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First Report on Vancomycin-Resistant *Staphylococcus aureus* in Bovine and Caprine Milk

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The present investigation was carried out to study the vancomycin resistance pattern of *Staphylococcus aureus* isolates ($n=274$) obtained from 352 milk samples of bovine (269) and caprine (63) clinical and subclinical mastitis from different districts of West Bengal, India. Of them, seven isolates (vancomycin-resistant *S. aureus* [VRSA] 1–7) exhibited resistance to vancomycin. Minimum inhibitory concentration of vancomycin (MIC_{van}) for VRSA2 and VRSA3 was ≥16 μg/ml; thus categorized as VRSA. For rest of the isolates, MIC_{van} was 8 μg/ml and they were grouped as vancomycin intermediate *S. aureus* (VISA). Even though all the isolates were resistant to cefoxitin and oxacillin and possessed *mecA* gene, none of them carried vancomycin resistance gene. Furthermore, all the seven isolates were subjected to *Staphylococcal* cassette chromosome mec (SCC*mec*) typing, *Staphylococcal* protein A (*spa*) typing, and enterobacterial repetitive intergenic consensus polymerase chain reaction. All the isolates except VRSA3 and VRSA4 from Kolkata district exhibited diverse genetic lineage, irrespective of their host and antibiotic resistance pattern. These two isolates showed clonal similarity (MRSA-SCC*mec*-V-*spa* t267) with methicillin-resistant *S. aureus* (MRSA) strains previously reported in human and animal infection. Isolation of VRSA and VISA could probably be due to intensive use of vancomycin in healthcare premises, which might have led to the development of glycopeptide-resistant strains and thereafter, further disseminated in the environment, including livestock farms. Detection of VRSA in milk is a serious concern as it may further cause health problems in the consumers. This is the first ever report of VRSA in food animals, even though the pathogen is otherwise prevalent in humans.

Introduction

Staphylococcus aureus is responsible for a wide spectrum of diseases, including pyoderma, osteomyelitis, endocarditis, septicemia, surgical wound complications, pneumonia in human beings, and mastitis in diary animals.[1,2] With the emergence of methicillin-resistant *S. aureus* (MRSA) and its increasing resistance to antibiotics of various groups such as penicillins, cephalosporins, fluoroquinolones, aminoglycosides and macrolides, vancomycin has been the antibiotic of the last resort to treat the hospitalized patients critically infected with MRSA or other Gram-positive organisms like *Clostridium difficile*.[3,4]

Vancomycin is a glycopeptide antibiotic, which acts against Gram-positive bacteria by inhibition of cell wall synthesis. However, intensive use of vancomycin over the years in healthcare premises has eventually led to the development of glycopeptide-resistant strains of *S. aureus.* After its first report during 1997 in Japan, vancomycin-resistant *S. aureus* (VRSA) and vancomycin intermediate *S. aureus* (VISA) were reported from various countries, including United States,[5] United Kingdom,[6,7] Germany,[8] Portugal,[9] Brazil,[10] China,[11] Bangladesh,[12] and Jordan.[13] Most of these reports were concerned with hospitalized human patients with preexisting MRSA infection.

In contrast, there is hardly any report available on the occurrence of VRSA in animals possibly due to the fact that glycopeptides are not regularly used in veterinary practices. Nevertheless, contamination of the environment or pasture land situated in and around the human healthcare premises with VRSA and its further colonization in the domestic animals cannot be ruled out. In recent past, VRSA isolates of farm animal origin have been reported from South Africa.[14] Again, this group of workers have reported reduced vancomycin

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susceptibility of MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE) isolates from bovine mastitis.[15] At the same time, reports on the occurrence of VRSA in hospitalized human patients from different parts of India are on the rise over the last few years.[16–20] To test our hypothesis that these pathogens might enter the livestock population, we investigated the VRSA isolates obtained from milk samples of clinical and subclinical bovine and caprine mastitis from different districts of West Bengal, India.

Materials and Methods

Bacterial isolates

A total of 352 milk samples were collected from bovine (269) and caprine (83) clinical and subclinical mastitis during 2012 to 2015 from different districts of West Bengal, India. From affected quarter of the udder, milk or secretions were collected. If more than one quarter was affected, pooled samples were considered for the study. The samples were transported in the laboratory maintaining cold chain. In the laboratory, 10 µl of each sample was incubated overnight in trypticase soy broth (BD, BBL) at 37°C and inoculated in Baird Parker agar (BD, BBL) with egg yolk emulsion and mannitol salt agar (MSA; HiMedia). Colonies surrounded by bright yellow zone in MSA or black, shiny, and convex colonies surrounded by a clear zone in Baird Parker agar were primarily selected as *Staphylococcus.* Single-isolated colonies were taken in nutrient agar (HiMedia) slant and were further processed for confirmation as *S. aureus* using standard tests such as Gram's staining, catalase, coagulase, oxidase, indole, methyl red, urease, Voges–Proskauer, lecithinase production, mannitol, and glucose fermentation.[21] Out of 352 milk samples, 216 were found positive for *S. aureus*. Finally, 274 colonies were confirmed as *S. aureus* encompassing single colony from 168 milk samples and two colonies from 48 milk samples. Furthermore, all the 274 isolates were confirmed by polymerase chain reaction (PCR)-based detection of 16S ribosomal RNA, thermonuclease (*nuc*), *Staphylococcal* protein A (*spa*), details of which is described later. Of the 274 *S. aureus* isolates, 211 isolates were obtained from bovine and 63 from caprine milk.

Isolation of VRSA

For isolation of VRSA, we followed three step procedure. First, all the *S. aureus* isolates were inoculated in Mueller Hinton Agar (MHA; HiMedia) containing 2.0 mg/L vancomycin (Sigma-Aldrich) and incubated at 35°C for 48 hours. Further, minimum inhibitory concentration of vancomycin (MIC_{van}) for all the isolates that grew on vancomycin supplemented MHA plates was determined using both Ezy MIC paper strips (HiMedia) coated with vancomycin (0.016–256 µg/ml) and agar dilution method.

For MIC determination using Ezy MIC paper strips, colonies were picked up after overnight incubation on nutrient agar plate to prepare suspension of 0.5 McFarland standards in sterile normal saline and inoculated on MHA. Finally, Ezy MIC paper strips were placed on the inoculated plates and incubated at 35°C for 24 hours. MIC was read where the ellipse intersected the MIC scale on the strip. In agar dilution method, MHA was prepared with vancomycin hydrochloride (0.5–256 µg/ml; Sigma-Aldrich) and spotted with the isolates. Following 24 hours incubation at 35°C, the plates were checked for any visible growth. For all the isolates, the test was repeated thrice in duplicate. In agar dilution method, gradient plates of *S. aureus* ATCC 29213 strain were used as vancomycin susceptible control.

Based on the results, isolates with MIC_{van} values ≥16 µg/ml were classified as VRSA, whereas the isolates with MIC_{van} in the range of 4–8 µg/ml were termed as VISA.[22] For further confirmation, the isolates showing vancomycin resistance (≥8 µg/ml) were checked for their growth in vancomycin screen agar (containing 6.0 mg/L vancomycin; BDL Difco).

Determination of methicillin/oxacillin resistance

All the *S. aureus* isolates exhibiting resistance to vancomycin were inoculated into MeReSa Agar Base (containing cefoxitin 6.0 mg/L and methicillin 4.0 mg/L; HiMedia) and methicillin–oxacillin screen agar (containing oxacillin 2.0 mg/L and polymyxin B 50,000 IU/L; HiMedia) and incubated at 35°C for 24 hours as per instructions of the manufacturer. Any visible growth in the respective medium indicated the isolates as methicillin/oxacillin resistant. Furthermore, MIC of oxacillin (MIC_{oxa}) and MIC of cefoxitin (MIC_{cef}) for all these isolates were measured by Ezy MIC paper strips (HiMedia) coated with oxacillin (0.016–256 µg/ml) following the guidelines of the manufacturer. MIC_{oxa} and MIC_{cef} were also confirmed for all the VRSA and VISA isolates following agar dilution method using the MHA plates supplemented with oxacillin sodium salt monohydrate and MHA plates supplemented with cefoxitin sodium salt (0.5–256 µg/ml; Sigma-Aldrich). The *S. aureus* isolates with MIC_{oxa} ≥4 µg/ml and MIC_{cef} ≥8 µg/ml were categorized as methicillin/oxacillin resistant.

Antibiotic susceptibility testing

The isolates exhibiting resistance to vancomycin were further checked for their drug resistance profile by disc agar diffusion technique using commercially available discs (HiMedia) against the following antibiotics—amoxyclav (30/10 µg), amikacin (30 µg), co-trimoxazole (25 µg), clindamycin (2 µg), ciprofloxacin (5 µg), gatifloxacin (5 µg), tobramycin (10 µg), polymyxin B (300 U), levofloxacin (5 µg), tetracycline (15 µg), cefpodoxime (10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), and piperacillin+tazobactam (100/10 µg). MHA plates were streaked with 0.5 McFarland standard inoculum of the purified isolates, and the zone of inhibition was measured after incubation with antibiotic discs at 37°C for 18–24 hours following CLSI guidelines.[22]

PCR-based screening of the VRSA

Bacterial DNA was isolated from all the *S. aureus* isolates resistant to vancomycin using the commercially available QIAamp DNA Mini Kit (QIAGEN). All the oligonucleotide primers were procured from Metabion, Germany, and the reagents for PCR were procured from QIAGEN and Fermentas. The PCR amplification of 16s rDNA, *spa*, *nuc*, *mecA*, *mecC*, *VanA*, and *VanB* was performed in Veriti 96-Well Thermal Cycler (Applied Biosystem) in 25 µl of reaction volume consisting of 2.5 mM $MgCl_2$, 200 µM of each dNTPs, 10 pmol of each primer, and 1 U of *Taq* polymerase as per the cycle conditions described earlier.[20,23–26] The amplified

products obtained were visualized by the gel documentation system (UV Mini Bis Bio-imaging System) after electrophoresis in 2% (W/V) agarose gel containing ethidium bromide (0.5 μg/ml; Sigma-Aldrich).

Cloning and sequencing

Amplified *mecA*, *nuc*, and *spa* genes were purified using the gel purification kit (Fermentas) and cloned in pGEM-T Easy Vector (Promega) following standard protocol. The plasmids containing the expected insert were sequenced using the BigDye Terminator® v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) in an automated sequencer (Applied Biosystems 3130 Genetic Analyzer) following the manufacturer's instructions. After obtaining the sequencing results, homology searches were conducted using the BLAST algorithm available at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Enterobacterial repetitive intergenic consensus-PCR

All the *S. aureus* isolates resistant to vancomycin were subjected to enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) in 50 μl reaction volume containing 50 pmol of each primer, 400 μM of each dNTPs, 2 U of *Taq* polymerase, 5 μl of 10× PCR buffer, and 1.5 mM $MgCl_2$. Following initial denaturation at 95°C for 10 minutes, *Taq* polymerase was added to the reaction mixture and PCR was conducted with 35 cycles consisting of 95°C for 1.5 minutes, 55°C for 2.0 minutes, and 72°C for 10 minutes with a final extension for 20 minutes at 72°C.[27] The amplified product was visualized as described earlier. For interpretation of ERIC-PCR data, all the images taken by the gel documentation system were analyzed using the Doc-itLS Image Analysis Software supplied with the system (UVP) as per manufacturer's instruction. Phylogenetic relationship among the isolates was established comparing the differences in the banding pattern to determine the degree of genetic divergence and similarity among the VRSA isolates.

Staphylococcal *cassette chromosome* mec *and* spa *typing*

All the VRSA isolates, which showed oxacillin and cefoxitin resistance, were subjected to multiplex PCR for subtyping of *Staphylococcal* cassette chromosome *mec* (SCC*mec*) as described previously.[28] The polymorphic X region of the *spa* of all the VRSA/VISA isolates was amplified, and the amplicons were cloned and sequenced as described earlier.[29] The sequencing information was submitted in the online software http://spatyper.fortinbras.us/ for obtaining the *spa* types.

Results

Out of 352 milk samples collected, 216 samples (61.36%) were found positive for *S. aureus*. All the 274 *S. aureus* isolates were mannitol fermenting, positive in tube coagulase test, and produced brown black colonies with opalescence in Baird Parker agar. All the *S. aureus* isolates were also positive for 16S rDNA and *nuc* gene by PCR.

Of these, seven isolates exhibited resistance to vancomycin as revealed by Ezy MIC test strip and agar dilution method. Amongst these, two isolates were from milk samples of goat and five were of bovine milk origin obtained from five different districts of West Bengal, India (Table 1). All the VRSA isolates were obtained from different animals.

The *S. aureus* isolates (VRSA2 and VRSA3) detected with MIC_{van} values of 16 and 32 μg/ml were classified as VRSA. For rest of the isolates (VRSA1, VRSA4, VRSA5, VRSA6, and VRSA7), the MIC_{van} value was 8 μg/ml (Table 1). These were accordingly classified as VISA as per CLSI breakpoints.[22] Furthermore, all the isolates were methicillin resistant as they were resistant to oxacillin and cefoxitin as revealed by MIC_{oxa} and MIC_{cef} values (Table 1). For two isolates (VRSA2 and VRSA6), MIC_{oxa} was more than 256 μg/ml. It is interesting to note that both the VRSA isolates (VRSA2 and VRSA3) exhibited high MIC values (64 and 256 μg/ml) for both oxacillin and cefoxitin (Table 1).

All the seven VRSA and VISA isolates were phenotypically resistant to oxacillin and methicillin and carried *mecA* gene as well. BLAST search with the amplified *mecA* gene revealed 100% homology with several sequences available in the database. The sequences were submitted to GenBank (Accession nos. KF895401.1, KJ872636, KT824865, KT824863). The *mecA* variant (*mecC*) was not detected in any of the isolates. Furthermore, all the isolates were negative by PCR for *vanA* and *vanB* genes despite being phenotypically resistant to vancomycin.

The VRSA and VISA isolates exhibited resistance to various antibiotics tested, including—all the beta-lactam compounds (cefpodoxime, cefepime, ceftazidime, ceftriaxone, piperacillin/tazobactam, and clavulanic acid potentiated amoxycillin), co-trimoxazole, polymyxin B, and clindamycin. However, most of the isolates were sensitive to amikacin, tobramycin, chloramphenicol, tetracycline, levofloxacin, ciprofloxacin, and gatifloxacin indicating the efficacy of fluoroquinolones and aminoglycosides against VRSA and VISA isolated from animals (Table 1).

All the VRSA and VISA isolates were subjected to ERIC-PCR, SCC*mec*, *and spa* typing to determine the clonal diversity and phylogenetic relationship among them. The isolates produced amplified products ranging from 166 to 3,342 bp in ERIC-PCR and were grouped into four major clusters namely A–D (Table 1). In *spa* typing, five isolates belonged to four *spa* types—t267, t527, t800, and t3626, whereas two isolates were nontypeable (Table 1: KT824864, KT803047, KT803046, KT824862, and KT764111). Of the seven isolates, three could not be typed by SCC*mec* typing, whereas three belonged to SCC*mec* type V (VRSA2, VRSA3, and VRSA4) and one isolate to type IV (VRSA7).

Although no specific ERIC-based cluster was observed according to the genotype or antibiotic resistance pattern of the isolates, VRSA strains isolated from mastitic cows of Kolkata district (VRSA3 and VRSA4) were clustered together in clad-B along with an isolate from Nadia district (VRSA1). Interestingly, both the isolates—VRSA3 and VRSA4 belonged to *spa* t267.

Discussion

In India, reports on occurrence of VRSA in humans, especially in the patients from intensive care unit or with prolonged MRSA infection and vancomycin therapy, are emerging.[16,17,20,30] However, occurrence of vancomycin-resistant MRSA strains is rarely reported in animals, as it is not used in veterinary practice in many countries, including India. Furthermore, there is no report available from India to support the present findings of vancomycin resistance

TABLE 1. CHARACTERISTICS OF VANCOMYCIN-RESISTANT AND INTERMEDIATE *STAPHYLOCOCCUS AUREUS* ISOLATED FROM MILK OF BOVINE AND CAPRINE CLINICAL AND SUBCLINICAL MASTITIS

				Genetic characterization							*Antibiotic resistance profile*[a]						
Sl. no.	*Isolates*	*Districts*	*Host*	*mecA*	*mecC*	*coa*	*nuc*	*spa*	*vanA*	*vanB*	*Resistant to*	MIC_{van} (μg/ml)	MIC_{oxa} (μg/ml)	MIC_{cef} (μg/ml)	*SCC*mec	*ERIC profile*	*Ridom/*spa *type (allelic variation)*
1	VRSA1	Nadia	Cow	P	N	P	P	P	N	N	AK (I), AMC, CTR, CIP (I), PB, PIT, TET	8.0	64	16	NT	B	NT[b]U1:K1:G1: J1:A1:E1r07:r16: r12:r23:r02:r13
2	VRSA2	Malda	Goat	P	N	P	P	P	N	N	AK (I), AMC, Co, CTR (I), PB,	32.0	>256	64	V	A	t527U1:J1:G1:F1:M1: B1:B1:B1:B1:B1: P1:B1r07:r23:r12: r21:r17:r34:r34:r34: r34:r34:r33:r34
3	VRSA3	Kolkata	Cow	P	N	P	P	P	N	N	AK, AMC (I), Co, CTR(I), Cd	16.0	64	64	V	B	t267U1:J1:G1:F1:M1: B1:B1:B1:P1:B1 r07:r23:r12:r21:r17: r34:r34:r34:r33:r34
4	VRSA4	Kolkata	Cow	P	N	P	P	P	N	N	Co, PIT (I)	8.0	32.0	16.0	V	B	t267U1:J1:G1:F1:M1: B1:B1:B1:P1:B1 r07:r23:r12:r21:r17: r34:r34:r34:r33:r34
5	VRSA5	North 24 Parganas	Cow	P	N	P	P	P	N	N	CTR (I), Cd	8.0	32.0	32.0	NT	C	t3626U1:J1:G1:F1: M1:B1:B1:B1:P1 r07:r23:r12:r21:r17: r34:r34:r34:r33
6	VRSA6	Nadia	Cow	P	N	P	P	P	N	N	AMC (I), Cd, Co, CTR(I)	8.0	>256	16.0	NT	D	t800U1:K1:G1: J1:A1:G1: J1:A1:B1r07:r16: r12:r23:r02:r12:r23: r02:r34
7	VRSA7	Hooghly	Goat	P	N	P	P	P	N	N	AMC, Co, CTR (I),PIT	8.0	32	32	IV	A	NT [b]B1:B1:B1:P1:B1 r34:r34:r34:r33:r34
8.	*S. aureus* ATCC 29213										Reference strain	0.5	0.5	1.0	—	—	t021W1:G1:K1:A1: K1:A1:O1:M1:Q1 r15:r12:r16:r02:r16: r02:r25:r17:r24

[a]All the isolates were sensitive to gatifloxacin and levofloxacin, but resistant to cefpodoxime, cefepime, and ceftazidime.
[b]A sequence that is a *spa* repeat, but does not exist in database.

CIP: ciprofloxacin (5 μg), TET: tetracycline (15 μg), AMC: amoxyclav (30/10 μg), CTR: ceftriaxone (30 μg), PIT: piperacillin+tazobactam (100/10 μg), AK: amikacin (30 μg), TOB: tobramycin (10 μg), PB: polymyxin B (300 U), Co: co-trimoxazole (1.25/23.75 μg), Cd: clindamycin (2 μg).

I, intermediate as per CLSI guidelines; MIC_{van}, MIC for vancomycin; MIC_{oxa}, MIC for oxacillin; MIC_{cef}, MIC for cefoxitin; NT, nontypeable; ERIC, enterobacterial repetitive intergenic consensus; P, positive; N, negative; MIC, minimum inhibitory concentration; VRSA, vancomycin-resistant *S. aureus.*

among *S. aureus* in animals, barring a single report from this group of workers on reduced susceptibility of *S. aureus* and *S. epidermidis* isolates towards vancomycin.[15] Development of such resistance could probably be due to contamination of pasture land or environment with the vancomycin-resistant isolates originated from human patients residing in close proximity. In general, milching of animal is mostly practised manually in all these areas and it may have substantially increased the possibility of infection or transfer of pathogens from the milkman to cows as recorded by other workers.[31] Added to this, presence of VRSA and MRSA in human population in this zone has also been previously documented.[18] Recent reports suggested that hospital-associated discharge/effluent is a convenient vehicle for dissemination of antibiotic resistance in MRSA and VRSA strains.[32] Furthermore, it is also possible that use of other cell wall acting antimicrobials may lead to vancomycin resistance in MRSA strains due to alteration of cell wall structure.[33,34]

In the present study, all the VRSA and VISA isolates were found resistant to several antibiotics of various groups like penicillin, third- and fourth-generation cephalosporins, clindamycin, co-trimoxazole, and glycopeptides, which are in corroboration with earlier reports.[18–20] Furthermore, all these isolates harbored *mecA* gene encoding for modified penicillin-binding protein (PBP2a). Probably because of this, these isolates were frequently resistant to beta-lactam drugs. In contrast, most of the isolates in the present study were sensitive to fluoroquinolones (ciprofloxacin, gatifloxacin, and levofloxacin) and aminoglycosides (amikacin and tobramycin). This is in contrast to the finding of earlier workers who reported that VRSA isolates of human origin from northern India were resistant to amikacin, netilmicin, gentamicin, tobramycin, norfloxacin, and ciprofloxacin.[20] This could possibly be due to the fact that previous exposure to such antibiotics might have led to selection of fluoroquinolone- or aminoglycoside-resistant strains. However, sensitivity of the VRSA/VISA isolates to tetracycline as recorded in the present study was also previously noted by a group of workers from Hyderabad, India.[19]

Of all the VRSA and VISA isolated in the present study, none was positive for *vanA* and *vanB* gene. Adegoke and Okoh could not detect *vanA* and *vanB* genes in VRSA isolates from animals.[14] Likewise, Tiwari and Sen also could not detect *van* cluster in the VRSA isolates.[20] Possession of *van* gene is not essential for vancomycin resistance as it can also be associated with increased cell wall thickness with more layers of peptidoglycans and murein monomers in the bacterial cell wall.[35] However, increased cell wall thickness or modified peptidoglycan synthesis is mostly reported for low level of vancomycin resistance in *S. aureus* isolates. Contrary to this, two isolates (VRSA3 and VRSA4) in the present study exhibited MIC_{van} of 16 and 32 μg/ml and such resistance in human isolates was mostly reported as a result of acquisition of *van* cluster.[10,18,19,30] Besides, there is also possibility of some other resistance mechanisms instrumental for the emergence of VRSA in animals.

In this study, all the VRSA and VISA isolates resistant to methicillin/oxacillin also carried *mecA* gene in PCR. Of them, four isolates (VRSA1, VRSA2, VRSA3, and VRSA6) exhibited high degree of resistance to oxacillin with an MIC_{oxa} of 64 or >256 μg/ml indicating overexpression of *mecA* or hyperproduction of modified PBP2a, which might have led to the development of vancomycin resistance in these isolates.[36] Various factors are known to influence antibiotic susceptibility pattern or MIC level when tested *in vitro*, such as bacterial inoculum, pH of the media, moisture, thymidine, or thiamine concentration. Even, certain divalent cations or metals are also known to antagonize some antimicrobials resulting in higher MIC levels.[37] However, very high levels of resistance to vancomycin, oxacillin, and cefoxitin as recorded in the present study are very unlikely to be influenced by such factors.

Although the VRSA and VISA isolates characterized with ERIC-PCR produced different band patterns, no distinct pattern or similarity in ERIC profile was noticed among them based on the criteria of animal species, antibiotic resistance pattern, or genotype. This is in contradiction with earlier findings of Ye *et al.* who reported a clear correlation between ERIC-PCR and resistance patterns in some strains.[27] In this study, two caprine isolates namely VRSA2 and VRSA7 were clustered together by ERIC-PCR. However, they belonged to two different *spa* types (Table 1). Interestingly, isolates VRSA3 and VRSA4 obtained from bovine milk from Kolkata district and clustered together by ERIC-PCR were also typed as t267 by *spa* typing. This indicates that these isolates were from the single-genetic lineage. This *spa* type (t267) was previously reported in VRSA isolates from bovine milk in China[38] and Japan.[39] Other than t267, the isolates namely VRSA2, VRSA5, and VRSA6 belonged to t527, t800, and t3626 types, respectively, whereas two isolates (VRSA1 and VRSA7) were nontypeable. The *S. aureus spa* type t800 was previously reported from ICU patients of the Netherlands,[40] UAE, and several other European countries. The other two types—t527 and t3626 were most infrequently reported from Finland, Croatia, and Iran (http://spa.ridom.de/frequencies.shtml). Three isolates (VRSA2, VRSA3, and VRSA4) of the present study were found to carry SCC*mec* type V and one isolate (VRSA7) from goat milk carried SCC*mec* type IV. The SCC*mec* type V was mostly reported as a novel gene cassette in community-associated MRSA strains. Likewise, SCC*mec* type IV is also community acquired SCC*mec* type detected in MRSA strains. Both the types (IV and V) were previously reported in bovine methicillin-resistant non-*S. aureus*.[41] Again, workers from Finland have also reported both SCC*mec* type IV and type V in MRSA and MRSE isolates from bovine mastitis.[42] Of the three isolates with SCC*mec* type V strains, two isolates were with *spa* type t267. MRSA strains with such genetic lineage of MRSA-SCC*mec*V-*spa* t267 were recently reported from bovine mastitis in China.[38] Although MRSA isolates with such genetic background are also recorded from human patients,[43] this kind of genetic characteristics has not been reported in VRSA and VISA strains till date. Nevertheless, the available reports indicate that isolates with such genetic background may cause infection both in animals, as well as human beings.

This is the first ever report of VRSA in food animals, especially in India, where the pathogen is otherwise prevalent in human. Isolation and detection of MRSA in goat milk is a cause of concern, as this was not previously detected and reported.

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Disclosure Statement

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