Molecular determination of methicillin resistance mecA and virulence coa genes in Staphylococcus aureus from pyogenic clinical cases of companion animals in India

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Abstract: Out of 100 pyogenic clinical cases of companion animals, 40 samples revealed Staphylococcus aureus confirmed by genomic amplification of 280-bp amplicons of the nuc gene. The presence of resistance and virulence determinants was confirmed for the genomic and plasmid mec, van, and coa genes. Out of 40 isolates of S. aureus, 23 isolates revealed amplification of 533-bp mecA genes on genomic DNA. These included 4, 14, and 5 isolates of cattle, buffalo, and dogs, respectively. On amplification for the coa gene, 19 isolates revealed amplicons of variable size and number on genomic DNA. The numbers of coa-positive isolates were 4, 12, and 3 of cattle, buffalo, and dogs, respectively. Among coa-positive isolates, thirteen were mecA-positive isolates while the other six isolates were mecA-negative.

Key words: Methicillin-resistant Staphylococcus aureus, resistance, virulence

1. Introduction
Staphylococcus species are among the most common pathogens of human and animals. Staphylococcus aureus naturally resides in the anterior nares of wild and domestic animals and humans (1) and is found to be involved in cases of severe necrotic lesions, abscesses, and bacteremia (2,3). The pathogenicity of staphylococci is traditionally attributed to the ability to produce coagulase and coagulase-negative staphylococci are considered as minor infectious pathogens (4). Coagulase is encoded by the coa gene, which possesses a repeated polymorphic and conserved region (5). These bacteria are the source of a wide variety of resistance genes and more than 40 resistance genes have been reported in staphylococci from animals (6). Out of these, methicillin resistance has emerged as a serious problem in human medicine (7). In recent years there has been an apparent increase in the number of methicillin-resistant Staphylococcus aureus (MRSA) infections in companion animals and it has been reported from cow with mastitis, dogs, horses, chickens, sheep, cats, and pigs (8). Resistance of MRSA to β-lactam antibiotics including penicillinase-stable β-lactam is mediated by the mecA gene (9), whereas the vanA gene acquired from vancomycin-resistant enterococci is responsible for a high level of resistance against vancomycin (10). Many of resistant genes are reported to be located on mobile genetic elements like plasmids and transposons and play a major role in the transfer of resistance to other bacteria (6,11). These resistant staphylococci harboring drug-resistant genes come into close contact with many other pathogenic and nonpathogenic bacteria of the same animal hosts as well as humans in close contact (12) and may act as recipients or donors of resistance genes (11). The presence of resistance to commonly used antibiotics and the ability to transfer resistant genes is of great concern as the animals carrying these bacteria may serve as reservoirs for MRSA infection of humans (7). Hence, in the present study, clinical pyogenic samples of skin infections and wounds of animals were screened for the presence of S. aureus containing methicillin resistance genes and their relation with the presence of the coa gene.

2. Materials and methods
A total of 100 pus samples were collected during the study from different clinical cases such as abscesses, postoperative wounds, wounds, mastitis, and metritis from the cattle, buffalo, and dogs presented at the Teaching Veterinary Clinical Complex (TVCC) of the institute, from nearby Goushalas, and during clinical camps with the help of sterilized swabs (HiMedia, Mumbai, India). The collected swabs were immediately transported to the laboratory, transferred to tryptone soya broth (HiMedia)

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with 6.5% NaCl (Merck, Bengaluru, India), and incubated aerobically at 37 °C overnight. The culture growths obtained were subjected to isolation and identification of S. aureus based on cultural, morphological, and biochemical characteristics as per standard procedures (13). All the presumptive S. aureus were confirmed by genomic DNA-based PCR amplification of species-specific 280-bp nuc genes using a previously reported set of primers and procedure (14).

The genomic and plasmid DNAs from all the S. aureus isolates were extracted employing the phenol-chloroform and alkaline lysis method, respectively (15). The genomic as well as plasmid DNAs of all the isolates were subjected to amplification of the mecA (16) and coa (17) genes using a previously reported set of primers (Table 1) and protocols with slight modification. PCR amplifications were performed in a total volume of 25 µL made up of the template DNA (genomic or plasmid DNA), primers specific for each gene, 2X EmeraldAmp MAX HS PCR Master Mix (Takara Bio Inc., Tokyo, Japan), and nuclease-free water to make up the volume. The amplified PCR products were subjected to agarose gel electrophoresis in 1.5% agarose gel (Genei, Bengaluru, India) containing ethidium bromide for the visualization under a UV transilluminator (Spectroline, Westbury, NY, USA).

### 3. Results

Based on cultural, morphological, and biochemical characteristics followed by positive PCR amplification of nuc genes, out of 100 pus samples collected, 40 were found positive for S. aureus. These included 24 isolates from buffalo (B1–24) and 8 each from cattle (C1–8) and dogs (D1–8). Out of those 40 isolates, all 23 isolates showing methicillin resistance in the drug sensitivity assay revealed amplification of the desired length of the 533-bp mecA gene fragment (Figure 1), while the remaining 17 isolates that were negative for methicillin resistance in the drug diffusion test were found negative for the amplification of mecA genes. The mecA gene-positive 23 isolates included 4, 14, and 5 isolates of cattle, buffalo, and dogs, respectively.

The PCR amplification carried out for detection of coa genes using gene-specific primes revealed amplicons of variable size and numbers for coa genes in 19 isolates (Figure 2). These included 4, 12, and 3 isolates of cattle, buffalo, and dogs, respectively. Among coa-positive isolates, thirteen were mecA-positive while the rest were mecA-negative.

The mecA- and coa-positive and -negative isolates are summarized in Table 2.

### 4. Discussion

Recently, S. aureus has been a focus of attraction in medicine not only for its pathogenicity but more for its ability to overcome the antimicrobial effects of multiple drugs (18). Furthermore, due to the development of drug resistance and ability to attain resistance to multiple drugs, S. aureus has been designated as a superbug (9). The production of nuclease is considered as an indicator of potentially pathogenic staphylococci and nuc genes encoding for thermostable nuclease are considered highly specific for S. aureus (14). Thus, nuc was used for the confirmation of S. aureus in the present study and all the isolates presumptive for S. aureus based on cultural, morphological, and biochemical characters were found positive in the amplification of 280-bp fragments of nuc genes.

There has been exhaustive work on the resistance pattern and development of drug resistance and based on these resistance patterns MRSA is the term used for any strain of S. aureus that has developed resistance to β-lactam antibiotics. Since the first report in veterinary science from mastitic cows (19) it has been reported in almost all domestic species including dogs, horses, chickens, sheep, cats, and pigs (8). Moreover, it has been reported from healthy horses (20), dogs and cats (21), and healthy canine hair coats (22). In the present study, 23 out of 40 S. aureus isolates showed the presence of the mecA gene, which is supposed to be responsible for the methicillin resistance of S. aureus (23). Similarly, all the methicillin-resistant isolates revealed the amplification of the desired length of amplicons of 533 bp corresponding to the mecA gene. The prevalence of MRSA among the S. aureus isolates was 50%, 58.3%, and 62.5% in cattle, buffalo, and dogs, respectively (Table 2). There is scanty information available on the prevalence of MRSA among animals in India. However,
13.1% prevalence of MRSA has been reported in cattle in India (24). In recent reports higher incidences of \textit{S. aureus} (56% and 44.25%) and MRSA (9.61% and 5.11%) were recorded in acute clinical mastitis of cows in India (23,25). The overall prevalence in total pyogenic clinical cases is higher in the present study with 19.05% in cattle, 22.22% in buffalo, and 31.25% in dogs. The higher rate might be due to the recent trend of increase in resistance of \textit{S. aureus} against methicillin all over the world (20,26).

With the emergence of MRSA, methicillin was replaced by vancomycin as the drug of choice (26). However, the excessive use of vancomycin against MRSA led to the emergence of two types of glycopeptide-resistant \textit{S. aureus} (GRSA) (27): vancomycin-intermediate \textit{S. aureus} (VISA) and vancomycin-resistant \textit{S. aureus} (VRSA) (19). VRSA is attributed to the \textit{vanA} gene of enterococci (10). Thus, all 40 isolates were also assessed for drug resistance against vancomycin by drug diffusion test and none of the isolates showed resistance against vancomycin.

Other than resistance to antibiotics, the virulence of \textit{S. aureus} is also attributed to the presence of coagulase-producing ability (28). In PCR-based determination of \textit{coa} gene presence, 19 isolates revealed the amplicon product of desired size with multiple amplicons in a few isolates (Table 2; Figure 2). These included five non-MRSA and 14 MRSA. The pattern was the same irrespective of species and all three species had both MRSA and non-MRSA isolates positive for \textit{coa} genes. These findings suggested that the presence or absence of coagulase-producing ability is not related to drug resistance in \textit{S. aureus}. The presence of more than
one amplicon in some isolates is attributed to the repetitive presence of the coa gene at different loci (17). Furthermore, the findings of the study suggest that coagulase-negative staphylococci, which were traditionally considered to be minor infectious pathogens, are also becoming common (4) and need attention in veterinary medicine as these are currently considered as emerging pathogens of bovine mastitis (8).

To conclude, the present report exposed a glimpse of the urgency required to be addressed in regard to the S. aureus superbug, particularly in reference to MRSA. It is not only increasing in its prevalence but also spreading in more geographical areas. The MRSA prevalence in S. aureus has also increased in the recent decade in India. Based on the findings of this study it can be said that there is an urgent need for more exhaustive study with larger sample sizes as well as more geographical areas. Further regular monitoring of drug resistance patterns with genotyping to assess the genetic modifications and diversity attained by MRSA is also recommended.

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References


