Associations between dru Types and SCCmec Cassettes

Mette D. Bartels1*, Kit Boye1, Duarte C. Oliveira3,4, Peder Worning1, Richard Goering5, Henrik Westh1,2

1 Department of Clinical Microbiology, Hvidovre Hospital, Hvidovre, Denmark, 2 Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, 3 Centro de Recursos Microbiológicos (CREM), Faculdade de Ciências e Tecnologia, Univ. Nova de Lisboa, Caparica, Portugal, 4 Instituto de Tecnologia Química e Biológica (ITQB), Univ. Nova de Lisboa, Oeiras, Portugal, 5 Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska, United States of America

Abstract

Molecular typing is an important tool in the investigation of methicillin resistant Staphylococcus aureus (MRSA) outbreaks and in following the evolution of MRSA. The staphylococcal cassette chromosome mec (SCCmec) contains a hypervariable region with a variable number of 40 bp repeats named direct repeat units (dru). The dru region has been suggested as a supplementary typing method for MRSA and an international nomenclature exists. The purpose of this study was to investigate the diversity and variability of the dru region in a diverse collection of MRSA. We studied 302 MRSA isolates harbouring SCCmec types I to VI. The isolates represented a broad genetic background based on Staphylococcal protein A (spa) typing and multilocus sequence typing (MLST) and included 68 isolates (68 patients) from an outbreak with t024-ST8-IVa and 26 isolates from the same patient. Sequencing identified 53 dru types (dt) in 283 isolates, while eighteen isolates contained no dru repeats and one isolate resisted sequencing. The most common dru type, dt10a, was present in 53% of the sequenced isolates and was found in all SCCmec types, except type II. Seven (10%) of the 68 epidemiologically related patients had isolates with dru type variants indicating that dru typing is not useful as a first line epidemiological typing tool. However, MRSA isolates cultured from a single patient over a three year period exhibited a single dru type. The finding of dt10a in most SCCmec types suggests that dru and mecA originate from the same Staphylococcus species.

Introduction

Our understanding of MRSA evolution and outbreaks has substantially increased as sequence-based typing methods have been more commonly used [1]. Multilocus sequence typing (MLST) is preferred for evolutionary studies, but is time consuming and expensive. Sequencing of the Staphylococcal protein A (spa) gene is a commonly used sequence-based typing method for local MRSA outbreak investigations and has excellent interlaboratory reproducibility [2,3]. Typing of the staphylococcal cassette chromosome mec (SCCmec) adds further information and is predominantly done by PCR fragment analysis [4–6] but sequence-based typing methods based on structures in the SCCmec, such as ccrB typing and dru typing have been introduced [7,8]. SCCmec typing, when combined with MLST and/or spa typing, is central in MRSA typing as it allows identification of international MRSA clones [1,9].

In 1991, Ryffel et al [10] described the region between IS431mec and the mecA gene in SCCmec. This region was named the hypervariable region (HVR) due to DNA length polymorphisms. Ryffel and colleagues sequenced the HVR of one MRSA strain and identified ten direct repeat units (dru) of 40 bp each. Most initial studies compared the gel band sizes or hybridization patterns of dru PCR products [11–16] while a few smaller studies sequenced the dru region [17,18]. The sequencing of the dru region of 277 EMRSA-15 and EMRSA-16 led to a universal nomenclature [7,19] and since then many new dru types have been reported [20–22]; www.dru-typing.org.

We studied the dru region of 302 MRSA isolates of global geographic distribution representing a broad range of genetic backgrounds and the SCCmec types I to VI. The collection included sixty-eight isolates from epidemiologically related Danish patients with t024-ST8-IVa and 26 isolates over a four-year period from the same patient. The purpose of our study was to investigate the diversity of the dru region in relation to different SCCmec cassettes and to evaluate its variability in both an epidemiological outbreak and over time in the same patient.

Materials and Methods

MRSA Isolates

Three-hundred and two MRSA isolates were included. Seventy-three of the isolates were from the International collection of Herminia de Lencastre and 229 were isolated in Denmark. The collection contained 65 spa types. The isolates harboured SCCmec types I to VI plus several SCCmec subtypes (Table 1). Only one isolate per patient was included except for one patient where 26 isolates, cultured from May 2005 to November 2009, were examined for longitudinal dru diversity. To test variability of the dru region during a seven year long MRSA outbreak we included 68 isolates of the same spa and SCCmec type from 68 patients that were epidemiologically linked.

Typing: All isolates were spa typed and SCCmec typed as previously described [4]. Some SCCmec type IV, type I and type III isolates from the International collection were subtyped [6,23]. Multilocus sequence typing (MLST) was available on all the
confirmed by resequencing. Chromas Pro (Technelysium Pty, Australia). New repeats were primary PCR. Sequences were analyzed and aligned using and sequenced on both strands using the same primers as in the present study, the bin distance was set to 0.5%, (i.e., the distance between two entries with 99.5 to 100% similarity was set at 0) on the MST, and the distance between two entries with 99 to <99.5% similarity was 1. Using the MSTs, dru types were deemed to belong to different subgroups if they were separated by an MST distance of =2 (i.e., if they showed ≤90.5% similarity). Therefore, if two dru types were at an MST distance of <2, they were considered to be closely related (i.e.,they formed a subgroup).

### Results

Two-hundred and eighty-three of the 302 isolates (94%) had a dru type and were all identified by the first primer set. Additionally one isolate was PCR positive by the first primer set but resisted sequencing. Seventeen isolates were dru negative by all three primer sets and one isolate had a double band with the primer set druGR/druGF but when sequenced contained no dru repeats. The 19 dru sequence negative isolates harboured SCCmec I (6 isolates), SCCmec II (11 isolates), SCCmec IV (1 isolate) and one isolate had lost part of SCCmec when retrieved from the −80°C freezer. In one dru PCR negative isolate (spa type 003, SCCmec II), the region between the mec and IS431 was sequenced and contained no dru repeats. Among the 283 dru sequenced isolates we found 53 dru types. The dru types contained from two to 14 repeats with the majority of dru types having 10 repeats (13 sequence variants) (Figure 1). The most common dru type, dt10a, was found in 53% (161) of the sequenced isolates and identified in all SCCmec types except type II. In Figure 1, dt10a is compared to the other 52 dt types. When excluding the SCCmec non-typeable (NT) isolates, forty-five dru types were restricted to one SCCmec type. However, 36 of these dru types were only found in one or two isolates each. The remaining nine dru types were found in three to 14 isolates with two to five spa types in each group belonging to two or three different CCs (Table 2). Seven dru types were found in at least two SCCmec types when excluding the non-typeable cassettes (Table 3).

The patient with 26 MRSA isolates over a four-year period had her first MRSA isolated in May 2005, where we found different spa types and dru types in five samples taken on the same day: t024-SCCmecV-dt11p (throat and groin), t024-V-dru negative (wound), t024-V-dt8l (axilla) and t292-V-dt11p (axilla). In October 2005 a t024-V-dt11b isolate was isolated from her groin. Just one month later, t024-I-Va-dt10a was found in three samples and continued to be the type cultured from the patient throughout 2006 (seven samples), 2007 (one sample), 2008 (three samples) and 2009 (three samples), except for one sample from January 2006 that was similar to the October 2005 t024-V-dt11b isolate.

To test the variability of the dru region in an outbreak situation, 68 epidemiologically related isolates of spa type t024-Iva from 68 patients were analyzed. These patients had either been admitted to the same hospital at the same time or lived in the same nursing homes. Sixty-one of the 68 isolates had dt10a, while the remaining seven isolates had dt2a (1), 7h (1), 7i (2), 8l (3). Two of the dt8l and dt7h were isolated in 2004, dt2a in 2005, the two dt7i isolates in

### Table 1. SCCmec type of the 302 isolates.

| SCCmec type | Number of isolates |
|-------------|--------------------|
| I           | 14                 |
| IA          | 5                  |
| I-VA        | 1                  |
| II          | 17                 |
| III         | 6                  |
| IIIA        | 11                 |
| IIB         | 1                  |
| IV          | 15                 |
| IVa         | 136                |
| IVb         | 1                  |
| IVc         | 28                 |
| IVd         | 3                  |
| IVe         | 1                  |
| IVg         | 5                  |
| IVh         | 7                  |
| IV-NTa      | 1                  |
| V           | 35                 |
| VI          | 3                  |
| NTb         | 12                 |
|            | 302                |

*as not been subtyped.
*Subtyping gave no result.
*non-typeable.

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International isolates and was performed on at least one isolate of each spa type in the Danish collection [24]. For dru PCR, the primers HVR1:5' ACTATTCCCTGAGGTGTC 3' and HVR2:5' GGAATTTAGTACGTC 3' were used [11]. Isolates that were dru PCR negative by this primer set were tested with an additional two primer sets to determine whether the dru region had been deleted:

druF2:5'CAAAGATCCCTGAGGTGTC 3’ and druGF:
5'GTAACATTATTCCCTGAGGTGTC 3’ and druGR:
5'GCCGATTCGTAGGTGTC 3’ and druGR:
5'TTCAGATTACAACACTTCACCAGG 3’. In one dru PCR negative isolate the region between mecA and IS431 was sequenced using the following primers: 5’CGGCTACAGTGATAACATCC 3’ and 5’TCCAGATTACAACACTTCACCAGG 3’. All dru PCR products were enzymatically purified as previously described [25] and sequenced on both strands using the same primers as in the primary PCR. Sequences were analyzed and aligned using Chromas Pro (Technelysium Pty, Australia). New repeats were confirmed by resequencing. dru repeats (dr, 40 bps) and dru types (dt, the combination of dru repeats) were named according to the DSI model and used to construct a minimum spanning tree (MST); the type with the greatest number of related types is assigned as the root node, and the other types derive from this node. In the present study, the default parameters were used for alignment of sequences. The software creates groups of certain distance intervals or similarity values (which BioNumerics terms bins) and converts the data into distance units. Because of the highly clonal nature of the MRSA isolates investigated in the present study, the bin distance was set to 0.5%, (i.e., the distance between two entries with 99.5 to 100% similarity was set at 0) on the MST, and the distance between two entries with 99 to <99.5% similarity was 1. Using the MSTs, dru types were deemed to belong to different subgroups if they were separated by an MST distance of =2 (i.e., if they showed ≤90.5% similarity). Therefore, if two dru types were at an MST distance of <2, they were considered to be closely related (i.e.,they formed a subgroup).
2006 and one dt8l in 2008. Within the same nursing home three different dts were found (10a, 7h and 8l).

**Discussion**

In this study we found 53 dru types in 281 MRSA isolates suggesting a high diversity in the dru region. Thirty-three new dru types were found. We believe that the main reason we identified so many new dru types was that our collection was selected to be very diverse in regard to genetic background, SCCmec types and geography. Identical SCCmec types were often found to have different dru types and the same dru type was sometimes found in different SCCmec types. A single dru type, dt10a, dominated (53%) both in the Danish and

![Figure 1. Minimum Spanning Tree generated using the BioNumerics software program representing the 53 dru types observed in the study isolates.](image)

Numeral values on the branches indicate the similarity (MST distance) between different dru types. BioNumerics software creates similarity values (termed bins) and converts these data into distance units. The bin unit distance was set to 0.5% (i.e., dru types at a distance of 1 on the MST have between 99 and 99.5% similarity, types at a distance of 2 have between 98.5 and 99% similarity, etc.). The dru types were assigned to the same (colored) cluster if they were separated by an MST distance of <2 (i.e., if they showed >98.5% similarity).

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![Table 2. dru types found in more than two isolates and restricted to one SCCmec type.](table)

| dru type | SCCmec type | spa types (ST/CC) | Number of isolates |
|----------|-------------|-------------------|--------------------|
| dt9g     | IVa         | t008 (8/8)        | 14                 |
| dt11p    | V           | t008 (8/8), t024 (8/8), t084 (15/15), t292 (8/8), t127 (748/1) | 11                 |
| dt11a    | V           | t018 (36/30), t024 (8/8), t123 (45/45), t591 (1/1) | 7                  |
| dt9a     | II          | t002 (5/5), t018 (36/30) | 5                  |
| dt8l     | IVa         | t024 (8/8), t126 (72/72) | 5                  |
| dt11    | III         | t037 (239/8), t421 (239/8) | 5                  |
| dt10u    | IVc         | t1798 (88/88), t1919 (30/30), t975 (30/30) | 4                  |
| dt17     | IVa         | t127 (1/1), t024 (8/8) | 3                  |
| dt8h     | I           | t001 (228/5), t041 (1/1) | 3                  |

*SCCmec non-typeable isolates are excluded.

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International isolates as has been observed by others [7,19,20]. The Danish isolates in this study originated from the years 2003 to 2010 and we found dt10a in the entire period. The International isolates were collected over several decades and 45% had dt10a (33 of 73). We propose that this dru type is the ancestor dru type for most MRSA lineages and that the other dru types have evolved from it as is depicted in Figure 1. However, the lack of dt10a in SCCmecII isolates and the strain specific association of some dru-SCCmec types (e.g., the majority of t008 SCCmecIva USA300 isolates are dt9g) seen here and elsewhere [7,26,27] are interesting exceptions. A blast search of staphylococcal genomic sequences reveals that dt10a is widespread in different MRSA strains including the archaic MRSA stain COL from 1965 and MRSA ST398 associated with livestock [28]. Methicillin resistant coagulase-negative staphylococci (CNS) have also been shown to carry dt10a [26] although additional studies comparing the dru types of human CNS to MRSA would be of interest. The frequent association of dt10a with the different SCCmec types included in this study supports evolution of the dru region after the insertion of the SCCmec into MSSA. However, because the dru region and the insertion sequence IS431 are in close proximity within SCCmec it is possible that they may jointly insert downstream of mecA during the SCCmec assembly process. In addition, the stable association of dru types and SCCmec ccrB sequences has yielded interesting information regarding the potential movement of SCCmec elements in staphylococcal populations and MRSA phylogenetic interrelationships [26,29].

In studies on EMRSA-15 and EMRSA-16 dru typing was useful to distinguish between subtypes of the same clone [7,19,20]. In the present study, seven patients had four dru types (dt2a, 7h, 7i and 8I) other than dt10a among 68 isolates from 68 epidemiologically related patients with MRSA t024-Iva. While these minor dru types are not closely related to dt10a based on MST (Figure 1) we believe that all 68 patients had related MRSA based on outbreak epidemiology. The dt2a and dt7i isolates were found in patients that had all been admitted to the same hospital, but not concomitantly. Sixteen of the 68 isolates were included in a recent study by our group where variations in the J3 region of the Danish t024-STR3-SCCmec Iva clone were identified [30]. One dt10a isolate and the dt7i isolate were from two persons living in the same nursing home and these two isolates had identical J3 variations (ccr2AB4 and the arc gene cluster within J3). One of the patients with a dt8l isolate lived in another nursing home together with a person with a dt10a isolate both of which exhibited an alternative J3 variation (the arc gene cluster but no ccr2AB4). The presence of the same J3 variation in isolates with different dru types suggests their derivation from a common ancestor. While the separation of the J3 and dru regions by IS431 suggests independent evolution of the two regions [30], one cannot rule out the possibility that J3 deletion may have also influenced dru repeat structure.

We studied 26 isolates over a four-year period from a patient who seemed to have acquired several MRSA types over time. In the first year, the patient had five t024-V isolates with either dt8l, dt11p or dt11bz, and one isolate with a 292-V-11p, 292 is one repeat shorter than t024 and we believe that a deletion in the spa region is the most likely explanation for this difference [31]. The finding of three different dru types at the same time in the same genetic background could reflect evolution in the dru sequence in the very rare t024-V clone. An alternative explanation would be multiple acquisitions of t024 MRSA with different dru types which is consistent with the observation, five months later, that the patient had acquired MRSA t024 with a SCCmec IVa instead of SCCmec V. The shift from t024-V to t024-Iva in this patient was probably caused by replacement of one strain with another rather than the excision of SCCmec V and insertion of SCCmec IV. We base this assumption on the fact that the patient was living in a nursing home with an outbreak of t024-Iva-dt10a. However, whole genome sequencing of the type IV and type V isolates reveals that they were closely related (data not shown) and therefore an excision/insertion event of the SCCmec cannot be ruled out. Over a four-year period we found 20 t024-Iva isolates from this patient of which 19 had dt10a and one dt11bz suggesting a stable dru region, however, the mutation rate in the dru region is unknown. From each positive sample we only typed one dru type for most MRSA lineages and that the other dru types have evolved from it as is depicted in Figure 1. However, the lack of dt10a in SCCmecII isolates and the strain specific association of some dru-SCCmec types (e.g., the majority of t008 SCCmecIva USA300 isolates are dt9g) seen here and elsewhere [7,26,27] are interesting exceptions. A blast search of staphylococcal genomic sequences reveals that dt10a is widespread in different MRSA strains including the archaic MRSA strain COL from 1965 and MRSA ST398 associated with livestock [28]. Methicillin resistant coagulase-negative staphylococci (CNS) have also been shown to carry dt10a [26] although additional studies comparing the dru types of human CNS to MRSA would be of interest. The frequent association of dt10a with the different SCCmec types included in this study supports evolution of the dru region after the insertion of the SCCmec into MSSA. However, because the dru region and the insertion sequence IS431 are in close proximity within SCCmec it is possible that they may jointly insert downstream of mecA during the SCCmec assembly process. In addition, the stable association of dru types and SCCmec ccrB sequences has yielded interesting information regarding the potential movement of SCCmec elements in staphylococcal populations and MRSA phylogenetic interrelationships [26,29].

### Table 3. dru types present in more than one SCCmec type.

| dru type | SCCmec types | spa types (ST/CC) | Number of isolates |
|----------|--------------|------------------|--------------------|
| dt10a    | I, IVAR, IIIA, IVa, IVb, IVc | 30 different spa types | 160 |
|          | IVd, IVg, IVh, V, VI | | |
| dt7f     | I, IIIA, IVa, IVh | t008(8/8), t459(239/8), t128(1/1), t036(8/8), t009(254/–) | 6 |
| dt7j     | IVa, IVc | t128(1/1), t008(8/8) | 5 |
| dt9m     | IVa, IVc | t127(1/1), t044(80/80) | 3 |
| dt10d    | IA, IIIA | t051(247/8), t037(239/8) | 2 |
| dt10h    | IVh, V | t022(22/22), t002(5/5) | 2 |
| dt10t    | IVa, IVc | t186 (88/–), t137 (–/–) | 2 |
| Total    |            |                  | 181 |

*SCCmec non-typeable isolates are excluded.

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the dru region is similar, it is not surprising that we did not find genetic changes in the dt10a of the patient.

We found no dru repeats in 18 isolates. dru negative isolates have also been found in other studies [7,33]. Four of the 18 dru negative isolates belong to ST225. In a study by Nübel et al [34] a deletion of the dru region was found in all isolates belonging to ST225. This indicates a spread of one MRSA clone rather than several episodes of acquisition of an SCCmec lacking the dru region into the same genetic background.

In conclusion, we found no correlation between dru type and SCCmec type, spa type or Sequence type. Therefore dru typing is not a first line epidemiological typing method for MRSA. In our study, the dru region seems rather stable in an outbreak situation, although isolates from seven of 68 patients had variations in the dru region. Therefore, a change in dru type in otherwise identical isolates is not enough to separate patients in an outbreak situation, but might be informative for epidemiological subtyping. Furthermore, dru typing can add interesting information on the evolution of SCCmec. Although this study includes 52 dru types it is worth noting that the dru database (www.dru-typing.org) contains 421 dtUs as of February 2015 and the conclusions drawn in this study are therefore based on a subset of the total dru database.

Over a seven year outbreak the dru type was retained in 90% of isolates, but the same common dru type, dt10a, was also found in several unrelated isolates. The finding of dt10a in so many different SCCmec types suggests that the dru region and the mecA originate from the same Staphylococcus species, while SCCmec I might have a different evolutionary pathway.

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Author Contributions

Conceived and designed the experiments: MDB KB DO PW HW. Performed the experiments: MDB KB PW. Analyzed the data: MDB KB PW RW. Contributed reagents/materials/analysis tools: MDB KB PW DG RW HW. Wrote the paper: MDB KB DO PW RW HW.

References

1. Moreno S, Coombs G, Shore MC, Coleman DC, Akpaka P, et al (2011) A field guide to pandemic and epidemic sporadic clones of methicillin-resistant Staphylococcus aureus. PLoS One. 6: e17936.
2. Aires-de-Sousa M, Boye K, de LH, Depalo A, Enright MC, et al (2006) High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. J Clin Microbiol. 44: 619–621.
3. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearnns AM, et al (2012) Methicillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents.
4. Boye K, Bartels MD, Anderson ES, Moller JA, West H (2007) A new multiplex PCR for easy screening of methicillin-resistant Staphylococcus aureus. SCCmec types I-V. Clin Microbiol Infect. 13: 725–727.
5. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, et al (2007) Combination of multiplex PCRPs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, cox, and major differences in junkyard regions. Antimicrob Agents Chemother. 51: 264–274.
6. Oliveira DC, de Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 46: 2155–2161.
7. Goring RV, Morrison D, Al-Doori Z, Edwards GF, Gemmell CG (2008) Usefulness of mec-associated direct repeat unit (dru) typing in the epidemiological analysis of highly clonal methicillin-resistant Staphylococcus aureus in Scotland. Clin Microbiol Infect. 14: 964–969.
8. Oliveira DC, Milheirico C, Vinga S, de LH (2006) Assessment of allelic variation in the ccrAB locus in methicillin-resistant Staphylococcus aureus clones. J Antimicrob Chemother. 58: 23–30.
9. Faria NA, Carrico JA, Oliveira DC, Ramirez M, de LH (2008) Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains. J Clin Microbiol. 46: 136–144.
10. Ryllf CJ, Bacher RJ, Kayser FH, Berger-Bach B (1993) The Staphylococcus aureus mec determinant comprises an unusual cluster of direct repeats and codes for a gene product similar to the Escherichia coli sn-glycerophosphoryl diester phosphodiesterase. J Bacteriol. 173: 7416–7422.
11. Nishi J, Miyahara H, Nakajima T, Kitaigama I, Yoshinaga M, et al (1993) Molecular typing of the methicillin resistance determinant (mec) of clinical strains of Staphylococcus aureus based on mec hypervariable region length polymorphisms. J Lab Clin Med. 126: 29–35.
12. Nishi J, Yoshinaga M, Miyahara H, Kawanaka M, Kawabata M, et al (2002) An epidemiologic survey of methicillin-resistant Staphylococcus aureus by combined use of mec-HVR genotyping and toxin genotyping in a university hospital in Japan. Infect Control Hosp Epidemiol. 23: 506–510.
13. Salmenlinna S, Vuopio-Varkila J (2003) Recognition of two groups of methicillin-resistant Staphylococcus aureus strains based on epidemiology, and molecular, toxicological susceptibility, hypervariable-region type, and ribotype in Finland. J Clin Microbiol. 39: 2243–2247.
14. Salmenlinna S, Lytyikainen O, Vuopio-Varkila J (2002) Community-acquired methicillin-resistant Staphylococcus aureus, Finland. Emerg Infect Dis 8: 602–607.
15. Schmitz JI, Steier M, Tichy HV, Hofmann B, Verhoef J, et al (1998) Typing of methicillin-resistant Staphylococcus aureus isolates from Dusseldorf by six genotypic methods. J Med Microbiol. 47: 341–351.
16. Senna JP, Pinto CA, Carvalho LP, Santos DS (2002) Comparison of pulsed-field gel electrophoresis and PCR analysis of polymorphisms on the mec hypervariable region for typing methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 40: 2254–2256.
17. Nalvi MD, Fitzgibbon JE, John JF, Duhin DT (2001) Sequence analysis of dru regions from methicillin-resistant Staphylococcus aureus and coagulase-negative staphylococcal isolates. Microb Drug Resist. 7: 1–12.
18. Witte W, Werner G, Cuny G (2001) Typing of MRSA isolates belonging to a widely disseminated clonal group by polymorphism of the dru sequences in mec-associated DNA. Int J Med Microbiol. 291: 57–62.
19. Shore AC, Rosney AS, Kinnvey PM, Brennan OM, Cramer E, et al (2010) Enhanced discrimination of highly clonal ST22-methicillin-resistant Staphylococcus aureus IV isolates achieved by combining spa, dru, and pulsed-field gel electrophoresis typing data. J Clin Microbiol. 48: 1839–1852.
20. Cramer E, Shore AC, Rosney AS, Dolan A, Sherlock O, et al (2012) Transmission of endemic ST22-MRSA-IV on four acute hospital wards investigated using a combination of spa, dru and pulsed-field gel electrophoresis typing. Eur J Clin Microbiol Infect Dis.
21. Ghaznavi-Rad E, Goering RV, Nor SM, Weng PL, Sekaizh Z, et al (2011) mec-associated dru typing in the epidemiological analysis of ST239 MRSA in Tanzania. Eur J Clin Microbiol Infect Dis. 30: 1365–1369.
22. Lam KT, Hauflah YA, Yunos MY, Goering RV, Thong KL (2012) Temporal changes in the genotypes of methicillin-resistant Staphylococcus aureus strains isolated from a tertiary Malaysian hospital based on MLST, spa, and mec-associated dru typing. Diagn Microbiol Infect Dis.
23. Milheirico C, Oliveira DC, de LH (2007) Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother. 60: 42–48.
24. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol. 38: 1008–1015.
25. Bartels MD, Boye K, Larsen AR, Skow R, Westh H (2007) Rapid Increase of Genetically Diverse Methicillin-Resistant Staphylococcus aureus, Denmark. Emerg Infect Dis. 13: 1333–1340.
26. Smyth DS, Weng A, Robinson DA (2011) Cross-species spread of SCCmec IV subtypes in staphylococci. Infect Genet Evol. 11: 446–453.
27. Tenover FC, McDougal IK, Goering RV, Killipere G, Projan SJ, et al (2006) Characterization of a strain of community-associated methicillin-resistant Staphylococcus aureus widely disseminated in the United States. J Clin Microbiol. 44: 100–118.
28. Wester A, Scott G, Keddie L, Ehricht R, Moncke S, et al (2010) Characterization of methicillin-resistant Staphylococcus aureus ST398 from cases of bovine mastitis. J Antimicrob Chemother. 65: 619–625.
29. Smyth DS, McDougal IK, Gran FW, Manoharan A, Enright MC, et al (2010) Population structure of a hybrid clonal group of methicillin-resistant Staphylococcus aureus Clonal Complex 8 and Unrelated Lineages. Antimicrob Agents Chemother. 55: 3932–3935.
30. Boye K, Westh H (2011) Variations in mec types found in consecutive MRSA isolates from the same patients. FEMS Microbiol Lett. 314: 101–105.
32. Kahl BC, Mellmann A, Deisick S, Peters G, Harmsen D (2005) Variation of the polymorphic region X of the protein A gene during persistent airway infection of cystic fibrosis patients reflects two independent mechanisms of genetic change in Staphylococcus aureus. J.Clin.Microbiol. 43: 502–505.

33. Oliveira DC, Wu SW, de LH (2000) Genetic organization of the downstream region of the meca element in methicillin-resistant Staphylococcus aureus isolates carrying different polymorphisms of this region. Antimicrob Agents Chemother. 44: 1906–1910.

34. Nübel U, Dördel J, Kurt K, Strommenger B, Westh H, et al (2010) A timescale for evolution, population expansion, and spatial spread of an emerging clone of methicillin-resistant Staphylococcus aureus. PLoS Pathog. 6: e1000835.