SURVEY THE PROPORTION AND ANTIBIOTIC SENSITIVITY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG PATIENTS IN DANANG ONCOLOGY HOSPITAL

Le Ngoc Uyen Thi[1], Le Ngoc Phu Thien[1], Le Hai Tan[1], Duong Nguyen Hoa Bao[1], Nguyen Gia Phuc[1], Nguyen Thanh Hoi[2], Giang Thi Kim Lien[1], Nguyen Pham Thanh Nhan[3], Nguyen Thi Thuy Duong[1]*

[1]The University of Danang - VNUK Institute for Research and Executive Education, Danang, Vietnam
[2]The University of Danang - Center for Continuing Education, Danang, Vietnam
[3]Danang Oncology Hospital, Danang, Vietnam

*Corresponding author: duong.nguyen@vnuk.edu.vn

(Received: June 15, 2023; Revised: August 04; Accepted: August 20, 2023)

Abstract - In this study, 134 patients including 80 adult men and 54 women treated at Danang Oncology Hospital from January 2019 to July 2021 had specimen culture results found *Staphylococcus aureus* was investigated. All study patient data was collected according to an established uniform form on Microsoft Excel spreadsheet software and processed by Python programming language. We found that 76.1% of patients infected with *S. aureus* during this time were MRSA. Of which, 100% MRSA and MSSA isolates were resistant to Amoxicillin, 61.2% to Cefuroxime. Multi-resistance and broad-spectrum multi-resistance to other antibiotics belonging to the Carpapenem, Quinolone and Aminoglycoside groups were recorded in most isolated *S. aureus*. The results showed that *S. aureus* causing bacterial infection at Danang Oncology Hospital during the survey period had a high antibiotic resistance rate.

Key words - *Staphylococcus aureus*; MRSA; MSSA; antibiotics; antibiotic resistance

1. Introduction

Staphylococcus aureus (*S. aureus*) is an immobile, coagulating Gram-positive cocci in the family of membrane-bound bacteria (Firmicutes phylum). They are one of the common members of the microbiome in the body, which is found in the mucous membranes of the upper respiratory tract and on the skin. *S. aureus* is a facultative anaerobic bacterium that can grow without oxygen, usually positive for catalase and nitrate reduction [1]. When the mucous and skin barrier is damaged, *S. aureus* can penetrate the layers of subcutaneous tissue or blood and cause infection. Patients with invasive medical devices such as central and peripheral venous catheters or immunocompromised patients are at risk for *S. aureus* infection [2].

S. aureus is the cause of many diseases, from mild skin infections, such as boils, impetigo, boils, cellulitis, folliculitis, boils, skin burn syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, sepsis, and sepsis [3].

It remains one of the five most common causes of nosocomial infections and is often the cause of postoperative wound infections. Each year, approximately 500,000 hospitalized patients in the United States become infected with staphylococcus, mainly due to *S. Aureus* [4].

In Asia, methicillin-resistant *S. aureus* (MRSA) is prevalent in most healthcare settings and constitutes a large infectious disease burden in this region. The incidence of the disease varies considerably between different countries and has changed over time [5]. Molecular epidemiological studies have demonstrated that most hospitals acquiring-MRSA strains from different countries share the same genotype, indicating the international prevalence of several healthcare-associated clones in this area. However, most of the reports come from high-income countries, including Japan, Taiwan, Singapore, South Korea, and the Hong Kong Administrative Region. For South and Southeast Asian countries, the data are often sporadic or lack statistical information. This has significantly limited understanding of the epidemiology of staphylococcal diseases in these regions [6].

In Vietnam, although the studies are still sporadic, the obtained data show the common problem that the isolates of *S. aureus* strain 61.43% are MRSA, most of the MRSA strains are resistant to common antibiotics. The rate of antibiotic-resistant bacteria has increased dramatically in recent days, therefore choosing antibiotics for treatment of bacterial infections is more and more difficult [7].

Currently, good and appropriate diagnosis and treatment are the leading strategies in the treatment of hospital-acquired infections. The delay in treatment or inappropriate antibiotic treatment is strongly related to the rate of treatment time, cost of treatment, and even increased mortality of inpatients [8]. Therefore, in public health and health management, circulation control and the response of MRSA to the remaining antibiotic options will be medical issues of primary concern.

2. Materials and methods

2.1. Research subject

One hundred and thirty-four sample sizes of bacterial cultures resulting in *S. aureus* were recorded in the Clinical Microbiology laboratory, Danang Oncology Hospital from January 2019 to July 2021. Ethical approval was received from both The University of Danang - VN-UK Institute for Research and Executive Education, and Danang Oncology Hospital.

The analysis focused on information of isolates classified *S. aureus* as obtained from specimen collection. *S. aureus* was identified based on Gram positive cocci arranged in bunches and confirmed biochemically tests

such as Coagulase (+), Mannitol (+), Catalase (+) and morphological observation. Danang Oncology Hospital was applying the threshold standard Antibiotic resistance and diagnostic criteria for MRSA according to CLSI 2021 for determining Minimal Inhibitory Concentration (MIC) and antimicrobial susceptibility test. Methodological details of this multicenter, cross-sectional study involved patients whose samples were taken from sites of infection in duration. Patients with a culture positive for *S. aureus* comprised the methicillin-susceptible *S. aureus* (MSSA) carriage group and methicillin-resistant *S. aureus* (MRSA).

A diagram of diagnosis of MRSA and MSSA is shown in Figure 1.

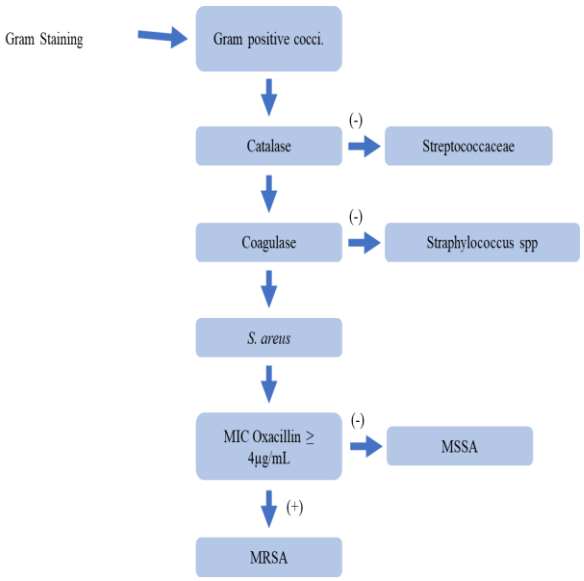

Figure 1. *Diagram of diagnosis of MRSA and MSSA*

2.2. Methodology

Study Design: Cross-sectional.

Description Research sample size: All the results of bacterial culture were established as *S. aureus*.

Sampling method: Convenience sampling.

Method of data collection: Retrospective survey of patients' medical records with service indications such as “Bacteria culture and identification by automatic system”, “Cultivation and identification of bacteria identification by manual method", "Quality drug-resistant bacteria" with results *S. aureus* culture results and antibiotic results. The missing information in the medical record is looked up directly in the files stored in the General Planning Department.

For *S. aureus* acquired patients with laboratory results with no interpretive value of MIC oxacillin when performed by culture system and identification by automatic system were excluded.

The medical records of inpatients managed by the hospital PC service following comprehensive patient characteristics were obtained: date of birth, sex, body mass index (BMI), blood types, types of cancer, stages of cancer, treatment unit, chronic underlying disease, antibiotics use, and other histopathological characteristics including metastasis (yes/no), catheter inserted (yes/no) and chemotherapy (yes/no).

Only patients with histologically confirmed diagnosis of cancer and evidence of incurable disease were included. The study covered patients receiving anticancer treatment with curative intent.

Sociodemographic-related data and associated risk factors were entered into Microsoft Excel and analyzed by using Python version 3.8.

3. Results and discussions

3.1. Characteristics of patients

During the specified study period, we established 158 clinicopathological characteristics patients, in which 14 patients were excluded from data analysis due to their insufficient index. For the remaining 134 patients, their characteristics were shown in Table 1.

Table 1. *Characteristics of patients*

	Characteristics	N	%
Age Median = 59	>59	65	48.5
	<59	62	46.3
	= 59	7	5.2
Gender	Male	80	59.7
	Female	54	40.3
BMI	<18,5	44.0	32.8
	18.5 - 24.9	88.0	65.7
	25 - 29.9	1.0	0.7
	≥30	1.0	0.7
Blood type	O	50.0	37.3
	A	41.0	30.6
	B	38.0	28.4
	AB	5.0	3.7
Catheter insertion	Yes	129	96.3
	No	5	3.7
Treatment unit	Internal medical Unit	69.0	51.5
	Surgical Unit	26.0	19.4
	Radiation therapy Unit	20.0	14.9
	ICU	9.0	6.7
	Chemotherapy Unit	1.0	0.7
	Outpatient	5.0	3.7
	Other	4.0	3.0
Types of cancer	Solid organ cancer	87.0	65.0
	Hematologic caner	29.0	21.6
	Other	18.0	13.4
Stages of tumor	I	78	58.2
	II	30	22.4
	III	17	12.7
	IV	9	6.7
Metastasis	Yes	76	56.7
	No	58	43.3

Table 1. presented the proportion of age of patients infected with *S. aureus* (including MRSA, MSSA), with the age of getting infection remaining around 59 years old. Regarding gender, a high risk of infection in men was reported with 80/134 patients (59.7%), higher than in women 54/134 patients, accounting for 40.3%. Patients whose BMI values were from 18.5 - 24.9 were also at a greatly increased risk of *S. aureus* infection. The incidence

of *S. aureus* in the healthy weight group was 88 per 134 people. That could be understood due to *S. aureus* being part of the normal human flora that normally resides in or on humans [9].

In our study, staph infection was dominant in persons that blood types A and O with 30.6% and 37.3% among investigated patients, respectively. This high risk of staphylococcal acquiring in the overall pool can be interpreted by the inability to elaborate an antibody which may cross-react with their own red blood cells. [2]. In the setting of injection of material into the bloodstream, additional factors contributing to the high incidence of *S. aureus* was the presence of an intravascular access device, in particular the use of a catheter. 129 per 134 patients had catheter insertion which was 25.8 times higher than those who had non-available injections. Another risk factor was the distribution of *S. aureus* by the clinical department. The highest rate of *S. aureus* was found in the Department of Internal Medical Unit (51.5%), Surgical Unit (19.4%), and Radiation Therapy Unit (14.9%).

Based on statistics for common types of cancer, we found that patients with solid tumors were much more common than hematologic malignancies prone to *S. aureus* infection [10]. The number of strains of *S. aureus* bacteria is most found in cancers of the solid organ cancer with 87 per 134 cases, accounting for 65%, followed by hematologic cancer and other types of cancer with rates of 21.6% and 13.4%, respectively.

Stages of cancer were a powerful determinant of staph incidence, the highest rates of infection occurring at stage I (58.2%). This rate gradually decreased in stage II- and stage III patients at 22.4% and 12.7 % but only 6.7% of patients had positive with *S. aureus*.

Among staphylococcal-infected individuals, there were 56.7% metastatic cases compared to 43.3% non-metastatic cases were reported to have *S. aureus* infection. Despite the disparity between cancer cell spreading and staphylococcal invasion their relationship has not yet been investigated and fully explained [11]. In our study, metastatic and non-metastatic patients were likely to have the same chance of bacterial acquiring.

3.2. Antimicrobial susceptibility of S. aureus isolates

The overall resistant rate for *S. aureus* to Beta lactam antibiotic group ranged from 35.8% (Piperacillin) to 100% (Amoxicillin) and was consistently higher than those in other antibiotic groups including carbapenem, quinolone, aminoglycoside, glycopeptide, and lincosamides (Table 2) Across the spectrum, *S. aureus* isolates shown complete resistance to Amoxicillin, but it was susceptible to many groups of antibiotics and susceptible to a very high percentage such as Amikacin (82.8%), and 100.0% susceptible to Lincomycin. Compared to other studies around the world, the percentage of Amikacin had a higher susceptible rate [12], [13]. *S. aureus* isolates revealed resistance pattern up to 14/15 of antimicrobials tested. Apart from Lyncomycin (100% susceptible), *S. aureus* has gotten frighteningly resistant to Cefuroxime (61.2% each), Kanamycin (43.3%). Intermediate resistance was found against Cefoperazone (38.1%), Levofloxacin (36.6%), Piperacillin (35.8%). Low resistance was found against Ciprofloxacin (29.1%), Meropenem (26.1%), Vancomycin (20.9%), and other antibiotics in Carbapenem and Quinolone groups. Notably, Vancomycin has long been considered as a standard therapy and the last-resort treatment for MRSA infections, the resistant rate to Vancomycin was extremely high in regimens in our study.

Table 2. *Antibiotic resistance pattern of S. aureus isolates*

Antibiotics		**NO of resistant isolate (n)**	**NO of resistant isolate (%)**	**NO of susceptible isolate (%)**
Groups	**Types**			
Beta lactam	Amoxicillin	134	100.0	0.0
	Oxacillin	102	76.1	23.9
	Cefepime	60	44.8	55.2
	Cefoperazone	51	38.1	61.9
	Piperacillin	48	35.8	64.2
	Cefuroxime	82	61.2	38.8
Carbapenem	Ertapenem	53	39.6	60.4
	Imipenem	19	14.2	85.8
	Meropenem	35	26.1	73.9
Quinolone	Levofloxacin	49	36.6	63.4
	Ciprofloxacin	39	29.1	70.9
Aminoglycoside	Amikacin	23	17.2	82.8
	Kanamycin	58	43.3	56.7
Glycopeptide	Vancomycin	28	20.9	79.1
Lincosamides	Lincomycin	0	0.0	100.0

Table 3. *Antibiotic resistance pattern of MRSA isolates*

Antibiotics		**R**	**%**	**S**	**%**
Groups	**Types**				
		R	S	R	S
Beta lactam	Amoxicillin	102	100	0	0.0
	Cefepime	46	34.3	56	65.7
	Cefoperazone	40	29.9	62	70.1
	Piperacillin	39	29.1	63	70.9
	Cefuroxime	62	46.3	40	53.7
Carbapenem	Ertapenem	45	33.6	57	66.4
	Imipenem	16	11.9	86	88.1
	Meropenem	27	20.1	75	79.9
Quinolone	Levofloxacin	35	26.1	67	73.9
	Ciprofloxacin	32	23.9	70	76.1
Aminoglycoside	Amikacin	15	11.2	87	88.8
	Kanamycin	45	33.6	57	66.4
Glycopeptide	Vancomycin	23	17.2	79	82.8
Lincosamides	Lincomycin	0	0.0	102	100.0

**R: resistant isolate; S: susceptible; %: percentage*

Notably, among 134 *S. aureus* harboring, 76.1% of cases of resistance (MRSA) were reported. This finding is similar to the results reported from other studies about nonsusceptibility to Oxacillin in MRSA at rates of 83% (in Ghana) [14], [15]. In which, the incidence of multiresistant antibiotics was found between MRSA and Cefepime (34.3%), Ertapenem (33.6%), Kanamycin (33.6%).

To secure an accurate understanding of drug-resistance profile of 102 MRSA isolates (Amoxicillin resistant strains), we continued to define percentage of MRSA that has acquired resistance to β-lactam, Carbapenem, Quinolone, Aminoglycoside, Glycopeptide and Lincosamides antibiotics (Table 3). MRSA strains had similar resistance rates with Cefuroxime, Cefepime and Kanamycin as *S.aureus* isolates. However, further statistical tests such Chi-square or Fisher exact test will be required to finalise the association of antibiotic susceptibility ad resistance between MRSA and *S.aureus* isolates.

4. Conclusion

Basically, all patients with *S. aureus* infection require prolonged treatment antibiotics. It was understandable that the was frighteningly resistant rate of *S. aureus* to many common antimicrobials. Based on data collection, patients in Danang Oncology hospital were likely to acquire staphylococci when they had metastatic tumors and were treated in Internal Medical Unit. This chance to get risk also was higher if they were male and blood types O and A. Those with the highest probability for cancer types belong to solid. Our results revealed that *S. aureus* has gotten frighteningly resistant to the major antibiotics in Beta lactam group. This resistant incidence had a significant high to Ertapenem, Kanamycin and Ertapenem. In contrast, 100% of MRSA and MSSA strains were still susceptible to Lincomycin in Lincosamides group. In summary, *S. aureus* infection can also affect patients with no age restriction. MRSA bacteremia may lead to sudden death without rapid diagnosis and treatment. Hence, we suggest strengthened antibiotic management and surveillance programs, which strategically manage the rational use of antibiotics by the guidelines for the user management of antibiotics in hospitals.

Acknowledgments: This research was financially supported by The University of Danang - VN-UK Institute for Research and Executive Education (Project code 03.2021). Professor Nguyen Duc Hoang (Ho Chi Minh City University of Science, Vietnam) and Dr. Pham Thi Kim Lien (RIKEN Institute, Japan) have participated in this project.

The authors thank all reviewers for carefully revising the manuscript.

REFERENCES

[1] E. J. M. Raineri *et al.*, "Staphylococcus aureus populations from the gut and the blood are not distinguished by virulence traits - a critical role of host barrier integrity", *Microbiome*, vol. 10, no. 1, pp. 1-23, 2022, doi: 10.1186/s40168-022-01419-4.

[2] S. Y. C. Tong, J. S. Davis, E. Eichenberger, T. L. Holland, and V. G. Fowler, "Staphylococcus aureus infections: Epidemiology, pathophysiology, clinical manifestations, and management", *Clin. Microbiol. Rev.*, vol. 28, no. 3, pp. 603-661, 2015, doi: 10.1128/CMR.00134-14.

[3] E. J. G. Pollitt, P. T. Szkuta, N. Burns, and S. J. Foster, "Staphylococcus aureus infection dynamics", *PLoS Pathog.*, vol. 14, no. 6, pp. 1–27, 2018, doi: 10.1371/journal.ppat.1007112.

[4] J. M, K. Kulhankova, C. S. Stach, B. G. Vu, and W. Salgado-pabón, "Phenotypes and Virulence among Staphylococcus aureus USA100, USA200, USA300, USA400, and USA600 Clonal Lineages", vol. 1, no. 3, pp. 1–15, 2016, doi: 10.1128/mSphere.00071-16.Editor.

[5] F. Schaumburg, A. S. Alabi, G. Peters, and K. Becker, "New epidemiology of Staphylococcus aureus infection in Africa", *Clin. Microbiol. Infect.*, vol. 20, no. 7, pp. 589–596, 2014, doi: 10.1111/1469-0691.12690.

[6] 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", *Crit. Care*, vol. 21, no. 1, p. 211, 2017, doi: 10.1186/s13054-017-1801-3.

[7] L. V. Nguyen *et al.*, "Appropriate antibiotic use and associated factors in vietnamese outpatients. Healthcare", *Healthcare*., vol. 9, no. 6, pp. 693–705, 2021, https://doi.org/10.3390/healthcare9060693

[8] J. Mensa *et al.*, "Recommendations for antibiotic selection for severe nosocomial infections", *Rev. Esp. Quimioter.*, vol. 34, no. 5, pp. 511–524, 2021, doi: 10.37201/req/126.2021.

[9] A. S. Lee *et al.*, "Methicillin-resistant Staphylococcus aureus", *Nat. Rev. Dis. Prim.*, vol. 4, no. 5, pp. 1–23, 2018, doi: 10.1038/nrdp.2018.33.

[10] K. V. I. Rolston, "Infections in Cancer Patients with Solid Tumors: A Review", *Infect. Dis. Ther.*, vol. 6, no. 1, pp. 69–83, 2017, doi: 10.1007/s40121-017-0146-1.

[11] N. Arora, A. Saroch, N. Verma, and P. Gupta, "Metastatic seeding in Staphylococcus infection", *BMJ Case Rep.*, vol. 14, no. 2, pp. 2020–2021, 2021, doi: 10.1136/bcr-2020-240301.

[12] S. Tiwari, M. Sahu, B. Rautaraya, T. Karuna, S. R. Mishra, and S. Bhattacharya, "Prevalence of methicillin-resistant Staphylococcus aureus and its antibiotic susceptibility pattern in a tertiary care hospital", *J. Indian Med. Assoc.*, vol. 109, no. 11, pp. 800–801, 2011.

[13] Y. Hu *et al.*, "Combinations of β-lactam or aminoglycoside antibiotics with plectasin are synergistic against methicillin-susceptible and methicillin-resistant Staphylococcus aureus", *PLoS One*, vol. 10, no. 2, pp. 1–15, 2015, doi: 10.1371/journal.pone.0117664.

[14] C. K. S. Saba, J. K. Amenyona, and S. W. Kpordze, "Prevalence and pattern of antibiotic resistance of Staphylococcus aureus isolated from door handles and other points of contact in public hospitals in Ghana", *Antimicrob. Resist. Infect. Control*, vol. 6, no. 1, pp. 1–6, 2017, doi: 10.1186/s13756-017-0203-2.

[15] E. Kayili and P. Sanlibaba, "Prevalence, characterization and antibiotic resistance of Staphylococcus aureus isolated from traditional cheeses in Turkey", *Int. J. Food Prop.*, vol. 23, no. 1, pp. 1441–1451, 2020, doi: 10.1080/10942912.2020.1814323.