RMJ Ramathibodi Medical Journal

Rama Med J | Original Article

Assessment of the Antibiotic Resistance Characteristics of *Staphylococcus aureus* Isolated at Da Nang Hospital for Women and Children, Vietnam

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Background: Infections caused by multidrug-resistant *Staphylococcus aureus* (*S. aureus*), typically from methicillin-resistant *S. aure*us (MRSA) have become a global problem, including Vietnam.

Objectives: To assess the prevalence of *S. aureus* infections, pathogenic characteristics, and evaluate antibiotic resistance profiles.

Methods: This was a cross-sectional descriptive study of 191 *S. aureus* isolates cultured from clinical samples with antibiotic resistance profiles at the Department of Microbiology, Da Nang Hospital for Women and Children, Vietnam, from July 2021 to September 2022. Strain identification and susceptibility tests were performed by using an AST-GP Card in VITEK 2 Compact.

Results: The proportion of *S. aureus* infection was high in females (56.02%) and children under 6 years (65.97%). The major clinical sample of *S. aureus* was pus (63.87%) and most commonly isolated in the Outpatient Department (31.41%). *S. aureus* showed resistance to over 70.00% of the antibiotics in group A, with lower resistance (1.05% - 48.69%) towards those in groups B and C. The rates of isolation of vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus* were 1.57% and 1.05%, respectively, but no isolates resistant to linezolid was found. The proportion of MRSA was found to be 78.01%. Prevalence of multidrug-resistance MRSA (MDR-MRSA) was markedly high (79.87%).

Conclusions: The presence of *S. aureus* with varying antibiotic sensitivity patterns was found, as well as multidrug resistance. The prevalence of MRSA and MDR-MRSA was noticeable in this study.

Keywords: *Staphylococcus aureus*, Antibiotic resistance, Multidrug resistance, MRSA

Rama Med J: doi:10.33165/rmj.2023.46.4.266177

Received: November 4, 2023 **Revised:** December 4, 2023 **Accepted:** December 20, 2023

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Introduction

Staphylococcus aureus (*S. aureus*) is one of the common pathogens that cause nosocomial infection and community infection.[1] *S. aureus* bacterium is often encountered in human pathology and is the causative agent of a wide variety of infections, including life-threatening toxic shock syndrome, pneumonia, and sepsis.[2] *S. aureus* was isolated quite commonly in hospitals and medical facilities, causing a serious impact on human health, despite efforts to get rid of bacteria.[3-5]

Currently, there have been many studies showing that *S. aureus* is increasingly resistant to antibiotics and spread of multidrug resistant (MDR). Notably, methicillin-resistant *S. aureus* (MRSA) is a global health problem, causing many difficulties in treatment and prognosis. In the last 2 decades, MRSA infection has been emerging as a major cause of morbidity and mortality in healthcare settings worldwide.[6] The rate of community-acquired MRSA infection has been increasing, which is an important opportunistic pathogen in Europe and most frequently identified worldwide in Asia and the United States.[7, 8] Previous studies also have shown that the prevalence of MRSA strains is higher than that of methicillin-susceptible *S. aureus* (MSSA). [4, 6] In Vietnam, the prevalence of MRSA has been reported to be considerably higher than that in other countries.[3, 5, 9] In 2019, over 100 000 deaths were attributed to resistance bacteria worldwide, with MRSA accounting for a large proportion of these deaths.[10] MRSA has become one of today's most serious treatment challenges due to its simultaneous resistance to multiple drugs and rapid global spread, leading to more than 450 000 infections each year.[5] Infections caused by multidrug-resistant *S. aureus* have become a global problem and are a real clinical and financial burden. Therefore, regular monitoring of bacteria and their sensitivity to antibiotics would help clinicians treatment more effectively and reduce costs, at the same time, take measures to control and prevent their spread.

Da Nang Hospital for Women and Children, Vietnam has been a key hospital, welcoming many patients every day for examination and treatment. Due to the common occurrence, urgency, serious consequences of drug resistance of *S. aureus* strains, this study aimed to survey some pathogenic characteristics of *S. aureus* strains isolated at Da Nang Hospital for Women and Children, Vietnam, and evaluate some antibiotic resistance characteristics of those strains.

Methods

Participants and Study Design

The study included clinical samples from patients who were laboratory diagnosed to have *S. aureus* infection. This observational and cross-sectional study was conducted at the Department of Microbiology, Da Nang Hospital for Women and Children, Vietnam, from July 2021 to September 2022. A nonprobability, purposive sampling in consecutive 191 clinical samples that satisfied the inclusion criteria for the study, and were accessible during the study period.

Ethics

The study was approved by the Ethics Council in Biomedical Research of Da Nang University of Medical Technology and Pharmacy, Vietnam, (No.01/BB-HDDD on January 12, 2023) and approved by the leadership of Da Nang Hospital for Women and Children, Vietnam (on June 1, 2023). Data were collected for research purposes only.

Variables of Interest

The outcome variables used in the study were *S. aureus* both MRSA and MSSA, whereas age, gender, clinical samples, group antibiotic, and antibiotic sensitivity outcomes were used as predictor variables.

Clinical Samples Collection, Isolation, and Identification of *S. aureus*

All 191 *S. aureus clinical* isolates were collected from the records of the Department of Microbiology, Da Nang Hospital for Women and Children, Vietnam.

S. aureus isolates were recovered from different clinical samples, collected from inpatients and outpatients admitted to the hospital, including pus, blood, fluid, sputum, and other clinical samples (urine, catheter, umbilical artery, and umbilical vein) from departments (Outpatient, Pediatric, Neonatal, Surgical, and Obstetrics and Gynecology). Bacterial isolates were identified as *S. aureus* by using ID-GP Card in VITEK 2 Compact (BioMérieux, Marcy-l'Etoile, France). Only unique isolates were included for analysis, in case the isolates from different patients, or from a patient with different susceptibility results. If the isolates contributed by a patient showed the same susceptibility results at different times for retesting, they were excluded from the study.

Antimicrobial Susceptibility Testing and Detection of MRSA

Antimicrobial susceptibility testing was performed using AST-GP Card in VITEK 2 Compact (BioMérieux, Marcy-l'Etoile, France). Identification of pathogenic bacteria was based on the colorimetric method to identify the biological and chemical properties of the bacteria through the color change of the environmental wells available in the identification card. The level of antibiotic resistance of *S. aureus* was divided into 3 levels: susceptible, intermediate, and resistant, according to the Clinical and Laboratory Standards Institute (CLSI)[11] guidelines, and was divided into groups A, B, and C. MRSA and MSSA were determined by testing with cefoxitin antibiotic discs using the automatic antibiogram technique on the VITEK 2 Compact. MDR isolates were defined as nonsusceptibility to at least 1 agent in 3 or more antimicrobial categories.[12]

Statistical Analysis

Data were analyzed and interpreted by SPSS version 20.0 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp; 2011). A chi-square test was applied to compare carriage rates between different wards and different clinical samples, *P* value less than .05 was considered statistically significant.

Results

Pathogenic Characteristics of *S. aureus*

Of the 191 patients who had the *S. aureus* infection (male, 84 [43.98%]; female, 107 [56.02%]), *S. aureus* was most commonly observed in children under 6 years (126 [65.97%]). The highest number of isolates were collected from the Outpatient Department (60 [31.41%]) and mainly from pus samples (122 [63.87%]) (Table 1).

Antimicrobial Resistance Profiles of *S. aureus*

Among group A antibiotics, *S. aureus* isolates were highly resistant to penicillin (187 [97.91%]) and quite highly resistant to other antibiotics such as oxacillin (149 [78.01%]), erythromycin (142 [74.35%]), clindamycin (141 [73.82%]). In group B, bacteria were resistant to tetracycline (93 [48.69%]). Notably, this study a small

Table 1. Pathogenic Characteristics of *S. aureus*

Characteristic	No. (%)
Gender	
Male	84 (43.98)
Female	107 (56.02)
Age, y	
< 6	126 (65.97)
6 - 15	40 (20.94)
16 - 30	19 (9.95)
> 30	6 (3.14)
Departments	
Outpatient	60 (31.41)
Pediatric	51 (26.70)
Neonatal	44 (23.04)
Surgical	20 (10.47)
Obstetrics and Gynecology	16 (8.38)
Clinical samples	
Pus	122 (63.87)
Blood	24 (12.57)
Fluid	22 (11.52)
Sputum	9 (4.71)
Others	14 (7.33)

proportion of vancomycin-resistant *S. aureus* (VRSA) (3 [1.57%]) and vancomycin-intermediate *S. aureus* (VISA) (2 [1.05%]). Bacteria were also susceptible over 70.00% to many antibiotics such as moxifloxacin, ciprofloxacin, and gentamicin in group C (Table 2).

Level of Multi-Antibiotic Resistance of *S. aureus* Strain

The 191 *S. aureus* isolates recovered in this study showed that 149 (78.01%) were MRSA and 42 (21.99%) were MSSA. There was a significant difference in the percentage of MRSA and MSSA from different clinical samples and departments. MRSA strains accounted for the largest proportion of pus (81.97%), followed by fluid (81.82%), blood (75.00%), other clinical samples (57.14%), and sputum (55.56%), respectively. However, the difference in the distribution of MRSA and MSSA according to clinical samples was not statistically significant ($P > .05$). Moreover, MRSA strains accounted for the largest proportion in the department of Obstetrics and Gynecology (93.65%), followed by the Outpatient (85.00%), the Surgical (80.00%), the Pediatric (78.43%) and the Neonatal (61.36%), respectively. The difference in the distribution of MRSA and MSSA according to departments was statistically significant ($P < .05$) (Table 3).

There there were differences in antibiotic resistance rates between MRSA and MSSA. MRSA strains were 100.00% resistant to penicillin and oxacillin, 85.23% erythromycin, and 84.57% clindamycin in group A antibiotics, and 61.07% resistant to tetracycline in group B. Resistance to the remaining antibiotics (1.34% - 21.48%), especially VRSA (2.02%) were detected. Meanwhile, MSSA strains were 90.48% resistant to penicillin in group A antibiotics, 4.76% to 38.10% resistant to remaining antibiotics, and bacteria resistant to oxacillin, rifampicin, and vancomycin had not been detected. Notably, MDR patterns were more prevalent in MRSA (79.87%) than in MSSA (11.90%) (Table 4).

Table 2. Antimicrobial Resistance Profiles of *Staphylococcus aureus* Strains

Antimicrobial Agent*	No. (%)		
	Susceptible	Intermediate	Resistant
Group A			
Penicillin	4 (2.09)	0	187 (97.91)
Oxacillin	42 (21.99)	0	149 (78.01)
Erythromycin	49 (25.65)	0	142 (74.35)
Trimethoprim/Sulfamethoxazole	156 (81.68)	0	35 (18.32)
Clindamycin	50 (26.18)	0	141 (73.82)
Group B			
Rifampicin	189 (98.95)	0	2 (1.05)
Vancomycin	186 (97.38)	2 (1.05)	3 (1.57)
Tetracycline	98 (51.31)	0	93 (48.69)
Linezolid	191 (100.00)	0	0
Group C			
Ciprofloxacin	155 (81.15)	0	36 (18.85)
Gentamicin	137 (71.73)	6 (3.14)	48 (25.13)
Moxifloxacin	155 (81.15)	4 (2.09)	32 (16.76)

* Antimicrobial agent was classified into 3 groups (A, B, and C), according to CLSI.[11]

Table 3. Isolation of MRSA and MSSA in Various Clinical Samples and Departments

Variable	No. (%)		*P* Value*
	MRSA (n = 149)	MSSA (n = 42)	
Clinical samples			
Pus	100 (81.97)	22 (18.03)	.28
Blood	18 (75.00)	6 (25.00)	
Fluid	18 (81.82)	4 (18.18)	
Sputum	5 (55.56)	4 (44.44)	
Others	8 (57.14)	6 (42.86)	
Departments			
Outpatient	51 (85.00)	9 (15.00)	.02
Pediatric	40 (78.43)	11 (21.57)	
Neonatal	27 (61.36)	17 (38.64)	
Surgical	16 (80.00)	4 (20.00)	
Obstetrics and Gynecology	15 (93.65)	1 (6.25)	

Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

* Significance for the test was determined at $P < .05$.

Table 4. Antibiotic Resistance Pattern of MRSA and MSSA to Various Antibiotics

Antimicrobial Agent*	No. (%)					
	MRSA (n = 149)			MSSA (n = 42)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Group A						
Penicillin	0	0	149 (100.00)	4 (9.52)	0	38 (90.48)
Oxacillin	0	0	149 (100.00)	42 (100.00)	0	0
Erythromycin	22 (14.77)	0	127 (85.23)	27 (64.29)	0	15 (35.71)
Trimethoprim/ Sulfamethoxazole	118 (79.19)	0	31 (20.81)	38 (90.48)	0	4 (9.52)
Clindamycin	23 (15.43)	0	126 (84.57)	27 (64.29)	0	15 (35.71)
Group B						
Rifampicin	147 (98.66)	0	2 (1.34)	42 (100.00)	0	0
Vancomycin	144 (96.64)	2 (1.34)	3 (2.02)	42 (100.00)	0	0
Tetracycline	58 (38.93)	0	91 (61.07)	40 (95.24)	0	2 (4.76)
Linezolid	149 (100.00)	0	0	42 (100.00)	0	0
Group C						
Ciprofloxacin	119 (79.87)	0	30 (20.13)	36 (85.71)	0	6 (14.29)
Gentamicin	114 (76.51)	3 (2.01)	32 (21.48)	23 (54.76)	3 (7.14)	16 (38.10)
Moxifloxacin	119 (79.87)	3 (2.01)	27 (18.12)	36 (85.71)	1 (2.38)	5 (11.91)

Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

* Antimicrobial agent was classified into 3 groups (A, B, and C), according to CLSI.[11]

Discussion

S. aureus is a common bacterial pathogen that affects everyone, both men and women. In this study, the distribution of *S. aureus* among gender was found to be higher in female than male patients (56.02% vs 43.98%). The finding resembled with the research conducted by Maharjan et al[4] and Pradhan et al.[13] The result obtained from the present study was contrast to previous studies[5, 14, 15] which showed the incidence of *S. aureus* was much higher in males than in females. The reason could be partly due to the infection rate between genders depending on geography and year of study. Therefore, the difference in the proportion of male and female patients infected with *S. aureus* only reflected patient characteristics at the time of the study and was not statistically significant.

The present study showed the majority of patients infected with *S. aureus* were under 6 years (65.97%), and over 30 years accounting for the lowest proportion (3.14%). The result was found nearly similar to previous study at the International Friendship Children's Hospital in Nepal, where they got a higher prevalence of *S. aureus* in ages group 1 to 5 years (51.07%).[16] The highest isolation rate of *S. aureus* in children under 6 years in the present study could be partly due to their resistance and immune system being weaker than the adults.

In this study, *S. aureus* strains were most frequently isolated from the Outpatient Department (31.41%), and lowest in the Obstetrics and Gynecology Department (8.38%). This result was different to a previous study from Vietnam by Dinh Binh et al[17] who reported *S. aureus* isolated from the Surgical Department was the highest and other departments had a relatively low rate. With high prevalence of *S. aureus* in the Outpatient Department in this study, the risk of spreading the infection in the community even before arrival at the hospital is very possible and could be a challenge to the infection control effort of the hospitals.

The highest prevalence of *S. aureus* was found in pus and blood, similar to other studies.[18-20] The highest isolation rate of *S. aureus* in pus and blood in this study could be partly due to *S. aureus* is a normal flora of skin that can enter the body through cracks, abrasion, cuts, surgical incisions, burn, and intravenous catheter and causes pyogenic infection.[21]

The antimicrobial susceptibility testing in the present study demonstrated high resistance profiles of *S. aureus* isolates. The results showed that *S. aureus* was highly resistant to group A antibiotics, including penicillin (97.91%), oxacillin (78.01%), erythromycin (74.35%), and clindamycin (73.82%). This finding was consistent with previous studies in Vietnam.[22, 23]

In group B, bacteria had low resistance to rifampicin (1.05%) and there were no cases of resistance to linezolid. Notably, this study found 3 cases of VRSA (1.57%) and 2 cases of VISA (1.05%). The resistance to vancomycin noted in this study was also supported by Mahmood et al[24] (10.3%) and Rasmi et al[15] (13.5%). In general, vancomycin was one of the last resorts and the drug of choice to treat infection caused by MRSA strains. The emergence of VRSA strains could be an urgent public health warning. Through survey results on antibiotic resistance of *S. aureus* and comparison with some domestic and foreign research results, this study found that *S. aureus* strains isolated at Da Nang Hospital for Women and Children were resistant to most antibiotics at a fairly high rate with the appearance of VRSA and VISA strains. Therefore, it was necessary to pay attention to infection control in hospitals, report regularly, and prescribe antibiotics according to antibiogram guidelines.

Multiple studies indicate that MRSA is a serious threat to hospitalized patients globally and it now represents a challenge for public health, as community-associated infections appear to be on the increase in both adults and children in different regions and countries.[25] Available studies suggest that the prevalence rate of MRSA in other regions of the country was not uniform, and a significant variation in its prevalence has been documented in different settings throughout the world. This study revealed a prevalence of 78.01% MRSA, which was higher than previous studies conducted in other

regions, including 25.4% in China,[5] and 36.69% in Nepal.[16] However, several other studies have shown an increase in MRSA strains over time. The result of this study was almost comparable to the percentages recorded in many previous studies from different countries, for instance, Nepali study of Dhungel et al,[26] Egyptian study of Alfeky et al,[27] and Iranian study of Goudarzi et al,[6] revealed the percentages of MRSA isolates were 81.2%, 87.2%, and 93.3%, respectively. These variations could be due to variances in the infection control procedures, and trends for antibiotic prescription in various hospital settings.

There was a significant difference in the percentage of MRSA and MSSA from different clinical samples and departments. Of the total 191 *S. aureus* strains isolated, the prevalence of MRSA isolated from pus was the highest in comparison to other clinical samples. This result was consistent with previous studies in different countries.[13, 21] Moreover, MRSA strains accounted for the largest proportion in the Obstetrics and Gynecology Department (93.75%). The result was different with a previous study carried out in Southwest Ethiopia, which showed that MRSA strains accounted for the largest proportion in the Surgical Department.[28] There was no significant relationship between MRSA/MSSA, and clinical samples, whereas it could be concluded that there was the difference between MRSA and MSSA regarding departments. This difference could be due to multiple hospital-related risk factors such as prolonged hospital stay, antibiotic treatment, deterioration of underlying immunity, hospital environment, instruments, and use of other invasive devices.

This study found a higher prevalence of resistance to antibiotics in MRSA when compared to MSSA. The present investigation depicted that all MRSA and MSSA in this study were found to be entirely susceptible to linezolid and were highly resistant to penicillin, but MRSA was more resistant to most antibiotics than MSSA except gentamicin. There was a significant difference in the resistance of MRSA and MSSA was observed in the case of oxacillin, rifampicin, and vancomycin. In a previous study conducted in the Kingdom of Bahrain by AlSaleh et al,[29] significant differences in antibiotic resistance between MRSA and MSSA were observed, only strains resistant to oxacillin and rifampicin were detected in MRSA with rates of 100% and 1%, respectively. Similarly to this, Zheng et al[5] noted that there was a substantial difference in the resistance of MRSA and MSSA isolates to oxacillin and rifampicin.

Given the fact that *S. aureus* could be transmitted from one person to another by direct or indirect contact, the rising prevalence of MDR-MRSA is becoming a grave issue in the current state of medical care. In this study, MDR patterns were more prevalent in MRSA (79.87%) than in MSSA (11.90%). Epidemiological studies have indicated a varying rate of MDR in MRSA in the world. For instance, in China, Zheng et al[5] noted that a majority of MRSA (71.1%) isolates were MDR. Additionally, the study of Adhikari et al[19] from clinical samples at a Tertiary Care Teaching Hospital in Nepal showed that 94.05% of the isolates were MDR in MRSA. In various contexts in Egypt, up to 79% of MDR-MRSA has been documented.[27] The appearance of these MDR strains showed that the issue of controlling MDR bacteria was an urgent issue, and it was necessary to strengthen measures to limit the spread of these bacterial strains.

Conclusions

S. aureus mainly causes infections in children under 6 years and was most commonly isolated in the Outpatient Department, typically from pus and blood samples. The bacteria showed resistance to over 70.00% of the antibiotics in group A, with lower resistance towards those in groups B and C. The rates of isolation of VRSA and VISA were 1.57% and 1.05%, respectively, but no isolates resistant to linezolid were found. The proportion of MRSA strains was 78.01%, while MSSA strains accounted for only 21.99%. The rate of antibiotic resistance and MDR among MRSA (79.87%) was higher than that of MSSA (11.90%). There was no significant relationship between MRSA/MSSA, and clinical samples, whereas it could be concluded that there was the difference between MRSA and MSSA regarding patient departments.

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การประเมินลักษณะการดื้อยาปฏิชีวนะของเชื้อ *Staphylococcus aureus* ที่แยกได้จากโรงพยาบาลดานังเพื่อสตรีและเด็ก ประเทศเวียดนาม

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บทนำ: เชื้อ *Staphylococcus aureus* (*S. aureus*) เป็นแบคทีเรียก่อโรคติดเชื้อ และเกิดการดื้อยาปฏิชีวนะหลายชนิด โดยเฉพาะเชื้อในกลุ่ม Methicillin-resistant *Staphylococcus aureus* (MRSA) ที่เป็นปัญหาทั่วโลก รวมถึงประเทศเวียดนาม

วัตถุประสงค์: เพื่อประเมินความชุกของการติดเชื้อ *S. aureus* ลักษณะที่ก่อให้เกิดโรค และประเมินการดื้อยาปฏิชีวนะ

วิธีการศึกษา: การศึกษาเชิงพรรณนาแบบตัดขวางในตัวอย่างทางคลินิกที่ติดเชื้อ *S. aureus* ที่ดื้อยาปฏิชีวนะ จำนวน 191 ตัวอย่าง จากแผนกจุลชีววิทยา โรงพยาบาลดานังเพื่อสตรีและเด็ก ประเทศเวียดนาม ตั้งแต่เดือนกรกฎาคม พ.ศ. 2564 ถึงเดือนกันยายน พ.ศ. 2565 การตรวจหาสายพันธุ์และทดสอบความไวต่อยาปฏิชีวนะโดยใช้แผ่นทดสอบ AST-GP ด้วยเครื่อง VITEK 2 Compact

ผลการศึกษา: สัดส่วนการติดเชื้อ *S. aureus* ในตัวอย่างเพศหญิง คิดเป็นร้อยละ 56.02 และในเด็กอายุต่ำกว่า 6 ปี คิดเป็นร้อยละ 65.97 ตัวอย่างทางคลินิกที่สำคัญของเชื้อ *S. aureus* คือ หนอง คิดเป็นร้อยละ 63.87 และพบมากในแผนกผู้ป่วยนอก คิดเป็นร้อยละ 31.41 เชื้อ *S. aureus* มีความต้านทานต่อยาปฏิชีวนะในกลุ่ม A มากกว่า ร้อยละ 70.00 และมีความต้านทานต่อยาปฏิชีวนะในกลุ่ม B และ C ต่ำกว่า (ร้อยละ 1.05 - 48.69) สัดส่วนเชื้อ *S. aureus* ดื้อยา Vancomycin ในระดับสูงและระดับปานกลาง คิดเป็นร้อยละ 1.57 และร้อยละ 1.05 ตามลำดับ แต่ไม่พบเชื้อดื้อยา Linezolid สัดส่วนของเชื้อ MRSA คิดเป็นร้อยละ 78.01 และพบความชุกของเชื้อ MRSA ที่ดื้อยาหลายขนาน (Multidrug-resistant MRSA, MDR-MRSA) สูงขึ้น คิดเป็นร้อยละ 79.87

สรุป: เชื้อ *S. aureus* มีรูปแบบความไวต่อยาปฏิชีวนะที่หลากหลาย รวมถึงการดื้อยาหลายขนาน และพบความชุกของเชื้อ MRSA และ MDR-MRSA สูงขึ้น

คำสำคัญ: เชื้อ *Staphylococcus aureus* การดื้อยาปฏิชีวนะ การดื้อยาหลายชนิด เชื้อ MRSA

Rama Med J: doi:10.33165/rmj.2023.46.4.266177

Received: November 4, 2023 **Revised:** December 4, 2023 **Accepted:** December 20, 2023

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