-negative staphylococci (CoNS). Overall, nine different species of CoNS were identified as: 44 *Staphylococcus haemolyticus* (40.7%), 17 *Staphylococcus chromogenes* (15.7%), 11 *Staphylococcus epidermidis*, *Staphylococcus warneri* and *Staphylococcus cohnii* each (10.2%), 6 *Staphylococcus simulans* (5.5%), 4 *Staphylococcus hominis* (3.7%), 3 *Staphylococcus capitis* (2.7%) and 1 *Staphylococcus xylosum* (0.9%). *S. haemolyticus*, *S. chromogenes* and *S. warneri* were the only species identified from clinical mastitis. No significant difference in staphylococcal IMI was found among the studied herds and regions. This study demonstrated that CoNS, especially *S. haemolyticus* and *S. chromogenes*, were predominant and thus be considered as emerging pathogens causing mastitis in the North West of Iran. Our results also revealed that the *gap* PCR-RFLP was useful for identifying staphylococcal isolates derived from bovine mastitis at species level.

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## 1. Introduction

Staphylococci are the main etiological agents of mastitis in dairy cows [1], comprises 45 species and 21 subspecies [2]. Although *Staphylococcus aureus* has been described as one of the most important mastitis pathogens in cattle, coagulase-negative staphylococci (CoNS) are increasingly becoming

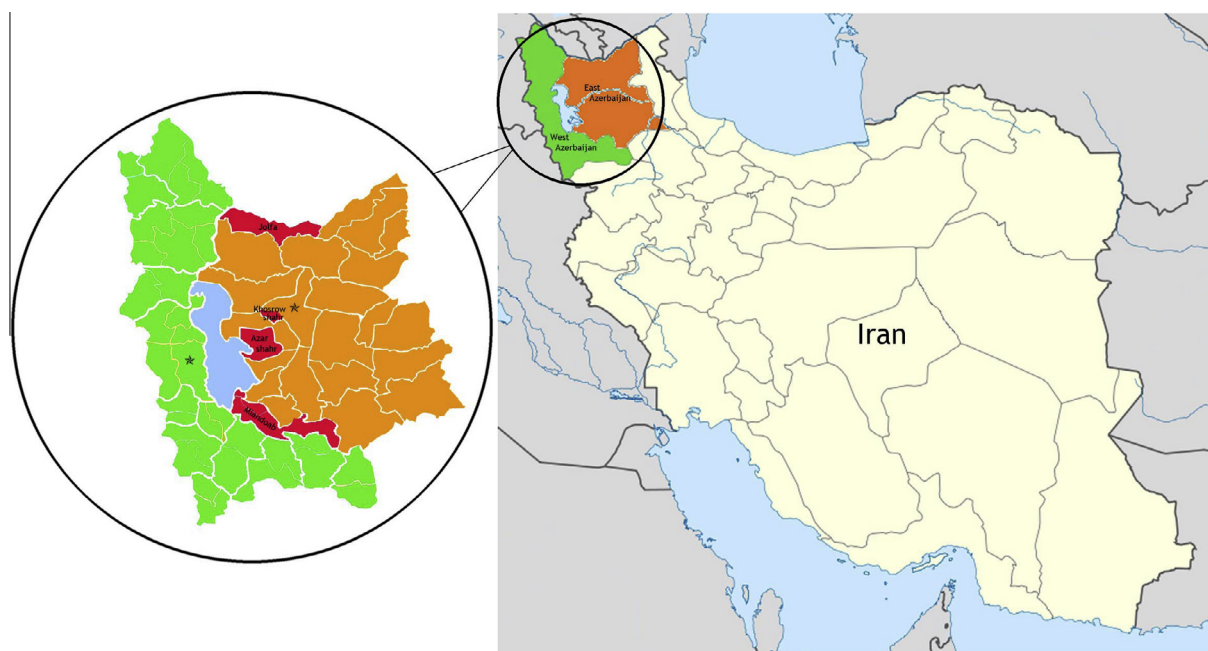
recognized as etiologic agents associated with intramammary infections (IMI) in most countries [3,4]. However, their clinical/pathogenic relevance when cultured from milk remains a point of discussion. Some consider them as true mastitis pathogens with important virulence factors [5], a high level of antimicrobial resistance [6], and the ability to cause chronic infections [7]. Others regard them as minor pathogens in dairy cows [8,9]. Today more than 15 CoNS species have been identified that cause IMI in dairy cows, but *Staphylococcus chromogenes*, *Staphylococcus simulans*, *Staphylococcus xylosum*, *Staphylococcus epidermidis*, *Staphylococcus hyicus*, and *Staphylococcus haemolyticus* are the most commonly isolated CoNS from bovine mastitis [10,11]. A number of other species have also been reported. Because of the increasing significance of CoNS in bovine mastitis, identification of CoNS at the species level is needed to develop effective control strategies for CoNS mastitis [12]. Also, evaluating the epidemiology of individual CoNS species is of great importance to understand their respective significance [13]. Currently the identification methods for staphylococci are under reassessment and molecular based methods are reported to be accurate alternatives and are superior to phenotypic identification of CoNS [14,15]. DNA sequence-base species identification is currently the most accurate method for CoNS identification and is considered as the gold standard [16], but is expensive and time consuming. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay of *gap* gene, which encodes glyceraldehyde-3-phosphate dehydrogenase, has proved to be a simple, more reliable and reproducible method for differentiating staphylococcal species and allow the identification of 28 species within the genus [17,18]. Although the role of *S. aureus* as etiological agents of bovine mastitis has been elucidated previously [19,20], studies on the

relative importance of specific CoNS in bovine mastitis have not been undertaken in Iran. Therefore, the overall aim of the present study was to improve the knowledge on prevalence and significance of different staphylococci species with emphasis on distribution of CoNS species.

## 2. Material and methods

### 2.1. Herds and cows

In total, 158 milk samples were collected from 31 clinical (recognized by physical examination and observable changes in the udder and the presence of abnormalities in the milk) and 127 subclinical (determined by CMT and bacteriological culture) mastitis of dairy cows during 2012. Seven herds (labeled A–G) located in four areas (including Jolfa, Khosrowshahr, Azarshahr, and Miandoab) of East and West Azerbaijan provinces from North West of Iran (Fig. 1) were selected for this study: A (29 cows), B (24 cows), C (26 cows), D (23 cows), E (22 cows), F (12 cows), and G (22 cows). The farms were representative of Iranian dairy herds regarding cow number, milk production, and management routines (housing, feeding, and milking). In the year of the study, cows were in 3–4 parity, the mean herd size was 48 with a range from 25 to 70 Holstein cows, the average milk production was 27.8 kg/cow per day (range 25.2–30.4 kg/d). Cows on each herd were milked twice daily in a milking parlor. On all 7 farms, milkers did not wear gloves during the milking process. Post milking teat disinfection and dry cow therapy were practiced in all herds. Cows were housed in open shed with concrete floor and dried manure solids (DMS) were used as bedding for dairy cows.



**Figure 1** Different sampling locations of bovine mastitic milks from the north west of Iran; Jolfa, Khosrowshahr, Azarshahr (located in East Azerbaijan province) and Miandoab (located in West Azerbaijan province). ☆, the capitals of East Azerbaijan (Tabriz) and West Azerbaijan (Urmia).

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