



Full Length Research Paper

Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin susceptible *Staphylococcus aureus* (MSSA) cause dermonecrosis and bacteremia in rats

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ABSTRACT

Inserting a contaminated vascular catheter during the surgical procedure is a factor that might promote biofilm formation on the catheter, possibly leading to bacteremia. The use of experimental models to evaluate organic changes allows a better understanding of infectious processes of multiple organs. The aim of this study was to investigate the influence of methicillin resistance based on bacterial genetic differences in induced infection and subcutaneous catheter colonization, when comparing MRSA and MSSA infections outcomes. Subcutaneous infections with MRSA and MSSA bacterial suspension were performed in 15 Wistar rats divided into three groups as following: saline solution control group, MRSA and MSSA suspension at a 1×10^5 CFU/mL for infection. Subcutaneous polyestilene catheters were surgically inserted into the skin of the animals before the infection. After 72 hours, subcutaneous implants, heart, spleen and kidneys were harvested and evaluated. The animals presented a typical auto resolutive case of bacteremia. The catheters and organs presented the same *S. aureus* isolate genotype as inoculated with evident biofilm formation. Our results showed both MRSA and MSSA bacteremia can compromise multiple organs within 72 hours of infection.

Keywords: *Staphylococcus aureus*, bacteremia, infection, biofilm and animal model.

INTRODUCTION

Staphylococcus aureus are Gram positive coccus commonly found colonizing skin and nasal cavities of healthy individuals (Cassetarri et al., 2005; Pereira, 2002; Koneman, et al., 2001). *S. aureus* has several features as virulence factors that supposedly contribute to its pathogenicity and its ability to colonize the host (Lutz et al., 2003; Michelim et al., 2005; Oliveira et al., 2001). Their virulence factors can be classified into three categories: (1) factors related to adherence to host cells or extracellular matrix like fibronectin, collagen, fibrinogen, coagulase; (2) factors related to evasion of host defense: staphylococci enterotoxin, toxic shock syndrome toxin (TSST), protein, lipase, and capsular

polysaccharide types 1, 5 and 8; (3) factors related to invasion of host cell and tissue penetration as: Pantone-Valentine leukocidin (PVL) (Diep and Otto, 2008) and alpha, beta, gamma and delta hemolysin toxins (Velazquez-Meza, 2005). Many factors contribute to the development of the disease complexity such as sepsis, endocarditis and necrotizing pneumonia, as patient's peculiarity, comorbidities, late diagnosis and treatment. The study of bacteria and its negative effects on the human organism is very important, since the understanding of how the secreted molecules by these microorganisms influence inflammatory processes are not totally elucidated.

Staphylococcus aureus infection enables the development of life threatening diseases such as endocarditis and necrotizing pneumonia. Initially, bacteremia is the result of bacteria biofilm detachment within bloodstream followed by multiple organs infection and sepsis. The risk factors for bacteremia include advanced age, ongoing chemotherapy and invasive procedures (Lowy, 1998). Regarding organ infections, the worst prognosis is for the lung. Bacteria could have been introduced as pericarditis from an injured site, mainly caused by penetrating injury by the use of central venous catheter. In the case of the lungs, it may cause pneumonia resulting from aspiration and also osteomyelitis and septic arthritis (Weems, 2001).

Staphylococcus aureus carries a mobile genetic element, described as Staphylococcal Cassette Chromosome mec (SCCmec), which contains determinants of antimicrobial resistance genes (Lowy, 1998; Ito et al., 2001). The multidrug-resistant MRSA is commonly found in hospital environments. In addition, antibiotic resistance β -lactam antibiotics, still have resistance to other classes of agents such as aminoglycosides (gentamicin), sulfamethoxazole/trimetoprim (SMZ-TMP), quinolones, tetracyclines, clindamycin and erythromycin. The resistance to many types of antimicrobial agents might be due to selective pressure imposed by usage, which classify these isolates as MRSA associated with the hospital environment (HA-MRSA, "Hospital-Associated MRSA") (Iain et al., 2003).

The transmission of *S. aureus* may occur through the handling of contaminated objects by health care professionals, runny nose, and especially in contact between individuals showing open infected wounds (Bradley, 1997). The ease of transmission makes the staphylococcal infections a difficult to treat and major problem in public health (Safdar and Maki, 2002).

MATERIALS AND METHODS

This study has been approved by the Committee of Ethics in Animal Experimentation from Fluminense Federal University, by the protocol number 439. Fifteen rats (Wistar) were randomly divided into three groups (table 1).

Bacterial samples used in the study were obtained from a collection of the laboratory of Molecular Epidemiology and Biotechnology - Fluminense Federal University. The colonies were grown in broth TSA (trypticase soy agar) for 24 hours, diluted in sterile saline (0.85% NaCl) at a concentration of 1×10^5 CFU/mL.

Surgical procedure

Animals were anesthetized by isoflurane inhalation under saturation (Forane® Abbott). The dorsal skin of rats were

shaved and cleaned with 10% polyvinylpyrrolidone-iodine solution. The subcutaneous insertion of the polystyrene was made under the dorsal midline of each rat with 1.5 cm incision. Aseptically, a catheter fragment (Becton Dickinson-Arentina SRL), measuring 0.5 cm was deployed in sterile bag. Bacterial suspension at a 1×10^5 CFU/mL concentration (30 μ L) was subsequently inoculated subcutaneously at one inch away from the catheter where the catheter was done, the control group was inoculated subcutaneously with sterile saline.

The bags were sealed with synthetic GLUBRAN® surgical Glue 2 (Sr GEM I-Italy). The animals returned to individual cages and were examined every six hours. After 72 hours of observation, the euthanasia of the animals was performed by an overdose of isoflurane anesthetic (Forane® Abbott). All catheter s, heart, spleen and left kidney were explanted for analysis.

Assessment of catheter colonization and bacteremia

Explanted organs, heart, spleen and kidney were harvested macerated were harvested from the five animals of each group, macerated in esteril saline solution and the extract was transferred to a sterile microtube and homogenized. A fraction of 100 μ L of the suspension was plated onto blood agar medium. The catheter s were explanted and grown directly onto blood agar plates. All samples were cultured for 24 hours at 37°C. The colonies that showed hemolysis halo were subject to tests for *S. aureus* identification.

Phenotypic methods for *S. aureus* Identification

The samples were plated onto mannitol salt agar, subjected to catalase and coagulase tests. The positive samples were cultured for antimicrobial susceptibility using oxacillin and cefoxitin disks (CLSI, 2012) and genotypic analysis to detect the presence of *mecA* gene, using DNA extraction method described by Aguiar-Alves et al. (2006) and polymerase chain reaction, as described by Oliveira and Lencastre (2002).

RESULTS AND DISCUSSION

None of the animals died after 72 hours of infection or have had clinical signs of infection, as fever, weight loss, dehydration or any behavioral change. Groups inoculated with both *S. aureus* strains presented a swelling in the area of insertion of the catheter, with skin color change from red to purple, as well as local detachment of the layers of the epidermis (dermonecrosis) (Figure 1).

The animals in the control groups did not present any colonization at the site of the catheter insertion, contrary to what was observed in MRSA and MSSA groups. The catheters plated onto blood agar also showed a sick layer of bacterial growth (Figure 2).

Table 1. Number of rats for each groups

Study design.		
Groups	Inoculum 1 x 10 ⁵ CFU/mL	N
MRSA	<i>mecA</i> +	5
MSSA	<i>mecA</i> -	5
Control	Sterile saline	5

The study was divided into three groups, each containing five animals, the control group inoculated with saline sterile group inoculated with MRSA bacterial strain Methicillin-resistant *S. aureus* and MSSA group inoculated with strain sensitive to methicillin. The bacterial concentration was inoculated 1 x 10⁵ CFU / ml.

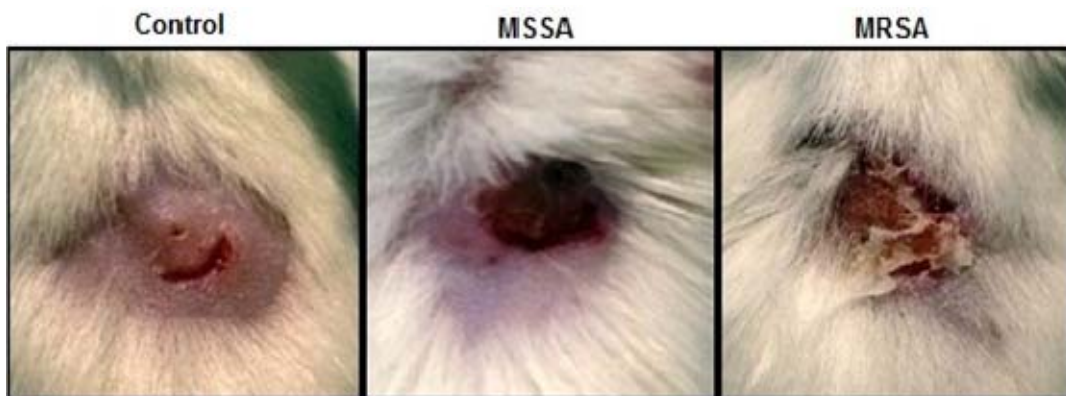


Figure 1. Rats dorsal region of each group (control, MSSA, MRSA). The images show the place where the catheter's fragments were inserted and the skin damage associated to it, after 72 hours of infection.

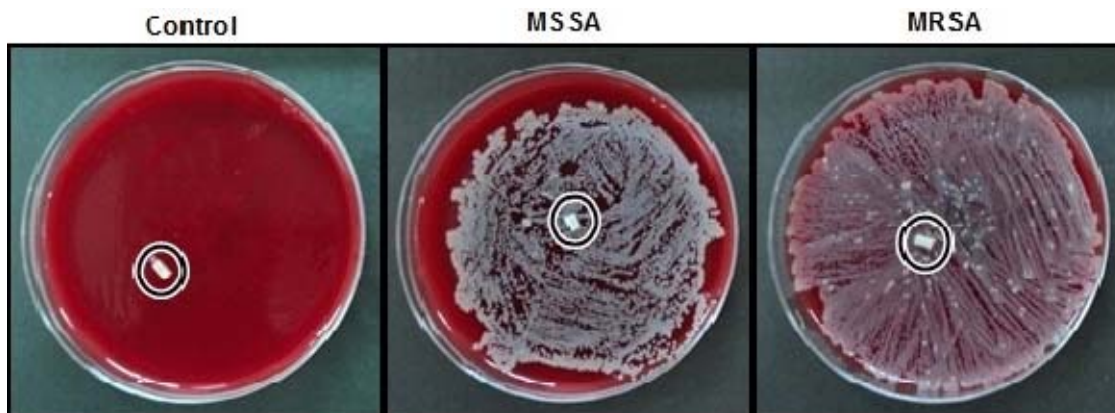


Figure 2. The images show the harvested catheter's fragments of each group (control, MSSA, MRSA). The catheters were incubated at 37°C onto blood agar plate. The open circles show the catheter's fragments and the colonies formation after overnight incubation.

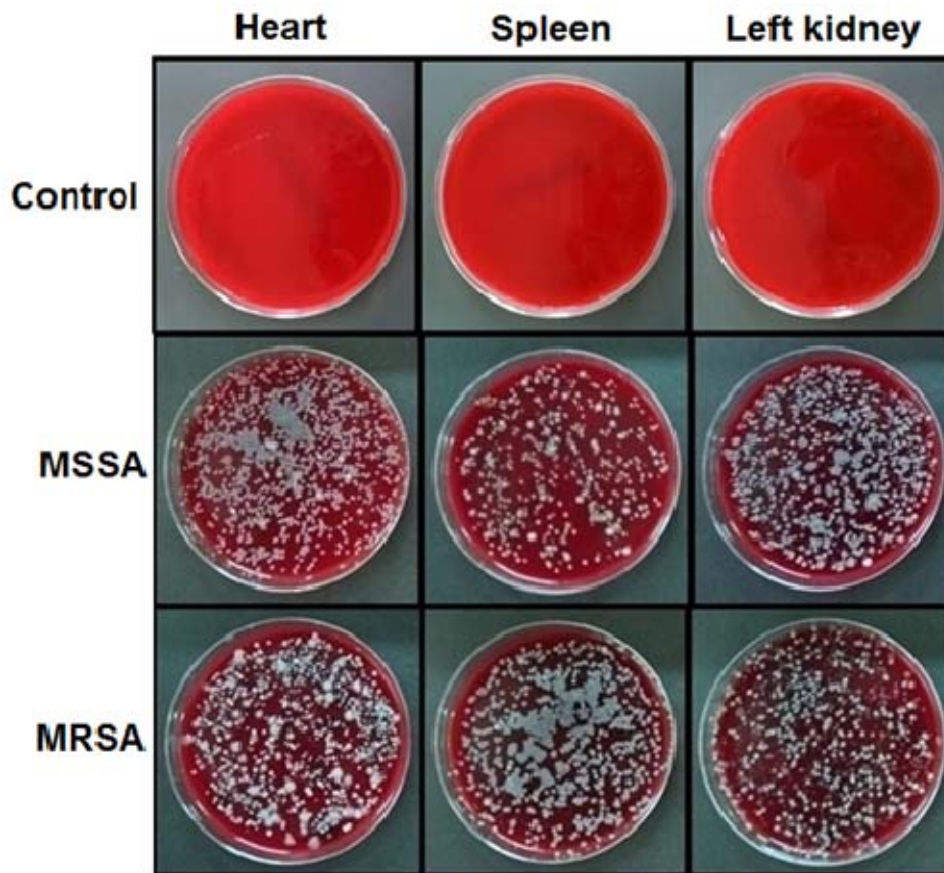


Figure 3. Heart, spleen and the left kidney were harvested from each group (control, MSSA, MRSA). The images show the colonies formation after 24 hours of incubation at 37°C onto blood agar plate after 72 hours of infection. The images show the colonies formation after 24 hours of incubation at 37°C.

Colonies were identified as MRSA and MSSA by sensitivity to antimicrobial susceptibility test for methicillin and ceftazidime, as well as the presence of *mecA* gene by PCR tests, respectively. The presence of MRSA strains on catheters confirmed its participation in biofilm formation. (Table 2; Figure 4).

Organ extracts of animals infected by MSSA and MRSA groups presented the development of bacteremia by presence of colonies in blood agar plates (Figures 3 and 4).

In this study, we observed that the formation of the catheter allowed biofilm bacteremia by inoculation of *S. aureus* in rats dorsal skin. This species were chosen because of its close correlation to humans inflammatory reactions to infections. Current studies show that sepsis is the leading cause of acute renal failure in hospitalized patients in recent years. Its incidence is approximately 19% in septic patients, 23% in patients with severe sepsis and 15% of hospitalized patients with septic shock, compromising multiple organs and systems (Lima et al., 2011).

Sepsis after infection of catheters has been mentioned as one of the main complications in vascular surgery. Vascular catheters are susceptible to infection by direct contamination during surgery procedures. Methicillin-resistant *S. aureus* has been mentioned as a major problem in cardiovascular surgical units' contamination during catheter insertion (Antonios et al., 2006; Earnshaw, 2002).

Limitations of this study were observed on the attempt to keep animals in control group free of contamination by other microorganisms, different from *S. aureus*. A second limitation was shown to be not using the ELISA method for the identification of specific antibodies in these animals which identify an anticipated settlement, prior to the beginning of the study. No difference in colonization with MSSA and MRSA was observed with the methodology used, possibly a change in inoculum preparation allow a change in the expression of results.

According to the type of material used to make the catheters, its adherence to microorganisms can be increased, which may cause biofilm formation. It was

Table 2. Identification of *S. aureus* species after 72 hours challenge

Groups	Inoculum	Material extracted for analysis	Number of colonies <i>S. aureus</i> found (CFU)
MRSA	mecA +	Catheter	>1000
		Heart	>1000
		Spleen	>1000
		Left kidney	>1000
MSSA	mecA -	Catheter	>1000
		Heart	>1000
		Spleen	462
		Left kidney	>1000
Control	Sterile saline	Catheter	0
		Heart	0
		Spleen	0
		Left kidney	0

In the group MRSA strains carrying the *mecA* gene colonized organs, heart, spleen, left kidney and the catheter inserted subcutaneously into the back of the animals with a quantity > 1000 CFU. A similar result was observed in the group inoculated with MSSA strains do not carry the *mecA* gene. What was different in the control group where no catheter colonization and organs.

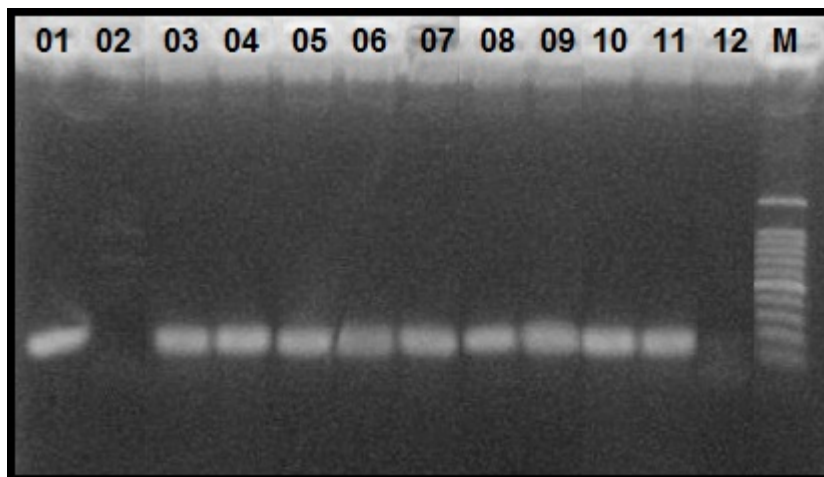


Figure 4. Result of Polymerase Chain Reaction Test and electrophoresis on agarose gel 1% for detection of the *mecA* gene in samples of *S. aureus* - lane 01: positive control; lane 02: negative control; lane 03 and 04: isolates from catheter; lane 05, 06 and 07: isolates from cardiac tissue; lane 08 and 09: isolates from spleen; lane: 10 and 11: Isolated samples of the left kidney; lane 12: reagents used for amplification of the *mecA* gene; lane M: molecular weight marker (100 bp) DNA ladder.

possible to observe the above-mentioned microorganisms forming biofilms. For these factors, antibiotic prophylaxis can be used to prevent such infections, however many of them, such as *S. aureus* can present beta lactam resistance, which will make imperative the use of a broad spectrum of antimicrobial profilaxys. The observation of organs infected by the inoculated *S. aureus* strain confirmed that this model is suitable for the study of this type of infection and biofilm formation method to study the pathogenesis of staphylococcal infections. More studies need to be performed to elucidate the different steps in the biofilm formation and sepsis by *S. aureus*.

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