Identification of virulence factors in 16S-23S rRNA intergenic spacer genotyped *Staphylococcus aureus* isolated from water buffaloes and small ruminants

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ABSTRACT

Staphylococcus aureus is an important human and animal pathogen, and is regarded as an important cause of intramammary infection (IMI) in ruminants. *Staphylococcus aureus* genetic variability and virulence factors have been well studied in veterinary medicine, especially in cows as support for control and management of IMI. The aim of the present study was to genotype 71 *Staph. aureus* isolates from the bulk tank and foremilk of water buffaloes ($n = 40$) and from udder tissue ($n = 7$) and foremilk ($n = 24$) from small ruminants. The method used was previously applied to bovine *Staph. aureus* and is based on the amplification of the 16S-23S rRNA intergenic spacer region. The technique applied was able to identify different *Staph. aureus* genotypes isolated from dairy species other than the bovine species, and cluster the genotypes according to species and herds. Virulence gene distribution was consistent with genotype differentiation. The isolates were also characterized through determination of the presence of 19 virulence-associated genes by specific PCR. Enterotoxins A, C, D, G, I, J, and L were associated with *Staph. aureus* isolates from buffaloes, whereas enterotoxins C and L were linked to small ruminants. Genes coding for methicillin resistance, Pantón-Valentine leukocidin, exfoliative toxins A and B, and enterotoxins B, E, and H were undetected. These findings indicate that RNA template-specific PCR is a valid technique for typing *Staph. aureus* from buffaloes and small ruminants and is a useful tool for understanding udder infection epidemiology.

Key words: *Staphylococcus aureus*, water buffalo, small ruminant, virulence factor

INTRODUCTION

Staphylococcus aureus is a worldwide pathogen causing several severe diseases. In ruminants, it is the most common etiological agent of subclinical and clinical mastitis (Watts, 1988), with relevant losses in the dairy industry, as it reduces milk quality, milk production, and increases the cost of production through increased culling. *Staphylococcus aureus* strains produce several virulence factors such as (1) surface proteins that promote colonization of host tissues, (2) proteins that promote bacterial spread in tissues (e.g., leukocidin, kinases, and hyaluronidase), (3) biochemical properties that enhance their survival in phagocytes (e.g., carotenoids and catalase production), (4) surface factors that inhibit phagocytic engulfment (e.g., capsule, protein A), (5) immunological “target like” (e.g., protein A, coagulase, clumping factor, biofilm production), (6) membrane-damaging toxins that lyse eukaryotic cell membranes (e.g., hemolysins, leukotoxin, and leukocidin), (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease [e.g., staphylococcal enterotoxins SEA to SEQ, toxic shock syndrome toxin-1 (TSST-1), and exfoliative toxins (ET)], and (8) mechanisms of resistance to antimicrobial agents.

Some virulence factors are expressed by genes that are located on mobile genetic elements called pathogenicity islands (e.g., TSST and some enterotoxins; Novick, 2003) or lysogenic bacteriophages [e.g., Pantón-Valentine leukocidin (PVL); Narita et al., 2001] and factors associated with suppressing innate immunity, such as the chemotaxis inhibitory protein and staphylokinase, which are integrated in the bacterial chromosome. All these factors can contribute in differ-

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ent ways to *Staph. aureus* pathogenicity and, therefore, influence the management of the disease (Dinges et al., 2000; Peacock et al., 2002). The molecular mechanisms responsible for the disease manifestation are not completely understood but differences in gene content and allelic variations between *Staph. aureus* strains are presumed to influence the pathogenesis of infections in cattle (Peacock et al., 2002). The importance of evaluating the combination of *Staph. aureus* virulence factors has been repeatedly emphasized both in human and veterinary medicine (Jarraud et al., 2002; von Eiff et al., 2004), and knowledge about the genetic variability within different *Staph. aureus* populations would help in the design of efficient therapeutic approaches. Actually, the genotype involved affects the prevalence of *Staph. aureus* and the number of infected quarters within a herd (Fournier et al., 2008). Although staphylococcal virulence factors have been identified in many *Staph. aureus* collections isolated from cases of bovine IMI, it is still unknown which factors are specifically associated with water buffalo and small ruminant IMI. The aim of the present study was to genotype and characterize *Staph. aureus* isolated from the milk of water buffaloes, small ruminants, and from udder tissue of sheep.

MATERIALS AND METHODS

Farm Selection and Isolates Identification

In the context of a pilot project focused on the improvement of welfare and control of SCC in water buffaloes and small ruminants in different areas of Latina County (Italy), 212 herds were sampled with the following distribution: 162 water buffaloes, 37 sheep, and 12 goats. Thirty-eight (18%) of these were positive for *Staph. aureus* in bulk tanks and 18 farms decided to collaborate for further analyses. From those farms [5 sheep (A to E), 2 goats (F to G), and 11 buffaloes (H to T)], a total of 71 *Staph. aureus* isolates, originating from water buffaloes ($n = 40$), sheep ($n = 22$), and goats ($n = 9$), were collected. All isolates were selected and stored at Istituto Zooprofilattico Sperimentale Lazio Toscana (IZSLT, Rome, Italy) Latina section, which is the main reference laboratory for the bacterial analyses on those species in the Lazio Region. All milk samples were collected and kept at 4°C until bacteriological analyses were performed according to the National Mastitis Council (NMC, 1999). A total of 7 sheep udder tissues from herd A were also examined. Parenchymal and cisternal tissues were collected from udder for bacteriological investigations and sections were sampled with a sterile swab that was rotated inside a 2-cm-long cut over a surface of 1 cm² and then streaked on a sheep blood agar plate and Baird-Parker

agar plates. After incubation of 24 to 48 h at 37°C, suspect colonies were picked up from culture plates according to their cultural features (colony appearance and hemolysis) and subcultured on sheep blood agar. Then, their presumptive identification as *Staph. aureus* was confirmed by a tube coagulase test.

DNA Extraction

The coagulase-positive isolates were inoculated in brain-heart infusion broth (Oxoid Ltd., Basingstoke, UK) and incubated aerobically overnight at 37°C. The DNA was extracted from 1 mL of culture broth (about 10⁸–10⁹ cfu/mL), following the instructions described in Cremonesi et al. (2006), starting from step 2. The quantity and quality of DNA samples were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). The DNA samples were then stored at –20°C until use.

16S-23S rRNA Intergenic Spacer Genotyping

The method of Jensen et al. (1993), rearranged by Fournier and coworkers (2008), was used for RNA template-specific PCR (**RS-PCR**) genotyping. This method is based on the amplification of the 16S-23S rRNA intergenic spacer region. In a total volume of 25 µL, each reaction contained 1× HotStarTaq Master Mix (Qiagen GmbH, Hilden, Germany), an 800 nM concentration of each primer (G1:GAAGTCGTAACAAGG and L1:CAAGGCATCCACCGT), and 30 ng of DNA. The amplification protocol was 95°C for 15 min, which was followed by 27 cycles at 94°C for 1 min, a 2-min ramp to reach the final temperature, and annealing at 55°C for 7 min. After a further 2-min ramp to reach the final temperature, extension was done at 72°C for 2 min. The PCR products were then analyzed using an Agilent 2100 Bioanalyzer with a DNA 7500 LabChip kit (Agilent Technologies Inc., Palo Alto, CA). For interpretation of the results, 2 patterns were considered different if 2 or more peaks of the electropherogram differed in size. Groupings of the RS-PCR profiles were obtained with the BioNumerics 5.0 software package (Applied Maths NV, Sint-Martens-Latem, Belgium) using the unweighted pair group method cluster analysis.

Molecular Strain Characterization

All DNA samples were analyzed by PCR to determine the presence of genes encoding enterotoxins (from *sea* to *sel*), leukotoxins (*lukE*, *lukS*/F-PV, *lukE*, *lukD*, and *lukM*), acquisition of methicillin resistance (*mecA*) and other virulence genes using primers and protocols described in the literature and listed in Table 1. The

Table 1. Target genes, primer sequences, and PCR amplification products

Target gene	Primer sequence (5'–3') ¹	Amplification size (bp)	Reference
<i>lukM</i>	TGGATGTTACCTATGCAACCTAC GTTCGTTTCCATATAATGAATCACTAC	780	Jarraud et al. (2002)
<i>lukE-lukD</i>	TGAAAAAGGTTCAAAGTTGATACGAG TGTATTTCGATAGCAAAAGCAGTGCA	269	Jarraud et al. (2002)
<i>cna</i>	AAAGCGTTGCCTAGTGGAGA AGTGCCTTCCCAAACCTTTT	192	Zecconi et al. (2006)
<i>scn</i>	ATACTTGCGGGAACCTTAGCAA TTTTAGTGCTTCGTCAATTTTCG	320	Sung et al. (2008)
<i>chp</i>	TTTTTAACGGCAGGAATCAGTA TGCATATTTCATTAGTTTTTCCAGG	404	Sung et al. (2008)
<i>sak</i>	TGAGGTAAGTGCATCAAGTTCA CCTTTGTAATTAAGTTGAATCCAGG	403	Sung et al. (2008)
<i>fmbb</i>	AATGAAGATGCGAATCATGTTG CATCCATTTTTGTTTGCGTAGA	725	Sung et al. (2008)
<i>lukE</i>	AATGTTAGCTGCAACTTTGTCA CTTTCTGCGTAAATACCAGTTCTA	831	Fournier et al. (2008)
<i>lukS/F-PV</i>	ATCATTAGGTAAAATGTCTGGACATGATCA GCATCAAGTGTTATGGATAGCAAAAAGC	433	McClure et al. (2006)
<i>mecA</i>	GTAGAAATGACTGAAACGTCCGATAA CCAATTCCACATTGTTTCGGTCTAA	310	McClure et al. (2006)
<i>coa</i>	ATAGAGATGCTGGTACAGG GCTTCCGATTGTTTCGATGC	Polymorphic size	Akineden et al. (2001)
<i>clfA</i>	GGCTTCAGTGCTTTGTAGG TTTTTCAGGGTCAATATAAGC	1,000	Akineden et al. (2001)
<i>spa</i>	CAAGCACCAAAAAGAGGAA CACCAGGTTTAAACGACAT	Polymorphic size	Akineden et al. (2001)
<i>tsst</i>	ATGGCAGCATCAGCTTGATA TTTCCAATAACCACCCGTTT	300	Akineden et al. (2001)
<i>eta</i>	CTAGTGCATTTGTTATTCAA TGCATTGACACCATAGTACT	120	Akineden et al. (2001)
<i>etb</i>	ACGGCTATATACATTCAATT TCCATCGATAATATACCTAA	200	Akineden et al. (2001)
<i>seb</i>	TCGCATCAAACTGACAAAACG GCAGGTACTCTATAAGTGCC	478	Pinto et al. (2005)
<i>see</i>	TACCAATTAACCTTGTGGATAGAC CTCTTTGCACCTTACCGC	170	Monday and Bohach (1999)
<i>sea</i>	TAAGGAGGTGGTGCCTATGG CATCGAAACCAGCCAAAGTT	180	Cremonesi et al. (2005)
<i>sec</i>	ACCAGACCCATATGCCAGATG TCCCATTATCAAAGTGGTTTCC	371	Cremonesi et al. (2005)
<i>sed</i>	TCAATTCAAAAAGAAATGGCTCA TTTTTCCGCGCTGTATTTTT	339	Cremonesi et al. (2005)
<i>seg</i>	CCACCTGTTGAAGGAAGAGG TGCAGAACCATCAAACCTCGT	432	Cremonesi et al. (2005)
<i>seh</i>	TCACATCATATGCGAAAGCAG TCGGACAATATTTTTCTGATCTTT	463	Cremonesi et al. (2005)
<i>sei</i>	CTCAAGGTGATATTGGTGTAGG CAGGCAGTCCATCTCCTGTA	529	Cremonesi et al. (2005)
<i>sej</i>	GGTTTTCAATGTTCTGGTGGT AACCAACGGTTCTTTTGAGG	306	Cremonesi et al. (2005)
<i>sel</i>	CACCAGAATCACACCGCTTA CTGTTTGATGCTTGCCATTG	240	Cremonesi et al. (2005)
<i>nuc</i>	AGTTCAGCAAATGCATCACA TAGCCAAGCCTTGACGAACT	400	Cremonesi et al. (2005)

¹For each target gene, the first primer is the forward primer and the second primer is the reverse primer.

amplified PCR fragments were distinguished by 2% agarose gel electrophoresis (GellyPhor; EuroClone SpA, Milan, Italy), stained with ethidium bromide (0.05 mg/mL; Sigma-Aldrich, Milan, Italy), and visualized by UV transilluminator (BioView Ltd., Nes Ziona, Israel). A 100-bp DNA ladder (Finnzymes, Espoo, Finland) was included in each gel electrophoresis.

RESULTS

RS-PCR Genotyping

Water Buffalo. The genotyping analysis of the 40 isolates from milk samples, revealed the presence of 23 different profiles from 11 herds (Figure 1). One of these

profiles (XXI) was common for 6 *Staph. aureus* isolated from bulk tank milk of the same herd (herd O), which was nonenterotoxigenic and with amplicons longer than 100 and 640 bp for the x-region of protein A and *coa* genes, respectively. In the same herd, 4 other profiles (XX, XXII, XXIII, and XXIV) were detected among bulk tank isolates. Another profile (XXXII) was common to 4 isolates from foremilk in herd S and was the only one detected in that herd. The remaining profiles resulted from the analysis of 1, 2, or 3 isolates from samples collected in the same or in different herds, revealing the presence of up to 3 different strains in only 1 herd (P).

Ovine. As described in Figure 1, analysis of the 16S-23S rRNA intergenic spacer of 22 isolates from milk and udder halves in 5 different herds revealed 7 diverse profiles. Out of the 7 diverse profiles, 2 were the most frequent, as they made up 9 (41%) and 6 (27.2%) isolates, respectively; both were isolated from the same farm. In the first profile, the isolates were obtained both from milk and udder tissue and were positive for *sec* and *sel* genes, with the amplicon for the x-region of protein A gene longer than 100 bp and the *coa* amplicon at 640 bp. Two of 9 isolates were negative for *lukE* and 4 were negative for *clfA* genes. Within the second profile, 5 out of 6 isolates were enterotoxigenic (positive for *sec* and *sel*), with amplicons for the x-region of protein A gene and *coa* longer than 100 and 640 bp, respectively. The other 5 RS-PCR profiles resulted from the analysis of 1, 2, or 3 strains isolated from samples collected in different herds.

Caprine. Analysis of 16S-23S rRNA intergenic spacer for the 9 isolates collected from foremilk in 2 different herds revealed 4 different profiles, 3 of which belonged to the same cluster, with a similarity of 80% (Figure 1). In herd F, a single *Staph. aureus* isolate (CAP664) appeared more related to the ovine profiles. It was nonenterotoxigenic and negative for *tsst*, *LukE-LukD*, *LukM-LukF/PV*, and *clfA* genes, showing a different profile from typical isolates from caprine samples (Table 2).

Molecular Characterization

All isolates were positive for coagulase (*coa*), thermonuclease (*nuc*) and membrane protein associated with antibiotic resistance (*fntB*) genes and negative for genes involved in host-cell invasion (*eta* and *etb*), in methicillin resistance (*mecA*), for Panton-Valentine leukocidin *lukS/F-PV* and for SEB, SEE, and SEH enterotoxins.

Polymorphic Genes. The analysis of the coagulase gene (*coa*) and of the x-region of protein A gene (*spa*) produced amplicons of different size, which allowed for

grouping the isolates in clusters based on the length of fragments obtained. Fourteen out of 40 (35%) *Staph. aureus* water buffalo isolates gave a *coa* amplicon longer than 640 bp and 26 out of 40 (65%), an amplicon of 640 bp. The *spa* gene analysis yielded amplicons longer than 250 and 100 bp, respectively, for 17/40 (42.5%) and 20/40 (50%) isolates; 3 isolates analyzed did not produce an amplification product. For ovine isolates, amplification of the *coa* gene yielded amplicons longer than 640 bp for 5/22 (22.7%), lower than 640 bp for 1/22 (4.5%), and 640 bp for 16/22 (72.7%) isolates, whereas the analysis of *spa* genes produced amplicons longer than 100 bp for 20/22 (90.9%) and longer than 250 bp for 2/22 (9.1%) isolates. For caprine isolates, the *coa* gene produced amplicons of 640 bp for 7/9 (77.7%) and longer than 640 bp for 2/9 (22.2%) of isolates; contemporary *spa* gene analysis revealed amplicons longer than 100 and longer than 250 bp, respectively, for 7/9 (77.7%) and 2/9 (22.2%) isolates.

Enterotoxins. Seventeen out of 40 (42.5%) water buffalo isolates were enterotoxigenic, harboring at least 1 of the genes coding for A, C, D, G, I, J, and L enterotoxins. The most frequent genes detected were *sea* (11/17) and *sed* (7/17). A single isolate (BUF275) harbored 4 enterotoxin genes (*sea*, *sec*, *sei*, and *sel*); 4 (BUF250, BUF251, BUF252, BUF253) and 3 (BUF269, BUF271, BUF273) other isolates harbored 3 (combination of *sea*, *sed*, and *sej*; or *sed*, *seg*, and *sei*; or *sea*, *sej*, and *sei*) and 2 genes (*sea* and *sed*), respectively.

Seventeen out of 22 (77.2%) ovine isolates were enterotoxigenic and coded for enterotoxins C and L. The same result was obtained from *Staph. aureus* isolates of caprine origin; all the isolates, except for 1 nonenterotoxigenic unrelated isolate (CAP664), harbored *sec* and *sel* genes.

Other Virulence Genes. As reported in Table 2, the *lukE* gene was detected in 90.9, 100, and 85% of ovine, caprine, and water buffalo isolates, respectively, whereas 77.2 (ovine), 88.8 (caprine), and 75% (water buffalo) of isolates were positive for the clumping factor gene (*clfA*). Eight out of 9 (88.8%) caprine isolates possessed the *tsst* gene that encodes the toxic shock syndrome toxin, whereas amplicons for these genes were not produced from water buffalo strains. Among the ovine samples, 14 out of 22 strains (63.6%) were positive for the *tsst* gene.

Twenty-one out of 22 isolates for ovines, all strains isolated from caprines, and 38 out of 40 isolates for water buffaloes harbored a gene coding for a new leukotoxin (*LukE-LukD*); contemporarily 17/22, 9/9, and 4/40 isolates respectively from ovines, caprines, and water buffaloes were positive for *LukM-LukF/PV*, which has been identified in *Staph. aureus* isolated from bovine milk (Jarraud et al. 2002). Among the isolates,

Figure 1. Dendrogram derived from the RNA template-specific PCR (RS-PCR) profiles generated during the analyses. The identities of the isolate, species (water buffalo, sheep, or goat), source (foremilk, udder, or bulk tank), herd (from A to T), and RS-PCR profile (from I to XXXIV) are reported for each sample.

Table 2. Virulence genes and their relative frequency (%) in water buffalo, ovine, and caprine isolates

Gene	Positive isolates [no. (%)]		
	Water buffalo (n = 40)	Ovine (n = 22)	Caprine (n = 9)
<i>lukM</i>	4 (10)	17 (77.2)	9 (100)
<i>lukE-lukD</i>	38 (95.0)	21 (95.4)	9 (100)
<i>cna</i>	31 (77.5)	10 (45.4)	1 (11.1)
<i>scn</i>	19 (47.5)	8 (36.6)	1 (11.1)
<i>chp</i>	18 (45.0)	4 (18.1)	1 (11.1)
<i>sak</i>	30 (75.0)	9 (40.9)	3 (33.3)
<i>lukE</i>	34 (85.0)	20 (90.9)	9 (100)
<i>clfA</i>	30 (75.0)	17 (77.2)	8 (88.8)
<i>spa</i>	37 (92.5)	22 (100)	9 (100)
<i>tsst</i>	0 (0)	14 (63.6)	8 (88.8)
<i>sea</i>	11 (27.5)	0 (0)	0 (0)
<i>sec</i>	1 (2.5)	17 (77.2)	8 (88.8)
<i>sed</i>	7 (17.5)	0 (0)	0 (0)
<i>seg</i>	6 (15)	0 (0)	0 (0)
<i>sei</i>	6 (15)	0 (0)	0 (0)
<i>sej</i>	2 (5.0)	0 (0)	0 (0)
<i>sel</i>	1 (2.5)	17 (77.2)	8 (88.8)

10 from ovines, 1 from caprines, and 31 from water buffaloes possessed the gene encoding the collagen-binding protein (*cna*).

Some of the tested isolates harbored genes carried on mobile genetic elements and usually present in strains involved in human infections (Sung et al. 2008). The gene encoding the anti-opsonin staphylokinase (*sak*) was present in 40.9, 33.3, and 75% of ovine, caprine, and buffalo isolates, respectively. In addition, the gene encoding the staphylococcal complement inhibitor (*scn*) was detected in 36.6, 11.1, and 47.5% of the isolates from ovines, caprines, and buffaloes. A positive reaction for the gene encoding the chemotaxis inhibitory protein (*chp*) was found in 18.1, 11.1, and 45% of the ovine, caprine, and buffalo isolates, respectively.

DISCUSSION

The epidemiology of *Staph. aureus* mastitis in dairy cattle has been studied using methods with high discriminatory powers during the past decade (e.g., pulsed-field gel electrophoresis, random amplified polymorphic DNA, *spa*-typing, and multilocus sequence typing; Haveri et al., 2007; Morandi et al., 2010). Despite the highly discriminative power, reproducibility, and portability that make them useful research tools, these methods are hardly applicable to daily practice. A simple technique based on the amplification of the 16S-23S rRNA intergenic spacer (RS-PCR) was used for genotyping in a short time numerous *Staph. aureus* isolates obtained from bovine herds in Switzerland (Fournier et al., 2008). In the current study, this technique was applied to the characterization of *Staph.*

aureus isolated from bulk tank milk and foremilk of water buffaloes and from the udder and foremilk of small ruminants from herds located in different areas of the Lazio Region.

At 95% similarity, the genetic diversity among isolates recovered from water buffaloes and small ruminants showed that the 71 isolates were distributed over 34 different profiles and, except for 2 isolates (CAP664 and OV657), the analysis revealed a clustering in accordance with species (Figure 1) and, to some extent, with herds. Among these types, 6 isolates for water buffaloes, 5 for goats, and 9 for sheep belonged to a single RS-PCR genotype for each species isolated from the same farm. The isolates belonging to each of these 3 main profiles (Table 3) shared the same *spa* and *coa* amplicon lengths and were enterotoxigenic (isolates from both small ruminants species were positive for *sec* and *sel*) and nonenterotoxigenic (isolates from water buffaloes). The other types were heterogeneous with regard to the presence of enterotoxin genes and *lukE* and *clfA* genes as well as in length of coagulase gene and *spa* gene amplicons. This is in agreement with the findings that *Staph. aureus* from ruminant IMI constitutes a heterogeneous group but only a few specialized *Staph. aureus* types were responsible for most of the cases of IMI (Fournier et al., 2008; Aires-de-Sousa et al., 2007) and that these profiles have a broad geographic distribution (Annemüller et al., 1999; Smith et al., 2005).

Moreover, it has been demonstrated that a link exists between virulence gene patterns and specific genotypes and their epidemiological and pathogenic properties (Fournier et al., 2008). Actually, some genotypes are particularly contagious and prone to spread from cow

Species	Frequency (%)	Gene												
		<i>lukM</i>	<i>lukE-lukD</i>	<i>lukE</i>	<i>lukS/F-PV</i>	<i>cna</i>	<i>sak</i>	<i>fmbB</i>	<i>coa</i> (bp)	<i>clfA</i>	<i>spa</i> (bp)	<i>tsst</i>	Enterotoxin	<i>nuc</i>
Ovine	9/22 (40.1)	+	+	+	—	—	—	+	640	+	>100	+	C, L	+
Caprine	5/9 (55.5)	+	+	+	—	—	—	+	640	+	>100	+	C, L	+
Water buffalo	6/40 (12.0)	—	+	+	—	+	+	+	640	+	>100	—	—	+

In our study, all the isolates from water buffaloes were negative for TSST, whereas 77.4% of the strains collected from small ruminants were positive for the *tsst* gene and for *sec* and *sel* genes as well, and all were located on the same bovine staphylococcal pathogenicity island (SaPI_{bov}). The genes encoding PVL and *mecA* were completely absent in the 71 isolates analyzed. This result is different from other observations about mastitis-related strains isolated in cows (Rainard et al., 2003; Zecconi et al., 2006). Though performed on a limited sample of isolates, the current survey records the absence of the *mecA* gene in *Staph. aureus* from dairy ruminants other than the bovine, and of a damaging leukocidin such as PVL. Leukocidins could play an overall important role in avoidance of immune response and thus pathogenesis, as reported by Rainard et al. (2003).

According to previous findings by Ikawaty et al. (2010), the 11 isolates (9 from ovine and 2 from caprine herds) encoding at least 2 virulence factors out of staphylococcal complement inhibitor (*scn*), chemotaxis inhibitory protein of *Staph. aureus* (*chp*), and staphylokinase (*sak*) may have a human origin, because these virulence factors show activity only against the human innate immune system. The recovery of human-associated *Staph. aureus* in bovine mastitis is common (Barlow et al., 2013) and their presence among water buffaloes and small ruminants isolates should be determined in future studies.

Knowledge about the epidemiology of *Staph. aureus* genotypes in dairy species and herds might help to formulate strategies for reducing the infection spreading and for focused treatments. Characterization of virulence gene profiles of these different *Staph. aureus* could help to predict clinical outcomes and especially to identify harmful strains. A linkage between virulence genes patterns and genotypes has been confirmed by the present study. To make decisions regarding infection management based on diagnosis and genotyping, a less-expensive and time-consuming method is required (Barlow et al., 2013). The findings of this study provide a contribution to the comprehension of *Staph. aureus* IMI in buffaloes and small ruminants and information to differentiate control measures according to pathogen and host features.

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