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Full Length Research Paper

Atypical manifestation in infection by methicillin-resistant *Staphylococcus aureus* carrier SCC*mec* IV and Panton-Valentine Leukocidin-producer in experimental sepsis model

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***Staphylococcus aureus* is considered an infectious agent of great clinical importance, responsible for many different types of infection. Strains of methicillin-resistant *Staphylococcus aureus* (MRSA), Panton-Valentine leukocidin producers, are considered more invasive, presenting clinical sequelae related to abscesses and infection in skin and soft tissues. The use of invasive techniques in hospital environment, such as the introduction of intravascular catheter in immunocompromised patients, has contributed to this microorganism spreading through the bloodstream, causing bacteremia, necrotizing pneumonia and increasing the number of septic patients in intensive care units with high mortality. In this report, atypical infections in Swiss mice using experimental model of sepsis was presented.**

Key words: methicillin-resistant *Staphylococcus aureus* (MRSA), mice infection, Panton-Valentine Leukocidin.

INTRODUCTION

Staphylococcus aureus is part of the natural microbiota of skin and nasal cavities (Kim et al., 2014; Lowy, 1998). Its capacity of colonization and pathogenicity is due to its virulence factors, important in adhesion and evasion of the host's immune system (Otto, 2010; Tavares, 2002). This microorganism is the most common agent causing pyogenic infections, having as primary site, the skin, and through bacteremia can infect several other organs (Andriolo, 2005; Boles and Horswill, 2008). The pathological features of *S. aureus* infections are due to

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the production of toxins, determinants factors of its virulence (Bukowski et al., 2010; Kim et al., 2014; Novick et al., 2010) which are commonly associated with purulent lesions and abscesses due to infiltration of neutrophils at infected site (Cheng et al., 2009; Kim et al., 2014).

The erroneous and constant use of antimicrobials in animals and humans led to selection of strains resistant to β-lactams and Cephalosporins being called methicillin-resistant *Staphylococcus aureus* (MRSA) (DeLeo and Chambers, 2009; Kim et al., 2014). By the acquisition of the chromosomal gene of *mec*A resistance which encodes structural modifications in receptor surface protein for β-lactams, there is promotion of dissemination of this microorganism in a hospital environment and making it difficult to treat infections (Atshan et al., 2012; Berger-Bachi and Rohrer, 2002).

An important cytotoxin secreted by *S. aureus* disseminated worldwide (Diep and Otto, 2008) is the Panton-Valentine leukocidin (PVL), commonly associated with community strains containing type IV staphylococcal cassette chromosome *mec* (SCC*mec*) (Diep et al., 2006; Vandenesch et al., 2003). Increasingly, community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains producing of PVL have been isolated from skin abscesses, soft tissue infection and necrotizing pneumonia in hospital settings (Gillet et al., 2002; Lina et al., 1999), being a major public health problem and more harmful and immunological weakness of infected patients. This leukocidin targets the polymorphonuclear leucocytes and macrophages. In high concentrations, this molecule forms pores in membrane, altering the permeability and allowing cations to enter (Ca^{2+}) causing degranulation and subsequent cytolysis, and in low concentration, promotes apoptosis by binding to the mitochondrial membrane, resulting in the release of oxygen in the reactive form (Genestier et al., 2005; Boyle-Vavra and Daum, 2007). The appearance of these strains in hospital infections may be related to preexisting colonization of the patient, which finds a port of entry during invasive surgical procedures (Enright et al., 2002; Maree et al., 2007), also through direct manual contact of health professionals with open lesions in infected patients in postoperative period. Because it is an easily transmitted microorganism, the eradication of staphylococcal infections in hospitals is becoming increasingly difficult and may be endemic in some countries (Michelim et al., 2005). For the treatment of infections caused by methicillin-resistant *S. aureus* from producer *pvl* (MRSA *pvl* (+)), the antimicrobials that proved to be effective were Vancomycin, Daptomycin, Linezolid and Teicoplanin (Lima et al., 2011; Kim et al., 2014; Liu et al., 2011).

This study aims to evaluate the pathogenicity of MRSA strains isolated from nasal colonization and infection in humans, and may help in research using experimental models that replicate the pathophysiology of the disease in humans.

MATERIALS AND METHODS

Experimental animals

Twenty-five (25) Swiss inbred mice were used in this study. The animals were young adults, six weeks of age, male weighing approximately 34 g and specific pathogenic-free (SPF). The animals mentioned were part of an experimental study approved by the Ethics Committee on Animal Research of the Pro-Rectory of Research and Postgraduate from Federal Fluminense University under the registration number, 439/2013.

Each experimental group was divided according to genotypic characteristics and colonization sites from which bacterial strains were isolated in humans. The animals were kept in collective and ventilated cages containing five animals in each group, which received commercial diet and filtered water *ad libitum*. Animals were exposed to light-dark cycles, at the temperature of 21 to 22°C (± 2) and 50 to 55% humidity.

Bacterial samples

Microbiological samples are part of collection of the Laboratory of Molecular Epidemiology and Biotechnology, Rodolpho Albino University Laboratory, Federal Fluminense University. Samples were preserved in brain heart infusion (BHI) medium containing 10% of glycerol and frozen at -80°C.

All bacterial samples were phenotypically tested in order to identify *S. aureus* using Gram staining, colonial morphology, fermentation of mannitol-salt agar (Zimbro et al., 2009), catalase production (Murray et al., 2007) and coagulase production (McDonald and Chapin, 1995). Thereafter, the species was confirmed by performing polymerase chain reaction (PCR) for 442 bp chromosomal DNA fragment, as protocoled by Martineau et al. (1998).

Methicillin resistance was identified using PCR for *mec*A gene according to the protocol of Oliveira and Lencastre (2002). The production of PVL as virulence factor was confirmed by *luk*F-PV and *luk*S-PV genes as established by Lina et al. (1999).

Bacterial samples selected for this study exhibited the following characteristics: methicillin-susceptible and PVL non-producing strains isolated from nasal colonization, *pvl* (-) MSSA; methicillin-susceptible and PVL-producing strains isolated from nasal colonization, *pvl* (+) MSSA; methicillin-resistant and PVL-producing strains isolated from peripheral blood of patients with severe pulmonary infection, *pvl* (+) MRSA.

Induction of infection

Bacterial colonies were cultivated for 24 h on trypticase soy agar (TSA) and suspended in sterile test tube containing 1000 µL of saline solution (NaCl 0.9%). Subsequently, serial dilutions were performed in order to obtain a density 1.0×10^7 colony forming units (CFU/mL).

The animals were anaesthetized through inhalation of Isoflurane FORANE® (2-chlorine-2-(difluorometoxy)-1.1.1-trifluor-ethane) in close campanula (Kiedrowski and Horswill, 2011), 50 µL of the bacterial suspension were intravenously inoculated through the tail vein, except in the control group (CG), which received the same volume of sterile saline solution. Animals were maintained in their respective cages for 96 h.

Histopathology

After 96 h, the animals were euthanized by overdose of Isoflurane

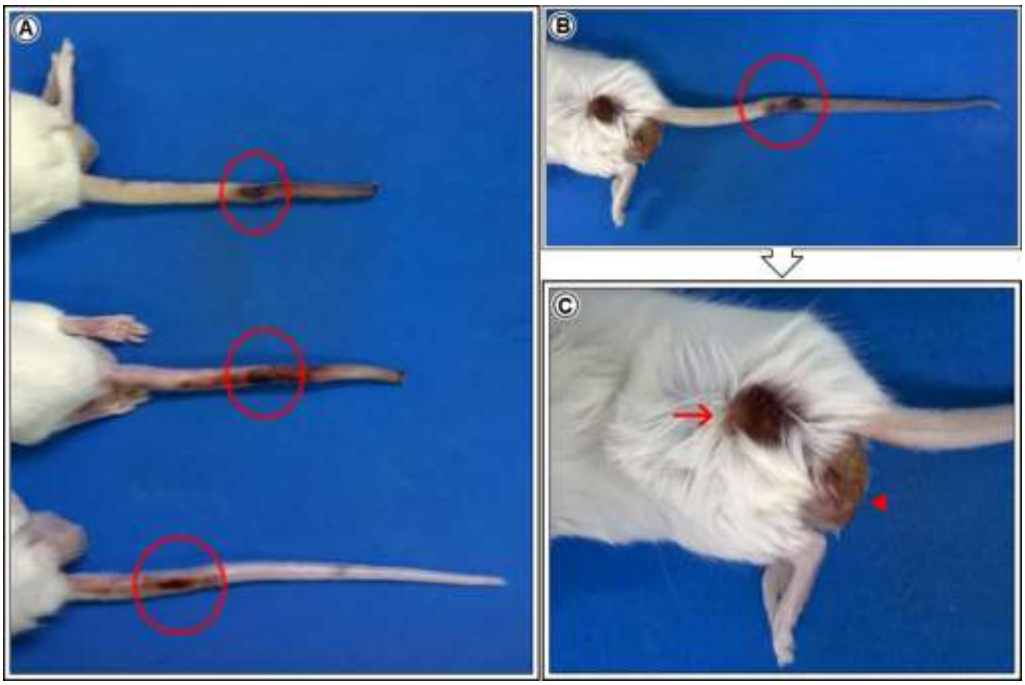

Figure 1. Infection in tail, skin and testicles. (A) Tails of mice inoculated with MSSA *pvl* (-) presenting ulceration and hyperemia, distal region with necrosis and region near base edematous. (B) Tails of mice inoculated with MSSA *pvl* (+) with ulceration and hyperemia, distal region with necrosis and region near the base edematous. (C) Posterior limb with skin infection (arrow), testicles with intense edema and hyperemia (arrowhead).

FORANE® (2-chlorine-2-(difluorometoxy)-1.1.1-trifluor-ethane). The eyeball was extracted by applying a pressure using tweezers around the orbital cavity. The samples were placed in cassettes and stored in 10% formaldehyde with pH between 0.6 and 0.7 during 48 h for the preparation of histological slides. Posteriorly, submitted to the processes of dehydration, diafanization and inclusion in paraffin, for the confection, 3-µm-thick sections were cut on a microtome (LAB-MR500), fixed on slides and stained with hematoxylin and eosin (H&E). The slides were observed in optical microscope (model LX 500) and photographed using iVm 5000 camera and ProgRes capture Pro 2.7 program to the description of inflammatory processes.

RESULTS

Among the results obtained, it was possible to observe pathological alterations not yet reported in an experimental model of systemic infection by this microorganism in mice. Three animals inoculated with β-lactam susceptible strain and not producing PVL: MSSA *pvl* (-), one animal inoculated with a strain producing PVL: MSSA *pvl* (+), and one animal inoculated with bacterial strain resistant to β-lactams and producer of PVL: MRSA *pvl* (+) isolated from peripheral blood in a patient afflicted by severe pulmonary infection; after 72 h of infection died of septic, with formation of ulceration and hyperemia at the site of inoculation (tail). The infectious process spread throughout the tail, causing end loss in distal region due to necrosis. In the region close to base of tail, pallor characteristic of edema was found (Figure 1A, B and Figure 2A). Only the animal inoculated with MSSA *pvl* (+) strains, presented a circular infectious region with hair loss and hyperemia on skin of right hind limb. Strong edema with hyperemia was also observed in both testes (Figure 1C). An unusual occurrence observed in the animal inoculated with MRSA *pvl* (+), was intense palpebral edema, with infection composed of purulent material, thick and yellowish covering the sclera in both eyes (Figure 2B). The histopathological analysis of the ocular globe revealed the presence of fibrinous inflammatory exudate adhered to the sclera, with cellular debris of necrotic tissue in the epithelium and some regions with leukocyte invasion (Figure 2C). CG animals did not present any anatomical alteration or inflammatory reaction at the inoculation site.

DISCUSSION

The observation of dermonecrosis in the skin of the right hind limb, tail necrosis and eye infection, using a model of systemic infection through the intravenous route, have not yet been reported in studies using a similar infection model. However, there are reports of same pathological processes in infections models skin using rabbit having

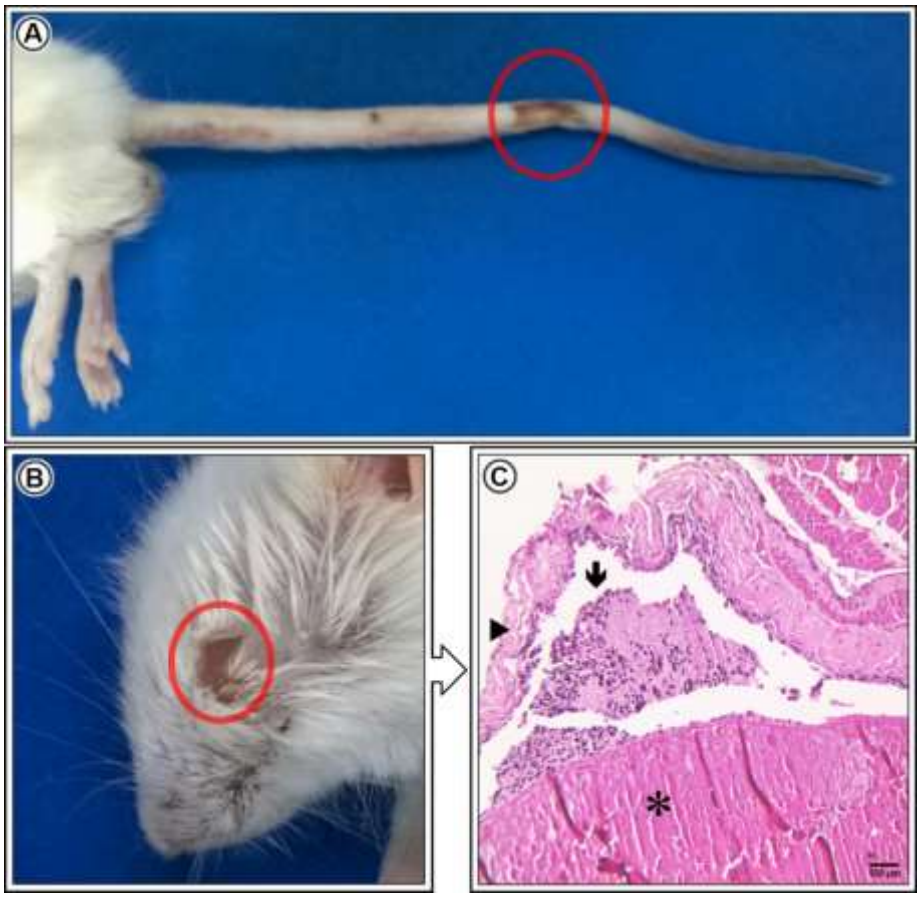

Figure 2. Tail and eye infection. (A) Tail of mouse inoculated with MRSA *pvl* (+) presenting ulceration and hyperemia, distal region with necrosis and region near the base with edema. (B) Inflamed eye with palpebral edema presenting thick yellowish material lining the sclera. (C) Photomicrography (100x), eyeball (asterisk) stained with H&E, presenting inflammatory exudate composed of polymorphonuclear leukocytes and fibrin (arrow), epithelium with necrosis region and leukocyte invasion (arrowhead).

local inoculation route (Diep and Otto, 2008). The fact that strains producing PVL are capable of causing dermonecrosis in rabbits and mice reinforces the hypothesis of a selective advantage in MRSA *pvl* (+) among healthy individuals. This report demonstrates the great importance in the sterilization of the hospital environment and surgical materials for the reduction of the dissemination of this microorganism, avoiding serious infections in hospitalized patients, because invasive surgical procedures and open cutaneous lesions are access doors for *S. aureus* to invade healthy tissues and through the bloodstream causes bacteremia and colonizes vital organs, leading to sepsis and death (Boyle-Vavra and Daum, 2007; Ward and Turner, 1980).

Infection models using animals can only partly reproduce the effect of pathogens on development of diseases in humans (Diep and Otto, 2008). Studies using PVL-purified in intravenous inoculation models demonstrated null effect in rats and rabbits, leading to the belief that action of PVL is associated with specific interactions with human neutrophils (Diep and Otto, 2008). However, in studies reported above it was possible to observe high intensity infections caused by strains producing PVL in Swiss mice having as an inoculation pathway, the vascular system.

The interactions between pathogen-host have not yet been well elucidated, mainly because MRSA strains do not secrete only PVL, as well as other exotoxins with leucolytic activities (Diep and Otto, 2008). In mice, intravenous inoculation with *S. aureus* triggers dissemination through the blood to other tissues and organs, where they establish lesions and abscess in skeletal muscle, in the vasculature, brain, lungs, heart, liver, spleen and kidneys (Cheng et al., 2009; Kim et al., 2014). In this study, *S. aureus* was able to cause intense local infection in skin and soft tissues, however, PVL-producing strains were more apt to migrate through the blood and cause infection in organs distant from inoculation site, as the eyes. This result reinforces the idea of PVL targeting polymorphonuclear leukocytes and

macrophages that are the first defense barriers of immune system, causing cytolysis and apoptosis in these cells, enabling the evasion of the immune system (Genestier et al., 2005; Boyle-Vavra and Daum, 2007).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Andriolo A (2005). Guides in outpatient medicine and hospital. UNIFESP/Medical Paulista School - Laboratory medicine. Coordenador Adagmar Andriolo. São Paulo: Manole.

Atshan SS, Shamsudin MN, Lung LTT, Sekawi Z, Ghaznavi-Rad E, Pei Pei C (2012). Comparative characterisation of genotypically different clones of MRSA in the production of biofilms. J. Biomed. Biotechnol. 2012(ID417247):1-7.

Berger-Bachi B, Rohrer S (2002). Factors influencing methicillin resistance in *Staphylococci*. Arch. Microbiol. 178(3):165-171.

Boles BR, Horswill AR (2008). *agr*-mediated dispersal of *Staphylococcus aureus* biofilms. PLoS Pathog. 4(4):e1000052.

Boyle-Vavra S, Daum RS (2007). Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. Lab. Invest. 87:3-9.

Bukowski M, Wladyka B, Dubin G (2010). Exfoliative toxins of *Staphylococcus aureus*. Toxins. 2:1148-1165.

Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM (2009). Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. FASEB J. 23:3393-3403.

DeLeo FR, Chambers HF (2009). Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat. Rev. Microbiol. 7(9):629-641.

Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, Lin F, Lin J, Carleton HA, Mongodin EF, Sensabaugh GF (2006). Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. Lancet 367(9512):731-739.

Diep BA, Otto M (2008). The role of virulence determinants in community-associated MRSA pathogenesis. Trends Microbiol. 16(8):361-369.

Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. 99(11):7687-7692.

Genestier AL, Michallet MC, Prévost G et al. (2005). *Staphylococcus aureus* Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. J. Clin. Invest. 115(11):3117-3127.

Gillet Y, Issartel B, Vanhems P (2002). Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 359:753-759.

Kiedrowski MR, Horswill AR (2011). New approaches for treating Staphylococcal biofilm infections. Ann. N. Y. Acad. Sci. 124:104-121.

Kim HK, Missiakas D, Schneewind O (2014). Mouse models for infectious diseases caused by *Staphylococcus aureus*. J. Immunol. Methods 410:88-99.

Lima JBA, Skare TL, Malafaia O et al. (2011). Sepsis inducing syndrome of multiple organ dysfunction: an experimental study in rats. Arq. Bras. Cir. Dig. 24(2):95-102.

Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J (1999). Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. 29(5):1128-1132.

Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, Rybak MJ (2011). Clinical practice guidelines by the infectious diseases society of america for the treatment of Methicillin-Resistant *Staphylococcus aureus* infections in adults and children. Clin. Infect. Dis. 52(3):285-92.

Lowy FD (1998). *Staphylococcus aureus* infections. New Engl. J. Med. 339(8):520-532.

Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG (2007). Community-associated methicillin-resistant *Staphylococcus aureus* isolates causing Healthcare-associated infections. Emerg. Infect. Dis. 13(2):236-242.

Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG (1998). Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. J. Clin. Microbiol. 36(3):618-623.

McDonald CL, Chapin K (1995). Rapid Identification of *Staphylococcus aureus* from blood culture bottles by a classic 2-hour tube coagulase test. J. Clin. Microbiol. 33(1):50-52.

Michelim L, Lahude M, Araújo PR, Giovanaz DS, Müller G, Delamare AP, Costa SO, Echeverrigaray S (2005). Pathogenicity factors and antimicrobial resistance of *Staphylococcus epidermidis* associated with nosocomial infections occurring in intensive care units. Braz. J. Microbiol. 36(1):17-23.

Murray PR, Baron EJ, Jorgensen JH, Landry MJ, Pfaller MA (2007). Manual of Clinical Microbiology. 9th Ed. Washington D.C.: ASM.

Novick RJ, Christie GE, Penadés JR (2010). The phage-related chromosomal islands of Gram-positive bacteria. Nat. Rev. Microbiol. 8(8):541-551.

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(7):2155-2161.

Otto M (2010). Looking toward basic science for potential drug discovery targets against community-associated MRSA. Med. Res. Rev. 30(1):1-22.

Tavares W (2002). Antibiotics manual and chemotherapeutic anti-infectives. cap 1: Classification of antimicrobials and cap 5: Bacterial resistance. 3th Ed. São Paulo: Atheneu.

Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J (2003). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. 9(8):978-984.

Ward PD, Turner WH (1998). Identification of staphylococcal Panton-Valentine leukocidin as a potent dermonecrotic toxin. Infect. Immun. 27(5):393-397.

Zimbro MJ, Power DA, Miller SM, Wilson GE, Johnson JA (2009). Manual of microbiological culture media. Difco™ & BBL™ Manual. BD. 2th Ed. United States of America. ISBN 0-9727207-1-5.