Molecular typing of methicillin-resistant *Staphylococcus aureus* based on PCR restriction fragment length polymorphism of the *Coagulase* gene

Ung Thi Thuy, Nguyen Hoang Bach, Le Van An
Department of Microbiology, Hue University of Medicine and Pharmacy, Vietnam

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most predominant agents that cause nosocomial infections. **Objectives:** To determine the rate of MRSA and the molecular characteristics of the coagulase encoding gene of these isolates based on polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). **Methodology**: A total of 100 strains of *S. aureus* were isolated from clinical samples. MRSA was investigated through the antimicrobial susceptibility testing. The *coa* gene was amplified by PCR and these products were digested by using *AluI* restriction enzyme. **Results:** In total, 100 *S. aureus* isolates were recovered from clinical samples, of which 59 isolates were MRSA. Three types of *coa* classes (550, 700, 750) are distinguished into 6 genotypic patterns, which were coded *coa* 1-6 and *coa* 2 was the most predominant (42.37%). The 700bp and 750bp amplicons formed two (*coa* 2 and 3) and three (*coa* 4, 5 and 6) patterns, respectively, whereas the 550bp fragments generated unique patterns designated *coa* 1. Only 2 isolates undigested by *AluI* restriction enzyme. **Conclusion:** Our results showed that 59% of MRSA strains are isolated with diverse genotype distributed in many different wards of hospitals by using PCR-RFLP

Keywords: *Staphylococcus aureus (S. aureus)*, *coa* gene, PCR-RFLP

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the major pathogens that caused nosocomial infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection has emerged in both hospitals and communities. Today, it is considered the most significant multidrug-resistant organism [1]MRSA isolates obtained from a tertiary care hospital in China were subjected to spa typing, SCCmec typing, multiple locus sequence typing (MLST. These strains are resistant to many antibiotics including methicillin and almost all β-lactam antibiotics. The spread of MRSA is associated with high morbidity and mortality rates [2]. Many molecular mechanisms related to methicillin - resistance in *S. aureus* have been studied [3]**.** Besides, virulence factors allow it to adhere to the surface, invade or avoid the immune system and cause harmful effects to the host. The enzyme coagulase is one of the virulence factors described earliest, which coagulates human and animal plasma. It is also the basis of the coagulase test widely used to distinguish *S. aureus* from other *Staphylococci* [4]. The *coa* gene, which encodes the coagulase enzyme, can be used for DNA-based diagnosis of *S. aureus* by its high polymorphism due to the difference in the sequence of the 3′ variable region. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) used to analyze the *coa* gene in many *Staphylococcus* species has shown diversity in different size and number of DNA fragments that result in characteristic banding patterns. The *coa* gen typing technique is simple, rapid, and useful to monitor variations in MRSA populations [5].

The purpose of this research was to investigate the rate of MRSA isolates at Hue University of Medicine and Pharmacy Hospital and identify the molecular characteristics of the coagulase encoding gene of methicillin-resistant *Staphylococcus aureus* based on polymorphic analysis of restriction enzyme cleavage DNA fragments (PCR-RFLP).

2. MATERIALS AND METHODS

2.1. Study design

This was a cross-sectional study

2.2. Bacterial strains

A total of 100 strains of *S. aureus* non-duplicate were collected from different clinical samples in period of November 2017 to August 2019 at Hue University of Medicine and Pharmacy Hospital. Identification of *S. aureus* from these samples was performed by standard microbiological methods

Corresponding author: Nguyen Hoang Bach, email: nhbach@huemed-univ.edu.vn *DOI: 10.34071/jmp.2020.7.7*
Received: 17/12/2019, Accepted: 23/12/2020

included gram staining, catalase test, mannitol fermentation and coagulase tests positive [6].

2.3. Antimicrobial susceptibility testing

Susceptibility to antimicrobial agents by disk diffusion method was performed on Mueller-Hinton agar (MH) (E&O Laboratories, Bonnybridge, Scotland) for all *S. aureus* strains by using a Kirby Bauer method. The isolates were identified phenotypically as MRSA via screening using a cefoxitin disk (30 µg) according to the Clinical and Laboratory Standards Institute (CLSI) guideline [7].

2.4. DNA extraction

Single colonies were picked up and cultured on BHI (Merck KgaA, Germany) at 37°C for 24 hours. The iVApDNA Extraction Kit (Viet A Technology Corporation, Ho Chi Minh City, Vietnam) was used for DNA extraction. A total 200 µl of bacterial suspension were treated as recommended by the manufacturer. DNA was eluted in a final volume of 50 µl TE buffer. Concentration and purity of total DNA were evaluated by using NanoDrop 2000 spectrophotometer (Thermo Scientific, Massachusetts, USA).

2.5. Amplifying the *coa* gene

The *coa* gene amplification was performed by using forward primer: (5'-ATA GAG ATG CTG GTA CAG G -3') and reverse primer (5'-GCT TCC GAT TGT TCG ATG C-3'). A 25µL PCR reaction mixture consists of 12.5 µL 2X DreamTaq™ Green PCR Master Mix (Thermo fisher, MA, USA), 0.4 µM of each primer and 100 ng genomic DNA. PCR amplification was profiled as follows: initial denaturation at 94 ° C for 5 minutes, followed by 36 cycles at 94°C for 1 minute, 55°C for 15 seconds, and 72°C for 15 seconds, final extension of 10 minutes at 72°C in Veriti® Thermal Cycler (Applied Biosystems, CA, and USA) [8][9]. The *S. aureus* ATCC 25923 was used as a positive control for the PCR reaction. The different sizes of PCR products ranging from 300bp to 800 bp were separated by electrophoresis on 1.5% agarose gel pre-colored with 1% GelRed™ 1X (Biotium Inc.) and digitized with GelDoc™ XR+ Gel Documentation System (BioRad, CA, USA).

2.6. PCR-RFLP of *coa* gene

The PCR product of *coa* gene were digested by *AluI* according to the manufacturer's instructions: 10 µl of the PCR product (~0.1-0.5 µg DNA) was digested by 1-2 µl *AluI* enzyme (10 U/µl)), 18 µl of nuclease-free water, 2 µl 10X tango buffer and incubate 37°C for 16 hours (Thermo Scientific, USA). The digested products were separated by electrophoresis on 1.5% agarose gel.

3. RESULTS

3.1. Identification species and MRSA

All 100 isolates were collected and identified as *S. aureus* according to biochemical tests. More than 80% of isolates were recovered from pus samples. The antimicrobial susceptibility testing by agar disc diffusion Kirby-Bauer method for *S. aureus* isolates determined that the percentage of resistance to cefoxitin were 59% MRSA and 41% were identified as MSSA. The proportion has been shown in Chart 1. Fifty nine isolates were considered MRSA and will be chosen for further genotyping tests.

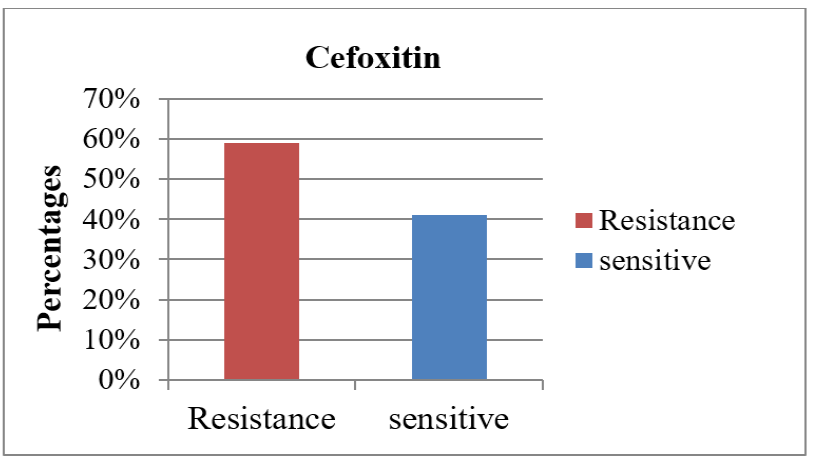
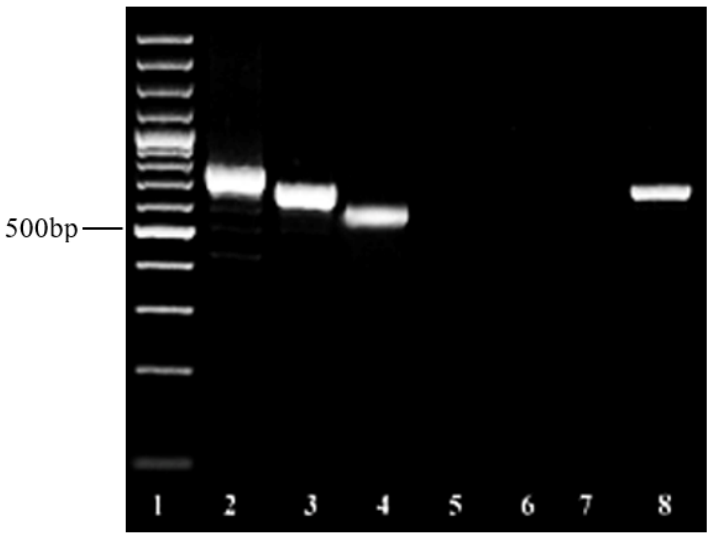


Chart 1: The rate of MRSA and MSSA in *S. aureus* species

3.2. *Coagulase* gene typing

The *co*a gene was amplified by PCR for 59 different MRSA isolates. All these phenotypically coagulase-positive *S. aureus* showed positive for *coa* gene by PCR analysis. Three different types of bands were found in the strains analyzed were 550, 700 and 750bp (figure 1). The 750bp band were the most predominantly noticed product sizes, accounting for 52.54% of the total *coa*-positive isolates, followed by 700 bp and 550bp with 45.76% and 1.69% respectively.

Figure 1: Amplification of *coa* gene by PCR.



Lane 1: 100bp DNA ladder; lane 2: 750bp; lane 3: 700 bp; lane 4: 550 bp; lane 5, 6: no band; lane 7: negative control; lane 8: positive control (*S. aureus* ATCC 25923)

AluI restriction enzyme digestion of the PCR products generated 6 different restriction patterns. Distinct PCR-RFLP *coa* profiles were named with a genotype code 1–6. The results showed that the 700bp formed 2 (*coa* 2 and 3) and 750bp amplicons formed 3 (*coa* 4, 5 and 6) patterns following *AluI* digestion, respectively, whereas the 550bp fragments generated unique patterns designated *coa* 1. The results of the different typing methods are shown in Table 1 (Figure 2). *Coa* gene RFLP pattern 2 was most common (25 (42.37%) of the isolates examined), followed by *coa* gene RFLP patterns 4 (19 isolates (32.2%)).

PCR products size (bp)	RFLP patterns (bp)	Genotype code	N (%)
550	230, 320	*Coa* 1	1 (1.69%)
700	180, 220, 300	*Coa* 2	25 (42.37%)
700	700 (Not digested)	*Coa* 3	2 (3.39%)
750	160, 190, 400	*Coa* 4	19 (32.20%)
750	130, 300, 320	*Coa* 5	5 (8.47%)
750	250, 500	*Coa* 6	7 (11.86%)

Table 1. Molecular typing of 59 MRSA isolates using PCR-RFLP of *coa* gene

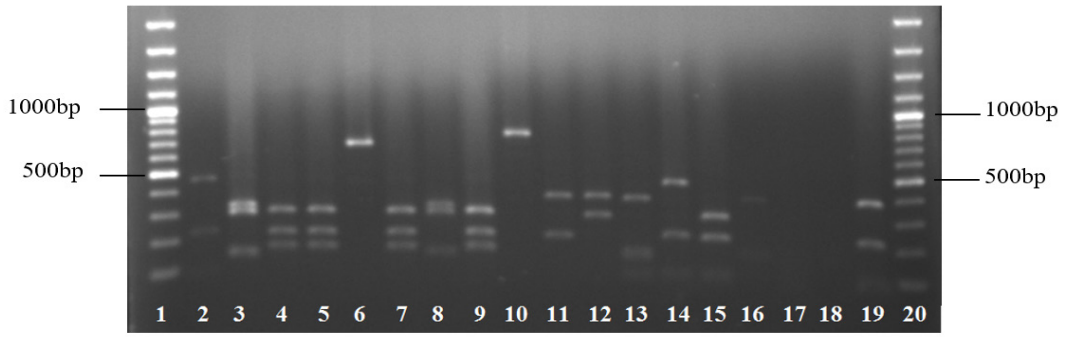


Figure 1. RFLP patterns with different *coa* gene amplicon size were digested by the restriction enzyme AluI. Lane 1,20: 100bp ladder; Lane 2, 14: 750bp (fragments 250 and 500bp); lane 3,8: 750 bp (fragments 130, 300 and 320bp); lane 9: 700 bp (fragments 180, 220 and 300bp); lane 6: 700bp (not digested by *AluI*); lane 4, 5, 7, 9, 10, 11, 12 fragments of MSSA isolates; lane 13: 750bp (fragments 160, 190 and 400bp); lane 15: 550bp (fragments 230 and 320 bp); lane 18: negative control, l ane 19: positive control (*S. aureus* ATCC 25923: 700bp (fragments 80, 220 and 400 bp)).

Besides, RFLP pattern results are shown the relationship between the genotypes and the source of the study isolates is summarized in Table 2 that circulated in the hospital. *Coa* 2 is the predominant genotype and present in 42.37% of MRSA isolates from different departments in the hospital. The traumatology surgery department is present with all 6 patterns in this study.

Departments	Floor	No. of isolates coa						
		Coa 1	Coa 2	Coa 3	Coa 4	Coa 5	Coa 6	Total
Pediatrics	5 (old-building)	0	0	0	0	1	0	1
Traumatology Surgery	4 (old-building)	1	15	1	12	3	6	38
Gastrointestinal Surgery	3 (old-building)	0	0	0	1	0	0	1
Anesthesia - Recuperate	2 (old-building)	0	1	0	0	0	0	1
Endocrinology - General	6 (41-building)	0	1	0	3	0	0	4
Cardiology	6 (41-building)	0	2	0	0	0	1	3
Intensive Care Unit	4 (41-building)	0	0	0	1	0	0	1
Urology Clinic	2 (41-building)	0	1	0	0	0	0	1
Urology- Neurosurgery	2 (41-building)	0	0	0	0	1	0	1
Obstetrics and Gynaecology	5 (51-building)	0	0	0	1	0	0	1
Gastrointestinal surgery clinic	4 (51-building)	0	0	1	0	0	0	1
ENT Clinic	4 (51-building)	0	1	0	1	0	0	2
Obstetrics and Gynaecology Clinic	3 (51-building)	0	1	0	0	0	0	1
Oncology	2 (G-building)	0	3	0	0	0	0	3
		1	25	2	19	5	7	59

Table 2. Circulation of *S. aureus* strains between departments in the hospital

4. DISCUSSION

MRSA strains are often associated with serious infections with high rates of drug resistance, difficult to treat and the ability to spread strongly. It is also one of the main causes of nosocomial infections. And coagulase is one of the earliest described virulence factors of *S. aureus*, which may contribute to its pathogenicity [4]. *Coa* gen is highly polymorphic at variant 3´ end which could be useful for differentiation of *S. aureus* isolates and determines the source of infection as well as the circulation of these strains [10]. And PCR-RFLP has proved a simple, adequate technique for the correct identification of almost all prevalent species. Furthermore, It is easier to use, less expensive, can be made within a short time with a large number of bacterial strains and less complex equipment than sequencing that suitable for epidemiological investigations in hospitals and communities [11].

In this study, a total of 100 *S. aureus* clinical samples were isolated and 59 MRSA isolates were studied to genotype the *coa* gene by PCR-RFLP. Results of *coa* gene typing showed 3 amplicons and six RFLP patterns. PCR products with different sizes of the *coa* gene were recognized in other researchers [8], [12]. However, in our study only 3 band classes (550, 700 and 750 bp) were observed and 52.54% of strains belonged to the 750 bp band class. The difference of coagulase type could be associated with geographical variation or can be alteration in the polymorphic repeated part of the *coa* gene because of point mutations [13]. *AluI* PCR-RFLP fragments in this study varied from one to three bands and these results similar to the previous studies [8], [12]. Besides, some studies indicated many results differ from this study, in which *AluI* PCR-RFLP fragments varied from one to five bands even the *coa* gene with multiple bands amplification products were detected. The reason may be the use of different primers as well as the geographic distribution of strains despite using the same restriction enzyme [14], [15].

Coa 1 pattern accounts for the lowest proportion of only about 1.69%. *Coa* 3 pattern which undigested by *AluI* restriction enzyme accounts for 3.39% similar to the other finding [16]. All the other samples were well digested by *AluI*. Especially, *coa* 2 pattern accounts for the majority of strains 42.37% (25/59) and popularly circulates in many different wards in the hospital, followed by *coa* 4 pattern 32.2%. And traumatology surgery is the only department with a present of all 6 patterns in this study. The presence of different *Coa* genotypes in different departments in the hospital indicated the circulation of these strains. The cause may be due to the movement of the patient from one department to another or some other problem related to the medical staff. The prevalence of MRSA was found to be significantly high in surgical ICU and the surgical wards that indicated in the other research [17]. Thus, our study emphasizes on the importance of MRSA positive coagulase molecular analysis. Polymorphism analysis by RFLP methodology in *coa* gen is extremely useful to trace the source of infection and routes of transmission in hospital as well as epidemiological investigations on its genotype in community.

5. CONCLUSION

The current study, 59% MRSA isolates were analyzed with more than one *coa* genotype and that only one genotype predominated. Based on PCR-RFLP, 6 distinct genotype codes of MRSA isolates were identified in this research; and *coa* 2 was the most predominant. This report highlights the rate of MRSA as a major cause of wound infections with much higher proportion in the Surgery department. Besides, it has also provided baseline information in assisting monitors on critical issues regarding nosocomial infection. Thus, continuous surveillance on resistance patterns of *S. aureus* in understanding emerging trends plays an important role. The information found in our study could be useful to prepare an efficient infection control measure.

REFERENCES

1. H. Kong, F. Yu, W. Zhang, X. Li, and H. Wang, “Molecular epidemiology and antibiotic resistance profiles of methicillin-resistant staphylococcus aureus strains in a tertiary hospital in china,” *Front. Microbiol.*, vol. 8, no. MAY, p. 838, 2017.

2. A. Hassoun, P. K. Linden, and B. Friedman, “Incidence , prevalence , and management of MRSA bacteremia across patient populations — a review of recent developments in MRSA management and treatment,” pp. 1–10, 2017.

3. S. J. Peacock and G. K. Paterson, “Mechanisms of

Methicillin Resistance in Staphylococcus aureus," *Annu. Rev. Biochem.*, vol. 84, no. 1, pp. 577–601, 2015.

4. A. Subramanian *et al.*, "Evaluation of Hiaureus™ coagulase confirmation kit in identification of Staphylococcus aureus," *J. Clin. Diagnostic Res.*, vol. 11, no. 2, pp. DC08–DC13, 2017.

5. M. González-Domínguez, C. Potel, C. Seral, L. Constenla, F. J. Castillo, and M. Álvarez, "Usefulness of PCR-RFLP coa gene for clonal classification of methicillin-resistant Staphylococcus aureus isolates in tertiary hospitals," *Scand. J. Infect. Dis.*, vol. 46, no. 10, pp. 719–722, Oct. 2014.

6. D. Burdass *et al.*, "Basic laboratory procedures in clinical bacteriology," *Nutrients*, no. 9, 2554.

7. R. D. Walker, *Standards for antimicrobial susceptibility testing.*, vol. 60, no. 9. 1999.

8. H. Mahmoudi, M. R. Arabestani, S. F. Mousavi, and M. Y. Alikhani, "Molecular analysis of the coagulase gene in clinical and nasal carrier isolates of methicillin-resistant Staphylococcus aureus by restriction fragment length polymorphism," *J. Glob. Antimicrob. Resist.*, vol. 8, pp. 41–45, 2017.

9. V. Sharma, S. Sharma, D. K. Dahiya, A. Khan, M. Mathur, and A. Sharma, "Coagulase gene polymorphism, enterotoxigenecity, biofilm production, and antibiotic resistance in Staphylococcus aureus isolated from bovine raw milk in North West India," *Ann. Clin. Microbiol. Antimicrob.*, vol. 16, no. 1, pp. 1–14, 2017.

10. R. Hakimi Alni, A. Mohammadzadeh, P. Mahmoodi, and M. Y. Alikhani, "Genotypic Analysis of Staphylococcus aureus Coagulase Gene using PCR-RFLP analysis," *Med. Lab. J.*, vol. 11, no. 6, pp. 12–17, 2017.

11. P. Standards Unit, Microbiology Services and Bacteriology, "UK Standards for Microbiology Investigations - Identification of Staphylococcus species, Micrococcus species and Rothia species Issued," *Bacteriology*, vol. B 55, no. 3, pp. 1–32, 2014.

12. A. O. Shittu and J. Lin, "Antimicrobial susceptibility patterns and characterization of clinical isolates of Staphylococcus aureus in KwaZulu-Natal province, South Africa," *BMC Infect. Dis.*, vol. 6, pp. 1–13, 2006.

13. P. Mohajeri, S. Azizkhani, A. Farahani, and B. Norozi, "Genotyping of coa and aroa genes of methicillin-resistant staphylococcus aureus strains isolated from nasal samples in western Iran," *Jundishapur J. Microbiol.*, vol. 9, no. 1, pp. 1–6, 2016.

14. M. Himabindu, D. S. Muthamilse, D. K. Bishi, and R. S. Verma, "Molecular Analysis of Coagulase Gene Polymorphism in Clinical Isolates of Methicilin Resistant Staphylococcus aureus by Restriction Fragment Length Polymorphism Based Genotyping," *Am. J. Infect. Dis.*, vol. 5, no. 2, pp. 170–176, 2009.

15. H. M. Abdulghany and R. M. Khairy, " The Frequency of Methicillin-Resistant Staphylococcus aureus and Coagulase Gene Polymorphism in Egypt ," *Int. J. Bacteriol.*, vol. 2014, pp. 1–6, 2014.

16. A. D. Anggraini, E. B. Koendhori, H. Pramono, and D. D. Wahyono, "Polymorphism Analysis of the Coagulase Gene in Isolates of Methicillin-Resistant Staphylococcus aureus with AluI Restriction Sites," *Heal. Sci. J. Indones.*, vol. 8, no. 1, 2017.

17. V. Aruna and S. Rajesh, "Prevalence of Methicillin Resistant Staphylococcus," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 6, no. 12, pp. 4204–4208, 2017.