Chronic Diseases Journal

DOI: 10.22122/cdj.v6i4.365 Published by Vesnu Publications

Staphylococcal protein A (spa) typing of Staphylococcus aureus isolates causing nosocomial infections

Rashid Ramazanzadeh[1], Mohammad Hosein Darehshiri[2], Mehdi Mirzaii[3]

1 Professor, Cellular and Molecular Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
2 Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran
3 Department of Microbiology, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran

Original Article

Abstract

BACKGROUND: Staphylococcal protein A (spa) typing is a typing method based on the DNA sequence analysis of staphylococcal protein A gene. The purpose of this study was to do molecular typing of Staphylococcus aureus isolated from patients in Toohid and Besat hospitals, Sanandaj, Iran, in 2014.

METHODS: Clinical specimens were collected from hospitalized patients over a period of 1 year. Staphylococcus aureus isolates were identified using culture and biochemical standard methods based on the Clinical and Laboratory Standards Institute (CLSI) guideline. spa gene patterns in Staphylococcus aureus isolates were identified using spa-typing techniques.

RESULTS: In total, 20 different patterns of spa gene were obtained in staphylococcus aureus isolates in this study, which included type t030 (6 cases), types t230, t459, and t701 (3 cases of each one), types t11332 and t304 (2 cases of each one), and types t325, t012, t1149, t1810, t197, t325, t7789, t808, t871, t937, t14896, t14913, t14928, and t14929 (1 case of each one). The highest prevalence belonged to types t030 (30.0%), and then, types t230, t459, and t701 (15.0% for each one). New types of t14896, t14913, t14928, and t14929 were identified during this study.

CONCLUSION: There were some well-known patterns of spa types, and also we identified new types that should be studied more to qualify. Analysis of these patterns can improve insight to design nosocomial infection control programs.

KEYWORDS: Staphylococcus Aureus, Epidemiology, Nosocomial Infections

Date of submission: 12 Sep. 2017, *Date of acceptance:* 15 Dec. 2017

Citation: Ramazanzadeh R, Darehshiri MH, Mirzaii M. **Staphylococcal protein A (spa) typing of Staphylococcus aureus isolates causing nosocomial infections.** Chron Dis J 2018; 6(4): 225-9.

Introduction

Staphylococcus aureus (S. aureus) is a commensal organism, and is responsible for a wide range of human diseases including serious nosocomial infections.[1-3] S. aureus is thought to be transmitted predominantly via direct contact, or via hands or droplet spreading, and indirectly through the fomites, and through the air in hospitals.[4]

S. aureus have several virulence factors such as surface immunoglobulin (Ig)-binding protein A (staphylococcal protein A or spa), that binds to IgG molecules, and therefore prevents phagocytosis of the bacterial cells by the host immune system.[5]

Different methods have been used to detect S. aureus strains such as spa typing, staphylococcal cassette chromosome mec (SCCmec) typing, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST).[6,7] It is shown that the spa type, in contrast to PFGE, can be used to study and determine both the molecular evolution as well as hospital outbreaks of S. aureus.[8]

Corresponding Author:
Rashid Ramazanzadeh
Email: rashid@muk.ac.ir

Typing the highly variable X region of the S. aureus surface protein A gene is one of the most common methods for genotyping.[9,10] This is due to the sequence data and ease of exchanging results via database available on the internet (http://www.spaserver.ridom.de).[11] spa typing is the method that has become increasingly popular during recent years.[12] spa typing has major advantages with the high discriminatory power, typing accuracy, speed, reproducibility, and ease of interpretation.[13-15] spa typing allows data comparison between clinical laboratories in the international and national levels.[16,17]

In the present study, the occurrence and characteristics of S. aureus isolates from the patients in different unit of hospitals was assessed using sequencing and spa typing method.

Materials and Methods

Bacterial isolates: In this cross-sectional study, clinical specimens were collected from hospitalized patients in Toohid and Beasat hospitals affiliated to Kurdistan University of Medical Sciences, Sanandaj, Iran, over a period of 1 year (in 2014).

Totally, 97 clinical specimens including urine, wound, abscess, blood, and cerebrospinal fluid (CSF) were gathered, and 40 S. aureus strains were analyzed in this study. Bacterial samples were cultured on sheep blood agar (Oxoid, UK), and were assessed using laboratory standard methods such as colony morphology and a positive plasma coagulase reaction, as well as standard biochemical methods.[18] Thermonuclease (nuc) gene was used as a gold standard for confirmation of S. aureus isolates.[4] For further analyses, the isolates were sub-cultured on tryptic soy broth (Oxoid, UK), and stored with glycerol at -20 ˚C and -70 ˚C.

Antibiotic susceptibility tests were used according to the Clinical and Laboratory Standards Institute (CLSI) guidelines with Kirby-Bauer disk diffusion method.[5] Antibiotic disks including erythromycin, clindamycin, gentamycin, ciprofloxacin, trimethoprim/ sulfamethoxazole, teicoplanin, mupirocin, and oxacillin (Rosco Diagnostica, Denmark) were used based on laboratory standards. Polymerase chain reaction (PCR) for mecA gene was used as a conformational test with using specific primers.

Genomic DNA was isolated from overnight culture with Cinna pure DNA protocol (kit for the isolation of DNA from Gram positive Bacteria) (Sinaclon, Iran). DNA template was prepared, purified, and stored at -20 °C until needed.

PCR and DNA sequence analysis (spa typing): Molecular spa typing is a PCR- and DNA sequence-based method has been used for epidemiological investigations.[18-20] This technique allows inter-laboratory exchange of information by means of a standard software analysis package and central internet depository (www.spaserver.ridom.de).[21] spa type is a repeated sequences which compose 24 repeated nucleotides (eight codons). In order to do typing of the polymorphic region of protein A, the X region of the spa gene was amplified using spa gene F primer (5′-TAAAGACGATCCTTCGGTGAGC-3′) and spa gene R primer (5′-CAGCAGTAGTGCCGTTTGCTT-3′).[22,23]

PCR reactions were performed in 25 µl final volumes containing 3µl of purified DNA, 1 µl of each primer, 12 µl of Master Mix (Sinaclon, Iran), and 8 µl of distilled water. The PCR amplification conditions for SPA primer were as: the initial denaturation at 95 ˚C, 5 minutes, and next 35 cycles consisting of a denaturation step at 94 ˚C, 30 seconds, annealing at 60 ˚C, 1 minute, extension at 72 ˚C, 1 minute, as well as a final extension step at 72 ˚C for 10 minutes, and storage at 4 ˚C at the end. Amplified products were sequenced by Macrogen (South Korea). Analysis of DNA sequences was done using Choromas Lite

software (Technelysium Pty Ltd, Australia). For this study, PCR amplification and sequence analysis of 40 spa products were performed with a software, and considered in 'very good' or 'excellent' grades.

Results

Antibiotic resistance: From 97 S. aureus strains, 52 (54.2%) were resistance to erythromycin, 49 (51.1%) to clindamycin, 39 (40.1%) to ciprofloxacin, 12 (12.5%) to teicoplanin, 35 (36.4%) to gentamycin, 18 (18.8%) to trimethoprim/ sulfamethoxazole, 58 (60%) oxacillin, and 17 (17.7%) to mupirocin.

Diversity of spa types: The genomic diversity analysis of 40 strains of S. aureus was carried out using spa typing method. Samples were sequenced with the same primers as used in PCR. Overall, we identified 20 different spa types among the 40 S. aureus isolates (Table 1). These isolates constituted 44.3% of all methicillin-resistant S. aureus (MRSA) isolates, but 55.7% of MRSA isolates in this study.

Table 1. Specification of 97 Staphylococcus aureus strains with spa typing method

spa type	spa type repeat succession (n = 40)	Number of repeats
t14896*	04-21-12-17-486-17-12-12-17	1
t14913*	11-10-21-17-34-24-34-22-676	1
t14928*	04-21-12-41-486-17-12-17	1
t14929	08-34-17-17	1
t030	15-12-16-02-24-24	6
t325	07-12-21-17-34-13-34-34-33-34	2
t7789	08-16-34-24-17	1
t701	11-10-21-17-34-24-34-22-25-25	3
t871	15-12-16-17-24-24	1
t304	11-10-21-17-34-24-34-22-25	2
t1149	08-16-34-24-34-17-17	1
t808	08-16-02	1
t1810	04-21-12-41-20	1
t012	15-12-16-02-16-02-25-17-24-24	1
t230	08-16-02-16-34	3
t459	04-34-17-66-66-32-17-23-24	3
t11332	04-21-12-41-486-17-12-12-17	2
t197	11-10-34-24-34-22-25	1
t937	08-16-34-24-34-34-17-17	1

* New types identified in this study, and registered in the spa server (www.spaserver.ridom.de).

Discussion

S. aureus, specially MRSA strains, is one of wide spread infections in hospital and community.[1,19,20] Typing and analysis of S. aureus strains responsible for serious infections is now routine in many parts of the world.[23] To establish communication survey of pathogen strains together, molecular methods such as spa typing, MLST, and PFGE can be used.[17] Typing methods are being used as a tool to identify strains on the genetic characteristics basis. These techniques can show the relationship between strains and clones.[9] Among these techniques, spa typing with high discriminatory power (99.5%), is fast, easy, and inexpensive, and is able to determine the lineage of strains, and classify them.[11,24-26]

Moodle et al. studied 320 S. aureus isolates collected from South Africa. They found that the five most common spa types were t012 (n = 68), t037 (n = 77), t045 (n = 25), t064 (n = 68), and t1257 (n = 31), which made up 84% of the isolates.[16] According to previous studies, the most prevalent spa types were recorded different such as t008 (31.9%) and t002 (27.6%),[2] t7685 (11.5%), t230 (8%), and t1149 (8%),[7] t003 (22%), t151 (16%), and t008 (12%),[27] and t030 (43%)and t037 (43%).[15] In this study, four novel spa type (t14896, t14913, t14928, and t14929) were detected and registered on spa server. The highest prevalence was related to t030 (30%) and t459, t701, and t230 (15% for each one). According to reports, most of S. aureus were colonized in hospital personnel and fomites.[27,28] Spreading occur via direct contact or handling of contaminated materials. Therefore, in nosocomial infection, clonal transmission is the prominent factor.

Conclusion

There are similar patterns of spa gene which represents a common source of infection in hospitals, and analysis of these patterns can

help to break the chain of infection transmission in hospitals.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

This is a part of a MSc thesis in Kurdistan University of Medical Sciences. The authors wish to extend their gratitude to the Deputy of Research and Technology of this university for financial support. We also thanks to Dr. Moradi for statistical analysis.

References

1. Abdollahi S, Ramazanzadeh R, Kalantar E, Zamani S. Molecular Epidemiology of Staphylococcus aureus with ERIC-PCR method. Bull Env Pharmacol Life Sci 2014; 3(3): 158-65.
2. Babouee B, Frei R, Schultheiss E, Widmer AF, Goldenberger D. Comparison of the DiversiLab repetitive element PCR system with spa typing and pulsed-field gel electrophoresis for clonal characterization of methicillin-resistant Staphylococcus aureus. J Clin Microbiol 2011; 49(4): 1549-55.
3. Basset P, Nubel U, Witte W, Blanc DS. Evaluation of adding a second marker to overcome Staphylococcus aureus spa typing homoplasies. J Clin Microbiol 2012; 50(4): 1475-7.
4. Brakstad OG, Aasbakk K, Maeland JA. Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. J Clin Microbiol 1992; 30(7): 1654-60.
5. Cockerill FR, Wikler MA, Bush K, Dudley MN, Eliopoulos GM, Hardy DJ, et al. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
6. Deurenberg RH, Stobberingh EE. The evolution of Staphylococcus aureus. Infect Genet Evol 2008; 8(6): 747-63.
7. Emaneini M, Khoramrooz SS, Taherikalani M, Jabalameli F, Aligholi M. Molecular characterization of Staphylococcus aureus isolated from children with adenoid hypertrophy: Emergence of new spa types t7685 and t7692. Int J Pediatr Otorhinolaryngol 2011; 75(11): 1446-9.
8. Enright MC, Knox K, Griffiths D, Crook DW, Spratt BG. Molecular typing of bacteria directly from cerebrospinal fluid. Eur J Clin Microbiol Infect Dis 2000; 19(8): 627-30.
9. Faria NA, Carrico JA, Oliveira DC, Ramirez M, de Lencastre H. Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains. J Clin Microbiol 2008; 46(1): 136-44.
10. Frenay HM, Bunschoten AE, Schouls LM, van Leeuwen WJ, Vandenbroucke-Grauls CM, Verhoef J, et al. Molecular typing of methicillin-resistant Staphylococcus aureus on the basis of protein A gene polymorphism. Eur J Clin Microbiol Infect Dis 1996; 15(1): 60-4.
11. Hallin M, Friedrich AW, Struelens MJ. spa typing for epidemiological surveillance of Staphylococcus aureus. Methods Mol Biol 2009; 551: 189-202.
12. Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 2003; 41(12): 5442-8.
13. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. spa typing method for discriminating among Staphylococcus aureus isolates: Implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol 2004; 42(2): 792-9.
14. Mellmann A, Weniger T, Berssenbrugge C, Keckevoet U, Friedrich AW, Harmsen D, et al. Characterization of clonal relatedness among the natural population of Staphylococcus aureus strains by using spa sequence typing and the BURP (based upon repeat patterns) algorithm. J Clin Microbiol 2008; 46(8): 2805-8.
15. Mirzaii M, Emaneini M, Jabalameli F, Halimi S, Taherikalani M. Molecular investigation of Staphylococcus aureus isolated from the patients, personnel, air and environment of an ICU in a hospital in Tehran. J Infect Public Health 2015; 8(2): 202-6.
16. Moodley A, Oosthuysen WF, Duse AG, Marais E. Molecular characterization of clinical methicillin-resistant Staphylococcus aureus isolates in South Africa. J Clin Microbiol 2010; 48(12): 4608-11.
17. Moosavian M, Wadowsky R. Analysis of genomic fingerprint patterns of coagulase-negative Staphylococci strains isolated from pediatric patients blood cultures using repetitive sequence-based PCR. Jundishapur J Microbiol 2007; 3(4): 147-53.
18. Petersson AC, Olsson-Liljequist B, Miorner H, Haeggman S. Evaluating the usefulness of spa typing, in comparison with pulsed-field gel electrophoresis, for epidemiological typing of methicillin-resistant

Staphylococcus aureus in a low-prevalence region in Sweden 2000-2004. Clin Microbiol Infect 2010; 16(5): 456-62.
19. Ramazanzadeh R, Narenji H. Molecular epidemiology of the Staphylococcus aureus by Rep-PCR method in Sanandaj hospitals. Life Sci J 2013; 10: 117-21.
20. Ramazanzadeh R, Salimizand H, Shahbazi B, Narenji H. Prevalence of mecA gene of methicillin resistant staphylococcus spp. isolated from nosocomial infections and environmental specimens in Sanandaj Hospitals, Kurdistan, Iran. Research in Molecular Medicine 2015; 3(3): 38-42.
21. Ruppitsch W, Indra A, Stoger A, Mayer B, Stadlbauer S, Wewalka G, et al. Classifying spa types in complexes improves interpretation of typing results for methicillin-resistant Staphylococcus aureus. J Clin Microbiol 2006; 44(7): 2442-8.
22. Shore AC, Rossney AS, Kinnevey PM, Brennan OM, Creamer E, Sherlock O, et al. Enhanced discrimination of highly clonal ST22-methicillin-resistant Staphylococcus aureus IV isolates achieved by combining spa, dru, and pulsed-field gel electrophoresis typing data. J Clin Microbiol 2010; 48(5): 1839-52.
23. Soliman RS, Phillips G, Whitty P, Edwards DH. Distribution of meticillin-resistant Staphylococcus aureus spa types isolated from health-care workers and patients in a Scottish university teaching hospital. J Med Microbiol 2009; 58(Pt 9): 1190-5.
24. Strommenger B, Braulke C, Heuck D, Schmidt C, Pasemann B, Nubel U, et al. spa Typing of Staphylococcus aureus as a frontline tool in epidemiological typing. J Clin Microbiol 2008; 46(2): 574-81.
25. Tille P. Bailey & Scott's diagnostic microbiology. Philadelphia, PA: Elsevier Health Sciences; 2013.
26. Votintseva AA, Fung R, Miller RR, Knox K, Godwin H, Wyllie DH, et al. Prevalence of Staphylococcus aureus protein A (spa) mutants in the community and hospitals in Oxfordshire. BMC Microbiol 2014; 14: 63.
27. Wisniewska K, Szewczyk A, Piechowicz L, Bronk M, Samet A, Swiec K. The use of spa and phage typing for characterization of clinical isolates of methicillin-resistant Staphylococcus aureus in the University Clinical Center in Gdansk, Poland. Folia Microbiol (Praha) 2012; 57(3): 243-9.
28. Xie Y, He Y, Gehring A, Hu Y, Li Q, Tu SI, et al. Genotypes and toxin gene profiles of Staphylococcus aureus clinical isolates from China. PLoS One 2011; 6(12): e28276.