Microbiological and Molecular Characterization of Staphylococcus hominis Isolates from Blood

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Abstract

Background: Among Coagulase-Negative Staphylococci (CoNS), Staphylococcus hominis represents the third most common organism recoverable from the blood of immunocompromised patients. The aim of this study was to characterize biofilm formation, antibiotic resistance, define the SCCmec (Staphylococcal Chromosomal Cassette mec) type, and genetic relatedness of clinical S. hominis isolates.

Methodology: S. hominis blood isolates (n = 21) were screened for biofilm formation using crystal violet staining. Methicillin resistance was evaluated using the cefoxitin disk test and the mecA gene was detected by PCR. Antibiotic resistance was determined by the broth microdilution method. Genetic relatedness was determined by pulsed-field gel electrophoresis (PFGE) and SCCmec typed by multiplex PCR using two different methodologies described for Staphylococcus aureus.

Results: Of the S. hominis isolates screened, 47.6% (10/21) were categorized as strong biofilm producers and 23.8% (5/21) as weak producers. Furthermore, 81% (17/21) of the isolates were methicillin resistant and mecA gene carriers. Resistance to ampicillin, erythromycin, and trimethoprim was observed in >70% of isolates screened. Each isolate showed a different PFGE macrorestriction pattern with similarity ranging between 0–95%. Among mecA-positive isolates, 14 (82%) harbored a non-typeable SCCmec type: eight isolates were not positive for any ccr complex; four contained the mec complex A ccrAB1 and ccrC, one isolate contained mec complex A, ccrAB4 and ccrC, and one isolate contained the mec complex A, ccrAB1, ccrAB4, and ccrC. Two isolates harbored the association: mec complex A and ccrAB1. Only one strain was typeable as SCCmec III.

Conclusions: The S. hominis isolates analyzed were variable biofilm producers had a high prevalence of methicillin resistance and resistance to other antibiotics, and high genetic diversity. The results of this study strongly suggested that S. hominis isolates harbor new SCCmec structural elements and might be reservoirs of ccrC1 in addition to ccrAB1 and mec complex A.

Introduction

Coagulase-negative staphylococci (CoNS) represent a group of opportunistic microorganisms commonly associated with infections of immunocompromised patients [1]. Among CoNS, Staphylococcus hominis is one of the three most frequently isolated species recoverable from the blood of neonates and immunosuppressed patients [2,3] and has been associated as a causal agent of bacteremia, septicemia, and endocarditis [3–7]. Nosocomial infections caused by CoNS are associated with the use of indwelling medical devices in combination with biofilm-forming potential of respective isolates [8–10]. However, among the CoNS, S. hominis strains are not typically categorized as a major biofilm producers [9,11]. It has been reported that some S. hominis isolates are resistant to methicillin that is conferred by protein PBP2a encoded by the mecA gene that resides within a mobile genetic element called the Staphylococcal Cassette Chromosome mec (SCCmec) [12]. At present, eleven SCCmec types (I–XI) of have been assigned for S. aureus based on the classes of the mec gene complex (A–E) and the mec gene complex (1–8) [http://www.sccmec.org/Pages/SCC_TypesEN.html]. Some studies have reported that SCCmec elements are more diverse in methicillin-resistant CoNS, with new variants of mec genes continually being identified [13–20].
A recent molecular epidemiologic study of S. hominis isolates conducted by Bouchami et al., 2011, demonstrated low clonality between isolates and the identification of isolates harboring the SCCmec type VI, VIII, and the new SCCmec type composed of mec complex A (in combination with ccrAB1). In addition, some isolates harbored the non-typeable SCCmec in the absence of the ccr complex and others expressed two ccr types (in the same isolate). Additionally, ccrB1 and ccrB4 were identified in mec-A-negative and mec-A-positive isolates with high nucleotide sequence homology to genes present in S. aureus isolates expressing SCCmec I, VI, or VIII, respectively (>95%) [21]. In agreement with a report by Hansen et al., 2004 staphylococcal strains from the same geographical region possess identical ccr genes that differ from sequences of strains from other regions. There is evidence of horizontal SCCmec gene transfer between CoNS and S. aureus [22,23]; therefore, characterization of SCCmec of S. hominis can provide useful information regarding the evolution and mobilization of this element from this species. The aim of this study was to characterize biofilm formation potential, antibiotic resistance, SCCmec type, and genetic relatedness of 21 S. hominis clinical isolates obtained from blood cultures.

Materials and Methods

Ethics Statement
This study was performed with the approval of the Local Ethics Committee of the School of Medicine of the Universidad Autónoma de Nuevo León (Approval MB11-006). Informed consent was not required since bacterial isolates were the subject of this study. Isolates, not human beings were studied. Thus, informed consent was not required by the local Ethics Committee.

Clinical isolates
S. hominis clinical isolates (n = 21) were collected between January 2006 and December 2011 from blood cultures from two hospitals in Mexico: Hospital Civil Fray Antonio Alcalde and Hospital Universitario Dr. José Eleuterio González. All isolates were causative agents of Laboratory-Confirmed Bloodstream Infection (LCBI) according to CDC criteria [http://www.cdc.gov/nhsn/pdfs/pscmanual/17pscnosinfdef_current.pdf]. Isolates examined met at least one of the following criteria: a) Patient had a recognized pathogen cultured from two or more blood cultures and organisms cultured from blood were not related to an infection at another site, b) Patient had at least one of the following signs or symptoms: fever (>38°C), chills, or hypotension and positive laboratory results not related to an infection at another site. Isolates were kept frozen in Brucella broth containing 15% glycerol at −70°C. Only one isolate per patient was included in this study.

Identification of isolates. Isolates were identified at the species level using API Staph galleries (bioMérieux, Inc., Durham, NC) according to the manufacturer’s instructions. Species identification was confirmed by partial sequencing of the 16S rRNA and the tuf genes as previously described [24]. Sequencing was performed at the Instituto de Biotecnología, Universidad Nacional Autónoma de México. DNA sequences were compared to gene sequences at the National Center for Biotechnology Information (NCBI) GenBank using the BLAST algorithm [http://www.ncbi.nlm.nih.gov/BLAST].

Phenotypic biofilm assay. Semi quantitative determination of biofilm formation was performed by crystal violet staining as previously described [10,25]. All isolates were tested in quadruplicate in two different experiments conducted on different days. These assays were conducted on polystyrene 96-well flat bottom, untreated plates with a low evaporation lid. Biofilm-forming capacity of all isolates was tested under two different growth conditions: in tryptic soy broth (TSB) supplemented with 1% glucose (TSBglu) or in TSB supplemented with 3% NaCl (TSB NaCl). Briefly, biofilm samples stained with crystal violet were dissolved in an ethanol-acetone mixture (70:30). The optical density of these solutions was subsequently measured at 550 nm. To simplify the data we used the ordinal classification for the level of biofilm production proposed by Christensen et al. Isolates with optical densities OD ≥0.25 were considered strong biofilm producers and isolates with optical densities between 0.15 and 0.24 were considered weak biofilm producers.

Staphylococcus saprophyticus ATCC 15305 (biofilm producer) and S. hominis ATCC 27844 (biofilm non-producer) were used as control organisms.

Methicillin resistance and susceptibility testing. Methicillin resistance was evaluated using the cefoxitin disk test and the mecA gene was detected by polymerase chain reaction (PCR) [26,27]. During the cefoxitin disk evaluation, isolates were considered resistant if measurements were ≥24 mm and susceptible if measurements were ≤25 mm [27]. Susceptibility testing was performed using the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [27]. The antibiotics tested were penicillin, ampicillin, amoxicillin-clavulanic acid, cefotaxime, vancomycin, daptomycin, gentamicin, erythromycin, tetracycline, ciprofloxacin, nitrofurantoin, trimethoprim, chloramphenicol, rifampin, and linezolid (Sigma Aldrich, Toluca, Mexico).

SCCmec and PFGE typing. SCCmec, ccr, and mec class typing was performed as previously described by Zhang et al. [26] and Kondo et al. [28] with modification to three primers as previously described Ruppe et al. [29]. All SCCmec typing experiments were performed in duplicate. As control strains we used for all PCR reactions isolates previously typed by Garza-González et al., 2010: Staphylococcus epidermidis JC-5, JC-6, JC-28, JC-30, JC-488, JC-1439 and Staphylococcus haemolyticus JC-2165 [14,30]. PFGE was performed as described for S. aureus [31] with modifications to the restriction enzymes used and running conditions were as previously described by Bouchami et al. [21]. S. hominis DNA samples were digested with the XbaI endonuclease and bands were separated using a CHEF-DRIII instrument (Bio-Rad Laboratories, Hercules, CA). Band patterns were generated by visual analysis using Labworks 4.5 software with 1% of tolerance. The similarity coefficients were generated from a similarity matrix calculated using the Jaccard coefficient (SPSS 20.0 software).

Results

Biofilm formation
By assay with TBSglu, 47.6% (10/21) of the S. hominis isolates were categorized as strong biofilm producers (defined by the cutoff values used in this study). Weak biofilm production was observed in 23.8% (5/21) of the isolates and 28.6% (6/21) were non-producers. Whereas by assay with TBS NaCl, 33.3% (7/21) were strong biofilm producers, 23.8% (5/21) weak producers, and 42.9% (9/21) non-producers (Table 1).

Methicillin resistance and susceptibility testing
Most isolates, 81% (17/21), showed methicillin resistance by the cefoxitin disk test, and all isolates tested positive for the mecA gene (Table 1). All S. hominis isolates were resistant to at least one of the non-β-lactam antibiotics tested. Resistance rates for penicillin, ampicillin, amoxicillin-clavulanic acid, erythromycin, trimethoprim, ciprofloxacin, tetracycline, chloramphenicol, gentamicin,
Table 1. Molecular and phenotypic characterization of *S. hominis* blood isolates.

| Isolate | Biofilm | FOX | meca | Zhang | Kondo | SCCmec type | Resistance profile |
|---------|---------|-----|------|-------|-------|-------------|--------------------|
|         | Glu NaCl | FOX | meca | ccr  | meca | ccr         |                    |
| 397     | Strong   | Strong | R | Pos  | A | 1-5 | A | 1-5 | UT1 | PEN, AMP, AUG, GEN, ERY, TET, TMP, RIF |
| 501     | Weak     | Neg | S | Neg | Neg | Neg | Neg | Neg | PEN, AMP, AUG, ERY, CIP |
| 1786    | Neg      | Neg | R | Pos  | B | Neg | B | Neg | UT | PEN, AMP, AUG, CTX, GEN, ERY, TET, CIP, TMP, CHL |
| 8115    | Neg      | Neg | R | Pos  | A | 1-5 | A | 1-5 | UT1 | PEN, AMP, AUG, ERY, CIP, TMP, CHL |
| 8122    | Neg      | Neg | R | Pos  | A | 1-5 | A | 1-4+5 | UT2 | PEN, AMP, AUG, ERY, TET, CIP, TMP, CHL |
| 8125    | Neg      | Neg | S | Neg | Neg | Neg | Neg | Neg | PEN, AMP, AUG, ERY, TMP |
| 8127    | Weak     | Neg | R | Pos  | A | Neg | A | Neg | UT | PEN, AMP, AUG, ERY, TET, TMP, DAP* |
| 8129    | Weak     | Weak | R | Pos  | A | 1 | A | 1 | New | PEN, AMP, AUG, GEN, ERY, TET |
| 8144    | Neg      | Neg | R | Pos  | A | Neg | A | Neg | UT | PEN, AMP, AUG, ERY, TET, CIP, TMP, CHL |
| 8179    | Neg      | Neg | R | Pos  | A | 3 | A | 3 | III | PEN, AMP, AUG, TMP, CHL, DAP* |
| 9241    | Strong   | Weak | S | Neg | Neg | Neg | Neg | Neg | PEN, AMP, AUG, ERY |
| 9989    | Strong   | Strong | R | Pos  | A | 1 | A | 1 | New | PEN, AMP, AUG, ERY, CIP, TMP, CHL |
| 9994    | Strong   | Weak | R | Pos  | A | 5 | A | 4+5 | UT3 | PEN, AMP, AUG, GEN, ERY, TET, TMP, CHL |
| 10866   | Weak     | Neg | R | Pos  | A | Neg | A | Neg | UT | PEN, AMP, AUG, ERY, CIP, TMP, DAP* |
| 11200   | Strong   | Weak | S | Pos  | B | Neg | B | Neg | UT | PEN, AMP, AUG, ERY, TET, CIP, NT, TMP, RIF, DAP* |
| 11477   | Strong   | Strong | S | Neg | Neg | Neg | Neg | Neg | PEN, AMP, AUG, ERY, DAP* |
| 11621   | Strong   | Strong | R | Pos  | A | Neg | A | Neg | UT | PEN, AMP, AUG, ERY, TMP, CHL, DAP* |
| 11628   | Strong   | Strong | R | Pos  | A | Neg | A | Neg | UT | PEN, AMP, AUG, GEN, ERY, CIP, TMP, DAP* |
| 11630   | Strong   | Strong | R | Pos  | A | 1-5 | A | 1-5 | UT1 | PEN, AMP, AUG, GEN, ERY, CIP, TMP, DAP* |
| 11631   | Strong   | Strong | R | Pos  | A | 1-5 | A | 1-5 | UT1 | PEN, AMP, AUG, CTX, GEN, ERY, TET, CIP, TMP, CHL, RIF, DAP* |
| 11634   | Weak     | Weak | R | Pos  | A | Neg | A | Neg | UT | PEN, AMP, AUG, ERY, DAP* |

1 Neg: biofilm non-producer; Glu: media with 1% glucose; NaCl: media with 3% NaCl.
2 FOX: cefoxitin test; R: resistant; S: susceptible.
3 Pos: meca gene present; Neg: meca absent.
4 Neg: not amplified.
5 SCCmec type III was assigned for *S. aureus* according to http://www.sccmec.org/Pages/SCC_TypesEN.html.
6 Resistance profile: PEN: penicillin; AMP: ampicillin; AUG: amoxicillin-clavulanic acid; ERY: erythromycin; TMP: trimethoprim; CIP: ciprofloxacin; TET: tetracycline; CHL: chloramphenicol; GEN: gentamicin; RIF: rifampin; NIT: nitrofurantoin; CTX: cefotaxime; DAP: daptomycin.

*Non-susceptible.

All isolates were susceptible to vancomycin and linezolid.

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rifampin, nitrofurantoin, and cefotaxime for all isolates were 95%, 95%, 95%, 95%, 76%, 52%, 43%, 38%, 33%, 19%, 10%, and 10%, respectively. Furthermore, 48% of isolates were daptomycin non-susceptible. None of the 21 isolates tested in this study were found to be resistant to vancomycin or linezolid. The 4 mecA-negative isolates showed resistance to at least one of the β-lactam antibiotics tested. No correlation was found between the level of biofilm production and the resistance phenotype.

SCCmec and PFGE typing

A high frequency of mec complex class A (88.2%), ccrAB1 (41.1%), and ccrC (35.3%) was observed among mecA-positive S. hominis isolates (Table 1).

Among the 17 mecA-positive isolates a high proportion were non-typeable (82%), eight were negative for ccr complex tested by both methods (UT); four isolates had a mec complex AccAB1 and ccrC (UT1), one isolate had the mec complex A, ccrAB4 and ccrC (UT2), and; one isolate had the mec complex A, ccrAB1, ccrAB4 and ccrC (UT3). Two isolates carried association mec complex A and ccrAB1. One strain had SCCmec type III described for S. aureus (Table 1) (mec complex A, ccr 3, and isolate 0179).

PFGE analysis of S. hominis isolates identified 21 different restriction patterns with at least 3 band differences between each isolate (Figure 1). Although a 100% similarity was not observed between isolates, two isolates had 95% similarity (11630 and 11631) and were categorized as strong biofilm producers, mecA-positive, mec class A, ccrAB1+ccrC, and only differed in their susceptibility pattern.

Discussion

Most studies examining the presence of SCCmec among CoNS isolates have included in their respective analyses few S. hominis clinical isolates recovered from catheters, the catheter insertion site, pus, wound secretions, cerebral spinal fluid, or blood [11,13,14,21,30,32–34]. S. hominis comprises part of the normal flora colonizing the skin and mucous membranes of humans and may be found as a culture contaminant. However, detection of S. hominis is indicative of an infection and a probable causative agent of bacteremia. In this study, we analyzed 21 S. hominis clinical isolates recovered from blood and were causative agents of Laboratory-Confirmed Bloodstream Infection (LCBI) according to CDC criteria. To our knowledge, this is the first report characterizing S. hominis isolates identified as causative agents of bacteremia recovered from the blood at the microbiological and molecular level.

A significant observation associated with the S. hominis isolates studied was the ability of almost half of these strains (47.6%) to produce biofilm (since S. hominis is not known as a major biofilm producer) [9,11]. This characteristic represents a significant virulence factor since biofilms facilitate bacterial adherence to biomedical surfaces (such as catheters), thereby facilitating their entrance into the bloodstream [8]. However, the polysaccharide or...
protein composition of S. hominis biofilms (or genes involved on its production) remains unknown to date. Among the mecA-positive isolates (81%), nearly half were carriers of a putative new SCCmec. In addition, most expressed the mec gene class A, ccr type 1, and others ccr type 5. This combination of mec-ccr complexes has been reported in this bacterial species before [11,14,21,32].

The mec-ccr complexes identified in this study were similar to those reported by Bouchami et al. that demonstrated that S. hominis could serve as a mec-ccr reservoir and also serve as a likely donor of mec type A to other bacterial species. Unlike that study, we found a higher proportion of non-typeable isolates (82%) and isolates harboring mec (29%).

The data regarding SCCmec diversity in CoNS presented in this study may be biased due to the typing methodology used that was in the interpretation of these data. Therefore, a variety of non-typeable elements in CoNS may be simply an indication that S. hominis elements are different enough from those of S. aureus that the present typing methods can not be applied to this CoNS.

Data presented in this report also demonstrated that most isolates with new or untypeable SCCmec were resistant to at least three antibiotic classes, and some isolates presented with two or three recombine complexes types, suggesting the presence of multiple SCCmec elements in tandem. However, to verify this, the S. hominis SCCmec cassette should be sequenced completely and compared to the S. aureus cassette. This analysis is currently underway in our laboratory.

We found that the 82% of mecA-positive isolates were untypeable and neither of the two methods used amplified any of the know recombine suggesting that these strains are therefore likely candidates for carrying novel SCCmec types. This observation was previously described for S. hominis [11,13,21,30,33,34] and may be explained by: a) that this cassette is a carrier of a new recombine not related to ccrAB or ccrC genes, b) they represent new ccr complex isotypes that cannot be amplified by currently utilized ccr primers, or c) ccr genes were not present [23].

In this study, we identified a high rate of methicillin resistance (81%) in addition to resistance to other antibiotics among the clinical isolates studied; an observation previously reported for S. hominis and other CoNS species [17,21]. All methicillin resistant isolates were also positive for SCCmec in addition to displaying resistance to most β-lactams antibiotics tested.

Among the S. hominis isolates collected in the present study none were clonal, therefore we concluded that infections caused by these isolates were not caused by dissemination of the same isolate throughout the hospital. Taking into account the fact that S. hominis is a component of the normal skin and mucous membrane flora, it is likely that these infections were endogenous.

In conclusion, our results showed that S. hominis is a biofilm producer and in combination with its high resistance rate to antibiotics, renders this species a serious threat for infections in immunocompromised patients. Finally, S. hominis isolates may possess different SCCmec types compared to those present in S. aureus.

Author Contributions
Revised the final version of the manuscript: SMO RMO EJR LDT SFT GMGG LV T EG. Conceived and designed the experiments: EGG RMO. Performed the experiments: SMO SFT. Contributed reagents/ materials/analysis tools: EJR LDT GMGG LV. Wrote the paper: SMO.

References
1. Hirata AI, Edwards JR, Patel J, Horan TC, Sievert DM, et al. (2000) NHSS annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol 29: 996–1011.
2. An WO, Houshi I, Rivera J, Cairo J, Hachem R, Raad I (2011) Comparing clinical and microbiological methods for the diagnosis of true bacteremia among patients with multiple blood cultures positive for coagulase-negative staphylococci. Clin Microbiol Infect 17: 569–571.
3. Chaves F, Garcia-Alvarez M, Sanz F, Alba O, Otero JR (2005) Nosocomial spread of a Staphylococcus hominis subsp. nov. biovar strain causing sepsis in a neonatal intensive care unit. J Clin Microbiol 43: 4877–4879.
4. Palazzo IC, d’Azevedo PA, Secchi C, Pignatari AG, Durini AL (2008) Staphylococcus hominis subsp. nov. biovar strains causing nosocomial bloodstream infection in Brazil. J Antimicrob Chemother 62: 1222–1226.
5. Sunbul M, Demirag MK, Yılmaz O, Yılmaz H, Öztrak R, et al. (2006) Pacemaker lead endocarditis caused by Staphylococcus hominis. Pacing Clin Electrohyd 29: 543–545.
6. Cunha BA, Eckrick MD, Larusso M (2007) Staphylococcus hominis native mitral valve bacterial endocarditis (SBE) in a patient with hypertrophic obstructive cardiomyopathy. Heart Lung 36: 300–302.
7. d’Azevedo PA, Trancesi R, Sales T, Monteiro J, Gales AC, et al. (2008) Outbreak of Staphylococcus hominis subsp. nov. biovar bloodstream infections in Sao Paulo city, Brazil. J Clin Microbiol 47: 253–257.
8. Fredheim EG, Klingenberg C, Rohde H, Frankenberger S, Gaustad P, et al. (2009) Biofilm formation by Staphylococcus haemolyticus. J Clin Microbiol 47: 1172–1180.
9. de Allori MC, Jure MA, Romero C, de Castillo ME (2006) Antimicrobial resistance and production of biofilms in clinical isolates of coagulase-negative Staphylococcus strains. Biol Pharm Bull 29: 1592–1596.
10. Christensen GD, Simpson WA, Younger J, Baddour LM, Barrett F, et al. (1985) Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol 22: 996–1006.
11. Garza-Gonzalez E, Lopez D, Pezina C, Munuet W, Bocanegra-Garcia V, et al. (2011) Diversity of staphylococcal cassette chromosome mec structures in coagulase-negative staphylococci and relationship to drug resistance. J Med Microbiol 59: 323–329.
12. Pi B, Yu M, Chen Y, Yu L, Li L (2009) Distribution of the SCCmecIII gene class A, ccr genes were not present [23].
24. Heikens E, Fleer A, Paauw A, Florijn A, Fluit AC (2005) Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. J Clin Microbiol 43: 2286–2290.
25. Klingenberg C, Aarag E, Ronnestad A, Soldal JE, Abrahamsen TG, et al. (2005) Coagulase-negative staphylococcal sepsis in neonates. Association between antibiotic resistance, biofilm formation and the host inflammatory response. Pediatr Infect Dis J 24: 817–822.
26. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM (2005) Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. J Clin Microbiol 43: 5026–5033.
27. CLSI (2012) M100-S22. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute.
28. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, et al. (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother 51: 264–274.
29. Ruppe E, Barbier F, Mesi Y, Maiga A, Cojocaru R, et al. (2009) Diversity of staphylococcal cassette chromosome mec structures in methicillin-resistant Staphylococcus epidermidis and Staphylococcus haemolyticus strains among outpatients from four countries. Antimicrob Agents Chemother 53: 442–449.
30. Garza-Gonzalez E, Morfin-Otero R, Liaca-Díaz JM, Rodríguez-Noriega E (2010) Staphylococcal cassette chromosome mec (SCC mec) in methicillin-resistant coagulase-negative staphylococci. A review and the experience in a tertiary-care setting. Epidemiol Infect 138: 645–654.
31. Muechau S, Kaufmann ME, Deplano A, de Ryck R, Struelens M, et al. (2003) Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant Staphylococcus aureus: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J Clin Microbiol 41: 1574–1583.
32. Bouchami O, Achour W, Mehni MA, Rolo J, Ben Hassen A (2011) Antibiotic resistance and molecular characterization of clinical isolates of methicillin-resistant coagulase-negative staphylococci isolated from bacteremic patients in oncohematology. Folia Microbiol (Praha) 56: 122–130.
33. Hansen AM, Solild JU (2007) Multiple staphylococcal cassette chromosome chromosomes and allelic variants of cassette chromosome recombinases in Staphylococcus aureus and coagulase-negative staphylococci from Norway. Antimicrob Agents Chemother 51: 1671–1677.
34. Ibrahem S, Salmenlinna S, Virolainen A, Kerttula AM, Lyytikainen O, et al. (2009) carriage of methicillin-resistant Staphylococci and their SCCmec types in a long-term-care facility. J Clin Microbiol 47: 32–37.