

Profile of SCCmec types in clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in a hospital in Southern Brazil.

Perfil dos tipos de SCCmec de isolados clínicos de *Staphylococcus aureus* resistentes a meticilina em um hospital do sul do Brasil.

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an opportunistic pathogen that affects public health representing the most common infections related to health care and community. MRSA infections are classified as health care-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) defined by the bacteria genetic profile. **Objective:** This study conducts a molecular characterization of MRSA isolates from a hospital in Porto Alegre, RS, Brazil, in order to determine the profile of SCCmec types. **Methods:** Eighty-one MRSA isolates from a hospital in Porto Alegre, RS, Brazil, collected from January to June of 2012 were included in the study. A multiplex PCR was performed to determine the SCCmec types. Results: From the 81 isolates, 24 (30%) were type I, 10 (12%) type II, 21 (26%) type III, 4 (5%) type IVa, 12 (15%) isolates were type IVc, 1 isolate was type I and IVc (1%) and 1 was type III and IVc (1%) simultaneously, while non-typable isolates corresponded to 8 isolates (10%). **Conclusion:** However most of the isolates were carrying SCCmec types related to HA-MRSA; the results reveal a change in the epidemiology, considering the decrease of the incidence of SCCmec type III and the increase of isolates being typed as SCCmec I.

Keywords: Methicillin-Resistant *Staphylococcus aureus*. Molecular Typing. Polymerase Chain Reaction

Resumo

Introdução: O *Staphylococcus aureus* resistente à meticilina (MRSA) é um patógeno humano oportunista que afeta a saúde pública sendo o representante mais comum em infecções relacionadas ao ambiente hospitalar e a comunidade. Infecções causadas por MRSA podem ser classificadas como associadas ao ambiente hospitalar (HA-MRSA) ou à comunidade (CA-MRSA) baseado em suas características genéticas. **Objetivo:** Este estudo refere-se a uma caracterização molecular de isolados de MRSA oriundos de um hospital de Porto Alegre, RS, Brasil, a fim de determinar o perfil de tipos de SCCmec presente nas amostras. **Métodos:** Oitenta e um isolados de MRSA oriundos de um hospital de Porto Alegre, RS, Brasil, coletados de Janeiro a Junho de 2012 foram incluídos no estudo. Uma PCR multiplex foi realizada para determinação dos tipos de SCCmec. Resultados: Dos 81 isolados, 24 (30%) apresentaram SCCmec tipo I, 10 (12%) tipo II, 21 (26%) tipo III, 4 (5%) tipo IVa, 12 (15%) isolados foram caracterizados como tipo IVc, 1 isolado apresentou tipo I e IVc (1%) e 1 com tipo III e IVc (1%) simultaneamente, enquanto isolados não tipáveis corresponderam a 8 isolados (10%). **Conclusão:** Apesar de a maioria dos isolados carregarem tipos de SCCmec relacionados a HA-MRSA, os resultados nos mostram uma mudança na epidemiologia, considerando a diminuição na identificação de SCCmec tipo III e o aumento na identificação de isolados carregando SCCmec I.

Palavras-chave: *Staphylococcus aureus* resistente à meticilina. Tipagem molecular. Reação de cadeia polimerase.

INTRODUCTION

Staphylococcus aureus is a microorganism responsible for a wide variety of infectious diseases¹. Methicillin resistance in staphylococci is mainly due to the expression of the *mecA* gene, which specifies penicillin binding protein 2a (PBP2a), a transpeptidase with low affinity for β -lactams². This gene is located on the staphylococcal cassette chromosome *mec* (SCCmec), a mobile genetic element³. The SCCmec has been classified in 11 types (I–XI) until now⁴; however, only type I–V is globally distributed².

Differences in SCCmec elements are classified and characterized according to combinations in *cassette chromosome recombinase* (*ccr*), the *mec* complex and the *junkyard* region^{2,5}.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an

important pathogen of public health representing the most common bacteria in infections related to health care and community^{1,4}. The rapid identification of the bacteria provides the successful control of the infection⁶.

Therefore, MRSA infections are classified as health care-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA)^{1,4}. Infections caused by CA-MRSA isolates are usually associated with healthy people and are epidemiologically defined depending on two situations: if collected from outpatients or if collected up to 48 hours after hospital admission⁴. Regarding to the genetic profile described, the most common SCCmec types are IV and V^{3,1,6}.

On the other hand, HA-MRSA it is associated with a longer

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period of hospitalization, previous use of antibiotic and epidemiologically related to SCCmec types I, II and III³.

This study evaluated the occurrence of SCCmec types I to V in clinical isolates of MRSA in a hospital in Southern Brazil.

METHODS

Eighty-one isolates of MRSA were collected at the Conceição Hospital Group (GHC) which is composed by hospitals Conceição, Criança Conceição, Cristo Redentor e Fêmeina in Porto Alegre, Rio Grande do Sul, Brazil.

The study included isolates from January to June of 2012 from different sites including blood, tracheal aspirate, sputum and secretions previously identified at the Central Laboratory of the hospital. The isolates were collected from different sites of patients who presented infection symptoms, regardless of age. Among the sites we can mention tracheal aspirate, sputum, urine, secretions of wounds, abscesses and blood.

Isolates were confirmed by genus and specie by phenotypic methods through the presentation of positive biochemical tests for coagulase and DNase. After this, we carried out DNA extraction for use in later steps; all MRSA isolates were submitted to a PCR for molecular confirmation of the presence of the genes investigated. The DNA extraction, a PCR to investigate the presence of *mecA* gene and a *multiplex* PCR using the SCCmec type I–V were made according to Zhang et al., 2005⁷.

Control strains for SCCmec type I NCTC10442, type II N315, type III NCTC85/2082, type IVa CA05, type IVb 8/6-3P, type IVc MR-108, IVd JCSC4469 and for type V JCSC3624 were used.

RESULTS

The isolates included in the study were positive for the *mecA* gene, confirming the resistance of bacteria to methicillin, which is given by the expression of this gene. Twenty-four isolates were type I (30%), 10 type II (12%), 21 type III (26%), 4 type IVa (5%), 12 isolates type IVc (15%), 1 isolated type I and IVc (1%) and 1 was type III and IVc (1%) simultaneously, while non-typable isolates corresponded to 8 isolates (10%). Isolates were considered non-typable when there was no amplification for the primers tested. Table I.

Table 1. SCCmec typing of MRSA isolates. NT: non-typable.

SCCmec	N(%)
I	24 (30)
II	10 (12)
III	21 (26)
IVa	4 (5)
IVc	12 (15)
I e IVc	1(1)
III e IVc	1(1)
NT	8 (10)
Total	81

DISCUSSION

Data demonstrated that infections related with HA-MRSA SCCmec types are still prevalent in health care, where type I was the most frequently found, followed by type III. Type IVc had a significant number of isolates and it is associated with infections caused by CA-MRSA.

Also related with isolates from Porto Alegre, Becker et al., 2012⁸ performed a study with thirty isolates of 2008. The incidence found was SCCmec type III with eighteen isolates (60%) clonally related with Brazilian epidemic clone, and eleven isolates (36.7%) harboring SCCmec type I closely related to the Cordobes/Chilean clone. Our study shows prevalence of others types of SCCmec in the clinical isolates from the studied hospital. This fact must be investigated more closely in order to determine if the prevalence of Brazilian epidemic clone is being replaced by other clones in hospitals from Porto Alegre.

A study involving the three largest hospitals in Porto Alegre (Clínicas Hospital, Conceição Hospitalar Group and Santa Casa de Misericórdia) was conducted by Perez et al., 2008⁹. In this study, 9 blood isolates from MRSA were obtained from August to December of 2004. About this isolates, 8 harbored SCCmec type III and one were non-typable. We could also observe the change occurred in the incidence of certain types of SCCmec in the hospital environment.

Reiter et al., 2010¹⁰ analyzed 364 isolates of *Staphylococcus aureus* from inpatients from Hospital de Clínicas in Porto Alegre, RS, Brazil. The study includes cystic fibrosis (CF) patients and non-cystic fibrosis. 104 (45%) *S. aureus* isolates were obtained from CF patients and 57 (45.5%) of these isolates were identified as MRSA. Among the isolates of non-CF patients, 89 (35%) were MRSA. All isolates of CF patients harbored SCCmec type III and one isolate harbored type I and II simultaneously. Isolates from non-CF patients, 31/89 (35%) harbored type I and 44/89 (49%) harbored type III with also one isolate harboring type I and II simultaneously. In the present study, we analyzed MRSA isolates from inpatients with the most distinct comorbidities not reported also causing infection. The molecular characterization also provided the observation of the change in the types of SCCmec circulating in the hospital. This study found incidence of isolates from GHC harboring SCCmec type I. It shows the importance of conducting epidemiological studies involving different hospitals in the same city to confirm the epidemiology change by the circulating SCCmec types.

Non-typable isolates probably occur when there is some variation in the disposition of genetic elements inserted in the SCCmec, causing variants of the most frequently types or even new types that have not been reported suggesting the need to analyze other SCCmec types reported to date (VI to XI)¹¹.

Predominance of SCCmec types related to HA-MRSA found in this study confirms that the infection occurred with circulating MRSA in the hospital environment, reinforcing the need of sanitizing hands and extreme caution in patient care.

Precautionary measures related to contact avoid outbreaks due to the spread of bacteria (6).

In conclusion, the molecular characterization of MRSA isolates added with the PFGE technique makes possible to determine the changes in the types of SCCmec found in MRSA isolates causing infections in Porto Alegre, Southern Brazil.

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