

**PROFILE, RISK FACTOR, GENOTYPE, AND OUTCOME OF
STAPHYLOCOCCUS AUREUS CATHETER RELATED BLOOD
STREAM INFECTION IN HOSPITAL UNIVERSITI SAINS
MALAYSIA**

DR TEY NIAN JYE

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE MASTER OF PATHOLOGY
(MEDICAL MICROBIOLOGY)



UNIVERSITI SAINS MALAYSIA

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Table of contents	
Acknowledgements	iii
Table of contents	iv
List of tables	vii
List of Figures	viii
List of appendices	ix
List of abbreviation	x
Abstrak	xi
Abstract	xiii
CHAPTER 1 Introduction.....	1
1.1 Background of Study	1
1.2 Epidemiology of Catheter Related Blood Stream infection.....	1
1.3 Pathogenesis of Catheter Related Blood Stream Infection	3
1.4 Microbiology Of <i>S. aureus</i>	4
1.5 <i>Staphylococcus aureus</i> Catheter Related Blood Stream Infection.....	5
1.6 <i>Staphylococcus aureus</i> virulence gene of interest	7
1.7 Rationale of the study.....	9
1.8 Objectives.....	10
To study the profile of CRBSI, as well as the risk factor, genotype and clinical outcome of <i>S. aureus</i> causing catheter related blood stream infection.	10
1) To determine the profile of CRBSI in HUSM	Error! Bookmark not defined.

2) To determine the association between <i>S. aureus</i> CRBSI with chronic kidney disease (risk factor) and death or intensive care admission (outcome).....	Error!
Bookmark not defined.	
3) To determine the proportion of virulence associated gene in <i>S. aureus</i> causing catheter related blood stream infection and its associated clinical outcome.	Error! Bookmark not defined.
1.9 Study flowchart	11
1.10 References	12
CHAPTER 2 Study Protocol.....	18
1.1 Title: Profile, risk factor, genotype, and outcome of <i>Staphylococcus aureus</i> catheter related blood stream infection in Hospital Universiti Sains Malaysia .	18
2.2 Objectives.....	18
2.3 Methodology	18
2.4 Data collection:	28
2.5 Statistical analysis	28
2.6 Ethical approval	28
2.7 References	29
CHAPTER 3 Manuscript	30
1.1 Title page.....	30
3.2 Title	32
3.3 Abstract	32
3.4 Introduction	34

3.5 Material and Methods	35
3.6 Results	38
3.7 Discussion	42
3.8 Conclusion	45
3.9 Conflict of Interest	45
3.10 Reference:	46

List of tables:

Table 2.1Primer used in the research.	25
Table 2.2: The volume of reagent in master mix.	26
Table 2.3: The PCR temperature cycling condition.....	27
Table 3.1Sequence for primer use in the research:	37
Table 3.2: Demographic data of CRBSI cases in HUSM (n=139).	38
Table 3.3 <i>S. aureus</i> CRBSI with its associated risk factors.	40
Table 3.4: <i>S. aureus</i> CRBSI with its associated outcome (death).....	41

List of Figures:

Figure 1.1Gram stain of <i>S. aureus</i>	5
Figure 1.2: Colony of <i>S. aureus</i> on blood agar.	5
Figure 3.1Proportion of <i>S. aureus</i> virulence gene (n=19).....	41
Figure 3.2: Proportion of <i>S. aureus</i> with complicated CRBSI(n=19).....	42

List of appendices:

Appendices 1: Proforma checklist

Appendices 2: USM ethical approval letter

Appendices 3: Surat Kebenaran Penggunaan Data Pesakit

Appendices 4: Bantuan Kewangan Geran Penyelidikan Sarjana Perubatan(GPSP)
2020

Appendices 5: Sequencing result for *S. aureus* virulence gene and housekeeping gene

Appendices 6: Gel electrophoresis result for *S. aureus* virulence gene and
housekeeping gene

Appendices 7: Poster presentation for 18th Asia Pacific Congress of Clinical
Microbiology and Infection (APCCMI) 2021

Appendices 8: Instruction for author on Journal Malaysia Society of Parasitology and
Tropical Medicine

Appendices 9: Raw data on SPSS

List of symbols, abbreviation or nomenclature:

Symbol	Meaning
$\leq$	Equal or less than
$<$	Less than
$\geq$	Equal or more than
$>$	More than
$^{\circ}\text{C}$	Degree Celsius
μl	Microlitre
μm	Micrometre
ALT	Antibiotic lock therapy
CKD	Chronic kidney disease
CLSI	Clinical and Laboratory Standard Institute
CRBSI	Catheter related blood stream infection
DNA	Deoxyribonucleic acid
ESRF	End stage renal failure
et.al	Et alia (and others)
HUSM	Hospital Universiti Sains Malaysia
IDSA	Infectious Disease Society of America
PCR	Polymerase chain reaction
<i>S. aureus</i>	<i>Staphylococcus aureus</i>

Abstrak:

Profil, faktor risiko, genotip dan hasil jangkitan *Staphylococcus aureus* jangkitan aliran darah disebabkan kateter di Hospital Universiti Sains Malaysia

Latar belakang:

Selain daripada menjadi organism yang biasa menyebabkan jangkitan aliran darah disebabkan kateter (JADDK), *S. aureus* juga menyebabkan jangkitan aliran darah disebabkan kateter rumit(JADDKR). Objektif kajian ini adalah untuk mengesan gen virulensi *S. aureus* yang diasingkan daripada kes JADDKR dan kaitannya dengan JADDKR. Profil JADDK, factor risiko dan hasil jangkitan yang berkaitan dengan *S. aureus* JADDK juga akan disemak.

Kaedah:

Kajian keratan rentas melalui semakan rekod retrospektif kes JADDK telah dijalankan dari Januari 2019 hingga Disember 2021. Sebanyak 139 kes JADDK telah disemak sepanjang tempoh kajian. Data klinikal pesakit, factor risiko dan hasil jangkitan *S. aureus* JADDK telah dianalisis. Statistik deskriptif digunakan dalam profil JADDK, manakala regresi logistic berganda digunakan untuk analisis factor risiko, dan Pearson Chi Square digunakan untuk analisis hasil jangkitan *S. aureus* JADDK. Sembilan belas sampel terbaru *S. aureus* JADDK telah dipilih dan tertakluk kepada tindak balas berantai polimeras molekul untuk mengesan kehadiran gen *Cna*, *Chp* dan *Cap8*. Hubungan antara pembentukan *S. aureus* JADDKR dan gen virulensi dianalisis menggunakan Pearson Chi Square.

Keputusan:

S. aureus merupakan penyumbang utama JADDK (31%, n=43). Faktor risiko untuk *S. aureus* JADDK adalah pesakit yang mengalami masalah buah pinggan (nilai $P < 0.001$). Insiden JADDKR adalah 37%. Gen virulensi yang paling kerap diasingkan di antara 19 sampel ialah *Cap8* (89.5%, n=17), diikuti oleh *Cna* (47.4%, n=9) dan terakhir sekali *Chp* (26.3%, n=5). Statistik analisis mendapati bahawa ketiga-tiga gen virulensi tidak dikaitkan dengan pembentukan JADDKR. *S. aureus* JADDK dikaitkan dengan kematian yang lebih tinggi (nilai $P = 0.035$)

Rumusan:

S. aureus adalah agen utama dalam pembentukan JADDK. Kadar *S. aureus* JADDKR adalah 37%, dan gen virulensi yang paling kerap dijumpai adalah *Cap8*. Penyakit buah pinggan kronik merupakan faktor risiko untuk membentuk *S. aureus* JADDK.

Kata kunci:

Staphylococcus aureus JADDK, faktor risiko, gen virulensi, JADDKR.

Abstract:

Profile, risk factor, genotype, and outcome of *Staphylococcus aureus* catheter related blood stream infection in Hospital Universiti Sains Malaysia

Background:

Apart from being a common organism causing CRBSI, *S. aureus* also causes complicated CRBSI. The objective of this study was to detect *S. aureus* virulence gene isolated from complicated CRBSI cases and its association with development of complicated CRBSI. The profile of CRBSI, the risk factors and outcome associated with having *S. aureus* CRBSI were also reviewed.

Method:

A cross sectional study involve retrospective record review of CRBSI cases was carried out from January 2019 till December 2021. Total of 139 CRBSI cases was reviewed over the study period. Patient clinical data, risk factors and outcome of *S. aureus* CRBSI were analyzed. Descriptive statistics were used for profile of CRBSI, while multiple logistic regression was use for analysis of risk factors, and Pearson chi square were used for analysis of outcome for *S. aureus* CRBSI. Nineteen latest sample of *S. aureus* CRBSI were selected and subjected for molecular PCR to detect the presence of the gene *Cna*, *Chp* and *Cap8*. The association between development of complicated *S. aureus* CRBSI and virulence gene were analyzed using Pearson Chi Square.

Result:

S. aureus was the predominant agent (31%, n=43) causing CRBSI. The risk factor for *S. aureus* CRBSI was having CKD or ESRF (P value <0.001). The incidence of complicated CRBSI was 37%. The most common virulence genes among those 19 samples were *Cap8* (89.5 %, n=17), followed by *Cna* (47.4%, n=9) and lastly *Chp* (26.3%, n=5). The 3 virulence gene was not found to be statistically significant with the development of complicated CRBSI. *S. aureus* CRBSI were associated with higher mortality (P value 0.035).

Conclusion:

S. aureus was the predominant agent for CRBSI. The incidence of complicated *S. aureus* CRBSI was 37%, and the most common virulence gene was *Cap8*. CKD or ESRF were the comorbid associated with development of *S. aureus* CRBSI.

Keyword:

Staphylococcus aureus CRBSI, risk factor, virulence gene, complicated CRBS

CHAPTER 1 INTRODUCTION

1.1 Background of Study

CRBSI is a common cause of nosocomial bacteraemia, with incidence of 8.5% in ICU admission (28). The condition as contribute to increase mortality, with rate can be as high as 21.8% (29). Even for those that survive, the condition result in increased medical expenses and prolong hospital stay. (30)

Staphylococcus aureus as one of the important organisms contributing to CRBSI, are second commonest pathogen base on United State National Safety Network (NHSN) 2011-2014 report. The organism is even more common among patient with CKD or ESRF (16). The occurrence of complicated CRBSI with either one of the below, including infective endocarditis, septic metastatic, septic thrombophlebitis, or persistent bacteraemia more than 72 hour by *Staphylococcus aureus* in some of the case of catheter related infection raise the question of possible molecular factor of the bacteria that contribute to complicated bacteraemia. The gene postulated to contribute to the development of *S. aureus* CRBSI complicate bacteraemia being the three genes including *Cap8*, *Cna* and *Chp*. (24).

1.2 Epidemiology of Catheter Related Blood Stream infection

Vascular catheter become bread and butter in modern medicine patient management. Their role ranges from conducting haemodialysis, to administration of chemotherapy and parenteral nutrition fluid. Various type of catheters is produced to meet the need. They can be divided based on the duration of the catheter into two group, short term or long-term catheter. Short term catheter can be peripheral venous or arterial catheter

or short term central venous catheter. Peripheral venous catheter is the most used vascular device that is inserted over the forearm for administration of fluid or medication, while peripheral arterial catheter is seen in critical care for monitoring of patient haemodynamic status or for blood gas determination. Short term central venous catheter is placed over the great vein like subclavian, jugular, or femoral vein. The catheter used is non cuff and will be inserted directly over the vein. They are usually seen in intensive care or operation theatre. While on the other hand long term catheter is cuff and will pass through a subcutaneous tunnel. The cuff that presents will adhere to the subcutaneous tissue and prevents the catheter from dislocation and reduces infection rates (1). They are useful to provide vascular access to patients who require haemodialysis while waiting for arteriovenous fistula creation, or prolonged chemotherapy.

Different type of infection can occur with the usage of vascular catheter, which include catheter colonization, tunnel infection and CRBSI. CRBSI being part of nosocomial bacteraemia, is important due to the burden of the disease to the healthcare system. They represent rather common adverse event for hospitalized patients, with prevalence is found to be 39.25% among patient admitted to acute care setting (2). In a cohort study in which infected patients in intensive care unit are matched to noninfected control subjects, attributable deaths have been as high as 35%, with extra hospital stay of 24 days, and incurring extra costs attributable to the infection averaged \$40,000 USD per survivor (3). In response to the magnitude of the problem, few measures have been taken include several states in United States that require the hospital by law to submit the incident of catheter related infection (4), and financial incentive program for healthcare facility that able to achieve the goal of catheter related infection (5).

1.3 Pathogenesis of Catheter Related Blood Stream Infection

The catheter related bacteremia starts with the microorganism invading and adhere to the surface of the catheter. The common point of access to either extraluminal or intraluminal surface of the catheter appears to vary between short term and long-term catheter. Organism causing CRBSI in short term catheter is mainly extraluminally acquired and derived from patient cutaneous flora (6). While electron microscope reveal bacterial colonization in long term catheter is mainly intraluminal (7), with the organism derived from catheter hub. Microbial adhesion on the catheter is affected by factors like catheter material, bacterial characteristic, and the formation of conditioning film. Intravascular catheter that inserted into large vessel and in contact with blood will inevitably condition or coated with organic macromolecule such as fibrinogen, pyruvate, and glucose. (8) These conditioning films change the surface of the catheter and enhance the adhesion of microorganism. The bacteria that attach to the catheter will subsequently synthesis and aggregate in extracellular polymeric substance to form biofilm (9). Extracellular polymeric substance consists of mainly polysaccharide with different physical and chemical properties that depend on the type of organism involve. In *S. aureus*, the biofilm production involves the development of *icaADBC*- encoded polysaccharide intercellular adhesin. (10) The final step in biofilm formation being the biofilm dispersal where microbiome from mature biofilm detach and dispersed to further spread the infection. Free living cell that also known as planktonic cell will be release from the biofilm and subsequently causing catheter related blood stream infection. (11) Quorum sensing system contribute to the dispersion by upregulating extracellular proteases that causes biofilm detachment. In *Staphylococcus*, this is under the control of *agr* (accessary gene regulator) quorum sensing system. (12)

1.4 Microbiology Of *S. aureus*

S. aureus is a common human pathogen belongs to Family *Micrococcaceae*, genus *Staphylococcus*. They are ubiquitous skin and mucosa colonizer, can be found to colonizes human nose. Human can be asymptomatic nasal carrier of the bacteria that later become the source of infection (13). The asymptomatic carrier can create problem when they were admitted to hospital and become the source of outbreak in ward. (14) They are frequent cause of human infection and hospitalization in susceptible individual. The clinical spectrum can be group into pyogenic disease, toxin related disease and invasive disease. The most common form of pyogenic infection was skin, soft tissue, and osteoarticular infection. Invasive infection includes infective endocarditis, lower respiratory tract infection, and community-onset bacteremia. While toxigenic disease can be food poisoning or *Staphylococcus* toxic shock syndrome that happen with prolong tampon use. The organism also seen in case of nosocomial infection, which include surgical site infection and nosocomial blood stream infection that usually associated with catheter use.

When stain with Gram stain, *S. aureus* showed Gram positive cocci in cluster due to its thick peptidoglycan cell wall (figure 1.1). When the specimen was inoculated into agar like blood agar, florid colony growth will form within 18-24 hour (figure 1.2). Colony morphology varies, with some showing beta hemolysis on blood agar. Some other phenotypic biochemical feature that helps for their phenotypic identification include catalase and coagulase positive (due to the present of enzyme coagulase and clumping factor), DNase test positive due to the present of enzyme DNase and giving red color on mannitol salt agar.

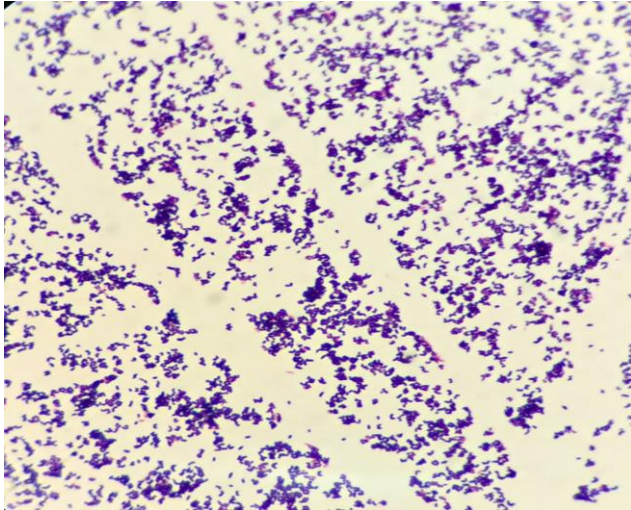


Figure 1.1 Gram stain of *S. aureus*

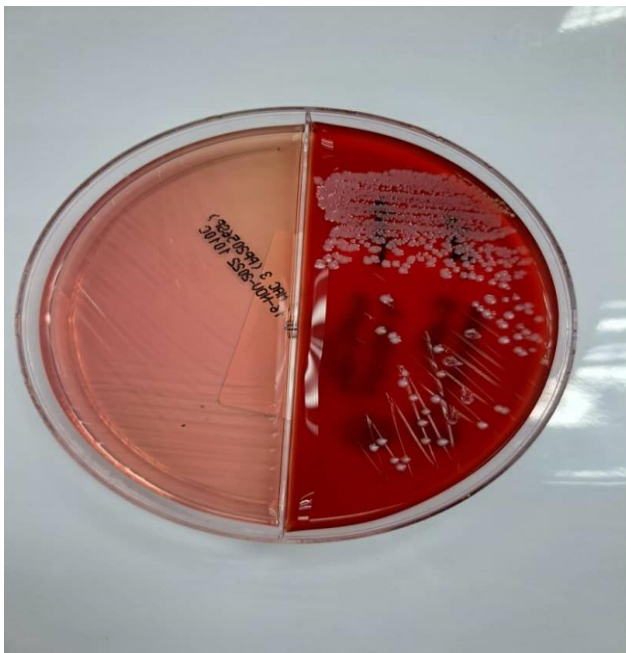


Figure 1.2: Colony of *S. aureus* on blood agar.

1.5 *Staphylococcus aureus* Catheter Related Blood Stream Infection

Nationwide surveillance study showed *S. aureus* to be common cause of CRBSI (15). When the specific condition or comorbid associated with developing *S. aureus* CRBSI were sought, CKD or ESRF were found to be the risk factor. This was shown from a

literature published by one of the local hospital universities (16), as well as a 12-year review from a tertiary referral hospital. (17)

The association between the two were related to the high nasal carriage of *S. aureus* in CKD or ESRF patient. Nasal carriage among dialysis patient can be found to be 41% in some cases. (18) This colonization eventually becomes a route of acquiring *S. aureus* CRBSI where the infection was endogenous. (19) The causal relationship between the two was shown in a genotypic study where more than 80% of cases of bacteraemia strain were genotypically the same as the patient *S. aureus* nasal carrier strain. (20) This correlation had led to the use of antimicrobials for nasal eradication of *S. aureus* reservoir, and a meta-analysis showed that the use of mupirocin for eradication was associated with a 59% (cpRR, 0.41; 95% confidence interval [CI], .36–.48) reduction in *S. aureus* infection among dialysis patients. (21)

Among the consequences of having *S. aureus* infection was their association with substantial mortality. In a study where nosocomial *S. aureus* bacteraemia that was predominantly catheter-related (61%), mortality was found to be 20% (22). In another 10-year prospective cohort study (23) showed that cases of CRBSI with *S. aureus* as the etiological organism were associated with significant mortality.

Other than its association with significant mortality, it also had the potential to develop complicated *S. aureus* CRBSI. Criteria for complicated *S. aureus* CRBSI include:

- a) development of endocarditis (according to modified Duke's criteria)
- b) septic thrombophlebitis (persistent *S. aureus* bacteraemia for >72hr after the initiation of active therapy associated with the documentation of a thrombus at the site of catheter insertion).

- c) Septic metastatic infection, including arthritis, spondylodiscitis, infection involving

vascular or osteoarticular prostheses or end organ hematogenous seeding to other location.

d) persistent bacteremia >72hr or

e) persistent fever for >4 day after extraction of the first blood culture yielding *S. aureus*, provided that alternative cause had been reasonably excluded.

The development of complicated *S. aureus* CRBSI in certain patient but sparing other had led to doubt the role of pathogen factor that affect the different in outcome. *San Juan et al* had demonstrated in this multicenter research (24) that pathogen factor for developing complicated *S. aureus* CRBSI was *Cap8*, *Cna* and *Chp* gene.

1.6 *Staphylococcus aureus* virulence gene of interest

From the research conduct by *San Juan et al*, the ranking of virulence gene starting from decreasing order of frequency would be *Chp* gene (81.6%), follow by *Cap8* (58.5), and finally *Cna* (56.6%). The role of the three virulence gene in the pathogenesis of infection and disease other than CRBSI had been an area of interest and research in other studies as well.

Cna gene encode collagen binding protein, the structure that is part of the microbial surface component recognizing adhesive matrix molecule (MSCRAMMS) (25) They are cell wall adhesin that function by binding to host collagen. The catheter placement into blood vessel with subsequent coating with patient serum that rich in fibrin, collagen will enhance collagen binding protein expression and contribute to the biofilm formation. (26)

While *Cap8* gene encode Capsule polysaccharide 8 that play role in evading human immune respond by its antiphagocytic function. (25)

Chp gene encode chemotaxis inhibitory protein that impair the neutrophil recruitment.
(27)

1.7 Management of CRBSI

The decision for catheter salvage versus catheter removal were important component in management of CRBSI. Catheter removal had the benefit of able to eliminate the source of infection, however they also come with the risk and cost associated with catheter replacement. Thus the selection of eligible cases was important. Clinical practise guideline on diagnosis and management of intravascular catheter related infection published by Infectious Disease Society of America (IDSA) (31) provide direction of decision making by looking into three main component, namely etiological agent of CRBSI, type of catheter used and type of CRBSI (complicated versus non complicated). Catheter salvage can be considered in non-complicated CRBSI patient with long term catheter when the causative organism was not *S. aureus*, *Pseudomonas aeruginosa*, *Bacillus spp*, *Priopionibacterium spp*, *Micrococcus spp*, fungi and Mycobacterium. While for patient with non-complicated CRBSI on short term catheter, catheter retention can be tried out if the organism were not *S. aureus*, *Enterococci spp*, gram negative bacilli, fungi, or mycobacterium. Catheter should be removed in those with complicated CRBSI regardless of causative organism or type of catheter. Catheter should also be removed when the cause organism for CRBSI was *S. aureus* as catheter salvage with antibiotic was associated with higher risk of cases relapse (33), as well as risk of developing complicated CRBSI. (34). While for the aforementioned group of patients that suitable with catheter salvation, they will require systemic antibiotic together with antibiotic lock therapy. The role of antibiotic lock therapy in treatment of catheter salvage was demonstrated in a systemic review that compare the outcome of patient treated with systemic antibiotic alone versus those on

systemic antibiotic and antibiotic lock therapy, where the latter group were found to be more superior as they required less catheter replacement. (32) In order to kill the sessile bacteria reside within the biofilm, antibiotic lock therapy will require a much higher concentration than regular intravascular antibiotic. For CRBSI cause by Gram positive organism like Coagulase negative Staphylococcus, vancomycin with concentration of 5 mg/mL and antibiotic lock fluid that changed at least every 48 hour was preferred to achieve lock therapy of concentration 11000 times the Staphylococcus MIC₉₀ during the entire dwell time (31). The systemic antibiotic of choice for Methicillin sensitive *S. aureus* CRBSI would be cloxacillin for four to six weeks. This treatment period followed the conventional treatment duration of *S. aureus* bacteraemia of four to six week that concern over development of abscess or infective endocarditis as the optimal treatment duration for *S. aureus* CRBSI would yet to be evaluate with randomized control trial. (31)

1.8 Rationale of the study

There was limited study done on the profile of CRBSI in HUSM, with last study conducted by *Katherason et al* in 2010. Much interest had raised on the trend of common organism, as well as its association with the risk factor and outcome. This will be able to provide knowledge and insight on CRBSI in HUSM that can be use as guidance for infection control policy and empirical antibiotic management.

Apart from that, the influence of pathogen-related molecular factors on the risk to develop complicated bacteraemia were also explored. The research on genetic profile of complicated *S. aureus* CRBSI and its correlation with clinical outcome can be useful as potential candidate for future diagnostic marker or vaccine development.

1.9 Objectives

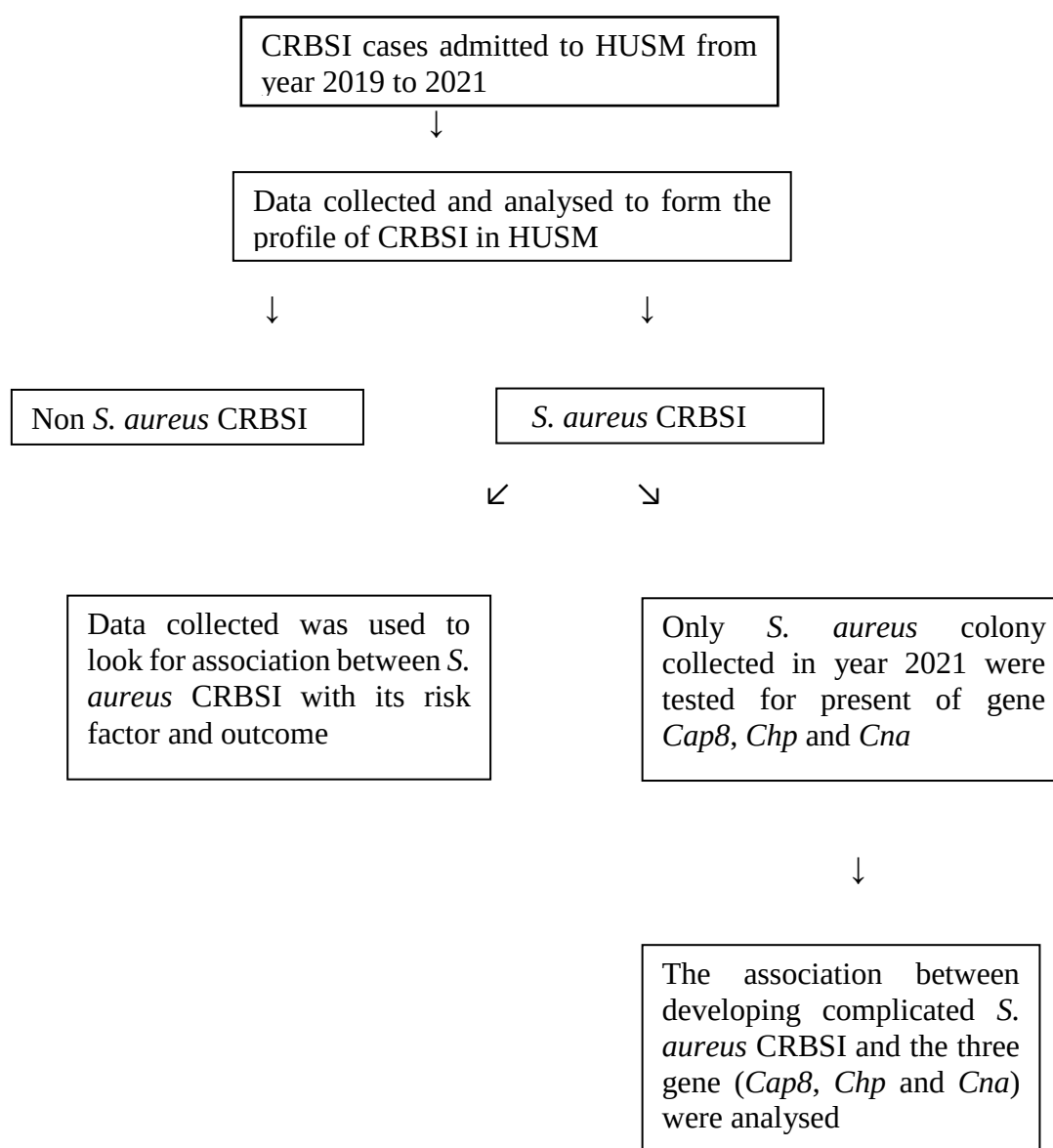
General:

To study the profile of CRBSI, as well as the risk factor, genotype and clinical outcome of *S. aureus* causing catheter related blood stream infection.

Specific:

- 1) To determine the profile of CRBSI in HUSM.
- 2) To determine the association between *S. aureus* CRBSI and its risk factor and outcome.
- 3) To determine the proportion of virulence gene in *S. aureus* CRBSI and its associated clinical outcome (complicated CRBSI).

1.10 Study flowchart



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CHAPTER 2 STUDY PROTOCOL

1.1 Title: Profile, risk factor, genotype, and outcome of *Staphylococcus aureus* catheter related blood stream infection in Hospital Universiti Sains Malaysia

2.2 Objectives

2.2.1 General objective

To study the profile of CRBSI, as well as the risk factor, genotype and clinical outcome of *S. aureus* causing catheter related blood stream infection.

2.2.2 Specific objective

- 1) To determine the profile of CRBSI in HUSM.
- 2) To determine the association between *S. aureus* CRBSI and its risk factor and outcome.
- 3) To determine the proportion of virulence gene in *S. aureus* CRBSI and its associated clinical outcome (complicated CRBSI).

2.3 Methodology

2.3.1 Study Design

The research was conducted using cross sectional study with convenience sampling of *S. aureus* isolate collected from patient diagnosed with *S. aureus* CRBSI from HUSM.

2.3.2 Study Period

The study was carried out from January 2021 until December 2021

2.3.3 Study Location

Data and samples of *Staphylococcus aureus* were collected from HUSM.

2.3.4 Reference population

All patients diagnose with CRBSI in HUSM from year 2019 to 2021.

2.3.5 Target population:

All patients diagnose with *S. aureus* CBSI in HUSM from year 2019 to 2021.

2.3.6 Source population

All patients diagnose with patient diagnose with *S. aureus* CRBSI with *S. aureus* isolated from culture sample in HUSM from January 2019 to December 2019.

2.3.7 Sampling frame

The sampling frame was patient diagnose with *S. aureus* CRBSI and eligible for data record in HUSM from January 2019 to December 2019.

2.3.8 Inclusion criteria

All microbiologically proven *S. aureus* CRBSI cases that send from 1st January 2019 until 31st December 2021.

2.3.9 Exclusion criteria

Presumptive/provisionally diagnose catheter related infection without fulfilling microbiological criteria.

Patient with repeated or duplicated culture.

Case with inadequate clinical data.

2.3.10 Sample size

Objective 1: To determine the profile of CRBSI in HUSM.

Variable being diabetes mellitus, using single proportion study, with p of dm= 25% (Hajjej et al 2014)
Precision being 6%,

Significant level= 0.05
Power(1-beta) = 0.8
Drop out= 10%
Sample size= 201
Sample size with drop out = 224

Objective 2: To determine the association between *S. aureus* CRBSI with chronic kidney disease (risk factor)

Using 2 proportion testing, with $p_0 = 22\%$ (Farrington et al 2019)
 $P_1 = 20\%$
Significant level= 0.05
Power= 0.08
Drop out= 10%
Sample size= 44
Sample size with drop out= 50

Objective 2: To determine the association between *S. aureus* CRBSI with death (outcome).

Using 2 proportion testing, with $p_0 = 99.7\%$ (Farrington et al 2019)
 $P_1 = 50\%$
Significant level= 0.05
Power= 0.08
Drop out= 10%
Sample size= 22
Sample size with drop out= 24

Objective 3: To determine the association between virulence gene in *S. aureus* CRBSI and its associated clinical outcome (complicated CRBSI).

Using 2 proportion testing, with $p_0 = 62\%$ (San-Juan et al 2017)
 $P_1 = 30\%$
Significant level= 0.05
Power= 0.08
Drop out= 10%
Sample size=57
Sample size with drop out=60
The calculation estimated 300 case that fulfil diagnosis of CRBSI from 1st January 2019 until 31st December 2021. However, the final number of cases that able to recruit into the study being 139. The insufficient in number of samples was largely due to cases with incomplete data and hence unable to include into the study.

A convenience sampling was applied to select the *S. aureus* CRBSI case from January 2021 until December 2021. The final number of colonies that was included into the

study was 19. The ongoing Covid-19 pandemic contribute to the scarcity of subjects and sample colony.

2.3.11 Sampling method

For objective 1: All microbiologically proven CRBSI from 1st January 2019 until 31st December 2021 will be included. Consent form is not required as it only uses secondary data. Clinical data will be collected by retrospective record review. The patients' identity will be made anonymous. All data will be kept confidential, available for researchers use only.

For objective 2: All microbiologically proven *S. aureus* CRBSI from January 2019 until December 2021 will be included. A checklist will be used to collect the studied variables.

For objective 3: Conventional sampling will be applied sample of *S. aureus* from 1st January 2021 until 31st December 2021. Sample will be collected from microbiology lab and store in cryotube. Confirmation of organism *S. aureus* will be perform using tube coagulase test and Staphaurex latex agglutination test. Genetic profiling will be performed via PCR on the organism using DNA primers *Cna*, *Cap8* and *Chp*.

2.3.12 Operational definitions

1) CRBSI: Paired central and peripheral blood sample with growth of same organism and sensitivity pattern, as well as differential time to positive of central sample 2 hour earlier than peripheral sample.

2) Complicated bacteraemia: present of one of five events after episode of *S. aureus* CRBSI:

- a) development of endocarditis (according to modified Dukes criteria)
 - b) septic thrombophlebitis (persistent *S. aureus* bacteraemia for >72hr after the initiation of active therapy associated with the documentation of a thrombus at the site of catheter insertion.
 - c) Septic metastatic infection, including arthritis, spondylodiscitis, infection involving vascular osteoarthritis prostheses or end organ hematogenous seeding to other location.
 - d) Persistent bacteraemia >72hr or
 - e) persistent fever for >4 day after extraction of the first blood culture yielding *S. aureus*, provided that alternative cause had been reasonably excluded.
- 3) Death: death within current admission, counted by reviewing clinical record and cause of death that is related to CRBSI.

2.3.13 Research methodology

2.3.13.1 Collection of bacterial isolates

The *S. aureus* isolate collected over the year were identified and confirm prior to further testing. The isolate was identified based on phenotypic characteristic: round convex colony that with or without beta hemolysis on blood agar, Gram stain showing gram positive cocci in cluster, catalase and coagulase positive; and positive with latex agglutination test Staphaurex Plus (Thermo Fisher Scientific, United State,2021). Only methicillin sensitive *S. aureus* (MSSA) will be included in the research. The antibiotic sensitivity testing for methicillin was carried out with disc diffusion Kirby Bauer method, with interpretive breakpoint provided by CLSI M100 version 31st.

2.3.13.2 Preparation of cell lysate

All the phenotypically confirm *S. aureus* colony were subjected to DNA extraction via cell lysate boiling method. Two loopful of the colonies was taken from blood agar plate that grow of *S. aureus* that incubated overnight in CO₂. It was mix into 100ml of distill water and stir adequately until no sediment seen. The mixture was then boiled at 100°C for 10 minutes, followed by centrifugation at 12,000 rpm for total of 5 minute to remove all the cell debris. The supernatant that forms after centrifugation was transfer into new tube while the cell debris was discard. The lysate will then be stored and kept in 4 °C while waiting for subsequent PCR analysis

2.3.13.3 Preparation of agarose gel

a. Tris Borate EDTA (TBE) Buffer (10x)

The buffer was prepared by dissolving 27.5g of boric acid, 54g of Tris base and 20ml of EDTA (pH 8.0) and mix with 500ml of sterile distilled water. The buffer solution was sterilized with autoclave and stored at room temperature.

b. Tris Borate EDTA (TBE) Buffer (0.5x)

The buffer was prepared by adding 950ml of sterile distilled water into 50ml of 10x buffer prepared earlier. After the dilution, mix the solution properly and stored it at room temperature.

c. 1.5% agarose gel

The gel was prepared by adding 1.5g of agarose powder into 100ml of 0.5X TBE buffer prepared earlier into a conical flask. Mix the solution and afterward heat it using

microwave for three minutes until it fully dissolved. The agarose solution was then cooled down to approximately 50°C, and 1.5µl of fluorescence dye (FluroSafe, First BASE Laboratory, Malaysia) was then added. The solution was then mixed well and poured into a gel casting tray with an appropriate gel comb. Any bubbles that present was cleaned, and the gel was left to cold down and solidified for 30 minutes at room temperature. Once the gel was solidified entirely, it was removed from the gel cast and was ready to be use.

2.3.13.4 Preparation and calculation for working primer solution

Primers were purchased from First BASE Sdn. Bhd. Malaysia and were supplied in lyophilized form. The vial was centrifuge at 9,000 rpm for 3 minutes. Preparation of 100µM stock primer concentration was done by adding the corresponding volume of sterile PCR water in accordance with the manufacturer's guideline. The required volume of 20µM working primer was determined using the following formula. The prepared working primer solution were stored at -20°C.

$$M_1V_1=M_2V_2$$

M1: Stock primer concentration

V1: Required stock primer volume

M2: Working primer concentration

V2 : Working primer volume

$$M_1V_1=M_2V_2$$

$$100\mu MV_1 = 20\mu M (100\mu l)$$

$$V_1 = \frac{20\mu M(100\mu l)}{100 \mu M}$$

$$V_1 = 20\mu l$$