Journal of Hospital Infection (2005) **59**, 19–26

Available online at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevierhealth.com/journals/jhin

Genomic characterization of oxacillin-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolated from Brazilian medical centres

A.P.F. Nunes[a], L.M. Teixeira[a], C.C.R. Bastos[b], M.G. Silva[a], R.B.R. Ferreira[a], L.S. Fonseca[a], K.R.N. Santos[a,*]

[a]*Departamento de Microbiologia Médica, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*
[b]*Hospital Naval Marcílio Dias, Rio de Janeiro, Brazil*

Received 26 June 2003; accepted 18 March 2004

KEYWORDS
S. epidermidis;
S. haemolyticus;
Oxacillin-resistance;
PFGE

Summary Studies on the genetic diversity of oxacillin-resistant coagulase-negative staphylococcal (CNS) isolates are important for the control and prevention of infections. The present study evaluated the clonal diversity of oxacillin-resistant *Staphylococcus epidermidis* (ORSE) and *Staphylococcus haemolyticus* (ORSH) strains, isolated from patients in nine Brazilian medical centres by using pulsed-field gel electrophoresis (PFGE) after digestion of bacterial DNA using *Sma*I. PFGE analysis of ORSE ($N=44$) and ORSH ($N=25$) strains showed the presence of 29 restriction profiles clustered in 16 PFGE types, and 21 distinct profiles in 15 PFGE types, respectively, indicating a large genetic diversity among isolates of both of these species. Among the ORSE isolates, 23 (52%) strains belonged to two predominant PFGE types (named A and B), which were observed in most of the hospitals assessed, indicating the spread of these PFGE types in hospitals located in Rio de Janeiro. The spread of PFGE types of ORSH was also detected in some of the hospitals investigated. The results show that PFGE is a suitable tool for epidemiological studies of oxacillin-resistant CNS, and can be used as a basis for infection control procedures for these multiresistant organisms.

* Corresponding author. Address: Laboratório de Infecções Hospitalares—sala I2-010, Departamento de Microbiologia Médica, Instituto de Microbiologia Prof. Paulo de Góes, CCS, Bloco I, UFRJ, Cidade Universitária, Rio de Janeiro, RJ, Brazil. Tel.: +55-21-2260-4193; fax: +55-21-2560-8344.
E-mail address: santoskrn@micro.ufrj.br

0195-6701/$ - see front matter © 2004 The Hospital Infection Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.jhin.2004.07.021

Introduction

Coagulase-negative staphylococci (CNS) are recognized as important nosocomial pathogens worldwide.[1] *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* are the most frequent species of CNS associated with nosocomial bloodstream infections and infections related to implanted medical devices.[2] Multiple drug resistance has been documented as a characteristic among such isolates.[3-5] Furthermore, a substantial increase in the frequency of oxacillin resistance in CNS isolates has occurred over the last decades, and this resistance trait has been detected in more than 70% of these isolates.[6] According to the results of the SENTRY study, about 80% of the CNS strains isolated from bloodstream infections in Brazilian hospitals possess oxacillin-resistance.[7] In a recent study, we found a frequency of 64% of oxacillin resistant CNS isolated from different clinical sources,[5] while strains of *S. epidermidis* and *S. haemolyticus* exhibit higher frequencies of oxacillin resistance, showing rates of 75 and 96%, respectively. The increasing number of infections due to oxacillin-resistant staphylococci makes the glycopeptides antibiotics an important choice for therapy of infections caused by these micro-organisms.[8] However, due to the emergence of vancomycin-resistant staphylococcal[8,9] and enterococcal[10] isolates, the judicious use of vancomycin is recommended.

The clonality of species of CNS has been investigated by different genotypic methods.[11,12] The use of pulsed-field gel electrophoresis (PFGE) for genetic typing of CNS, especially for *S. epidermidis* and *S. haemolyticus* strains, has been described in some reports, and has been shown to be a suitable method with regard to its discriminatory ability.[13,14] Nevertheless, the understanding of the dissemination of multiresistant CNS isolates and their epidemiology is not as clear as for methicillin-resistant *S. aureus* (MRSA) isolates.[15] A reduced genomic diversity has been found for MRSA strains, indicating the worldwide dissemination of a few prevalent clones.[15] Conversely, reports identifying oxacillin-resistant CNS clones that circulate in different hospitals are scarce.[14] The use of molecular epidemiology techniques as tools for tracking of clones of the most frequent CNS species could help the understanding and control the spread of resistance among staphylococci. The purpose of the present study was to evaluate the genotypic variability and dissemination of oxacillin-resistant *S. epidermidis* (ORSE) and *S. haemolyticus* (ORSH) clinical strains isolated from different Brazilian medical centres using PFGE.

Methods

Clinical isolates

Forty-four *S. epidermidis* and 25 *S. haemolyticus* distinct clinical isolates obtained from different clinical specimens (blood, $N=34$; surgical site, $N=11$; nares, $N=9$; and other sites, $N=15$), between 1994 and 1999, from patients at nine Brazilian hospital centres (HNMD, HUCFF, HBP, CB, HUAP, HCR, PB, HCUFU and HUFMS; Tables I and II) were tested. HUCFF, HUAP, HCUFU and HUFMS are teaching hospitals. HNMD, HUCFF, HBP, CB, HCR and PB are located in Rio de Janeiro city, while HUAP is located in Niterói city. Both cities are located in Rio de Janeiro state, 13 km apart. HCUFU and HUFMS are located 1000 and 1500 km from the Rio de Janeiro medical centres, respectively. Only one strain from each patient was analysed. All strains were identified to species level by conventional tests, including: Gram stain, detection of enzyme production (coagulase, catalase, phosphatase, ornithine, urease and pyrrolidonyl arylamidase), haemolytic activity, acid production from mannitol, mannose and trehalose, and resistance to polymyxin and desferrioxamine.[16-18] Isolates were kept frozen at $-20\,^{\circ}C$ in tryptic soy broth containing 20% (v/v) glycerol.

Oxacillin disk-diffusion test

Susceptibility to oxacillin was determined by the disk-diffusion method on Mueller–Hinton agar plates (Difco laboratories, Detroit, MI, USA) according to the guidelines of the National Committee for Clinical Laboratory Standards.[19] Disks containing 1 mg oxacillin (CECON, São Paulo, Brazil) were used. *S. aureus* ATCC 25 923 was used as a control.

Polymerase chain reaction (PCR)-based detection of the *mecA* gene

The *mecA* gene was detected by PCR using the specific primers MRS_1 (5′-TAGAAATGACTGAACGTCCG-3′) and MRS_2 (5′-TTGCGATCAATGTTACCGTAG-3′) (Life Technologies, São Paulo, Brazil) as described previously.[20] Staphylococcal DNA was extracted by boiling as described by Nunes *et al.*[21] Subsequently, 10 mL bacterial DNA was added to the PCR mixture (final volume of 50 mL) containing 250 mM of each dNTP (Life Technologies), 1.5 U *Taq* DNA polymerase (Life Technologies), 20 mM Tris–HCl, pH 8.4, 50 mM KCl and 2 mM $MgCl_2$. PCR assays were run in a programmable thermal controller (PTC-100; MJ Research, Inc.).