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Molecular characteristics of MRSA strains and patient risk factors in vascular surgery

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ABSTRACT

Methicillin-resistant Staphylococcus aureus *(MRSA) is one of the major pathogens in hospitals, and since the 1990s it has been recognized as an important pathogen in community infections. (1) The aim of this study was to analyze MRSA strains from a vascular surgery ward over a five-year period, since the vascular ward is considered to be a high-risk site for different multi-resistant pathogens, among which MRSA is very important. The method used for the microbiological identification and susceptibility testing of strains was the Vitek2 system. For the detailed characterization of the MRSA strains, we used the following molecular methods: SCCmec typing, pulse-field gel electrophoresis (PFGE), spa typing and Panton-Valentine leukocidin (PVL) detection. During the 5-year period, 77 MRSA strains were isolated. Antimicrobial susceptibility: 100% of MRSA isolates were susceptible to oxazolidinones and glycopeptides, 55% were susceptible to gentamycin, and 98% were susceptible to tetracyclines. SCCmec typing: 43 of 77 (55.8%) strains were typed as SCCmec I. The number of isolates with SCCmec II was 28 (36.4%). Three isolates carried SCCmec III.*

After the PFGE analysis, the isolates were grouped into six similarity groups: A-F. The largest number of isolates (80.6%) belonged to one of two groups: A: 35 (46.8%) and D: 25 (33.8%). Conclusion: The analysis of MRSA strains in the vascular surgery ward revealed high homogeneity among the strains, the majority of which belonged to SCCmec type I. This type, together with the susceptibility profile and PFGE grouping, is considered to be typical of Hospital-Acquired (HA) MRSA.

Key words: surgery, infection, molecular typing

Introduction

Staphylococcus aureus (S. aureus) can cause a wide range of human infections, including boils, furuncles, pneumonia, meningitis, sepsis, and postoperative surgical site infections (SSIs). (1,2) A resistant variant, methicillin-resistant *Staphylococcus aureus* (MRSA), can cause the same range of infections, and infections that are not treated in a timely manner can cause more severe consequences than infections that are properly treated. The common anatomic locations of MRSA carriage are the nares, groin, throat, and any wounds that are present, as for *S. aureus.* (3) The reduction of patient-to-patient MRSA transmission is an important aspect of the prevention of healthcare-associated MRSA infection. (4) Methicillin was first introduced in 1959, and the first *Staphylococcus aureus* isolates that are resistant to methicillin appeared in 1961 in the UK. (5) MRSA is one of the major pathogens in hospitals, and in the 1990s it was recognized as one of the most important pathogens in community infections. Several severe and fatal cases have been described. In the USA, this bacterium causes the majority of skin and soft tissue infections. (6) In Europe, the burden of community-acquired MRSA is much smaller, but patients with MRSA colonization or infection are still often found in hospitals, (1, 7) One other concern is raised by the occurrence of community strains and the introduction of those strains into hospitals. (8, 9) The main difference between HA and CA MRSA is in a genetic element called the SCCmec cassette, which carries the

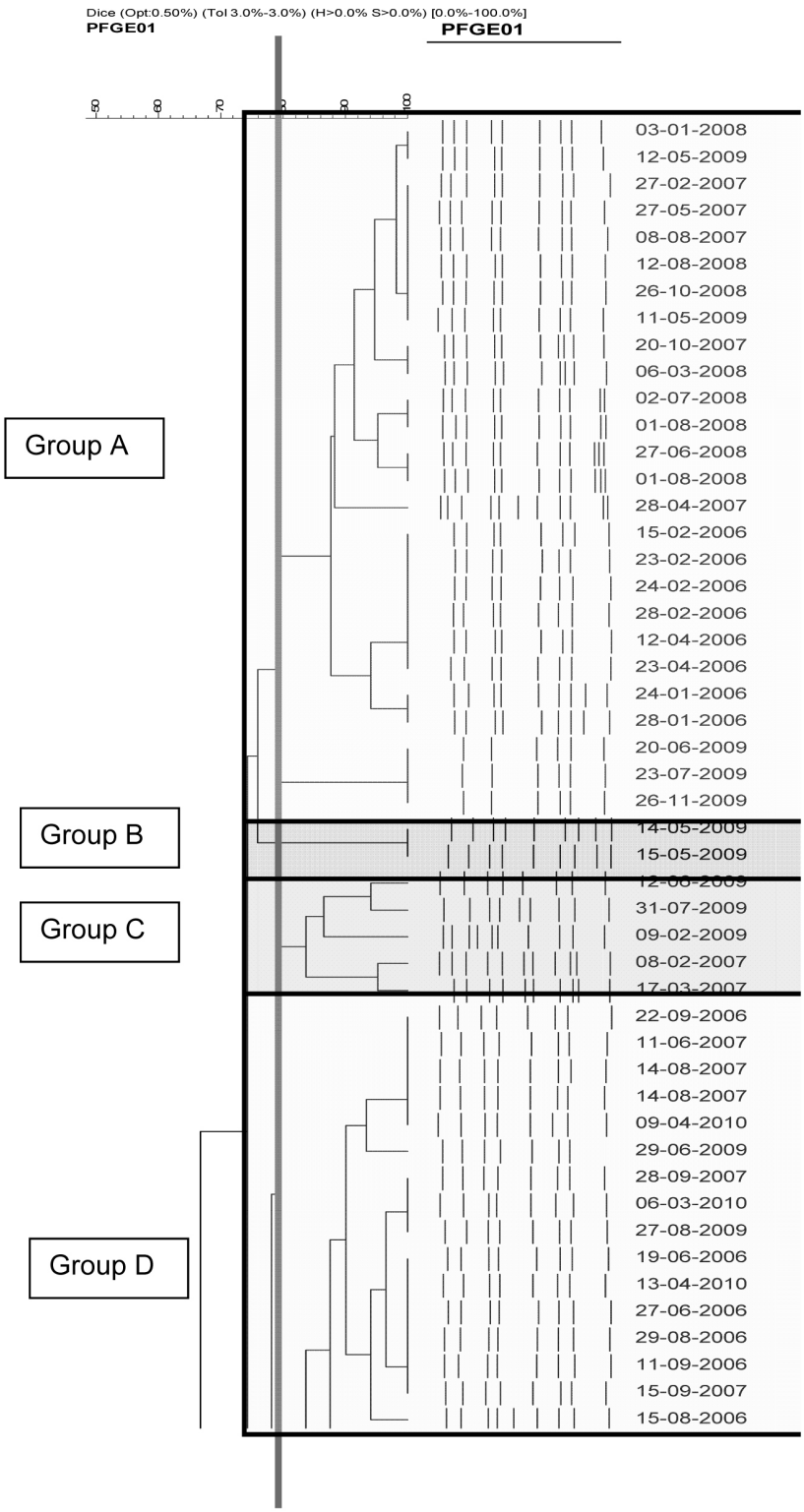

Figure 1. presents PFGE patterns of the MRSA clones observed in this study

mecA gene. The mecA gene encodes PBP2a, a mutated penicillin-binding protein (PBP), which is a marker of MRSA and has an active binding site for all penicillins and cephalosporins. (7) So far, there are 11 known and described types of SCCmec and numerous subtypes. The first 3, or I, II and III, are predominantly found in HA MRSA. Others, and especially types IV and V, are found in community strains, as are many other types (VI-XI). (10)

Because community-associated MRSA strains have certain virulence factors, such as Pantone-Valentine leukocidin (PVL), and the smaller mobile genetic element that carries the resistance gene mecA, called the SCCmec cassette, certain authors consider CA MRSA as a substantial threat, especially when introduced into hospitals. (1) The aim of this study was to analyze MRSA strains from one vascular surgery ward over a five-year period, since the vascular ward is considered to be a high-risk site for different pathogens, and especially MRSA. Vascular SSI occurs as a result of preoperative events that lead to bacterial colonization of both the surgical wound and, frequently, any underlying prosthetic graft that may be present. (11, 12) The prevention of SSI is a major concern for vascular surgeons, and reduction of the incidence of SSI requires an understanding of the changing epidemiological risk factors for the development of SSI and the use of effective patient care strategies. The strategies for the prevention of vascular SSI include surveillance, decolonization, antibiotic prophylaxis, specific surgical techniques, and methods that involve the application of impregnated grafts. (12) In Croatia, vascular surgery wards have historically had endemic MRSA strains, since the patients who are often hospitalized in these wards are patients at high risk of acquiring MRSA – such as the elderly, nursing-home patients, diabetics and patients with compromised circulation and tissue oxygenation. Due to this shift from HA MRSA to CA MRSA, we decided to investigate the genetic background and relatedness of MRSA in a vascular surgery ward over a four-year period and to determine whether our patients were typically carriers of or infected with HA or CA MRSA. If the dominant type is still HA MRSA, the therapeutic choice is limited to glycopeptides, linezolid and daptomycin.

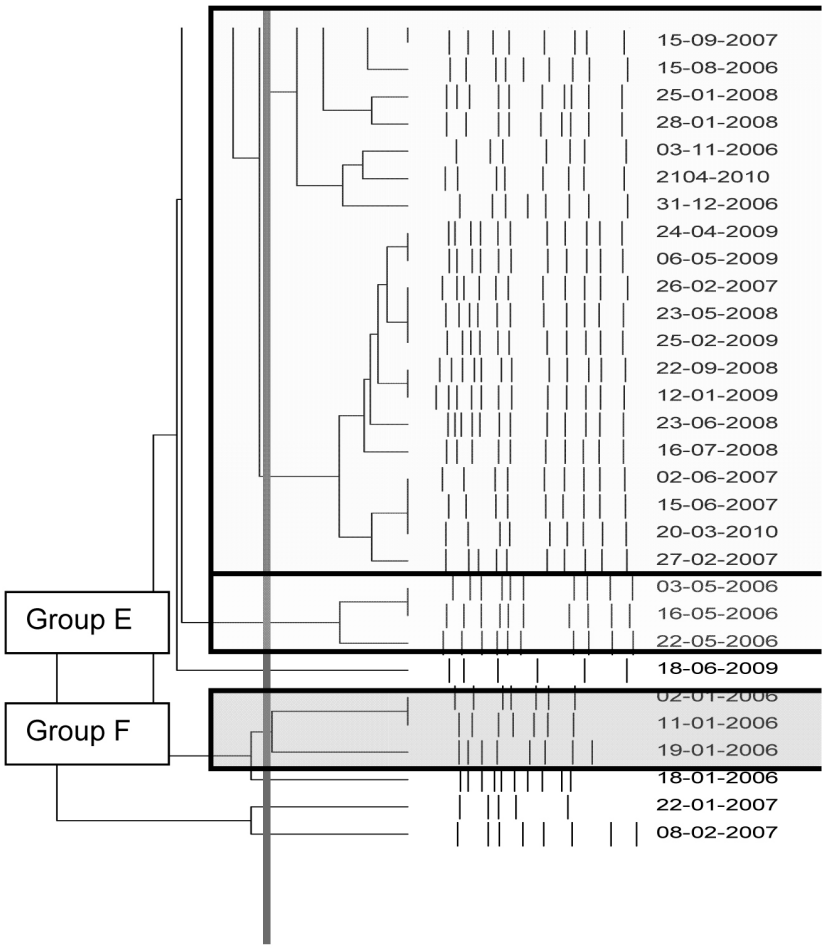

Figure 1. presents PFGE patterns of the MRSA clones observed in this study

But if there is a shift toward community strains, other therapeutic options should be possible. In addition, and in such cases, we will need to enhance our infection control programmes because transmission is occurring and continuing. Among high-risk patients and at admission to high-risk wards, it is important to perform screening if such screening is part of a hospital policy. Moreover, screening results should be available as soon as possible. Before that, a patient should be placed in a single room and treated as possibly colonized/infected. Given that there is a lack of single-bed hospital rooms, and because working hours for laboratories and attending physicians are limited, pre-emptive isolation and even screening are not routinely performed as this approach is costly, and MRSA carrier status information is not used fully. (12) One concern in investigations of MRSA is the introduction of community-associated MRSA strains into hospitals. Beyond SCCmec typing, the characterization of MRSA strains can be based on several other techniques. (13) If the long-term relation and origin of the strains are needed, then multi-locus sequence typing (MLST) is the method of choice. Based on this method, the majority of known MRSA strains are grouped into 5 major clonal complexes, evidencing the high clonality of MRSA strains and also showing which lineages were more successful than others when the introduction of the mecA gene into susceptible *S. aureus* occurred. (14) For hospital epidemiological purposes, relatedness between strains can be established by the use of pulse-field gel electrophoresis (PFGE) and spa typing. (15-17)

Materials and methods

MRSA was isolated from patient screening samples and clinical isolates. The screening policy included screening on admission and weekly screening at three sites: the nose, groin, and axillary area. The swabs were processed in a microbiology laboratory using clinical laboratory guidelines and were pooled in broth. Colonies with typical morphology were identified by standard microbiological tests (coagulase, DNase), and susceptibility testing was performed according to EUCAST standards. (18) MRSA was suspected if the cefoxitin zone was 21 mm or smaller. All fox-R strains were submitted to molecular confirmation of mecA, which is the gold standard for MRSA confirmation. A range of 19 antibiotics was tested for each isolate using the Vitek2 system and the disk diffusion method according to EUCAST.

Molecular analysis of the SCCmec cassette was performed using a method previously described by Oliveria et al. (19), with certain modifications described by Budimir et al. (20)

PVL was detected using PCR primers previously described by Lina et al. (21)

PFGE was performed as described in the study by Tenover et al. with some adjustments. (15,16) The band patterns were analyzed with Dice comparison and unweighted-pair group matching analysis settings with GelCompar II (Applied Maths, Sint-Martens-Latem, Belgium) according to the scheme by Tenover et al. (16) MRSA isolates with a similarity index of 0.70 or more were classified as a clonal group. (22). A relatedness of 80% or more was considered to be significant, and strains with at least 80% similarity were considered to be related to each other and were grouped together in a similarity group or genotype. Spa typing was performed according to previously published methods. (23,24) An analysis of sequence chromatograms was performed using Ridom StaphType™ (Ridom GmbH, Wurzburg, Germany).

Table 1. SCCmec types distribution among MRSA isolates in Vascular surgery ward

SCCmec type I	SCCmec type II	SCCmec type III	SCCmec type IV	SCCmec type V	Non-typeable NT
43 (55.8%)	28 (36.4%)	3 (3.9%)	0	0	3 (3.9%)

Table 2. Spa typing results

Spa type	Number of isolates	Percentage
t041	32	41.6%
t003	14	18.2%
t014	9	11.7%
t001	8	10.4%
t481	2	2.6%
t603	2	2.6%
t030	2	2.6%
t1003	1	1.3%
t127	1	1.3%
t127	1	1.3%
NT	4	5.2%

Results

A total of 77 patients were MRSA positive at least at one site. MRSA-positive isolates were found more frequently in men than in women (75% vs. 25%). MRSA was present in 67 wound swabs, four throat swabs, two nasal swabs, two perineal swabs, one blood culture, and one central vascular catheter. In total, 41 patients had diabetes, and 36 did not. Fifty patients were submitted to an operative procedure during their stay in the hospital, and 20 were not. There were no data on operative status for seven patients. Antimicrobial susceptibility: 100% of MRSA isolates were susceptible to oxazolidinones and glycopeptides, 55% were susceptible to gentamycin, and 98% were susceptible to tetracyclines. Additionally, all isolates were resistant to clindamycin and erythromycin. SCCmec typing: 43 of 77 (55.8%) strains were typed as SCCmec I. The number of isolates with SCCmec II was 28 (36.4%). Three isolates carried SCCmec III. Non-typeable strains were also found in 3.9% of cases. No isolates with SCCmec IV or V were found during this analysis. A summary of the results is presented in table 1. After the PFGE analysis, the isolates were placed together in six similarity groups: A-F. The largest number of isolates (80.6%) belonged to one of two groups: A (35; 46.8%) and D (25; 33.8%). Group C included 5.2% isolates, and E and F included 3, 9%. Four isolates were singletons, i.e., not similar to any of the other strains. Placement of strains into groups and clonal relatedness are shown in picture 1.

Discussion

Comparison grouping by PFGE and SCCmec typing was used to determine whether our strains belonged to HA or CA MRSA strains. The majority of isolates in PFGE group D had SCCmec type I, and fewer had SCCmec types II and III. According to our results, MRSA represents a significant causative agent of infections and patient colonization in a vascular surgery ward at the University Hospital Centre in Zagreb. This ward includes predominantly male patients, and they are often carriers of, or infected by, MRSA. (25) Certain diseases and conditions are also more often found in men, so they are found to have MRSA more often than women. Most of the patients were over 60 years of age, and men with MRSA were significantly younger than the female MRSA carriers. The risk factors for MRSA colonization/infection are previous MRSA colonization, previous hospitalization, chronic diseases, living in a care home, and previous antibiotic use. (9) In our patient group, only 10% of the patients were nursing-home residents, but more than 70% of patients had received antimicrobials in the year preceding the current hospitalization. It is well known from available literature that MRSA emergence is greatly promoted by the use of fluoroquinolones and cephalosporins. In our patient population, the majority of patients were receiving ciprofloxacin. It is important to note that there is no uniform policy for patient screening in the vascular surgery ward, so the majority of samples were clinically indicated samples, in the form of wound swabs. (12) Several swabs were obtained from chronic wounds, which are often colonized, especially by gram-negative flora, but are rarely infected. We also collected only one invasive isolate, obtained from a blood culture, which is a preferable sample type because the finding of MRSA in blood culture is often a sign of bacteremia and may not be treated or recognized as severe infection. Because this single isolate was obtained from a single blood culture from a symptomatic patient, and given that we did not have a properly collected sample, we can only assume that this patient was septicemic.

Analyzing all other risk factors important for vascular insufficiency and MRSA carriage/infection, we observed that more than half (53%) of the patients were diabetic, and the majority were 60

years and older, which is typical. These findings reflect the additional vulnerability of this population and their greater probability of infection. Other compromising factors, and often the main reasons for hospitalization, were peripheral occlusive illness and hypertension. In examining the susceptibility profiles of the tested isolates, we found typical HA MRSA profiles: a high percentage of resistance to gentamycin and 100% resistance to clindamycin and erythromycin. Additionally, all isolates were susceptible to glycopeptides and linezolid, as well as tigecycline. Results from Croatia in the same time period were in concordance with our results regarding antimicrobial susceptibility. (26) In efforts to reduce the MRSA burden in hospitals and to reduce the spread of multiresistant strains, strict infection control measures are advised. Contact precautions, the isolation of a patient in a single room and a decolonization regimen can be very successful. It is useful to apply a screening policy, especially in high-risk wards, but it is up to the infection control committee and microbiology service to choose the best method – which should be suitable, rapid and sufficiently inexpensive to not burden the hospital budget unduly. (4) Because the aim of the current study was to try to find community-associated MRSA in a vascular surgery ward, we used a combination of molecular characteristics and epidemiological definitions to identify these strains. As far as we are aware, there were no CA MRSA strains in this population of strains or in this population of patients. Because patients with this profile are often re-admitted to the hospital, re-admission could represent a risk factor for HA MRSA, more so than for CA MRSA. SCCmec typing revealed typical HA MRSA strains, which was clear even after susceptibility testing because the susceptibility profiles were typical of HA MRSA. The absence of genes encoding the toxin PVL also proves that our strains were CA MRSA.

Spa typing showed 10 spa types, which were also not as diverse as one would expect. Spa type t041 was most common in this study, as shown in certain previous publications from our country. This type is typical in Croatia and in connection to SCCmec I, which was also previously found in certain other European countries, such as Germany, Austria, the Czech Republic, Sweden and Switzerland, but not on other continents. (5, 20) Other spa types are also very well known in these areas, according to the literature and Ridom SpaServer. (3, 5, 20) Spa type t041 in MRSA, together with SCCmec I, is typically presented in Southern German MRSA clones. (20) The cost and benefit of this type of study should be considered in light of the new developments in MRSA epidemiology and the identification of certain new "strange" hypervirulent or community strains. The routine use of molecular techniques is preferable if a hospital can afford it, but traditional microbiological methods can also be very effective in local epidemiology. The results can be (at least preliminarily) communicated with wards very soon after the inoculation of strains in selective broth. In Croatia in 2005, MRSA represented 19.98% of all SA isolates, and the percentage was 233% in 2008. However, after that year the percentage slowly decreased as part of a decreasing trend in the global MRSA rate.

Conclusion

The molecular characteristics of MRSA strains in the vascular surgery ward over a 5-year period showed high genetic similarity. Using a combination of techniques, we were able to investigate the origin and genetic background of the strains. PFGE analysis revealed two large, genetically related groups of strains consisting of 60 isolates.

This finding could have been the result of inadequately strict adherence to infection control measures, failure to apply the screening policy, a lack of knowledge about multiresistant strains, or the local epidemiological situation, which should be the basis of antimicrobial empiric therapy for hospitalized patients.

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