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SDS-PAGE and Western/Immunoblot Studies on Methicillin-Resistant *Staphylococcus aureus* Isolates from Tamil Nadu, India

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ABSTRACT

Staphylococcus aureus is the most prominent species in medical microbiology. It causes various diseases in humans and animals. *S. aureus* have become resistant to various antibiotics, particularly methicillin. Methicillin-resistant *S. aureus* proteins were isolated and purified with 10% trichloro acetic acid (TCA) and estimated with the Lowry's method. In SDS-PAGE protein bands were found between 5.8 and 23.5 kDa regions. Polyclonal antibody was raised in normal healthy rabbits and it was used for an Immunoblot study, which showed a 5.8 kDa band.

Keywords: immunoblot, polyclonal antibody, MRSA, SDS-PAGE
Abbreviations: **MRSA**, methicillin-resistant *Staphylococcus aureus*; **SDS-PAGE**, sodium dodecyl sulphate-polyacrylamide gel electrophoresis

INTRODUCTION

Staphylococcus aureus is a group of Gram-positive bacteria. Its main habitats are the nasal membranes and skin of warm blooded animals (Kuroda *et al.* 2001). It causes mild and serious infections like sepsis, pneumonia, osteomyelitis and infectious carditis (Projan and Novick 1997). It can produce enterotoxin in humans and animals.

Modern medium faces a crisis as new strains of multidrug-resistant bacteria threaten advanced treatments and intensive care. Every year nearly five million people die due to infections that do not respond to antibiotics particularly multidrug-resistant *S. aureus* (MRSA) infection. Drugs, which were considered safe, have begun to fail because the bacteria are developing the ability to resist antibiotics. The application of more antibiotics against these bacteria has resulted in the development of a multi-drug resistance mechanism. Therefore, vaccines need to be prepared. Vaccine preparation needs knowledge about antigenic protein study. Julie *et al.* (2002) reported that the Frb family of Fe-regulated protein was present in the *S. aureus* cell wall. Gaston *et al.* (1998), during evaluation of electrophoretic methods for typing methicillin-resistant *S. aureus* strain plasmid profiles, reported on whole cell protein and immunoblotting methods.

S. aureus causes renal failure. Renal failure patients were significantly more likely to colonize and represent a potential source of MRSA (Price *et al.* 2000). *S. aureus* is the most frequent infectious agent in chronic mastitis of cattle. It may also cause acute infections. Chronic mastitis with clinical or sub-clinical manifestation is a serious economic problem all over the world (Baumgartner *et al.* 1984).

Antigenic protein was isolated from MRSA using a standard procedure and SDS-PAGE (sodium dodesyl sulphate-polyacrylamide gel electrophoresis) was performed for identification of the protein profiles. Western blot and immunoblot techniques confirmed the immunogenic capacity of isolated proteins.

MATERIALS AND METHODS

Preparation of cell surface antigenic protein

From MRSA isolates and MTCC 87 cell surface antigenic proteins were prepared from cultures which were grown in 5 ml Brain Heart Infusion broth incubated at 37°C for 18 hrs as described by Hussain *et al.* (2000). The antigenic cell surface proteins were suspended with 20 µl of Tris glycine buffer (pH 8.0), to which an equal volume of 10% trichloroacetic acid was added, and the precipitated proteins were collected. 20 µl of petroleum ether was added for removing the lipids.

Estimation of protein

Total antigenic protein concentration was determined by Lowry *et al.* (1951) procedure. The concentration of antigenic protein was read at 660 nm spectrophotometrically using a digital spectrophotometer (Spectronic 180) and compared with a standard plot drawn by known concentrations of bovine serum albumin.

SDS-PAGE

Samples were loaded in SDS-PAGE along with molecular weight markers (AB GENE, UK). After running for 45 min at 50 V, the gel was removed and stained with Coomassie Brilliant Blue (CBB) stain for 30 min and transferred to 7% glacial acetic acid as destaining solution until the excess stain got de-stained. The bands were observed with a trans-illuminator and subsequently measured.

Western/immunoblot

Western blotting was performed following the protocol of Towbin *et al*. (1979). Primary antibodies stored at –70°C in a deep freezer until the study, were raised in healthy male rabbits (*Oryctolagus cuniculus* L.) weighing more than 2kg following the procedure of Wolf (1954). The gel and (NCP) nitrocellulose filter paper (Millipore, HAWP) were kept in transfer buffer (pH 8.3) in an electro-

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Research Note

transfer apparatus. After the transfer the nitrocellulose filter paper was dried, it was floated on the surface of a tray of deionized water and allowed to wet from beneath by capillary action. Then it was submerged in the water for 5 min to displace trapped air bubbles. The filter was transferred to a tray containing working solution of Ponceau-S stain and incubated for 5-10 min with gentle agitation. When the protein bands were visible, the NCP was destained using deionized water and the positions of proteins were marked using the molecular weight marker as standards with water proof black ink. Immunological probing was then proceeded. The nitrocellulose filter was then transferred to blocking solution containing 5% (w/v) non-fat dried milk, 0.01% of antifoam A and 0.02% sodium azide in (PBS) phosphate buffered saline. It was incubated for 1-2 h at room temperature with gentle agitation on a platform shaker. The blocking solution was discarded and the filter was incubated with the primary antibody directed against the targeted protein. The filter was then washed and incubated in a sealed plastic bag with a secondary reagent which was the anti-antibody coupled to HRP (horse radish peroxidase) enzyme. The filter was transferred to a tray containing 200 ml of 150 mM NaCl and 50 mM TrisCl (pH7.5). The filter was incubated for 10 min at room temperature with gentle agitation. This step was repeated for three more times using fresh NaCl and TrisCl solution each time. Chromogenic substrate was added with enzyme-coupled antibodies. 10 ml of 30% H_2O_2 was added and mixed well; the mixture was used immediately. The reaction was stopped as soon as the bands were clearly visible.

RESULTS AND DISCUSSION

All MRSA isolates and standard strain MTCC 87 have 110-120 µg protein per 20 µl. In all MRSA isolates protein bands were found in between 5.8 and 23.5 kDa regions. MRSA isolates showed a different banding pattern when compared to the standard strain (positive control) MTCC 87 (**Fig. 1**). In the present study, MSSA (methicillin susceptible *S. aureus*, negative control) has not been used, since the study included MRSA standard (MTCC 87). The standard strain MTCC 87 was possessing 24.2 kDa band but not at the 23.5 kDa region. MRSA 1, 2, and 3 showed bands at the region of 23.5 kDa. MRSA 3 protein bands tightly bound to CBB stain, hence appears dark (**Fig. 1**). MRSA 4 did not show any clear band. The study has shown that the polyclonal antibody subjected was highly sensitive and specific to the cell wall antigenic protein.

S. aureus whole cell polypeptide SDS-PAGE evaluation could be used for taxonomic and typing tool analysis. The polypeptide patterns and comparison made by the use of coefficient of Dice showed minor differences in banding patterns among the same species strains and they have got banding pattern between 56 and 53 KDa regions (Clink and Pennington 1987). The current study showed minor band differences in between isolates. As per the banding pattern of this research, all MRSA isolates were classified into four groups. Julie *et al.* (2002) and Krikler *et al.* (1986) identified two conserved immunogenic *S. aureus* cell wall proteins of 40 and 87 kDa regions. Kawaharda (1985) described a method for typing *S. aureus* based on SDS-PAGE banding pattern of cell extracts with monospecific antisera encapsulated strains. In the present study polyclonal antibodies were used only for the identification to the immunogenic protein. A 5.8 KDa protein was found to have good immunogenic capacity.

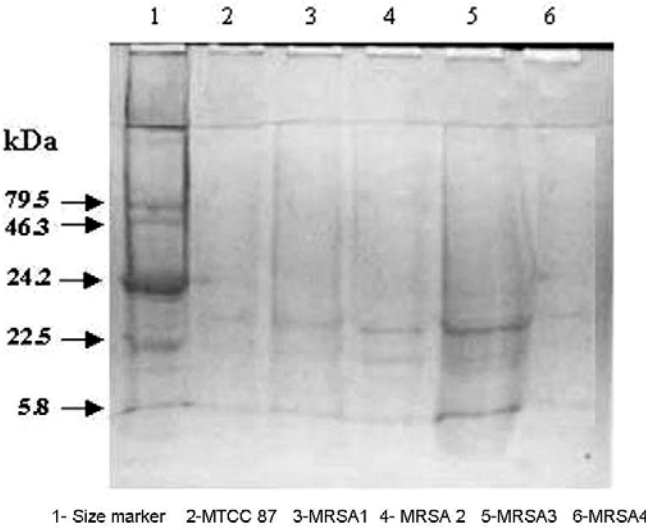

Fig. 1 SDS-PAGE protein profile of MRSA isolates.

REFERENCES

Baumgartner A, Nicolet J, Egginoann M (1984) Plasmid profiles of *Staphylococcus aureus* causing bovine mastitis. *Journal of Applied Bacteriology* **56**, 159-163

Clink J, Pennington TH (1987) Staphylococcal whole cell polypeptide analysis evaluation as a taxonomic and typing tool. *Journal of Medical Microbiology* **23**, 41-44

Gaston MA, Duff PS, Ellis K, Roberts JIS, Richardson JF, Cooke EM (1998) Evolution of electrophoretic methods for typing methicillin-resistant *Staphylococcus aureus*. *Journal of Medical Microbiology* **26**, 189-197

Hussain FM, Vavras B, Bethel CD, Daum RS (2000) Current trends in community acquired methicillin-resistant *Staphylococcus aureus* at a tertiary care pediatric facility. *Pediatric Infectious Diseases Journal* **19**, 1163-1166

Julie A, Menissey A, Hamcott J, Bishop K, Johnson AD, Hilland PJ, Williams (2002) Conservation, surface exposure and *in vivo* expression of the four family of Iron-regulated cell wall proteins in *Staphylococcus aureus*. *Infection and Immunity* **70**, 2399-2407

Kawaharda WA, Fouraid JM, Vann WF, Arbeit R, Schneerson RS, Robbins JB (1985) Method for the serological typing of the capsular polysaccharides of *Staphylococcus aureus*. *Journal of Clinical Microbiology* **36**, 445-447

Krikler SJ, Pennington TH, Petric D (1986) Typing of strains of *Staphylococcus aureus* by western blot analysis of culture supernates. *Journal of Medical Microbiology* **21**, 169-171

Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K (2001) Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *The Lancet* **357**, 1225-1240

Laemmli UK (1970) Cleavage of structured proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 683-685

Lowry OH, Brough R, Fan AL, Rendell RT (1951) Protein measurement with solin phenol reagent. *The Journal of Biological Chemistry* **193**, 263

Price MF, Carlini M, Houstons X, Gentry L (2000) Prevalence of nasal colonization with methicillin resistant infect control. *Hospital Epidemiology* **21**, 603-605

Projan SJ, Novick RP (1997) The molecular basis of pathogenicity. In: Crossley KB, Archer GL (Eds) *The Staphylococci in Human Disease*, Churchill Livingstone, New York, pp 55-61

Towbin H, Stachelin T, Gordan J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose: Procedure and some applications. *Proceedings of the National Academy of Sciences USA* **76**, 4350-4354

Wolf JW (1954) *Animal Inoculation* (Ed 183), Chanks C Thomaas, Springfield, IL, USA, 33 pp