TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2018) 42: 371-375

doi:10.3906/vet-1705-13

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

Ritika YADAV, Amit KUMAR*, Vinod Kumar SINGH, Jayshree SINGH, Sharad Kumar YADAV
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Uphadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura, India

Received: 05.05.2017 • **Accepted/Published Online:** 20.07.2018 • **Final Version:** 12.10.2018

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)

* Correspondence: balyan74@gmail.com

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,

Table 1. PCR primers used in this study

Target gene	Primer sequence 5′ to 3′	Product size (bp)	Reference no.
mecA	F- AAAATCGATGGTAAAGGTTGGC R- AGTTCTGCAGTACCGATTTGC	533	(16)
coa	F- AACAAAGCGGCCCATCATTAAG R- TAAGAAATATGCTCCGATTGTCG	Variable	(17)

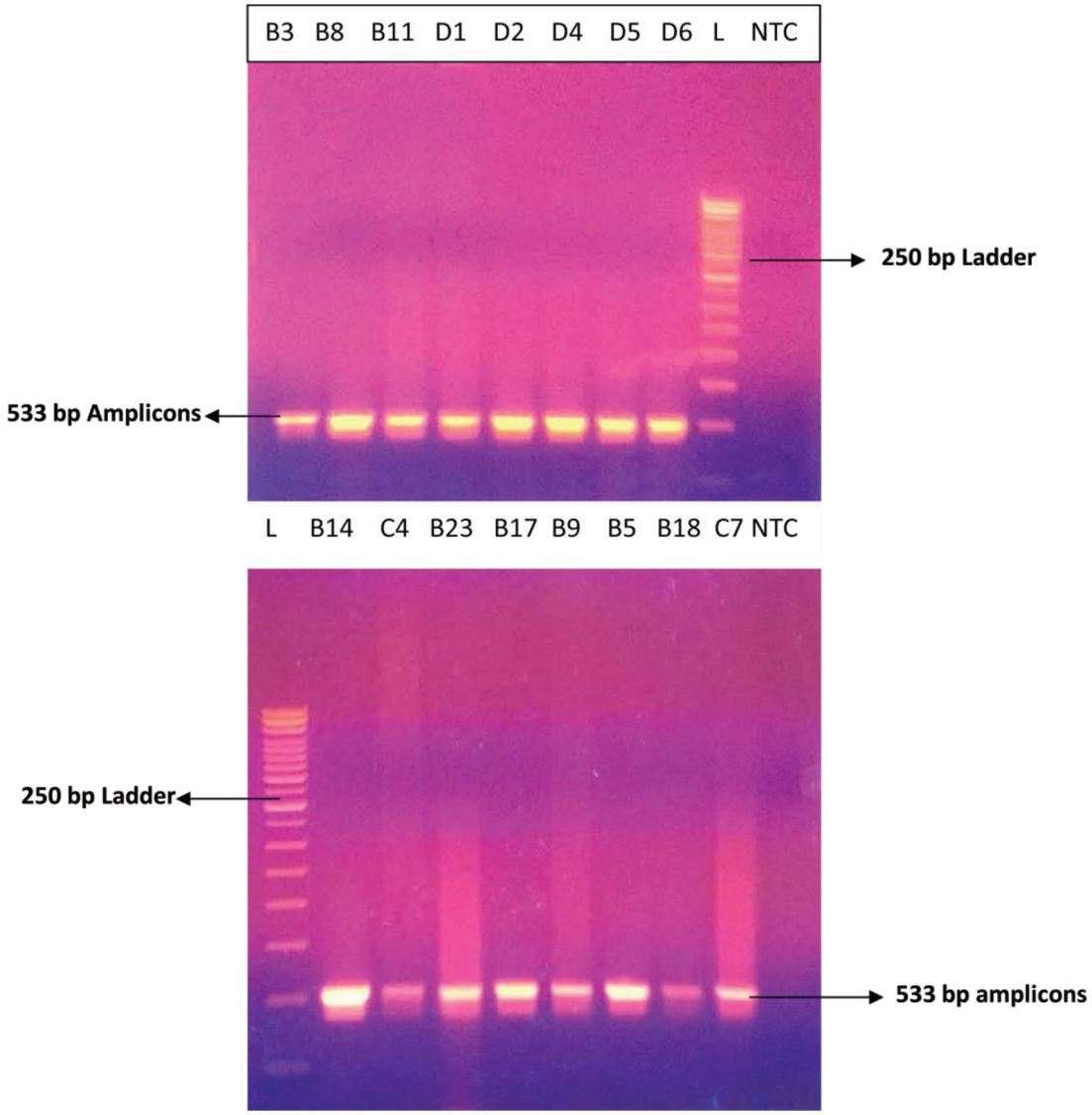

Figure 1. Amplification of the *mecA* gene of MRSA.

13.1% prevalence of MRSA has been reported in cattle in India (24). In recent reports higher incidences of *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 *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 *S. aureus* (GRSA) (27): vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) (19). VRSA is attributed to the *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 *S. aureus* is also attributed to the presence of coagulase-producing ability (28). In PCR-based determination of *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 *coa* genes. These findings suggested that the presence or absence of coagulase-producing ability is not related to drug resistance in *S. aureus*. The presence of more than

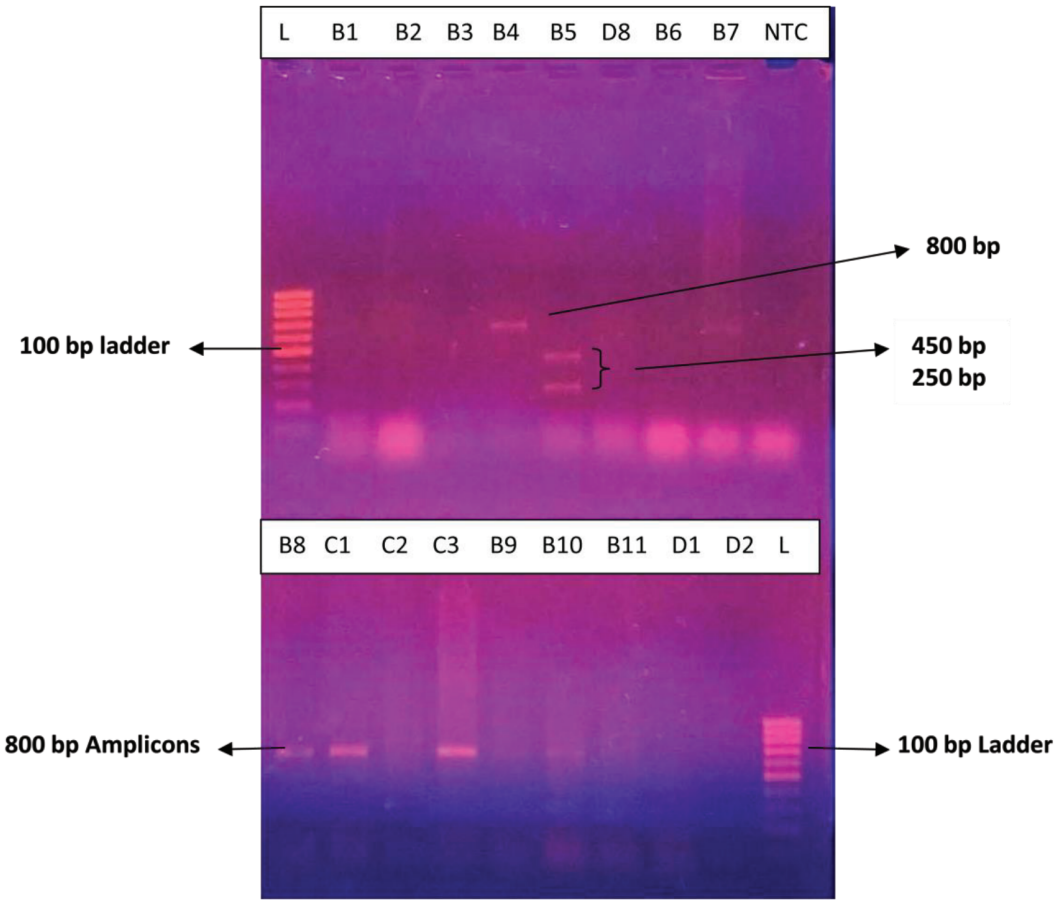

Figure 2. Amplification of the *coa* gene of *S. aureus* isolates.

Table 2. PCR amplification-based species-wise summary of different genes in *S. aureus* isolates.

Gene	Cattle isolates	Buffalo isolates	Canine isolates
mecA gene	C1, C4, C7, C8	B1, B3, B5, B8, B9, B10, B11, B14, B15, B17, B18, B20, B22, B23	D1, D2, D4, D5, D6
coa gene	C1, C2, C3, C7	B1, B3, B4, B5, B7, B8, B10, B11, B14, B15, B17, B23	D1, D2, D6

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.

Acknowledgment

The authors are very thankful to the Dean, College of Veterinary Science and Animal Husbandry, Vice Chancellor of Uttar Pradesh Pandit Deen Dayal Uphadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura, India, for providing all facilities and funding.

References

1. Cuny C, Kock R, Witte W. Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany. Int J Med Microbiol 2013; 303: 331-337.
2. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med 1998; 339: 520-532.
3. Reacher MH, Shah A, Livermore DM, Wale MC, Graham C. Bacteremia and antibiotic resistance of its pathogens reported in England and Wales between 1990 and 1998: trend analysis. Br Med J 2000; 320: 213-216.
4. Huxley JN, Greent MJ, Green LE, Bradely AJ. Evaluation of the efficacy of an internal teat sealer during the dry period. J Dairy Sci 2002; 85: 551-561.
5. Reinoso EB, El-Sayed A, Lammler C, Bogni C, Zschock M. Genotyping of *Staphylococcus aureus* isolated from humans, bovine subclinical mastitis and food samples in Argentina. Microbiol Res 2006; 163: 2200-2203.
6. Cuny C, Friedrich A, Kozytska S, Layer F, Nubel U, Ohlsen K, Strommenger B, Walther B, Wieler L, Witte W. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. Int J Med Microbiol 2010; 300:109–117.
7. Leonard FC, Markey BK. Methicillin resistant *Staphylococcus aureus* in animals: a review. Vet J 2008; 175: 27-36.
8. O'Mahony R, Abbott Y, Leonard FC, Markey BK, Quinn PJ, Pollock PJ, Fanning S, Rossney AS. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Vet Microbiol 2005; 109: 285-296.
9. Batabyal B, Kundu GKR, Biswas S. Methicillin-resistant *Staphylococcus aureus*: A brief review. Int J Biolog Sci 2012; 1: 65-75.
10. Courvalin P. Vancomycin resistance in gram-positive cocci. Clin Infect Dis 2006; 42: S25-34.
11. Kadlec K, Feßler AT, Hauschild T, Schwarz S. Novel and uncommon antimicrobial resistance genes in livestock associated methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 2012; 18: 745-755.
12. Kumar A, Rahal A, Dwivedi SK, Gupta MK. Prevalence and antibiotic profile of bacterial isolates from bovine mastitis in Mathura. Egyptian J Dairy Sci 2010; 38: 31-34.
13. Quinn PJ, Carter ME, Markey BK, Carter GR. Clinical Veterinary Microbiology. London, UK: Mosby; 1994.
14. Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J Clin Microbiol 1992; 30: 1654-1660.
15. Sambrook J, Russell D. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory; 2001.
16. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol 1991; 29: 2240-2244.
17. Montesinos I, Salido E, Delgado T, Cuervo M, Sierra A. Epidemiological genotyping of methicillin resistant *Staphylococcus aureus* by pulsed field gel electrophoresis at a university hospital and comparison with antibiotyping and protein A and coagulase gene polymorphisms. J Clin Microbiol 2002; 40: 2119-2125.
18. De Souza MMS, Coelho SMO, Pereira IA, Soares LC, Pribul BR, Coelho IS. Antibiotic resistance in *Staphylococcus species* of animal origin. In: Pana M, editor. Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium. Rijeka, Croatia: InTech; 2012. pp. 273-302.
19. Devriese LA, Vandamme LR, Fameree L. Methicillin (cloxacillin)-resistant *Staphylococcus aureus* strains isolated from bovine mastitis cases. Zbl Vet Med B 1972; 19: 598-605.
20. De Martino L, Lucido M, Mallardo K, Facello B, Mallardo M, Iovane G, Pagnini U, Tufano MA, Catalanotti P. Methicillin-resistant *staphylococci* isolated from healthy horses and horse personnel in Italy. J Vet Diagn Invest 2010; 22: 77-82.
21. Doyle ME, Hartmann FA, Lee Wong AC. FRI Food Safety Review: Methicillin-Resistant *Staphylococcus aureus* (MRSA): Implications for Our Food Supply? Madison, WI, USA: University of Wisconsin; 2011.
22. White SD, Ihrke PJ, Stannard AA, Sousa C, Reinke S, Rosser EJ, Jang S. Occurrence of *Staphylococcus aureus* on the clinically normal canine hair coat. Am J Vet Res 1983; 44: 332-334.
23. Kutar K, Kumar AK, Sharma B, Kumar A, Yadav SK. Analysis of *mecA* gene and antibiotic resistance in *Staphylococcus aureus* isolates from bovine mastitis. Ind J Comp Microbiol Immunol Inf Dis 2015; 36: 22-27.
24. Kumar R, Yadav BR, Singh RS. Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. J Biosci 2011; 36: 175-188.
25. Chandrasekaran D, Venkatesan P, Tirumurugaan KG, Nambi AP, Thirunavukkarasu PS, Kumanan K, Vairamuthu S, Ramesh S. Pattern of antibiotic resistant mastitis in dairy cows. Vet World 2014; 7: 389-394.
26. Ng ST, Lim CY, Tan CS, Abd Karim A, Haron H, Ahmad N. Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA). Webmed Central Inf Dis 2011; 2: WMC002787.
27. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 1997; 40: 135-136.
28. Harris LG, Foster SJ, Richards RG. An introduction to *Staphylococcus aureus* and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials: review. Eur Cells Mater 2002; 4: 39-60.