Bangladesh J Med Microbiol 2008; 02 (01): 3-7

Bangladesh Society of Medical Microbiologists

Original Article

Coagulase Typing and *spa* Typing of Methicillin Resistant *Staphylococcus aureus*: Relatedness Among Patients' and Carrier Strains

Sadia Afroz[1], ABM Bayezid Hossain[2], Afzalunnessa Binte Lutfor[1], Naima Muazzam[3]

[1]Department of Microbiology, Sir Salimullah Medical College, Mitford, Dhaka; [2]Department of Surgery, Sir Salimullah Medical College, Mitford, Dhaka; [3]Department of Microbiology, Dhaka Medical College, Dhaka

Abstract

This study was carried out to determine the types of Methicillin-Resistant *Staphylococcus aureus* (MRSA) by two typing systems, Coagulase typing and *spa* typing and to identify relationship between types of MRSA strains isolated from patients and hospital staff carriers. A total of 40 MRSA strains, 33 from cases of wound infection and 7 from anterior nares of hospital staffs were investigated. Coagulase typing showed that all the MRSA strains isolated from patients were coagulase type VI and all the strains isolated from carriers were coagulase type VII. In *spa* typing, seven *spa* types were detected such as S4, S6, S7, S8, S9, S10 and S11. Predominant *spa* type in patients was S7 detected in 15 (45.45%) MRSA isolates, followed by S4 in 8 (24.24%) strains and S6 in 5 (15.15%) strains. Other *spa* types detected in patients in small numbers were S8 (3.03%), S9 (3.03%), S10 (6.06%) and S11 (3.03%). While in strains of MRSA isolated from carriers, only two types S4 and S9 were detected. Both the *spa* types identified from carriers were also detected in a total of 9 (27.2%) of MRSA strains isolated from patients.

Key words: MRSA, Carriers of MRSA, Coagulase typing, *spa* typing

Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen worldwide and is potentially a great threat to medical therapy.[1] Infected and colonized patients provide the primary reservoir and transmission occurs mainly through hospital staffs.[2] Typing of MRSA strains is recognized to be important in understanding the epidemiology, transmission route of the organism, evaluating the effectiveness of infection control and antimicrobial therapy.[3] A number of typing methods based on biological properties and genomic polymorphisms have been designed and applied for typing of MRSA strains.[4] Typing methods include chemical resistograms, phage typing, ribotyping, biotyping, antibiograms, pulsed-field gel electrophoresis (PFGE) and PCR- based methods.[5]

Coagulase typing method has been used successfully in epidemiological investigations of Staphylococcal infections. This method is based on the difference in eight antigenic types (I to VIII) of coagulase produced by *S. aureus*. It is a simple, rapid, easy to perform and reproducible typing system.[6]

Genotyping methods based on DNA analysis has become the most trusted marker for MRSA typing. The sequencing of the polymorphic Xr region of the Protein A gene (*spa*), containing a variable number of 24-bp repeat regions, flanked by well-conserved regions and is the *spa* typing.[7] This sequence-based typing method detects the nucleotide and amino acid sequence

✉ ***Correspondence:***
Dr. Sadia Afroz
Clinical Pathologist, Department of Microbiology
Sir Salimullah Medical College, Mitford, Dhaka.

diversity of the Xr region of protein A. The *spa* typing combines a number of technical advantages such as rapidity, reproducibility, portability and higher discriminatory power, enabling its use for typing MRSA strains.[8]

The aim of this study was to investigate and compare the types of MRSA by coagulase and *spa* typing to identify source and transmission of MRSA and to differentiate genetic origin of strains from patients and nasal carriers.

Methods

The study was a cross-sectional study carried out in the department of Microbiology, Dhaka Medical College from January to December, 2004.

A total of 40 MRSA isolates, 33 from cases of wound infection and 7 from anterior nares of hospital staffs were investigated. Identification of MRSA was done by Oxacillin disc diffusion method using 1µg disc and MRSA latex agglutination test (Denka Seiken, Japan) following manufacturer's instructions. Coagulase typing of MRSA was done in the department of Microbiology, Mymensing Medical College. The identified MRSA strains were preserved at -20ºC and finally sent to Sapporo Medical University, Japan for *spa* typing.

Coagulase type was determined by a neutralization test using Coagulase type specific antisera (I-VIII) (Staphylococcal Coagulase antiserum kit, Denka Seiken, Inc., Tokyo, Japan). A 0.1 ml amount of each antiserum and normal rabbit serum (as a control) was added to 0.1ml of the suspension obtained from an overnight culture of each test isolate. This solution was incubated at 37ºC for 1 hour, after which 0.2 ml of rabbit plasma was added. Inhibition of coagulation after further incubation at 37ºC for at least 1 hour indicated the Coagulase type.[9]

For *spa* typing, bacterial DNA was extracted using achromopeptidase. DNA sequence including that of the Xr-region in the *S. aureus* gene was amplified by PCR using a pair of primers spa-2 and spa-5. (Table I)

The size of the PCR product was determined by using appropriate molecular weight markers in Electrophoresis in 2% NuSieve 3:1 Agarose stained with Ethidium bromide (FMC BioProducts) at 100 Volts for 1±5 hours. The PCR product contained additional 72 and 37 nucleotides at the 5' and 3' ends of the repeat region, respectively. Consequently, the relation of the repeat number of 24-base units to the size of the PCR product is expressed by the following formula: size (bp) of PCR product = (repeat no.) × 24+109.[10]

Table I: Sequence of oligonucleotide primers and their locations in protein-A gene

Primer name	Nucleotide sequence (5'-3')	Location* (nucleotide nos.)
spa-1	+CAAGCACCAAAAGAGGAA	1153-1170
spa-2	-CACCAGGTTTAACGACAT	1475-1492
spa-3	+GCTAAAAAGCTAAACGAT	1132-1149
spa-4	+CCTTCGGTGAGCAAAGAA	1102-1119
spa-5	+GACGATCCTTCGGTGAGC	1096-1113
spa-6	-TCAGCAGTAGTGCCGTTTGC	1516-1535

* Nucleotide number is described according to the protein-A gene sequence of *S. aureus* strain 8325-4.

Direct DNA sequencing of the PCR product from representative strains was performed by dideoxynucleotide chain termination method using Sequenase PCR product sequencing kit (United States Biochemical, Cleveland, Ohio, US), employing the primers listed in Table I. In addition to the DNA amplified with primers spa-2 and spa-5, PCR products generated with primers spa-5 and spa-6 were also used as templates for sequencing of all the strains. The *spa* types expressed by arrangement of repeat unit genotypes, was assigned to each isolated bacterial strain.[10]

Result

Out of the 40 MRSA strains investigated, 33 (82.5%) were isolated from patients with wound infection and 7 (17.5%) from hospital staff carriers. Among these strains, two coagulase types were detected: Coagulase type VI and Coagulase type VII. All MRSA strains isolated from cases of wound infections were Coagulase type VI and those from carriers were Coagulase type VII. (Table II).

Table II: Coagulase type of MRSA strains isolated from patients and carriers

MRSA isolates' source	Number of isolates	Coagulase type of the isolates
From patients	33 (82.5%)	Type- VI
From carrier	7 (17.5%)	Type- VII

The *spa* type of all the MRSA strains examined was expressed as series of repeat units, each of which represented 24 nucleotides. (Figure 1)

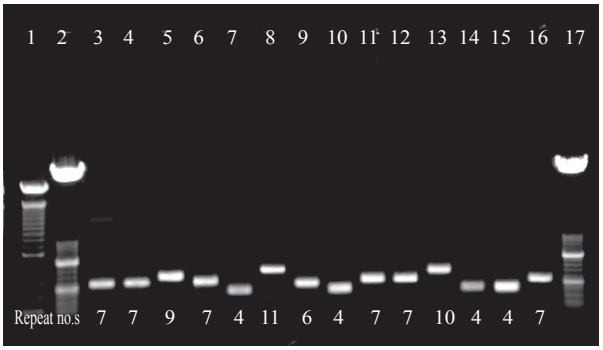

Figure 1: PCR products containing the whole Xr region of representative MRSA strains. Estimated number of repeat is indicated below each lane. Lane 1- Molecular weight marker (100 bp ladder), Lanes 2 &17- Molecular weight markers (50 bp ladder).

Consequently, 7 *spa* types were identified among the 40 strains isolated. The MRSA strains from wound infection cases belonged to the *spa* types S4, S6, S7, S8, S9, S10 and S11, and MRSA strains from carriers were *spa* types S4 and S9. In MRSA from patients, 7 repeat type (S7) was the predominant type. The highest number of *spa* types was S7 and was detected in 15 (45.45%) of MRSA strains, followed by S4 in 8 (24.24%), and S6 type in 5 (15.15%) strains. S10 was detected in 2 (6.06%) strains, while each of *spa* types S8, S9 and S11 was found in 1 (3.03%) strain. In carriers, S4 type was detected from 4 (57.14%) and S9 type from 3 (42.85%) of the isolated strains. (Table III)

Table III: *spa* types of the MRSA strains isolated from patients and carriers

	Number of isolates from-	
spa type	wound infection (n=33)	Carrier (n=7)
S4	8 (24.24)	4 (57.14)
S6	5 (15.15)	0 (0)
S7	15 (45.45)	0 (0)
S8	1 (3.03)	0 (0)
S9	1 (3.03)	3 (42.85)
S10	2 (6.06)	0 (0)
S11	1 (3.03)	0 (0)
Total	33 (100)	7 (100)

Figures within parentheses indicate percentages

Discussion

The Methicillin-Resistant *S. aureus* (MRSA) is one of the most significant healthcare-associated pathogens responsible for a wide range of hospital infections and is widely prevalent in Bangladesh.[11,12] Hospital staffs play an important role in transmission of MRSA to patients. Several studies has reported that nasal carriage of the hospital staffs act as a significant source of MRSA for new nasal acquisition by patients and contribute to development of infection.[13] Typing of MRSA strains is important for epidemiological monitoring and specially identification of the strains responsible for outbreaks. Typing is used to delineate the pattern of spread, identify source and vehicle of transmission and to monitor the reservoir of epidemic strains.[14]

In the present study, Coagulase types VI and VII were detected from the strains isolated from patients and carriers respectively. This finding differ from other reports on coagulase typing. This typing system was widely used in Japan, where predominant coagulase types were type II and type IV.[15,16] This difference in predominant coagulase types might be due to geographical variation.

In this study, coagulase types of the MRSA strains isolated from patients and carriers were different. It suggests that the infections were endogenous in origin. However, coagulase typing, being a phenotypic method have lower discriminatory power and can not differentiate genetic relatedness of MRSA isolates from different sources.[17] So, possibility of infection from exogenous sources can not be excluded. In *spa* typing of the MRSA strains, seven different genetic types, *spa* types S4, S6, S7, S8, S9, S10, S11 were identified. Strains showing different *spa* types can be regarded as different clones. Although coagulase types of the strains were identical, they might belong to different genetic types. Predominant *spa* types in Bangladesh (S7 and S4) was different from those reported from other countries. Studies has shown that dominant *spa* type varies in different countries. In Germany predominant *spa* type detected was S30, in Japan S10 and in Paraguay it was S5.[10,18,19]

The *spa* types from carriers (S4 and S9) were different from predominant *spa* type from the patients (S7) in this study. This suggests that majority of MRSA infections was

endogenous in origin or transmitted from patient to patient. In case of wound infection, high rate of endogenous source is well documented, which is explained by the fact that the normal colonizing flora in a patient changes within 24-48 hours under selective antibiotic pressure. In these patients, skin is often colonized with the same strain and disinfection is not effective in deeper layers of skin. So, these endogenous *S. aureus* becomes the source of wound infection.[20] However, *spa* types of carrier-strains were also detected in 9 (27.3%) of the patient-strains. This finding shows that there is a role of hospital staff carriers in transmitting MRSA infections to patients. Carriage of MRSA in healthcare provider has been associated with some hospital outbreaks and considered as a potential risk for MRSA infection among hospitalized patients.[21] Reliable detection and elimination of MRSA carriage in hospital staffs is, therefore, necessary for effective infection control intervention in a hospital.

It is apparent from the present study that the predominant MRSA types in Bangladesh is different from that of other countries, and hospital staffs, who act as carriers, play an important role in transmission of MRSA to patients. Regular screening of hospital staffs and elimination of carriage state may reduce the rate of MRSA infection in a hospital. Regular epidemiological monitoring and typing of MRSA to identify the changing trend of the strains should be carried out on a large scale for effective control of MRSA infections.

Acknowledgement

We acknowledge Professor Nobumichi Kobayashi (Head, Department of Hygiene, Sapporo Medical University, Japan) for his logistic support and technical assistance in *spa* typing of MRSA strains. We especially thank him for his encouragement and contributing valuable opinion for this study. We are grateful to Dr. AKM Shamsuzzaman and all other members of Department of Microbiology, Mymensingh Medical College for their help and support in performing coagulase typing done in this study.

References

1. Ayliffe GAJ. The progressive intercontinental spread of Methicillin resistant *Staphylococcus aureus*. Clin Infect Dis 1997; 24(1): 74-79.
2. McDonald M. The epidemiology of Methicillin resistant *Staphylococcus aureus*: surgical relevance 20 years on. Aust N Z J Surg 1997; 67: 682-685.
3. Lee JL, Suh JT, Kim YS, Lenz W, Bierbaum G, Schaal KP. Typing and antimicrobial susceptibilities of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated in a hospital in Korea. J Korean Med Sci 2001; 16: 381-385.
4. Schlichting C, Branger C, Fournier JM, *et al*. Typing of *Staphylococcus aureus* by pulsed field gel electrophoresis, zymotyping, capsular typing, and phage typing: resolution of clonal relationship. J Clin Microbiol 1993; 31: 227-232.
5. Murchan S, Kaufmann ME, Deplano A, *et al*. Harmonization of pulsed field gel electrophoresis protocols for epidemiological typing of strains of methicillin resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strain. J Clin Microbiol 2003; 41 (4): 1574-1585.
6. Ushioda H, Terayama T, Sakai S, Zen yozi M, Nishiwaki, Hidano A. Coagulase typing of *Staphylococcus aureus* and its applications in routine work. J Jeljaszewicz (ed.) 1981; 10: 77-83.
7. Frenay HME, Theelen JPG, Schouls LM, *et al*. Discrimination of epidemic and non-epidemic Methicillin resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. J Clin Microbiol 1994; 32: 846-847.
8. Shopsin B, Gomez M, Montgomery SO, *et al.* Evaluation of Protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol 1999; 37(11): 3556-3563.
9. Shimizu A, Fujita M, Igarashi H, Takagi M, Nagase N, Safaki A, Kawano J. Characterization of *Staphylococcus aureus* coagulase type VII isolates from Staphylococcal food poisoning outbreaks (1980-1995) in Tokyo, Japan by Pulsed field gel electrophoresis. J Clin Microbiol 2000; 38(10): 3746-3749.
10. Kobayashi N, Urasawa S, Uehara N, Watanabe N. Analysis of genomic diversity within the Xr-region of the protein A gene in clinical isolates of *Staphylococcus aureus*. Epidemiol Infect 1999; 122: 241-249.
11. Karim S, Ahmed S, Parvez M, *et al.* Emerging multi-drug resistant organisms in a tertiary care hospital of Dhaka city. Bangladesh J Med Sc 2002; 8: 9-13.
12. Jahan Y, Jahan F, Mamun KZ, *et al.* Emergence of Methicillin resistant *Staphylococcus aureus* associated with wound infections. Mymensingh Med J 2004; 13(1): 76-81.

13. Eveillard M, Martin Y, Hidri N, Boussougant Y, Joly-Guillou ML. Carriage of methicillin-resistant *Staphylococcus aureus* among hospital employees: prevalence, duration and transmission to households. Infect Control Hosp Epidemiol 2004; 25 (2) : 114-120.

14. Struelens MJ and Members of European society for clinical Microbiology and infectious diseases. Consensus guidelines for evaluation, appropriate use and interpretation of molecular epidemiological typing systems for bacterial pathogens. Clin Microbiol Infect 1996; 2: 2-11.

15. Nakae M, Sasaki H, Yasui H, *et al*. Epidemiological studies on drug resistance patterns, coagulase types and MRSA phage types. Jpn J Antibiot 1999; 52(4): 313-321.

16. Masaki H, Yoshimine H, Onizuku S, *et al*. Coagulase typing of *Staphylococcus aureus* in geriatric wards after introduction of preventive measures of hospital infections. Kanes Zas 1997; 71(3): 229-235.

17. Strandén A, Frei R, Widmer AF. Molecular typing of Methicillin-Resistant *Staphylococcus aureus*: Can PCR replace Pulsed Field Gel Electrophoresis? J Clin Microbiol 2003; 41(7): 3181-3186.

18. Strommenger B, Kettlitz C, Weniger T, Harmsen D, Friedrich AW, Witte W. Assignment of Staphylococcus isolates to groups by *spa* typing, *SmaI* macrorestriction analysis, and Multilocus Sequence Typing. J Clin Microbiol 2006; 44(7): 2533-2540.

19. Mayor L, Ortellado J, Menacho C. Molecular characterization of Methicillin-Resistant *Staphylococcus aureus* isolates collected in Asunción, Paraguay. J Clin Microbiol 2007; 45(7): 2298-2300.

20. Schentag JJ, Hayatt JM, Carr JR, Paladino JA, Birmingham MC, Zimmer GS. Genesis of Methicillin-Resistant *Staphylococcus aureus*. How treatment of MRSA infections has selected for vancomycin resistant enterococci & the importance of infection control. Clin Infect Dis 1998 ; 26: 1204-1214.

21. Lessing MP, Jordens JZ, Bowler IC. When should healthcare workers be screened for methicillin resistant *Staphylococcus aureus*? J Hosp Infect 1996; 34: 205- 210.