PATHOGENIC STUDIES OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES FROM BIOMEDICAL WASTES

Ganesh Manikandan S.*, Lakshminarasimhan C.², Thajuddin N.³ and Saravanan R.⁴

¹V.M.K.V. Engineering College, VMU, Salem-636308, Tamilnadu, India.
²PRIST University, Vallam, Thanjavur-613403, Tamilnadu, India.
³Bharathidasan University, Thiruchirappalli-620024, Tamilnadu, India.
⁴BMERF, Salem-636124, Tamilnadu, India.

Abstract: Staphylococcus aureus is naturally present in the nasal membranes and skin of warm blooded animals including human beings. It can grow in aerobic and anaerobic conditions, forming grape-like clusters. It causes a range of infections from mild, such as skin infections and food poisoning to life threatening, such as pneumonia, sepsis, osteomyelitis and infectious endocarditis. Methicillin Resistant Staphylococcus aureus (MRSA) isolated from clinical samples, band aid, cloth and cotton collected from hospitals and clinical laboratories in various parts of Tamilnadu. All MRSA isolates were transferred to nutrient agar containing 5% sodium chloride and 3 gm of oxacillin antibiotic. MRSA isolates were properly diluted with sterile saline and injected to tail vein of mice. After 14th day mice were dissected, liver and kidneys were selected for histopathological studies. MRSA injected mice group showed different pathogenic characters like lesions, white coloured patches, cellulitis and necrosis when compared with control group.

Keywords: Biomedical waste, Cellulitis, Necrosis, Methcillin resistant Staphylococcus aureus, Oxacillin

1. INTRODUCTION

Staphylococci are Gram positive bacteria, present in skin of human beings and warm blooded animals (Manikandan et al., 2009a). Methicillin-resistant Staphylococcus aureus (MRSA) is a type of bacteria that does not react to certain antibiotics and will normally cause skin infections, and MRSA can also cause other infections including pneumonia. MRSA can be fatal (Centre for Disease Control and Prevention, 2007). Staph infections occur most often among people in hospitals and healthcare facilities (such as nursing homes and dialysis centers) who have weakened immune systems. The infection can be spread by skin-to-skin contact, sharing or touching a personal item with someone with infected skin, or touching a surface or item that has been in contact with someone with MRSA (Centre for Disease Control and Prevention, 2007). The increasing prevalence of community-associated MRSA (CA-MRSA) has resulted in a change in the paradigm for the treatment of skin and soft-tissue infections (Sharif and Ashour, 2008). The CA-MRSA strains are more likely to carry the genes for the Panton-Valentine leukocidin toxin and have increased susceptibility to non-β-lactam antibiotics (Pradhan, 2008)

*Corresponding Author: E-mail: biotech27@gmail.com
and the mec-A gene present in all MRSA strains and which act as molecular marker (Manikandan et al., 2009a, b). S. aureus causes renal failure and chronic mastitis of cattle also. S. aureus and MRSA can survive up to 12 months in hospital dust, bedding and clothing. However, the role of the environment in the spread of MRSA in hospitals is still open to conjecture and routine sampling is not advised (BSAVA, 2010). Hand touch sites seem to be most important in contamination and transmission, but other sites could include floors, tables anaesthetic machines, taps, door handles, cages, clinical equipment (stethoscopes, otoscopes, endoscopes, etc.), and computer mouse and keyboards. The aim of the present study was to analyze the pathogenesis of MRSA isolates from biomedical wastes with suitable laboratory animals. In this study, ethical committee rules and regulation were strictly followed by the authors.

2. MATERIALS AND METHODS

The methods described hereunder were adopted from Paulhebeisen et al. (2001), Kaneko et al. (2003) and Calander et al. (2004).

2.1 Test Animal

Albino mice were obtained from the Department of Biotechnology, Center for Advanced Studies in Botany, Guindy, University of Madras Tamilnadu. The weight of the animals was between 30 and 36 g, and they were 3 weeks old while subjecting them for pathological studies.

2.2 Test Sample

Band-aid, cotton, guaze and hospital dusts were collected from various hospitals and medical laboratories in different parts of Tamil Nadu, India. The samples were transferred to Mannitol Salt Broth medium and incubated at 37°C for 12 hrs.

2.3 Inoculum Preparation and Inoculation

MRSA strains were cultured on a Tryptase Soy Agar-based Sheep Blood for 24 hrs at 37°C. The bacteria were suspended in endotoxin-free sterile saline and harvested by centrifugation (3,000 × g, 4°C, and 10 min). Then the microorganisms were resuspended in cold sterile saline and diluted to 1 – 3.5 × 10⁷ CFU/ml. The suspension (1.0 ml) was drawn into a 1.0-ml syringe, and rapidly injected with a 26-gauge needle into 49 ml of rapidly stirred ice-cooled sterile saline. Then the suspension was injected into the tail vein of each mouse (10 ml/g of body weight). The saline solution injected to tail vein of each mouse served as control.

2.4 Pathology

The mice were sacrificed on day 14th, and sera were obtained and kept frozen at –20°C until analysis. In each experiment, the mice were weighed and evaluated for signs of arthritis regularly after inoculation. At day14, they were sacrificed and observed for the symptoms in internal organs.
2.5 Histopathology
The kidneys and livers were chosen for histopathological studies. Thin sections of 5 μm of kidneys and liver were made with Microtome and the tissue sections were placed on glass slides, and then fixed with 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline). Paraffin wax was frequently used to fixation. The samples were transferred through baths of progressively more concentrated ethanol to remove the water, followed by a clearing agent, xylene, to remove the alcohol, and finally molten paraffin wax which replaces the xylene. Orcein stain was applied on tissue sectioned slides for five minutes, washed gently with sterile water and the results were observed under microscope.

3. RESULTS AND DISCUSSION
The test group animals showed low food intake. The maximum weight loss of 60% and 80% were noted in the mice inoculated with $2 \times 10^7$ and $3.5 \times 10^7$ CFU/ml MRSA respectively, in contrast to no weight loss in the un-inoculated control groups. It was also noted that the infecting MRSA in mice also mediated vascular leakage with lowered blood pressure compared with control group. The bacteriological study of re-isolation showed ‘positive’ in the experimental animals of test groups drawn from their nasopharyngeal secretions.

The hind limbs of the experimental animals showed rigidity. The legs showed lesion-containing pus. Mortality rate of 10% was noted. One animal among a test group of 10 died during the study period of 48 days. An MRSA dose of $3.5 \times 10^7$ CFU/ml was found to be lethal (to a tune of 10%). It was noted that the overall survival rate was high with $1 \times 10^7$ CFU/ml MRSA dose in injected mice compared with $3.5 \times 10^7$ CFU/ml MRSA dosage. The liver and kidney cells were highly damaged and showed necrosis. More number of white coloured lesion-like patches was also seen on MRSA inoculated mice when compared with the control group. The control group mice did not show any symptoms on the kidneys and livers.

S. aureus produces a wide variety of pathogenic factors that contribute to its ability to colonize and cause infection. Some bacteriologists (Hiramatsu et al., 2000) classified the factors into four categories: adhesions, exoenzymes, exotoxins and others. They identified almost all known S. aureus pathogenic factors within the strains, N315 and Mu 50. In the present study the results revealed a close resemblance to their results as revealed from MRSA isolates which expressed adverse clinical symptoms in experimental animals. Some researchers (Agalar et al., 2005) conducted experiments with mice and reisolated MRSA ($10^6$-$10^8$) from injected mice. The wound infection was created by MRSA strain. The result proved, thus, that S. aureus was in the experimental mice.

Kiser et al. (1999) reported that the samples collected from nasopharyngeal and lungs tissues of injected mice contained S. aureus. The number of bacteria found in the reisolated lung samples showed only minimal (1 to 6 CFU) occurrence. Surprisingly, S. aureus was not detected in samples of the blood of the experimental mice. S. aureus expressed various surface proteins, some of which contained adhesions that could act as specific receptors for extra cellular matrix proteins of the host tissue. Production of collagen binding protein is strongly associated with pathogenesis of osteomyelitis and septic arthritis.
Staphylococci were the most important cause of prosthetic joint infections and they are the significant pathogens in acute arthritis, affecting both native and prosthetic joints. Thus the encapsulated MRSA can cause various infections and produce toxins. The clinical observations are in well correlation with the above findings and perhaps the test group animals would have been suffering from the toxic effects of the MRSA isolates (Levy et al., 2001).

MRSA appears to be emerging as an important veterinary and zoonotic pathogen, and the epidemiology of MRSA in household pets may take a parallel course to that in humans. Ongoing MRSA surveillance in animals is required, including proper testing of specimens from clinically affected animals and surveillance for colonization. The potential for transmission of this clone between humans and pets should also be evaluated to clarify its epidemiology and to facilitate development of measures to reduce household transmission (Carlo et al., 2006). From the present study it is concluded that the biomedical wastes contain MRSA isolates which are able to cause infection in mice.

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References


