OUTCOMES OF STAPHYLOCOCCUS AUREUS BACTEREMIA

PRABHA PARTHASARATHY

(M.D MICROBIOLOGY, INDIA)

A THESIS SUBMITTED FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF MEDICINE

NATIONAL UNIVERSITY OF SINGAPORE 2009

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude and sincere thanks to my supervisor Associate Professor Paul Anantharajah Tambyah, Division of Infectious Disease, Department of Medicine for his profound interest, support and guidance throughout the period of my graduate stay at the National University of Singapore. I am also thankful to him providing me with the necessary funds to perform the laboratory work and for critically reviewing this thesis.

I would also like to express my gratitude to the entire staff of the Division of Infectious Disease including the consultants and the registrars. I am grateful to Dr. Hsu Li Yang for providing guidance in molecular typing and Dr. Dale Fisher, Dr. Arlene Chua and Dr. Sophia Archuleta for reviewing the patients in the trial and providing timely feedback.

I would also like to express my thanks to the Department of Microbiology, to Dr. Raymond Lin for his advise and support in regards to the molecular typing and Dr. Roland Jureen for helping me with the analysis of Spa typing results. I appreciate the help provided by the staff of Microbiology at different stages, Donald Chiang for providing me the Staphylococcus aureus strains, Leela and Zarinah for informing me about new cases and to Carol, Technical Head. I am deeply grateful to Dr. Jeanette Teo, postdoctoral fellow at the Department of Microbiology for helping me set up the molecular diagnostic facility.

I would also like to thank the Research Division of Infectious Disease, especially Dr. Anupama Vasudevan, Clinical Research Coordinator, for helping me with the statistical analysis and providing support for IT related issues. I would like to thank M.B.B.S students Dr. Jonathan Chia and Dr. Raji for helping me collect the data.

I owe a special thanks to the staff at the National University Hospital who cooperated a great deal for this study in spite of having a hectic and demanding schedule. And last and not the least, this study would have not been possible without the cooperation of the patients and the physicians. I hope the results of this study would translate to a better care and outcome for these patients.

My work would be incomplete without the help of my family, who have sacrificed a great deal during my study.

Finally, I am glad that The National University of Singapore provided me this opportunity and awarded me with the research scholarship to make this study happen.

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SUMMARY

Staphylococcus aureus bacteremia (SAB), a leading cause of community and healthcare associated bacteremias is well known for complications such as a high mortality rate, endocarditis, metastatic infections and recurrence. The epidemiology of SAB is different worldwide due to differing rate of Methicillin Resistant *Staphylococcus aureus* (MRSA) and different comorbidities in the population. While most of the reports are available from the Western World, there is scant information in the Asian context. Hence, we decided to undertake this study at the National University Hospital with the main aim to define the outcomes of SAB. In addition, we evaluated the effect of an Infectious Disease (ID) consultation in a randomized trial and performed genotyping by the Staphylococcus Protein A (Spa) Typing method on a subset of strains. We recruited 300 consecutive patients with SAB making this one of the largest cohorts of SAB patients to be studies in Asia. .

The SAB and MRSA bacteremia rate was 3.42 and 1.44 per 1000 discharges or deaths. The epidemiology was characterized by a high percentage of MRSA (42%) and underlying comorbidities(88.4%). The mortality, infective endocarditis and recurrence rate was 29, 14.5% and 9.9% of all SAB cases respectively. On a multivariate logistic regression, MRSA infection, elderly age, malignancies, history of skin disease, and a higher APACHE score were associated with mortality; persistent bacteremia and IV drug use was associated with metastatic infections. In MRSA patients, metastatic seeding was commonly isolated bony infection and infective endocarditis. 21% of the MRSA strains had a vancomycin MIC of 2 or higher. The higher MIC was associated with bony metastatic infection and persistent bacteremia.

An ID consultation when evaluated in a randomized trial was associated with a better standard of care; however, outcomes of mortality and recurrence were comparable. The results are still preliminary and further evaluation of other outcome parameters is needed before drawing conclusions.

Genotyping of MRSA revealed 9 Spa types, 89% of which belonged to t032 (ST22, E-MRSA15, 21%) and t037 (ST 239-241, 68%). There was only one case of C-MRSA. Spa type t032 was associated with more endocarditis and pneumonia, however, mortality and recurrence was similar to t037.

In conclusion, the epidemiology and outcomes at our center were similar to those reported from the Western World such as USA or UK. The high proportion of infections due to MRSA warrants an intensification of the current infection control practices. There is a need for use of scoring systems such as APACHE II And Charlson score to adjust for underlying comorbidities. SAB patients including MRSA cases are prone for metastatic infections; hence a high degree of suspicion and imaging, in particular Transesophageal echocardiography is warranted. Amidst concerns of rising rates of the emergent E-MRSA 15 (t032), the outcome of bacteremia due to this clone was not different from others. Spa typing is a convenient and a good screening molecular typing method to draw relevant epidemiological conclusions.

LIST OF ABBREVIATIONS

SAB	Staphylococcus aureus bacteremia
CDC	Center of Disease Control and Prevention, USA
CLSI	Clinical and Laboratory Standards Institute
C-MRSA	Community acquired Methicillin Resistant Staphylococcus aureus
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-Reactive Protein
CVC	Central Venous catheter
CVS	Cardiovascular disease
E-MRSA	Epidemic-Methicillin Resistant Staphylococcus aureus
HIV	Human Immunodeficiency Virus
H-MRSA	Healthcare acquired Methicillin Resistant Staphylococcus aureus
hVISA	Hetero VISA
ICU	Intensive care unit
ID	Infectious Disease
IE	Infective endocarditis
IQR	InterQuartile Range
IV	Intravenous
IVDU	Intravenous drug use

LOS	Length of stay
MLST	Multilocus sequence typing
MRSA	Methicillin-Resistant Staphylococcus aureus
MSSA	Methicillin Sensitive Staphylococcus aureus
OR	Odds ratio
PFGE	Pulse Field Gel Electrophoresis
PFTE	Polytetrafluoroethylene grafts
Spa	Staphylococcal Protein A
SSI	Skin/soft tissue infections
TEE	Transesophageal echocardiography
TTE	Transthoracic echocardiography
US	United States
USA	United States of America
VISA	Vancomycin Intermediate *Staphylococcus aureus*
VRSA	Vancomycin Resistant *Staphylococcus aureus*
PVL	Panton Valentine Leucocidin
AV	Arteriovenous
DIC	Disseminated intravascular coagulation
MIC	Minimum inhibitory concentration
H/O	History of

CHAPTER 1

REVIEW OF LITERATURE

1.1 INTRODUCTION

Staphylococcus aureus, the causative organism of *Staphylococcus aureus* bacteremia (SAB) are Gram-positive cocci that are arranged in clusters as seen on a gram stain. This bacterium is extremely hardy and can survive on dried clinical material for months. It is also easily transmissible from person to person. The bacterium grows on common culture media including nutrient agar and sheep blood agar. Its ability to grow in a high concentration of salt and ferment mannitol has been utilized to develop selective media such as Mannitol Salt Agar.

Staphylococcus aureus can be differentiated from other members of this genus such as *S.epidermidis* and *S.saprophyticus* by its appearance on blood agar plates and additional biochemical tests(1). The characteristic colonies of *Staphylococcus aureus* are round, 1-2mm, golden yellow in colour with a zone of complete hemolysis on sheep or horse blood agar. In addition, *Staphylococcus aureus* gives a positive coagulase test, ferments mannitol, is sensitive to novobiocin and produces DNAase.

Staphylococcus aureus is among one of the most pathogenic members in its genus. It is armed with a range of surface proteins, enzymes and toxins that can lead to an inflammatory reaction at the local site and in some instances a toxin mediated effects at a distant site. Infections due to *Staphylococcus aureus* are broadly categorized as pyogenic or toxin mediated disease. Among the pyogenic infections, skin infections such as impetigo, carbuncles, furuncles, cellullitis and blephritis are common. At times, the bacteria can seed the blood and cause bacteremias and endocarditis. The other pyogenic infections include osteomyelitis, post surgical wound infections and pneumonias. Toxin

mediated diseases include food poisoning due to enterotoxins, toxic shock syndrome and scalded skin syndrome.

Common antibiotics used in the treatment for Staphylococcus *aureus* include methicillin, cloxacillin, cefazolin, levofloxacin, cotrimoxazole and vancomycin. The therapy is largely directed by the antimicrobial susceptibility profile of the organism. Penicillin, a beta-lactam antibiotic, the first to be used for *Staphylococcus aureus* is no longer in common usage as almost 90% of the strains are resistant to it. Methicillin resistance in *Staphylococcus aureus* (MRSA) was first documented in 1961 and since then there has been a growing concern for various reasons. Firstly, MRSA strains are resistant to other classes of antibiotics, thus limiting the therapeutic options to few select agents such as vancomycin, daptomycin and linezolid. Secondly, MRSA is particularly notorious in hospital environments, commonly seen in patients with serious underlying comorbidities and those undergoing invasive diagnostic or therapeutic procedures during the hospital stay. Outcomes in these patients are poor with a high mortality rate and increased hospital costs and length of stay among the survivors. Thirdly, MRSA, although mainly a healthcare associated issue, seems to be increasingly recognized in the community causing infections among healthy young adults who have not had any contact with healthcare facilities previously. Finally, many countries have witnessed a dramatic rise in the number of Staphylococcal infections attributable to MRSA. In some countries this percentage is as high as 40%(2). Due to the nature of the problem, many healthcare institutes across the world have implemented active surveillance programs to curtail the spread. Some of the common measures include screening, isolation and eradication of carriage especially in high-risk patients.

Staphylococcus aureus bacteremia or SAB although rare, is one of the dreaded complications of *Staphyloccoccus aureus* infection. In addition to a high mortality rate anywhere between 20-30% of all cases. (3-7), SAB is also associated with complications such as endocarditis, metastatic seeding and recurrences. The epidemiology and outcomes of SAB vary in different countries and largely depend on the prevalence of MRSA and underlying risk factors. Although well recognized in the Western World (3, 7-10), there is a paucity of data from the Asian continent. Hence, this study was conducted to understand the epidemiology and outcomes of *Staphylococcus aureus* bacteremia in Singapore.

1.2 EPIDEMIOLOGY OF SAB

1.2.1 Incidence

Staphylococcus aureus is a leading cause of community and hospital acquired bacteremias. It is the second most important cause of Nosocomial and Community acquired bacteremias after Coagulase Negative Staphylococcus and *Escherichia coli* respectively(11, 12). In a retrospective review across 17 hospitals in Australia, 25% of all bacteremias were attributed to *Staphylococcus aureus*(10) .

Most of the *Staphylococcus aureus* bacteremia (SAB) data are available from Europe and North America. Over the previous two decades, many of these countries have recorded a marked increase in the incidence of *Staphylococcus aureus* infections. Finland noticed an increase from 11 per 100,000 in 1995 to 17 per 100,000 in 2001 (p value <0.001)(3) .In Denmark, on an average, there has been a 5.3% annual increase in *Staphylococcus aureus* bacteremia incidence rate from 1981 to 2000(4).

In a population-based study conducted in Calgary, Canada, the average incidence rate of *Staphylococcus aureus* bacteremia was estimated at 19.7 cases/100,000 population with no significant change from 2000-2006. However, during the same period the rate of MRSA bacteremia increased dramatically ($p<0.001$)(5). The National Nosocomial Infections Surveillance reported a rise in nosocomially acquired *Staphylococcus aureus* bacteremia in the US from 1980 to 1989 (p value <0.001)(13). Many countries have witnessed such a rise in specific subpopulations of SAB.

1.2.2 Mode of acquisition

Till late, SAB was commonly defined as "nosocomial" or "community acquired" according to the US Center of Disease Control and Prevention (CDC) definitions(14). This was based on the timing of the positive blood culture. A positive *Staphylococcus aureus* blood culture drawn in the first 48 hours of hospitalization from a patient was assumed to be a community acquired bacteremia while a positive blood culture after 48 hours of hospitalization is believed to be nosocomially acquired. Many authors and clinicians now feel the need to define a third category, namely Healthcare associated infection (8, 15-17). This category includes all those SAB episodes labeled as "community acquired" in patients with specific healthcare risk factors. The SAB infection in these patients was thought to be related to their healthcare exposure rather than a community acquired bacteremia. The need for this category arose with increasing number of patients seeking healthcare outside the hospital in particular, home dialysis and intravenous (IV) home therapy. Currently, there is no consensus on the definition of previous healthcare exposure. In a recent paper, Klevens and Colleagues used the following criteria(8).

- IV home therapy
- History of MRSA colonization
- History of surgery, hospitalization, dialysis or residence in a long term facility in the last one year

It appears that almost 2/3rds of all *Staphylococcus aureus* bacteremias are in patients with previous or ongoing healthcare exposure. In a retrospective review of SAB cases across 17 hospitals in Australia(10) , 51% were nosocomial, 34% community onset and 15% healthcare associated. In another population based surveillance conducted in Calgary, Canada from 2000-2006(5), 1542 bacteremic cases were reported with 39% nosocomial , 25% community onset and 36% healthcare associated infections.

The impact of various modes of acquisition on the outcome is described elsewhere.

1.2.3 Problem of MRSA bacteremia

The proportion of *Staphylococcus aureus* bacteremias caused by MRSA varies in different countries. It is documented to be as high as 40% in United Kingdom(2) to as low as 1% in the Scandinavian countries of Denmark and Sweden(4) . Most of the MRSA bacteremias (70-85%) are healthcare related (8, 10).

In recent years, there have been concerns about an increasing number of MRSA causing community onset infections. Genotypically, strains of MRSA causing community onset infections (C-MRSA) are known to be different from their hospital counterparts (H-MRSA). Although many infections are self-limiting, the infection can be severe at times and lead to bacteremias. Community MRSA bacteremia is usually seen in the younger patients with no serious comorbidities unlike healthcare associated MRSA which is seen in older patients with significant comorbidities(18).

The global extent of the problem of C-MRSA is not fully known. Nevertheless, many countries seem to be recognizing the problem. In certain countries, 15-30% of the community-acquired bacteremias are caused by C-MRSA(6, 19). A 10-year (1997-2007) retrospective review of SAB cases presenting at a tertiary care center in Perth, Australia revealed that almost 10% of all the MRSA bacteremic cases could be attributed to C-MRSA. Similar rates (12%) have been reported from the US(8). Among the Perth cohort, Intravenous Drug usage (IVDU) was the only risk factor associated with C-MRSA and the 7-day and 30-day mortality did not vary significantly between H-MRSA and C-MRSA bacteremia in this cohort (18) .

In Singapore, the problem of C-MRSA also seems to be increasingly recognized as well. At a tertiary care center, a search through the microbiology archives from 2001-2004 yielded only 8 possible C-MRSA cases, however, between May 2004 to June 2005, there were a further 37 isolates. All these cases appear to be imported through construction workers or maids and the mean age of these patients was 35 years. Although most of the cases (35 of 42) were cutaneous abscesses, there was one case of C-MRSA bacteremia in 2004 in a patient with IVDU(20). A genetic analysis of C-MRSA strains by Hsu and colleagues showed that majority of the community MRSA strains isolated from 2000-2005 showed the presence of Panton Valentine Leucocidin (PVL) toxin and belonged to multilocus type ST30 (21).

1.2.4 The Asian Scenario

Most reports from Asia come from experiences at tertiary care centers. Although these reports cannot be extrapolated to the general population, they seem to suggest a dynamic

epidemiology as seen in the other parts of the world. Highlighted below are the experiences from two countries, Taiwan and Singapore.

1. The Taiwan Experience:

- MRSA accounted for 53% to 83% of all *Staphylococcus aureus* clinical isolates in 12 major hospitals in 2000(22) .
- Proportion of infections attributed to MRSA has increased dramatically over the last decade or two. At a university hospital, this proportion was 39% in 1991 and 75% in 2003(23).
- The prevalence of C-MRSA has also increased over years. A study from one of the centers reported a rate of 32% per year from 2001 to 2006(24).
- As mentioned earlier, high proportions (30%) of community acquired SAB are MRSA(19) .

2. The Singapore Experience:

- At the National University Hospital, Singapore, a total of 100 patients presented with MSSA bacteremia within the first 48 hours of hospitalization between March 2005 to February 2006. Forty eight percent of these patients had previous healthcare exposure(25).
- Approximately 35% of all *Staphylococcus aureus* isolates are methicillin resistant (MRSA)(26).
- There are suggestions of an increasing number of C-MRSA isolates in hospitals. The experience of Singapore General Hospital is mentioned earlier(20) .

1.3 RISK FACTORS

SAB is frequently seen in the following group of patients(27)

- Elderly patients
- Patients with underlying comorbidities including Diabetes mellitus, Cardiovascular disease (CVS), Human Immunodeficiency virus(HIV), Carcinoma, Rheumatoid arthritis and rare blood disorders such as Job's syndrome, Chediak-Hegashi syndrome etc.
- Hemodialysis, peritoneal dialysis, and other hospital related invasive procedures
- Presence of a foreign body in particular, orthopedic devices, prosthetic heart valves and urinary catheters.
- Intravenous drug abusers
- Surgical site infection
- Patients residing in long term care facilities (including nursing homes)

The risk factors for SAB vary depending on the mode of acquisition. Patients with community-acquired infections tend to be younger and less likely to have comorbidities than nosocomial or healthcare associated infection. Community acquired SAB is commonly seen in IVDU's and the underlying source of infection is not usually clear(27). Patients with nosocomial SAB tend to be older and frequently have the above mentioned comorbid conditions. Intravascular catheterisation, surgical wound infection or hospital-acquired infections such as respiratory illnesses during the hospital stay, make these patients more prone to SAB.

Healthcare associated infections are encountered in patients receiving dialysis, home IV therapy or in residents of long term care facilities. The risk factors are similar to

nosocomial infections. The patients are older and usually have an identifiable focus like IV catheters or in the case of nursing home residents decubitus or foot ulcers.

MRSA bacteremia is more commonly seen in a healthcare set up, hence risk factors include a longer hospital stay(28), central venous line (CVC)(28, 29) ,surgical site infection(28, 29), prior antibiotic exposure(29, 30) and nosocomial bloodstream infection(30-32).

Some of the key risk factors of SAB are further elaborated below

Hemodialysis and SAB: The increasing use of intravascular catheters has been cited as one of the reasons of an increasing prevalence of hospital acquired SAB(27). *Staphylococcus aureus* is a common infectious complication in patients undergoing hemodialysis. In a prospective observational study conducted over 2 years at six teaching hospitals in USA, 127 consecutive episodes of bacteremia were evaluated in 118 patients undergoing hemodialysis. *Staphylococcus aureus* was found to be the commonest cause accounting for 31% of all bacteremias in this population.(33). In addition, *Staphylococcus aureus* was more likely to cause access site infection than other microbes (p=0.0001)

The overall incidence of SAB in hemodialysis patients has been estimated at around 0.5-1 episodes per 100 patient hemodialysis months (34-39). The attributable mortality in these patients range anywhere from 5% to 19 %. Complications such as infective endocarditis (IE) were thought to occur at a lower frequency in these patients. However a recent study reported a 14% prevalence of IE among SAB hemodialysed patients. The authors used the sensitive Transesophageal echocardiography (TEE) method for detecting infective endocarditis(34)

Hemodialysed patients are particularly prone to SAB episodes due to

- Breaks in the skin as occurs during intravenous catheterisation
- Colonization of skin , the vascular access site and the hub with the organism
- Impairment of the immune system due to uremia, malnutrition, iron overload and Diabetes mellitus.

The risk of SAB also increases with specific hemodialysis procedures. Central lines, permanent catheters and Polytetrafluoroethylene grafts (PFTE) are more commonly associated with bacteremias in comparison to arteriovenous (AV) fistulas (40-43)

Intravenous drug abuse: *Staphylococcus aureus* is the most common bacteria causing infectious complications such as skin/soft tissue infection, bacteremias or endocarditis in IVDU(44, 45) . In a study conducted at Detroit, Michigan, SAB accounted for 57% of all bacteremic episodes among IVDU. Of these 42% were caused by MRSA alone, making it the second most common bacterial etiology after MSSA(45)

Some of the risk factors which increase the chance of *Staphylococcus aureus* infection in IVDU are nasal colonization, use of contaminated needles, close personal contact such as the shooting galleries and subcutaneous or intramuscular injections(46).

The rate of infective endocarditis is higher in IVDU patients with SAB as compared to non IVDU patients with SAB(47) . Moreover, IVDU's suffering from infective endocarditis are also prone to thromboembolic events especially to the lung. In spite of the increased occurrence of endocarditis in IVDU, the prognosis of these patients is favorable, perhaps due to their younger age, lack of comorbidities and right-sided involvement.

Nasal colonization: Various studies suggest that patients who are colonized with *Staphylococcus aureus* are more prone to bacteremic episodes (48, 49). In a prospective study conducted in Netherlands from 1999-2001, 14008 non-surgical non-bacteremic patients were screened for *Staphylococcus aureus*. Of these patients, 24% of the patients carried *Staphylococcus aureus*. Nosocomial SAB was more common in carriers (1.2%) than the non carriers (0.4%)(48).

The relationship between carriage and infection has been more widely studied in regards to MRSA. It appears that patients with MRSA colonization are more prone to bacteremias than MSSA colonizers. In a prospective cohort study conducted in Spain, patients admitted to the Intensive care unit (ICU) over a year's time from 1991 to 1992 were screened for nasal carriage of *Staphylococcus aureus* and MRSA. These patients were subsequently followed up and any SAB episode was recorded. The rate of bacteremia among MRSA carriers, *Staphylococcus aureus* carriers and non carriers was 38%, 9.5% and 1.7% respectively(49). The increased rate of bacteremia in MRSA carriers was associated with an increased ICU stay, surgery and invasive procedures as seen in this set of patients.

1.4 PATHOPHYSIOLOGY AND CLINICAL MANIFESTATIONS

Around 80% of the general population is colonized with *Staphylococcus aureus* at some point or the other in their life(1). Around 20-30% are persistent colonizers. The commonest site of colonization is the anterior nares. The other sites are axilla, rectum and perineum .The rate of colonization has been observed to be higher in specific subgroups

such as health care workers, patients undergoing dialysis, diabetics, IV drug abusers and HIV infected individuals(1) .

From the anterior nares, the bacterium can go on to colonise the skin. However, infection does not follow due to the effective innate immunity in the form of the barrier of skin and mucous membrane. People become prone to skin/soft tissue infections (SSI) when this barrier is breached as a result of trauma. The bacterium can thus gain entry into the underlying tissue and cause an inflammatory response to be generated. What ensues is an abscess formation consisting of bacteria, necrotic tissue and phagocytes. In most instances, the inflammation is self-limiting to the skin. Rarely does the bacterium seed the deeper tissue or bloodstream. For MRSA infections, the proportion of SSI is almost eight times higher than bacteremia(50). As mentioned earlier, bacteremia is more common in patients with specific risk factors.

In addition to skin and soft tissue infections, the other portals of entry for SAB are surgical site infection, osteomyelitis, septic arthritis, pneumonia or an intravascular device like central venous lines and IV lines. Many a times, there is no obvious source for SAB. Such episodes are also labeled as primary bacteremias. Various studies have reported rates of primary bacteremia anywhere between 15 to 50% of all SAB (4, 5, 51-53). The clinical picture of SAB is governed by the underlying source. Specific signs and symptoms of bacteremia include fever with chills and rigor, drowsiness and in some cases, signs of septic shock such as hypotension and DIC. Specific clues for endocarditis like septic emboli to skin or a new cardiac murmur may be present

1.5 COMPLICATIONS AND OUTCOME

1.5.1 Prognosis

Although the proportion of deaths due to SAB has decreased after the availability of antibiotics, it still remains high. Various population-based studies have reported a mortality rate between 20-30% (3-8). Statistics from individual tertiary care centers have documented an in-hospital mortality rate of 20-40% (52-59) and 30-day mortality between 10-25% (52, 60). Very few studies determine the long-term mortality. At a tertiary care center at Germany (58), the 1-year mortality rate was observed as 37.6% among all SAB cases. Studies at different centers in the US report a 60-day mortality at 11.5%(60) and 90-day mortality at 57 %(61).

In Asia, most of the available information on mortality is from tertiary care centers and focus on subsets such as MRSA or community infections. In Singapore, the attributable mortality was 11% among 100 Community MSSA bacteremic patients presenting at the National University Hospital during 2005-2006(25). An older age (>65) and chronic pulmonary disease were predictive of mortality in this subset of patients ($p<0.01$). In another study conducted at Taiwan, 177 patients of MRSA bacteremia were evaluated for their outcome. The in-hospital mortality in this group of patients was 33.3% with 60% of the deaths taking place in the first 14 days of hospitalization(62). In yet another study in Thailand, a very high attributable mortality (48%) was noted among SAB patients(63). This rate was much higher than observed in the resource rich countries.

One of the strongest predictors of mortality is the age of the patient (3-5, 7, 52, 54, 62, 64-66). In a study conducted in Finland, the increase in incidence of SAB in elderly lead to an increase in annual mortality rate due to SAB from 2.6 to 4.2 deaths per 100,000

population per year(3). Other factors predicting mortality include MRSA infection (67), a lung origin(4, 5, 7, 52, 62) , unknown source , septic shock(64, 65), endocarditis (4, 7), persistent bacteremia(62) and metastatic infection(62) , a greater severity of illness and comorbidities (4, 7, 62, 68-70), inappropriate or a delay in institution of antibiotic (65, 71-73) and nosocomial infection(54). The impact of some of these factors have been elaborated further later in this review.

1.5.2 Endocarditis:

Staphylococcus aureus is a leading cause of infective endocarditis (IE) globally. In a prospective cohort study conducted in 39 sites across 16 countries from 2000-2003, *Staphylococcus aureus* was the etiological agent in 32% of all IE making it the commonest cause followed by viridans Streptococci (18%)(74). In this cohort, patients with *Staphylococcus aureus* IE were more likely to have Diabetes mellitus, a presumed intravascular source, IVDU and healthcare associated IE, as compared to the Non *Staphylococcus aureus* IE. The rate of embolisation and persistent bacteremia was also higher in the SAB cohort.

Traditionally SAB IE has been viewed as a community acquired problem, commonly MSSA in origin and encountered in young patients especially IVDU's. However, with the increasing use of prosthetic devices and IV home therapy, rising rates of MRSA infection and use of echocardiography for detection of vegetations, it appears that a significant proportion of infections are also acquired in the healthcare set up. This is particularly true in the US where almost 33-40% of all SAB IE are healthcare associated (60, 74). Epidemiologically, healthcare associated IE is more common in older patients

with MRSA infections. It is also fraught with the problem of persistent bacteremia and is associated with a higher mortality (60).

Worldwide, the incidence of IE in SAB varies anywhere from 5-15 % (4, 5, 7, 60, 65, 75). In Quebec, between 1993 to 2005, the cases attributable to endocarditis increased from 4% to 11%(7). The proportion of IE cases due to MRSA has been noted to be as high as 40% in certain regions(74).

Endocarditis portends a high mortality rate in patients with SAB. Most studies have reported a mortality rate > 30% (4, 7, 60, 70). Hence it is important to identify SAB patients who are at risk of IE and IE patients at risk of mortality. Some of the factors believed to be predictive of IE in patients with SAB are persistent bacteremia(60, 76), presence of a prosthetic valve(60, 76) prior endocarditis(60), IVDU(60), community acquisition(60) and unrecognized source(60, 76). Patients with MRSA are more prone for persistent bacteremia (60, 76) and both MRSA and persistent bacteremia show a strong trend towards mortality (60, 76).

Echocardiography is also being increasingly used to diagnose additional cases of endocarditis. Currently, two modalities are used for diagnosis of SAB IE: Transthoracic echocardiography (TTE) and Transesophageal echocardiography (TEE). TEE is more sensitive than TTE in detecting vegetations. In a series of 103 patients, valvular vegetations were obtained in 21% of patients with TEE as compared to 7% with TTE(77). In addition to overall sensitivity, TEE is particularly useful in picking up smaller size vegetations, vegetations on prosthetic heart valves and complications such as abscesses. In the past, most of the nosocomial SAB episodes never had an echocardiographic evaluation, as many believed this to be a low risk population.

However, a prospective evaluation showed that almost 25% of patients with catheter SAB had evidence of endocarditis as suggested by TEE(77). Hence, many believe in a routine echocardiographic evaluation for all patients with SAB.

Although more sensitive, TEE is not always routinely feasible due to the invasive nature of the procedure. Some centers recommend a routine TTE for all patients with SAB and reserve TEE in patients with a negative TTE especially where the risk of endocarditis is high, for instance, IVDU, community acquisition with unknown source, patients with prosthetic heart valves, persistent fever, persistent bacteremia or where a short course of antibiotics is being considered. (78).

1.5.3 Other complications and recurrence

As many as 30% of non-infective endocarditis SAB are associated with metastatic complications (79-82). Secondary metastatic infection can involve bone and joints and viscera like the lung, spleen, liver, kidney and brain. Among bony metastatic infection, vertebral osteomyelitis and septic arthritis especially of the knee joint and sacroiliac joints are common. (78). Hence, a high index of suspicion is needed when a patient with SAB presents with back pain. Pulmonary metastasis is common entity among IV drug abusers with Tricuspid valve endocarditis.

Bacteruria is another consequence of bacteremia. Many believe that is it the consequence of renal seeding of the bacteria. Whether it is a surrogate marker of bacteremia is uncertain(78). Many patients with concomitant bacteremia and bacteruria have underlying urinary pathology and in particular, a urinary catheter which could be the cause of bacteremia. Nevertheless, bacteruria in a SAB patient without a catheter could serve as a surrogate marker of metastasis and must prompt a detailed evaluation for other

metastatic sites (78). Metastatic infections usually need a longer course of antibiotics and at times a surgical intervention. In the absence of an adequate treatment, the secondary foci could lead to future relapses and recurrences.

The rate of recurrence in SAB infections appears to be anywhere between 2.5 to 12% (9, 53, 54, 64, 83-85). Recurrences can be due to infection with the same strain (relapse) or due to a different strain (reinfection). Some investigators believe that relapses occur earlier than reinfection. (70, 86). Some of the risk factors associated with relapse include persistent bacteremia(54), failure to remove source(54), vancomycin treatment (54, 85), native valve disease(70) and endocarditis(70)

1.6 METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AND OUTCOMES

The impact of MRSA on mortality has been studied extensively. Many studies report a higher mortality rate among patients with MRSA bacteremia as compared to MSSA bacteremia (5, 7, 56, 61, 87-90). However, it is not certain if this higher mortality is because of underlying comorbidities, a longer hospital stay and delay in antimicrobial stay in patients with MRSA bacteremia.

In order to understand the impact of MRSA on mortality, a meta-analysis was conducted by Cosgrove and her colleagues(67). They included thirty-one cohort studies from 1980 to 2000 that had cited numbers and mortality rates for patients with MSSA and MRSA bacteremia. Barring four studies, the rest of the studies observed an increased mortality among MRSA bacteremic cases. A statistically significant difference in mortality was observed in only seven studies. All studies combined, MRSA bacteremia was associated with a higher mortality with an OR of 1.93(CI: 1.54-2.42, p value<0.001). The increased

mortality was observed even after adjusting for comorbidities and severity of illness. The authors postulate that the increased mortality observed in MRSA could be a result of a delay in the institution of appropriate antibiotics or due to the decreased efficacy of vancomycin as compared to beta lactams.

One of the major limitations in understanding the impact of MRSA on the outcome is the lack of consensus on how to evaluate underlying comorbidities. Commonly studies employ comorbidity scores such as the Charlson comorbidity score (64), APACHE II (91) or McCabe Jackson score(92) to quantify the severity of illness. In regards to *Staphylococcus aureus* bacteremia, there is a wide heterogeneity in these indices used across studies. Hence, the varying results.

It appears that MRSA also has a significant impact on the length of hospital stay and on hospital costs (72, 93-98). The length of stay is almost 1.3-1.4 fold longer for MRSA patients as compared to MSSA patients(72). In the same study, MRSA bacteremic patients incurred a higher cost of hospitalization. On an average, patients with MRSA infection had a 1.3-1.8 fold or $3800- $10,000 higher infection related costs compared to MSSA patients (57, 72). Greater comorbidities, slower clinical response have been cited, as reasons for a longer hospital stay observed with MRSA.

The impact of MRSA on the length of stay and hospitalization costs seems to extend to bacteremias as well. In a matched case control study of nosocomial bacteremias, MRSA bacteremias had 3 fold increase in costs as compared to MSSA bacteremias(99).

1.7 REDUCED SUSCEPTIBILITY TO VANCOMYCIN AND OUTCOMES

The reduced susceptibility (Vancomycin Intermediate *Staphylococcus aureus*-VISA) and resistance to vancomycin (Vancomycin Resistant *Staphylococcus aureus*-VRSA) is

defined microbiologically based on minimum inhibitory concentration (MIC) to vancomycin. The Clinical and Laboratory Standards Institute (CLSI) revised MIC values for vancomycin susceptibility in January 2006 bringing down the cutoff for VISA from a MIC of 4 to an MIC of 2. This was largely based on a Working Group report which suggested poor outcomes in patients with an MIC of 4 (100). The CLSI breakpoints are given in Table 1 (101). Additionally, strains of *Staphylococcus aureus* are defined as hetero VISA (hVISA) if they are sensitive to vancomycin at a breakpoint of <=2, but show a higher MIC (4-8 ug/ml) when tested by other methods(101).

Unlike resistance to most of the other antibiotics such as penicillin and methicillin, resistance and a reduced susceptibility to vancomycin appeared late after the introduction of the antibiotic in 1956. In May 1996, the first report of MRSA with a reduced susceptibility was reported from Japan(102). Subsequently, many countries have reported cases of VISA(103-111). Frank resistance or VRSA is a rare event with scant reports worldwide(112-114).

The mechanism of resistance for VRSA is different from VISA. All the VRSA strains have shown the presence of van A gene which is commonly responsible for vancomycin resistance in enterococci. The van A gene is present on a plasmid and hence it is possible that *Staphylococcus aureus* might have acquired the gene through intraspecies transfer. Van A when present confers high level vancomycin resistance with MIC as high as 512 in strains.

VISA/hVISA appears to be an adaptive response of MRSA strains when subjected to prolonged exposure to vancomycin. In vitro data shows that phenotype of MRSA exposed to previous vancomycin is different from the vancomycin naïve MRSA. Isolates

with a prior exposure to vancomycin have higher MIC's and a decreased vancomycin killing at 24 hours(115).

Table 1 Breakpoints for vancomycin susceptibility/resistance*

Label	Vancomycin MIC breakpoint
Vancomycin susceptible *Staphylococcus aureus* (VSSA)	<=2
Vancomycin intermediate *Staphylococcus aureus* (VISA)	4-8
Vancomycin resistant *Staphylococcus aureus* (VRSA)	>=16
HVISA (Hetero VISA)	<=2 :on routine testing >=4: on testing using special diagnostic methods

*CLSI guidelines:Central Laboratory Standards Institute/NCCLS. Performance standards for antimicrobial susceptibility testing. Sixteenth Information Supplement M100-S16 ed: Wayne PA, CLSI; 2006

Unlike VRSA, no single gene can be attributed to the VISA phenotype. Although Agr genotype II is strongly associated with VISA, not all the VISA strains have this genotype and results are still preliminary in solely implicating the AGR operon and its malfunctioning as the cause of VISA(116). Instead the current understanding is that VISA seems to be a result of various genetic alterations that are inducible and thus can be switched on and off depending on the environment. The end result of these alterations is an altered cell wall physiology and structure. There is evidence of increased number of D-Ala D-Ala residues, which are false targets for vancomycin on the cell wall. Binding to these residues leads to a reduced diffusion coefficient and prevents vancomycin access to

the actual site of action. On the other hand, there also appears to be cell wall thickening due to reduced peptidoglycan crosslinking, reduced autolysis and lower cell wall turnover(117).

VISA/hVISA are becoming a concern as a result of the poor outcomes observed in patients with this phenotype. Even among VSSA (Vancomycin susceptible *Staphylococcus aureus*), there is mounting evidence of a poor outcome at higher MIC's (118-121). In a study conducted by Sakoulas and colleagues, patients with an MIC of <=0.5 have 56% efficacy with vancomycin in comparison to patients with vancomycin MIC 1-2, for which the noted efficacy was 9.5%(118). In another case control study involving patients undergoing hemodialysis, the outcome of patients with MRSA bacteremia with a vancomycin MIC >2 (n=17) was compared with a group of patients with MIC <=0.5 (n=33). The authors found that patients with a higher MIC had increased mortality (35% vs 24%) and increased hospitalization costs(119). Persistent bacteremia and endocarditis are also thought to be more commonly associated with hVISA/VISA(122-124).

Owing to the difficulties in detection and the heterogeneity in methodology of detection, the exact prevalence of VISA/hVISA worldwide is not exactly known. Worldwide rates vary anywhere between 0-50% (19, 103, 106, 122, 123, 125, 126). In one such study determining the prevalence in Asia, 1357 MRSA isolates from 12 Asian countries including 87 isolates from Singapore were tested for hVISA by the agar screen method containing 4mg/L of vancomycin(103). A positive growth was confirmed by population analysis which by and large is considered the best method for detection of hVISA (127). There were 58 (2.3%) hVISA in this cohort. The prevalence in different countries varied

from 0-8% and was noted be around 2.3% in Singapore. Using yet another method, the E Test Macromethod, heteroVISA was found to be increasingly in prevalence, with rates as low as 2.2% from 1986-1993 to 8% in 2003-2007 among isolates collected from a healthcare center in Detroit(126). Interestingly, in this study, almost 60% of the hVISA isolates were from the blood. Unlike hVISA, the rate of VISA was noted to be stable in the study and varied from 0.4-2.3%.

The problem of VISA is aggravated by the limited therapeutic options. One approach to treatment is to increase the dosage of vancomycin; however this is fraught by serious side effects. There is also new mounting evidence to suggest that the response of such patients does not necessarily improve with increasing the vancomycin dosage (124, 128). New drugs like linezolid, daptomycin, dalbavancin, oritavancin appear promising; however, emergences of resistance to these agents have already been documented.

1.8 INFECTIOUS DISEASE (ID) CONSULTATION ON OUTCOMES

Specialist consultation is known to improve the outcome of various illnesses (129, 130). However, it is not known if such an effect is seen with patients with *Staphylococcus aureus* bacteremia. Specialist ID consultation for SAB could help patients by recommending appropriate antibiotics, removing the source and detecting metastasis.

Few studies in the past have shown a beneficial effect of an ID consultation. In a study conducted by Fowler and colleagues(131) at the Duke Medical Center, ID recommendations were provided to 244 patients with *Staphylococcus aureus* bacteremia. Patients were followed for 12 weeks after their initial episode of bacteremia. At the end of 12 weeks, outcome parameters of death and recurrence were compared in two groups namely, patients for whom recommendations were followed (n=112) versus patients for

whom recommendations were not adhered to or partially followed (n=132). Patients for whom recommendations were followed were less likely to have relapses (6.3%) versus patients in the other group (18.2%). This was statistically significant. However, no difference in the mortality rates was observed for both groups.

In another recent study in 2005, patient's characteristics, outcomes and standard of care was compared for SAB cases before (n=127) and after the start of the policy of mandatory ID consultation (n=98)(132). They found that the number of ID consultations had increased from a prior 53% to 90% of all cases. Consultations occurred early in the course of infection. The standard of care was found to be better in the year following routine consultation. Echocardiography was more common (73 vs. 53%, p=0.01), more intravascular catheters were removed (89 vs. 73%, p=0.05) and appropriate antibiotics were more commonly instituted (92 vs. 67%, p=0.001). Recurrent bacteremia and overall mortality was lower after the policy change (10 vs. 4, 12 vs. 6, respectively). However, these results were not statistically significant.

1.9 ANTIBIOTIC THERAPY AND OUTCOMES

The choice and duration of antibiotics for *Staphylococcus aureus* bacteremia depends on the presence or absence of the following factors

- Whether the isolate is MRSA or not
- Whether there is a removable source of infection for example a central line
- Presence of complications like endocarditis
- Risk for developing complications or recurrence
- History of specific drug allergies

1.9.1 Drug of choice

The drug of choice for MSSA is penicillin (where the isolates are penicillin sensitive), oxacillin/nafcillin/flucloxacillin or cefazolin. The drug of choice for MRSA is vancomycin.

Vancomycin is not recommended as a definitive treatment for MSSA bacteremia as there is mounting evidence of its inefficacy as compared to nafcillin/oxacillin. In a prospective multicentre study conducted in six tertiary care hospitals in the US, nafcillin had fewer failure rates as compared to vancomycin for MSSA bacteremia (0 vs. 19%)(84). In another case control study, 27 cases of MSSA bacteremia receiving vancomycin were compared with 267 patients receiving nafcillin. The mortality rate of patients receiving vancomycin was higher as compared to the group receiving nafcillin (37% vs. 18%, OR 3.3, CI: 1.2-9.5, p value =0.02)(133). Few studies have also shown that a poor outcome in terms of mortality or delayed clearance is present even if an empirical vancomycin is switched later to a beta lactam agent (134, 135). In light of this information, vancomycin is recommended as the mainstay of treatment of MSSA bacteremia only in cases of serious allergies(136).

1.9.2 Dosage

Following are the recommended dosages for treating SAB(137)

Penicillin: 4 million units every four hours

Nafcillin or oxacillin (Cloxacillin is used in Singapore): 2gm every 4 hours

Cefazolin: 2gm every 8 hours

Vancomycin: 30mg/kg every 24 hours in two equally divided doses or renal adjusted doses.

Many clinicians prefer using such high doses so as to prevent metastatic seeding and recurrences. Also, there is evidence that a lower dosage may be associated with a poor outcome. In a evaluation of patients with SAB, Jensen et al found that a lower dosage of beta lactam (<4gm for cloxacillin) was associated with a higher mortality and recurrence rate (65)

1.9.3 Route of administration

The intravenous route is the preferred route of administration for MRSA or MSSA bacteremia(137). Compared to intravenous route, oral therapy has of limited bioavailability, poor compliance and gastrointestinal side effects, hence is not recommended for serious infection. Oral therapy is reserved for patients who refuse intravenous therapy or if the patient is keen on going home and cannot follow up in an outpatient set up where IV therapy can be provided.

1.9.4 Duration of therapy

For a long time, patients with SAB were routinely treated with a long course of antibiotics, presumably because SAB was believed to have a high rate of complications. However, later studies suggested a shorter course of antibiotics (10-14 days) to be suffice for a catheter related infection (83, 138). In 1998, investigators from the Duke University published their treatment recommendations(131). They believed that treatment depends on clinical findings, surveillance blood cultures and echocardiography. They recommended a longer duration of antibiotics for patients with metastatic seeding of bacteria, a deep source of infection such as a joint/prosthetic infection or infective endocarditis, a positive surveillance blood culture or a surveillance echocardiography suggestive of vegetations. For uncomplicated SAB, they recommended a 7-14 day

treatment. Although many centers have adopted such a policy for determining the duration, it is still not an absolute rule. At a recent Clinical consensus conference, 45% of the respondents (79/206) felt that uncomplicated SAB defined as catheter infections, with negative follow up blood cultures, negative TEE, absence of prosthetic device and lack of clinical evidence of metastatic infection could be treated with 7-14 days of antibiotics. Although many participants agreed on a 14 day treatment, they believed that such patients should be followed up regularly in order to prevent relapses(139). The duration of therapy is thus finally based on clinical judgment.

1.9.5 Optimal antibiotics and outcomes

The results of most studies which aim at looking at the role of optimal/non optimal antibiotic therapy on outcome measures such as recurrence or mortality are confusing mainly because of the lack of consensus on definitions for optimal antibiotics for SAB, differing mortality definitions used and the presence of serious underlying comorbidities . In general, MRSA bacteremic patients have a delay in treatment as compared to MSSA bacteremic patients (61, 71, and 88). However, only few studies find an association of this delay in treatment with mortality(71) , while others don't(61, 88, 140). Similarly, some studies show that optimal antibiotic is associated with lower mortality (9, 73, 141, 142), especially so with MRSA bacteremia, while other studies fail to find such a difference (31, 62, 89).

The effect of antibiotics on recurrence is even more unclear. Logically, it would seem that patients receiving a longer course of antibiotics would be less likely to relapse. However, two previous studies have failed to show such an association (54, 84). In one study, treatment for less than 14 days did not increase the relapse rate in patients having

short bacteremias (less than 3 days). Infact, in this study a 4-6 week antibiotic proved inadequate in many patients with delayed clearance of bacteremia receiving vancomycin therapy. In both studies factors such as failure to remove the source or endocarditis were associated with recurrences.

These findings do highlight the complexity of SAB, which cannot be tackled by antibiotic treatment alone. Indeed, management needs to addressed in a multipronged approach consisting of removal of the source of infection as early as possible, active search for metastasis and adequate management of such infections such as valve replacement for serious vegetations and effective control of underlying comorbidities. In view of such an approach, there would perhaps be a need for a combined care provided by the primary physician, Infectious disease physician and surgeons where needed.

1.10 REMOVAL OF SOURCE AND OUTCOMES

The role of the removal of source is highlighted by the fact that most line related infection couldn't be cured by antibiotic treatment alone. The presence of a catheter increases the chance of recurrence in such patients (85, 86). Indeed, in one study, catheter salvage was associated with a treatment failure in 68% of patients(143). In another retrospective study involving 238 patients, an eradicable source was associated with a lower mortality as compared to a noneradicable source. In this cohort, the mortality of MRSA and MSSA (11 vs. 13%) was similar in patients with eradicable focus of infection(144). Although the removal of the source is important to prevent recurrences, studies show that this might not prevent the development of complications (143, 145).

1.11 GENETIC ANALYSIS OF STAPHYLOCOCCUS AUREUS BACTEREMIA

1.11.1 Background

Typing methods are commonly used to define the epidemiology, determine clonality and assist in outbreak analysis of any infectious disease. A wide range of such methods is available for *Staphylococcus aureus* with genotypic methods gradually replacing the conventional phenotypic methods. Although we have a wide range of technological platforms to work with, no single method has all the ideal characteristics needed of a typing method. Hence the use of any method depends on the aim of typing, the properties of the method and logistics in terms of the cost and the technical expertise required.

In regards to MRSA typing, Pulse Field Gel Electrophoresis (PFGE) and Multilocus sequence typing/SCC Mec typing (MLST) are considered the reference methods against which newer genotypic methods are evaluated(146, 147). PFGE consists of whole chromosome analysis following restriction enzyme digestion with rare cutters such as Sma I. The resulting fragments are separated and analysed using a special form of electrophoresis. PFGE has a high discriminatory power and is currently considered the gold standard for many bacterial typing(148). Many prefer using it for an outbreak analysis as it has a good resolution for genetic microvariations(147). However, it is cumbersome, time consuming and does not allow interlaboratory comparison.

MLST is the analysis of mutations on the sequences of seven housekeeping genes of *Staphylococcus aureus*. MLST is increasingly becoming popular as this analysis enables intercountry and intercontinent comparison. Worldwide, an online website maintains the databases of all the MLST types, also called ST types (www.mlst.net). Although MLST has a lower discriminatory power than PFGE, the concordance rate between the two

methods is good. An international nomenclature using MLST typing in combination with SCC Mec typing has been widely used to establish the circulating clones worldwide. (149) SCC mec is a mobile genetic element found in all strains of MRSA. Currently seven types I-VII have been described in different strains and this is determined by molecular methods (146).

In the last decade, Spa Typing has also found wide acceptance because of its ease of performance, faster results and good interlaboratory comparison. Spa typing is the sequence analysis of the Spa gene, which codes for Protein A, a well-known surface protein of *Staphylococcus aureus.* . Strain differences in Spa gene is the result of a polymorphic X region which consists of a 24 base pair repeat unit, the number and the position of which varies in different strains of MRSA, largely due to deletion, duplication or point mutation. (150). Spa typing has a discriminatory power between that of PFGE and MLST. Hence, many believe that this typing can discern both micro and macrovariation and be used for both sudden outbreak analysis and establishing clonality. Spa typing, PFGE and MLST showed 100% typebilility in various studies and a good concordance was obtained for all three methods (147, 151-154). The discriminatory power (defined as the probability that a typing system will assign the same strain type to strains randomly selected from the same group) of Spa typing and PFGE was above 90% in most studies (147, 151, 153, 154)

1.11.2 Genotyping of MRSA and Staphylococcus aureus bacteremia

Genotyping of large worldwide collections of MRSA have revealed that MRSA strains are highly clonal(146). Table 2 shows the various clones circulating worldwide and their

Spa and MLST/SCC mec types. In a PFGE analysis of 864 isolates, 82% of which were from blood, two clones namely, USA 100 and USA 300 contributed to 87% of all MRSA invasive episodes (8). Many other countries have also been able to attribute majority of their MRSA isolates to one or two circulating clones (18, 22, 155-157). Interestingly, many countries have also witnessed a shift in the circulating clones of MRSA in the last decade or so. In the US, unlike a decade age, almost 38-49% of all healthcare infections seem to be due to community MRSA clone USA 300(8, 155, 158). This clone, typically sensitive to antibiotics including clindamycin and ciprofloxacin has been gradually replacing USA 100, a multidrug resistant MRSA clone. In Singapore, MRSA sensitive to cotrimoxazole and gentamicin was first documented in 1997 but increased dramatically in numbers in 2003. This clone of MRSA was identified by MLST as the E-MRSA 15 clone, a genotype commonly found in European countries (ST22-IV)(156). In 2004, E-MRSA 15 constituted 18% of all MRSA isolates. Similar shifts in epidemiology has also been documented in Taiwan, the prevalent ST239 being displaced with ST5(22).

Table 2 Overview of the major clones of healthcare acquired methicillin-resistant Staphylococcus aureus (MRSA)

(Adapted from**:** Deurenberg R, Vink C, Kalenic S, Friedrich A, Bruggeman C, Stobberingh E. The molecular evolution of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect. 2007 Mar;13(3):222-35)

Clone	MLST ST type	SCCmec type	Spa Type
Archaic	250	I	t008, t009, t194
Southern Germany	228	I	t001, t023, t041, t188, t201
UK EMRSA-3	5	I	t001, t002, t003, t010, t045, t053 , t062, t105, t178, t179, t187, t214, t311, t319, t389, t443
Iberian	247	I	t008, t051, t052, t054, t200
Irish-1	8	II	t008, t024, t064, t190, t206, t211
New York/Japan	5	II	t001, t002, t003, t010, t045, t053 t062, t105, t178, t179 ,t187, t214, t311, t319, t389, t443
UK EMRSA-16	36	II	T018, t253, t418, t419
Brazilian/Hungarian	239	III	T030, t037, t234, t387, t388
Berlin	45	IV	t004, t015, t026, t031, t038, t050 t065, t204, t230, t390
Paediatric	5	IV	t001, t002, t003, t010, t045, t053, t062, t105, t178, t179, t187 t214 t311, t319, t389, , t443
UK EMRSA-2/-6	8	IV	t008, t024, t064, t190, t206, t211
UK EMRSA-15	22	IV	t005, t022, t032, t223, t309, t310, t417, t420

Various studies have also have tried to ascertain if specific genotypes are associated with invasive infections including bacteremias. Results are conflicting. A study conducted on an oxforshire collection of MRSA isolates did not find any such association (159). However, in a recent study conducted at the Duke Medical Center MLST clonal complexes CC5 and CC30 and spa types 2 and 16 were more represented in the bacteremic group(160). Another group of investigators have reported a higher prevalence of enterotoxins in patients with an invasive disease (160-162). With the increasing use of typing methods especially technologies such as microarrays which are able to look at the expression of large number of genes, more associations can be studied and are likely to reported in the future.

1.12 AIMS OF THE STUDY

This study was conducted with the main aim to define the epidemiology and outcomes of *Staphylococcus aureus* bacteremia. Following were the specific aims of the study

1. To define the epidemiology of *Staphylococus aureus* bacteremia
2. To define the rates and predictors of adverse outcomes such as mortality, metastatic infections and a high vancomycin MIC
3. To ascertain if an Infectious Disease consultation (ID) is associated with a better outcome in patients with *Staphylococcus aureus* bacteremia
4. To ascertain the molecular types of MRSA and MSSA using the Staphylococcal Protein A (Spa) Typing and to look for epidemiological associations for major clones.

CHAPTER 2

MATERIALS AND METHODS

2.1 EPIDEMIOLOGICAL METHODS

Data was collected prospectively from patients hospitalized in National University Hospital- a 900-bed acute care tertiary care hospital in Singapore, from April 12 2007 to October 30, 2008. Cases notified after October 12 2007 also formed a part of a clinical trial.

2.1.1 Subjects for inclusion into the study

Patients were included in the study if one or more blood culture grew *Staphylococcus aureus*. Positive blood cultures were notified to the investigator by the hospital microbiology laboratory. Patients were excluded if the bacteremia was polymicrobial or age of the patient was less than a year. Each patient was included only once in the dataset. Any recurrence of bacteremia during the study period from a patient who was already recruited was not included again. All patients who met the criteria during the duration mentioned above formed a part of the case series analysis of *Staphylococcus aureus* bacteremia.

2.1.2 Subjects for inclusion in the clinical trial

A Randomized controlled trial was started on October 12 2007. The Hospital's Institutional Review Board had given approval for the study and the details of the trial are available on www.clinicaltrials.gov (NCT00622882). The objective of the trial was to ascertain if an early infectious disease consultation (within 72 hours of notification of bacteremia) improved outcomes of patients of *Staphylococcus aureus* bacteremia. For this trial, consecutive subjects of *Staphylococcus aureus* bacteremia (as notified from the laboratory) were recruited if they met the specific inclusion criteria as shown below.

Inclusion criteria

- Patient with a one or more blood culture positive for *Staphylococcus aureus*
- Age more than 1 year
- Patient admitted in hospital

Exclusion criteria

- Recurrent bacteremia in a patient already recruited in the trial
- Polymicrobial infection
- Age less than one year
- Patient not admitted in the hospital

2.1.3 Trial workflow

Figure 1 shows the workflow of the trial. *Staphylococcus aureus* bacteremia cases were notified to the single investigator (myself) by the laboratory. If the subject met the above criteria, they were randomised using a stratified block randomisation (Stratified by age cutoff of 65). Stratification was performed as various studies show that an older age group is associated with a higher mortality in patients with SAB. Subjects were randomised to one of the two arms as mentioned below

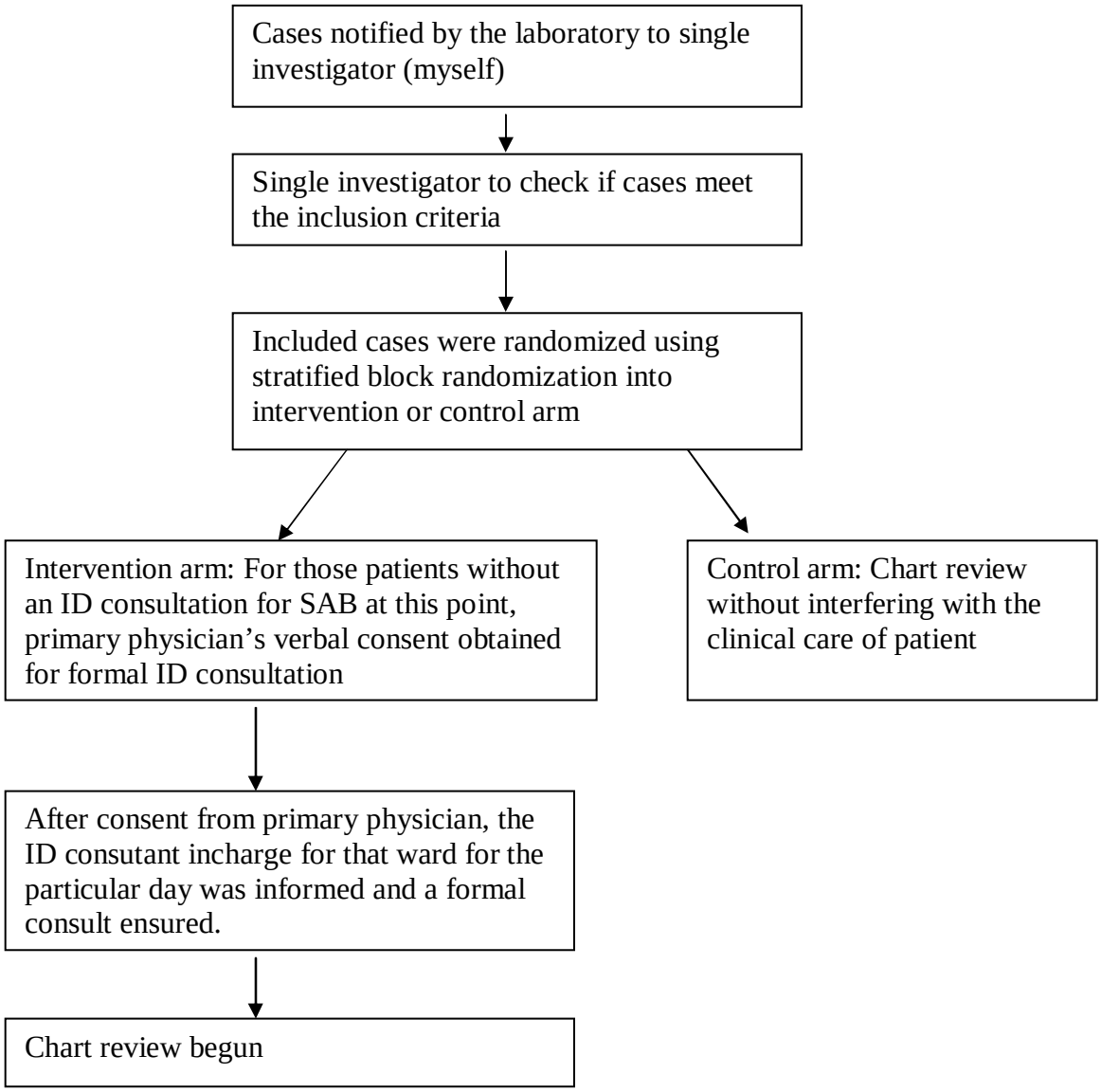
- Intervention arm: Subjects in this arm would receive an early ID consultation subject to physician's consent
- Control arm: The clinical management in this arm would not be altered by an ID consultation, unless requested by the physician in care.

We had obtained a consent waiver and hence before approaching the patient, only the primary physician’s consent was obtained. The ID consultation involved a thorough history taking, examination and recommendation by a consultant. Phone consults were

not considered an ID consult. Before starting the trial, the ID consultants agreed upon a set management protocol. However, the final management of the patient was on the treating physician's discretion, which would decide whether the ID recommendations need to be followed or not.

All cases recruited in this trial formed the dataset for the trial analysis and also the case series analysis. In addition, patients excluded from the trial analyses, which however, met the inclusion criteria for case series analysis were included.

Figure 1 Trial workflow



2.1.4 Chart review

A single investigator (myself) reviewed the medical records and charts of the patient (from the case notes and the patient information system). The data was collected at the time of notification and then regularly till the discharge of the patient on a pocket PC (HP iPAQ rx 1950 series) with a database software namely, HanDBase® for Pocket PC Professional 3.51 Build 1. (Figure 2). The data was then synchronised from the pocket PC to a base computer and the data was exported and stored in an excel chart. Microsoft access was used to merge relevant databases.

The relevant data included patient demographics, comorbidities, mode of acquisition, the source of bacteremia, vitals and comorbidity score (APACHE II and Charlson index), antibiogram of *Staphylococcus aureus*, MIC of every *Staphylococcus aureus* isolated from blood of each patient, relevant investigations, antibiotic details including the type, dose, route of administration and duration and the ID consultation details in particular the date, the recommendations and the adherence to recommendations. Certain data such as the hospital discharges and deaths in a year were collected from the Medical information systems available on the hospital intranet available to all staff.

2.1.5 Outcome measures

For the cohort and trial assessment, the primary outcome measure was mortality. The secondary outcomes were presence of metastatic infection, recurrence and 1-year mortality rate (based on review of patient information system). In addition for the trial, the standard of care received by patients for SAB was assessed by the following parameters

- Appropriateness of antibiotics
- Follow up blood cultures
- Echocardiography following SAB episode
- Relevant radiological examination

The relevant case definitions are given in the Table 3.

Figure 2 HP POCKET PC with a sample of the HanDBase® database

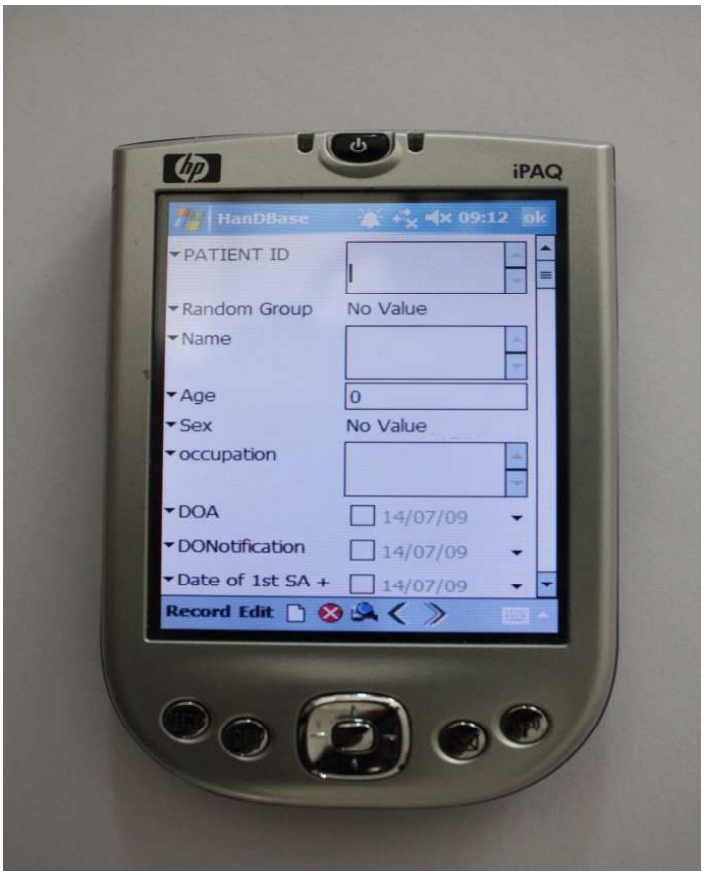


Table 3 CASE DEFINTIONS

Profile	Relevant case definitions
Mode of acquisition	**Nosocomial bacteremia** was defined if the positive blood culture was withdrawn 48 hours after hospitalization **Community acquired bacteremia** was defined as a positive blood culture withdrawn within 48 hours of hospitalization in a patient with no previous healthcare risk factors as defined below **Healthcare associated bacteremia** was defined as a positive blood culture withdrawn within 48 hours of hospitalization in a patient with the following healthcare risk factors; intravenous home therapy, hemodialysis, previous hospitalization in the last one year, nursing home resident, previous MRSA isolated in the last one year
Primary source of bacteremia	The source was determined by the presence of signs and symptoms of inflammation or isolation of *Staphylococcus aureus* at the site prior to the episode of bacteremia. When the source could not be determined, the bacteremia was considered as an unknown source or primary bacteremia
APACHE II	Relevant parameters collected on the date of withdrawal of blood culture (91)
Charlson comorbidity score	Relevant parameters collected on the date of withdrawal of blood culture (64)
Antibiogram	An isolate was labeled as MRSA by standard microbiological techniques ie VITEK.MIC to Vancomycin and other antibiotics was determined by VITEK
Investigations	TWBC and CRP data (if available) within 24 hours of time of withdrawl of blood culture was collected from the electronic medical information systems for the patient. Radiological and echocardiographic investigations was collected from the day of onset of bacteremia to the discharge or death of the patient
Specialist consultation details	Note was taken from the date of notification to the date of ID consultation. Adherence to recommendations was considered if the antibiotics and the removal of source recommendations were adhered
Antibiotic appropriateness	**Empiric antibiotic** was considered appropriate if the organism isolated was sensitive to it. **Appropriate definitive antibiotics** was defined as follows 1. IV cloxacillin (8gm/day) and iv cefazolin (6gm /day) were considered appropriate antibiotics for MSSA bacteremia 2.Vancomycin (2gm/day or trough adjusted dose) was considered appropriate for MSSA only if the patient was allergic to penicillin 3. Oral therapy with cloxacillin or quinolone/rifampicin combination was considered appropriate for MSSA if the patient was unable to take

	IV or was keen to go home 4. Vancomycin (2gm/day or trough adjusted dose) or oral/iv linezolid 600mg twice a day was considered appropriate for MRSA 5. A total duration of 10 days for uncomplicated infection and 4 weeks for complicated infection including endocarditis, persistent bacteremia, visceral or bony metastatic infections was considered appropriate
Primary outcome	In-hospital mortality was defined as death during hospital stay. Patients who left against medical advice were not included in analysis**.**
Secondary outcome	An extravascular site was considered a **metastatic** site if there were signs of inflammation /vegetation or isolation of *Staphylococcus aureus* during or after the development of bacteremia. This included endocarditis, bony sites for e.g., septic arthritis, vertebral osteomyelitis, discitiis, visceral sites such as the spleen, lung, brain and kidneys **Recurrence** was defined as a positive blood culture after resolution of signs of symptoms of bacteremia or after the receipt of appropriate antibiotics or after the documentation of negative blood cultures

2.1.6 Statistical analysis

Statistical tests were mainly used for

- Predicting the risk factors for mortality (death versus discharge)
- Predicting risk factors for metastatic infections (metastatic versus non metastatic infections)
- Predicting the risk factors for mortality in MRSA bacteremia (MRSA death versus MRSA survivors)
- Predicting risk factors and outcomes of a higher vancomycin MIC (Vancomycin MIC >2 vs. less than 2)
- Predicting factors responsible for not getting a Transthoracic echocardiography (those who got a TTE versus those who didn't)
- Comparing the baseline characteristics, standard of care and outcome in the randomized trial (intention to treat analysis, perprotocol and pretreatment, explained later in the results)

- Comparing the baseline characteristics and outcomes of different molecular clones of MRSA (Spa type t037 and t032, explained later in results)

For all categorical dependent variables, univariate categorical variables was analysed using the Chi-square test or the Fisher's exact test where needed and continuous variables were analysed by t-test or Wilcoxon rank sum test. Variables with a p value less than 0.05 on univariate analysis were tested with multivariate analysis where needed in a stepwise estimation using multiple logistic regression. For all statistical analysis, significance was assumed at an alpha level of 0.05. Analysis was performed using STATA version 10 (StataCorp, Texas, USA)

2.1.7 Microbiological methods

All blood cultures were processed with a commercial blood culture system (BacT/Alert 3D, Biomerieux, Durham, NC). Positive signals were then subcultured and reviewed after incubation for growth. If a colony was suspected to be Staphylococcus, it was speciated and confirmed as *Staphylococcus aureus* on the basis of a positive latex staphaurex agglutination and tube coagulase test. Every *Staphylococcus aureus* blood culture isolate was subjected to an antimicrobial susceptibility test using the VITEK method (which also records the MIC value for each antibiotic). The MIC values to serial *Staphylococcus aureus* isolates from each patient was recorded from the medical information systems.

2.2 LABORATORY METHODS

A subset of strains isolated from April 12, 2008 to 26 May 2008 was subjected to Staphylococcal Protein A (Spa) typing method as described below.

Strains of *Staphylococcus aureus* were procured from the Hospital Microbiology laboratory and freezed at -80^{o}C in glycerol BHI broths till further used. The steps of Spa (Staphylococcal Protein A) typing are elaborated below

2.2.1 DNA extraction

DNA was extracted from strains using the QIAGEN DNAeasy kit(Hilden, Germany). Briefly, a 1 ul loopful of growth was suspended in lysis buffer containing lysozyme and lysostaphin. After incubating at 37^{o}C for 30 min, the lysed solution was treated with Proteinase K and ethanol before applying it to a column. The sample was then eluted after undergoing repeated washing steps in the column. More details on the methodology is available in Appendix 1

2.2.2 Primer preparation

The forward and the reverse primer used for spa typing is given below(150).

Table 4 Primers used for PCR

Primer	Reference	Primer sequence
Forward Primer	**1095 F**	**5'-AGACGATCCTTCGGTGAGC-3'**
Reverse Primer	**1517 R**	**5'-GCTTTTGCAATGTCATTTACTG- 3'**

These primers were procured from a Commercial company (1st BASE, Singapore) in a lyophilised form and stored at -20°C till further use. 100 µM stock solution of the primer was made according to the data sheet provided by the company supplying the primers. The primers were finally diluted and aliquoted as 10uM solutions for downstream PCR reactions.

2.2.3 PCR Protocol

PCR reagents were procured form QIAGEN (Taq PCR core kit, Hilden, Germany). The reaction master mix was set up in the proportions as given in the QIAGEN manual and are mentioned in Table 5. 1 µl of DNA was added to each of the reactions tubes. The cycling conditions as adopted by Shopsin and colleagues were used and are mentioned in Table 6(150) .

Table 5 Master mix for PCR reaction

Reagents for Master Mix	Volume per reaction, l (1X)
Water	31.75
10X PCR Buffer	5
Q solution	10
DNTP's	1
Forward Primer	1
Reverse Primer	1
Taq polymerase	0.25

Table 6 Thermocycling conditions used for the PCR reaction

No. of Cycles	Steps	Temperature (°C)	Duration
1X	Initial Denaturation	95	10min
30X	Denaturation	95	45s
	*Annealing	52	30s
	Extension	72	45s
1X	Final Extension	72	10min

The PCR products were resolved on a 1.5% agarose gel incorporated with ethidium bromide. 5ul of the PCR products were loaded on the gel and products were resolved at 120V for 40 min.

A negative control in the form of sterile water was included in each PCR run to ensure no contamination.

2.2.4 Purification of PCR products

The PCR products were purified using QIAGEN PCR purification kit (Hilden, Germany) or the QIAGEN Gel extraction kit (Hilden, Germany) depending on the number of DNA bands visualised. When more than one band was seen per sample, an additional 20ul was loaded and resolved on the gel and each band was then cut off the gel and purified. More details are given in Appendix 2

2.2.5 DNA sequencing and analysis

The purified PCR products were sent to a commercial company for DNA sequencing (1st BASE, Singapore). The samples were coded and anonymous in order to maintain confidentiality. All the spa sequences were analysed using Bionumerics® Version 4.6. The variable region is analysed between two signature sequences (GCACCTAAA and TACATGTCGT as on the forward strand). Bionumerics® uses the Ridom nomenclature which assigns numbers to each unique repeat (r01, r02, r03….) and spa type (t01, t02, t03, t04….). A cluster analysis was generated using the Minimum Spanning Tree (MST). MST chooses the sample with the highest number of related samples as the root node and derives the relation of the other samples from this node.

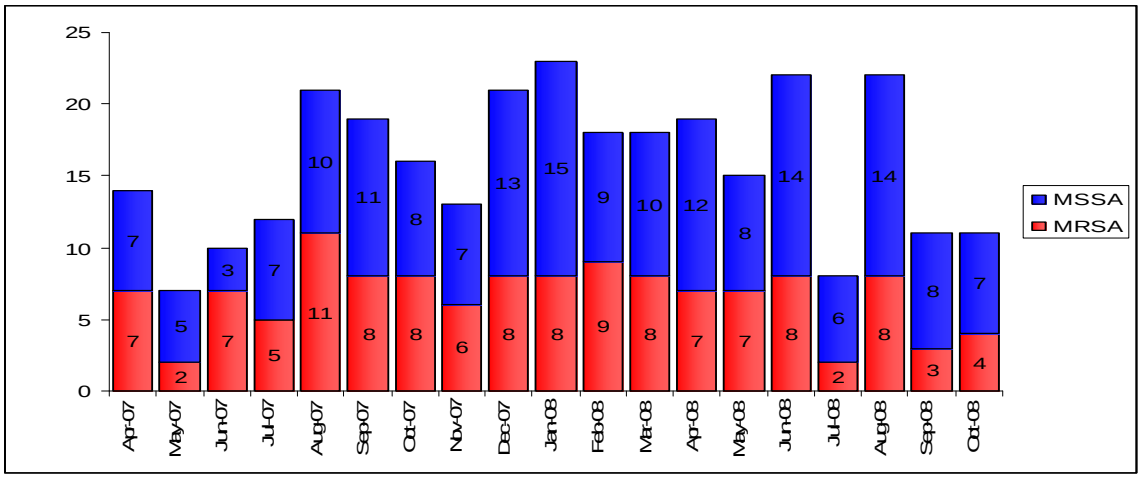
CHAPTER 3

RESULTS 1: EPIDEMIOLOGY AND OUTCOMES OF SAB AT A TERTIARY CARE CENTRE IN SINGAPORE

3.1 Overview

A total of 301 cases of SAB were notified by the laboratory from April 13, 2007 to October 30, 2008. These included 90 cases before the start of the trial on October 12, 2007 and 211 cases after the start of the trial. One case was duplicated hence, 300 cases (which included only the first episode of bacteremia for the duplicate case) were part of the case series analysis. The overall SAB rate was 3.42/1000 discharges and deaths. Of the 300 cases, 126 (42%) cases were attributed to MRSA and 174 (58%) to MSSA. The MRSA bacteremia rate was 1.44/1000 discharges and deaths. Figure 3 shows the monthwise distribution of all bacteremic cases. The highest numbers of cases were notified in January 2008 (n=24) and the lowest in May 2007 (n=7). There was no observable seasonal distribution of cases.

Figure 3: Monthly distribution of SAB cases



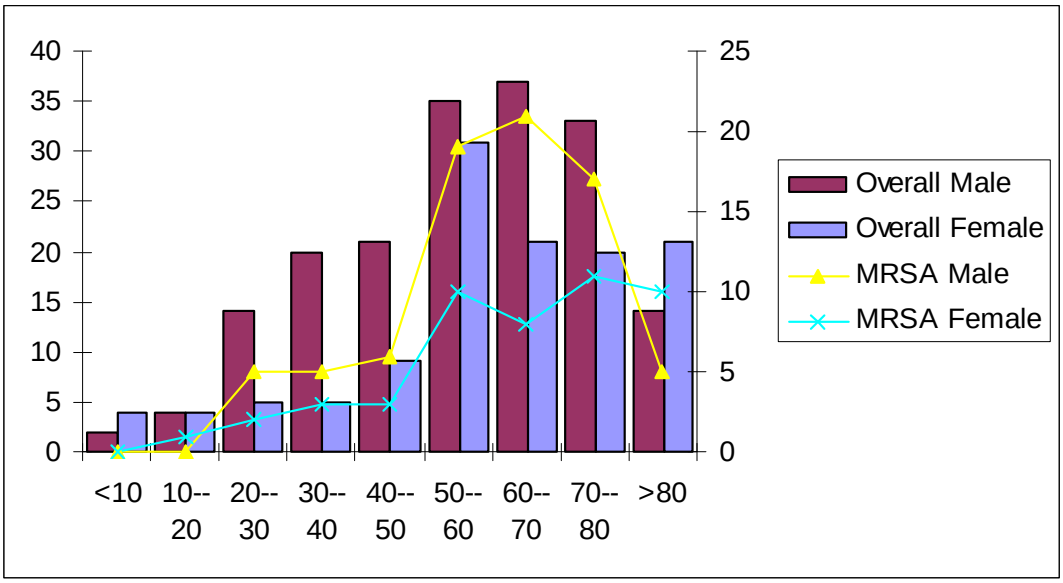
3.2 Age and sex distribution

The median age of patients was 58 (IQR: 21-73, range: 1-96) with 180 (60%) cases of SAB in males and 120 cases (40%) in females. Female patients and MRSA bacteremic

cases were older in comparison with males (median age: 61 vs. 56, p=0.03) and MSSA bacteremia (median: 64 vs. 54, p value**=0.004)** respectively**.**

Figure 4 shows the age distribution of SAB cases and MRSA cases in males and females. Overall, a male preponderance is observed in all age groups except the extreme age groups (more than 80 and less than 20). As seen in Table 7, there is an unequal distribution of cases across different specialities and this could possibly explain the male preponderance. The median age for MRSA bacteremic women was 68 (IQR: 55-78) vs. 62(IQR: 51-71) for males (p value: 0.07).

Figure 4: Age and sex distribution of Staphylococcus aureus bacteremia



3.3 Distribution in different disciplines

Table 7 shows the distribution of SAB in different disciplines. 234 (78%) of the cases were admitted in the medical wards at the time of bacteremia as compared to 66 (23%) in the surgical wards. Majority of the cases as expressed in numbers were admitted in Cardiothoracic vascular surgery, nephrology, oncology, general medicine, respiratory medicine and cardiology.

The proportion of cases caused by MRSA varied in the medical and surgical disciplines (35.8% vs. 63% p value<0.001, OR:0.3 CI_{95}:0.2-0.6} The rate of MRSA bacteremia/1000 discharges and death was highest in the orthopedics cases with 6.56 cases /1000 discharges/death.

Table 7 Distribution in different disciplines

Discipline	SAB Numbers	SAB rate/1000 discharges/death	MRSA Numbers	MRSA/1000 Discharges/death
Otorhinolaryngology	1	0.92	1	0.92
Plastic surgery	2	2.48	2	2.48
Urology	2	1.20	1	0.60
Geriatric medicine	3	6.98	0	0
Hepatobiliary medicine	3	2.34	3	2.34
Colorectal surgery	3	1.21	3	1.20
Trauma	3	3.43	3	3.43
Rheumatology	3	6.74	1	2.25
General surgery	5	1.87	4	1.50
Neurosurgery	5	2.23	3	1.34
Spinal surgery	5	3.58	2	1.43
Infectious Diseases	6	27.65	0	0
Endocrine medicine	8	6.48	3	2.43
Gastroenterology	8	2.94	4	1.46
Pediatrics	11	1.78	0	0
Cardiothoracic vascular surgery	17	7.29	13	5.57
Orthopaedics	21	13.79	10	6.56
Cardiology	25	2.97	9	1.07
Respiratory	27	6.81	11	2.77
Hematoloy/oncology	30	7.41	15	3.70
Nephrology	53	17.25	17	5.53
General medicine	57	6.79	21	2.50
Dermatology	1	83.3	0	0
Dental medicine	1	58.8	0	0
Total	300	3.42	126	1.44

3.4 Mode of acquisition

In our case series of 300 cases, 122 (41%) were healthcare associated, 113 (38%) nosocomial and 64(21%) community-acquired infections (Figure 5). The mode of acquisition of one case of MRSA bacteremia was not known. Among the healthcare

associated infections 50/122 (40.9%) were receiving hemodialysis and 78/122 (63.9%) patients had a hospitalization in the three months prior to bacteremia. The time from the date of admission to the onset of bacteremia for nosocomial infections ranged between 2-132 days (Figure 6). Almost 50% of the nosocomial SAB had the episode between 2-8 days of hospitalization.

IVDU was the risk factor in 27 of the 64 (42%) of the community acquired infections and all these strains were MSSA. An additional 18 of the 64 (28%) community acquired SAB had comorbidities in the form of diabetes (9), renal failure (5), structural heart disease (3), blood disorder (2) and history of orthopedic implants (1). No specific risk factor could be identified in 19 (30%) of the cases.

The proportion of cases attributable to different modes of transmission varied in the MRSA and MSSA cohort. Of the 125 MRSA and 174 MSSA cases, 81 (64%) vs. 32(18.3%) were nosocomial, 41(32.5%) vs. 81(46.5%) healthcare associated and 3 (2.3%) vs. 61 (35.2%) community acquired respectively. The difference in the proportions of nosocomial and community infections caused by MRSA and MSSA was statistically significant (MRSA vs. MSSA: for nosocomial 71 %vs. 28%, for community 4.6 %vs. 95% p value <0.001) (Figure 5).

Similar difference of proportions was seen among patients admitted in the medical and surgical wards. Surgical patients were more likely to have a nosocomial infection. In contrast, all three modes of transmission were equally represented among the patients admitted in medical specialties (p value<0.001, Figure 5).

Figure 5: Mode of acquisition of Staphylococcus aureus bacteremia

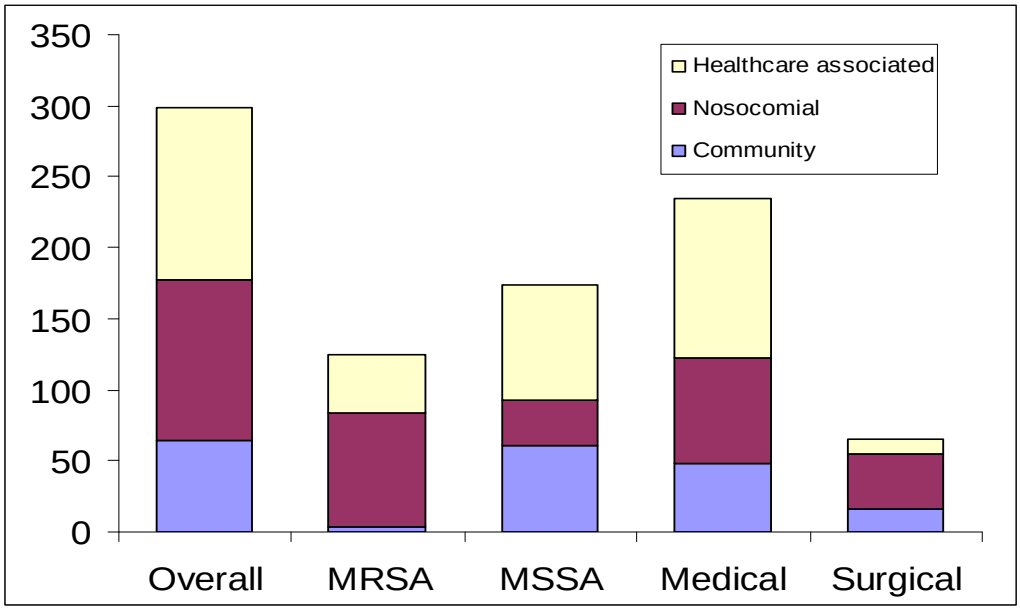
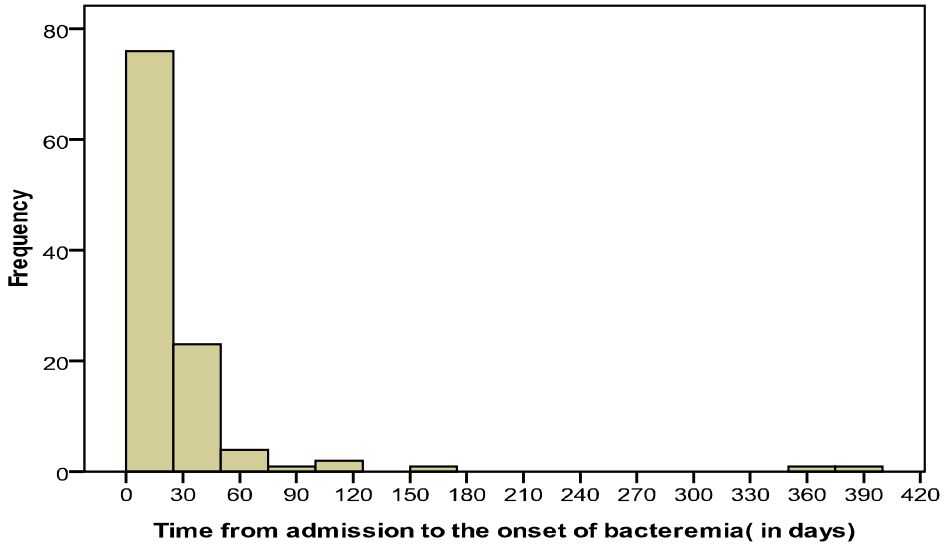


Figure 6: Timing of nosocomial infection



3.5 MRSA profile

Figure 7 and 8 below shows the antibiotic resistance of MRSA. Notably the resistance to ciprofloxacin and erythromycin exceeded 90% while the resistance to clindamycin, cotrimoxazole and gentamicin was between 60-70%. We do not have data on the dissociative resistance to erythromycin and clindamycin. No strain showed a frank

resistance to vancomycin when tested by the routine microbiological methods. However, 21% of the strains showed a MIC of 2 or 4.

Figure 7: Antimicrobial resistance profile of MRSA

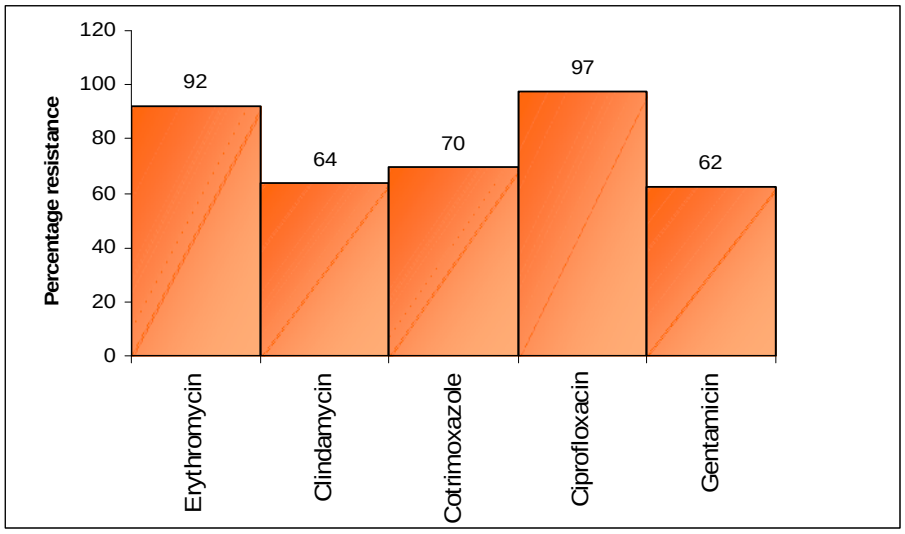
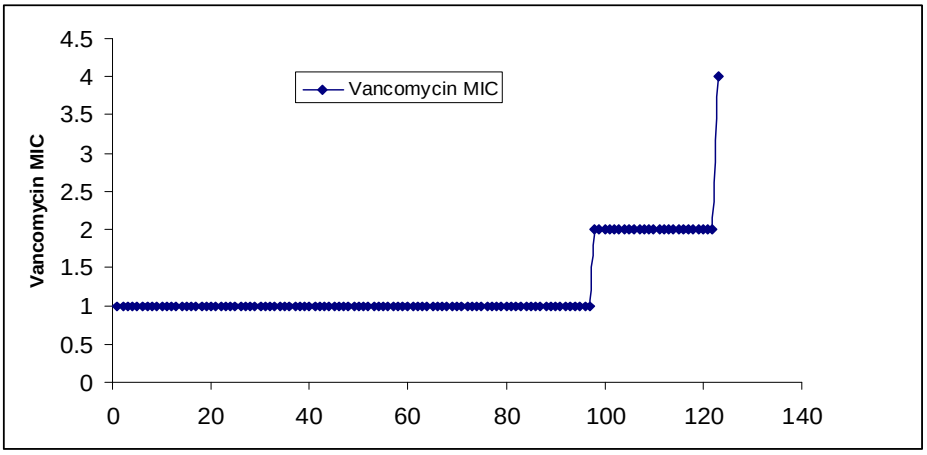


Figure 8: Vancomycin MIC distribution among MRSA (n=126)



3.6 Risk factors

Around 95% of the case series cohort had presence of one or more risk factors. These included previous surgery (83/27%), previous hospitalization (224, 74%), foreign implants (24, 8%), presence of long lines (83/27%), IV drug abuse (32/10%) and comorbidities in particular diabetes (129,43%), malignancy (49,16%), structural heart

disease (41, 13.6%), chronic liver disease (18, 6%), chronic renal disease (103, 34%), blood disorders (18, 6%), connective tissue disorder (123,4%), COPD (15,5%) and pneumonia (47,15%). Two or more risk factors were present in 81% of the cohort.

No identifiable comorbidity was present in 11.6% of the cohort. Among the rest, a single comorbidity was present in 36% (107), two comorbidities in 36%(109) and three or more comorbidities in 16% (49) of the cohort.

3.7 Portal of entry (Source of bacteremia)

The source of bacteremia was unknown or primary bacteremia in 36% (107/300) of the cases. The commonest observable source was a line source in 25% (75/300) of patients. The line infection represented a CVC line in 69 cases and a peripheral intravenous line in 6 cases. The other sources were superficial skin source in 19% (58/300) cases, deep tissue source including septic arthritis, mediastinitis and pyomyositis in 14% (43/300) and a lung source in 5%(14/300) cases. In two patients a combined source was suspected. This included pneumonia in both patients in addition to a sacral source and a CVP line infection.

3.8 Outcome at a glance

Of the 300 cases, the outcome of 10 patients was unknown as they were discharged against medical advice (n=10). Of the remaining 290 cases, 201 (69.3%) patients were discharged after an improvement in the condition, 85(29.3%) died during the hospital stay, 3 were transferred to another healthcare after two weeks stay in the hospital (15,17 and 18 days) and 1 patient was still in hospital. Among those discharged, 17 patients (17/201; 8.5%) subsequently died within a year of the bacteremic episode.

3.9 Mortality profile

The crude in-hospital mortality rate was 29.3%. The 7 -day, 30- day and 60- day mortality was 41/294 (13.9%), 74/293 (25.3%) and 85/291(29.2%) respectively. One year crude mortality rate could be evaluated at the end of 2008 for the cohort of patients presenting with bacteremia in the year 2007. The 1-year mortality rate in this cohort was 33 %(44/133).

Of the 85 in hospital deaths, 53/85 (62.4%) had MRSA bacteremias. Eighty seven percent (74/85) of the deaths were in patients with previous or ongoing healthcare exposure (also classified as nosocomial and healthcare associated infection). The time from the onset of bacteremia to death ranged from 1- 156 days. Approximately 20% of the deaths took place in the first 2 days, 40% in the first 4 days and 80% within 21 days of bacteremia (Figure 9).

For analyzing the predictors of in-hospital mortality, we compared 85 such patients with 201 patients who were discharged after the bacteremic episode. Table 8 and Figure 10 shows the results of this analysis. In univariate analysis, patients who died were older (median: 71 vs. 54, p value <0.01) and more ill as suggested by a higher Charlson (median: 6 vs. 4, p value <0.01) and higher APACHE II score (median 16 vs. 12, p value<0.01). Other predictors of mortality were MRSA infection (OR 3.34, CI_{95}: 1.97-66 p value <0.01), ICU stay (OR 2.4 CI_{95}:1.29-4.46, p value <0.01), chinese ethnic group (p value<0.05), nosocomial infection (p value<0.05), source such a lung (p value<0.05), malignancy (OR 2.28 CI_{95}: 1.2-4.36, p value=0.01), persistent bacteremia (OR 2.77 CI_{95}: 1.32-5.85, p value=0.01), history of widespread skin disease (OR 3.3 CI_{95}: 1.64-6.74 p=0.001) and pneumonia (OR 3.98 CI_{95}: 2.07-7.66, p value < 0.01).

For the multivariate analysis, all the above-mentioned significant factors were included except persistent bacteremia (as this data was not available for patients who died in the first 48 hours of bacteremia), ethnicity, IVDU and an ICU stay (as we limited the numbers of factors analysed to 8 or less owing to small numbers of outcome measures i.e. 85 deaths). Using the multiple logistic regression, the predictors of in-hospital mortality were age (p value<0.001), MRSA infection (p value=0.002), malignancy (p=0.002), history of skin disease (p value <0.001) and Apache score (p value =0.008). A skin and line source was associated with lower mortality when compared to unknown source as a reference (line source, p value=0.04, skin source, p value=0.022)

Figure 9: Time from onset of bacteremia to death

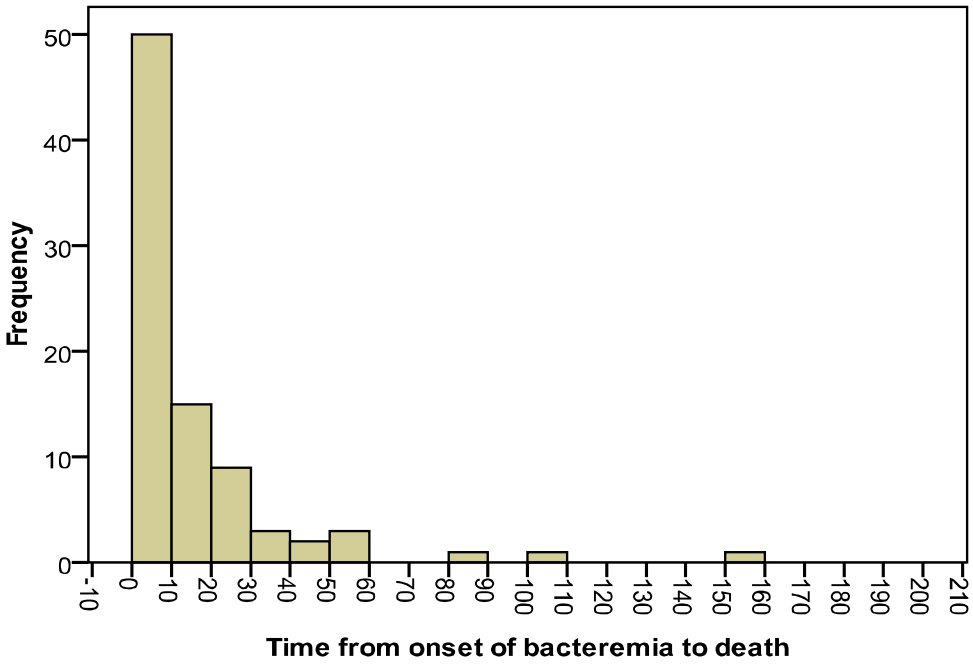


Figure 10: Age, APACHE Score, Charlson index for patients who died versus survivors

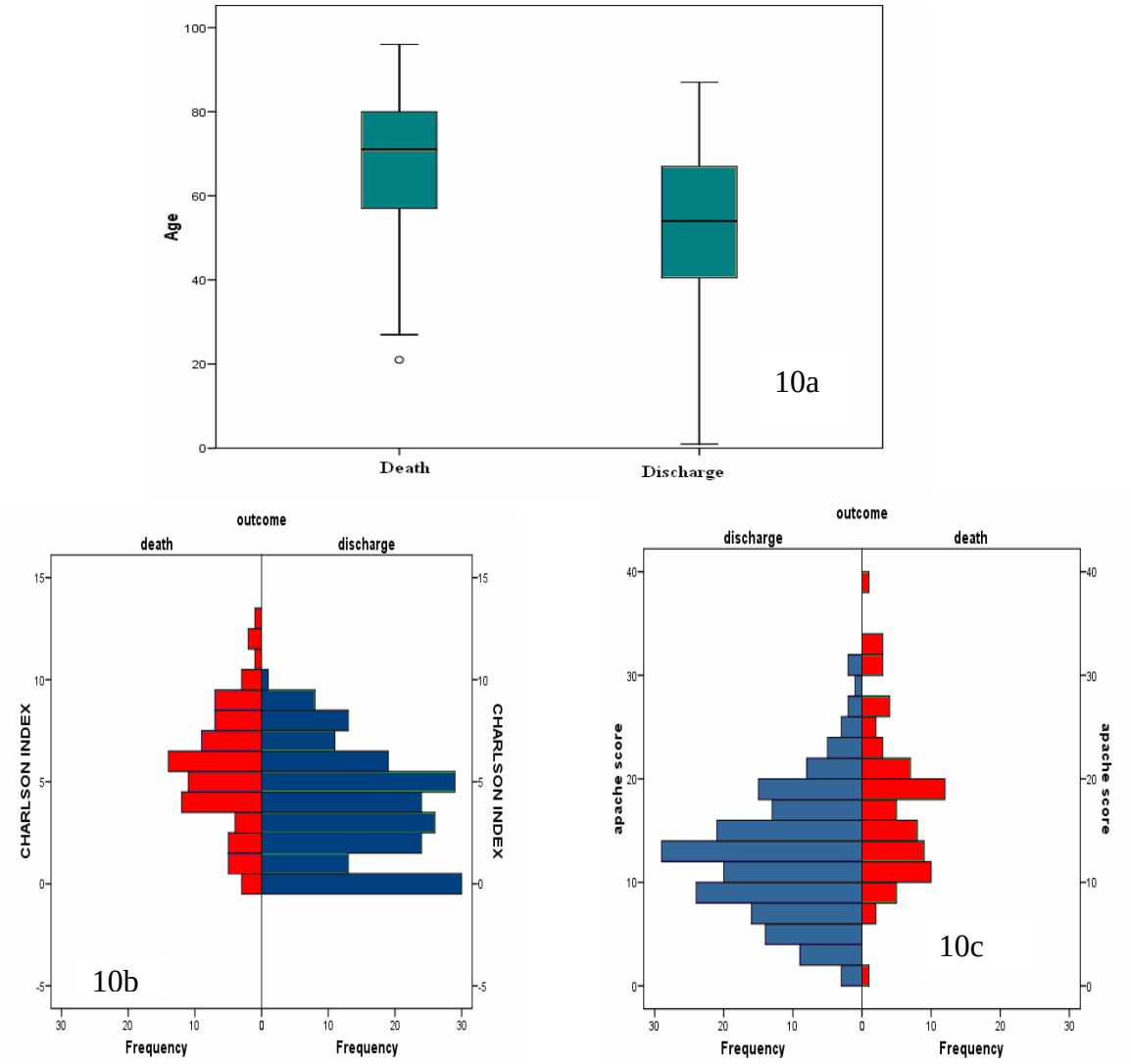


Figure 10a: Age distribution of patients who died vs. those who survived
Figure 10b: Charlson score of those who died vs. survived
Figure 10c: APACHE score of those who died vs. survived

Table 8 Analysis of predictors of all cause in hospital mortality

	Discharge (201) No. (%)	**Death (85) No.(%)**	**ODDS RATIO**	***P VALUE Uni*** [ß] ***(multi*** [γ] ***)***
Age	54(40-67)*	71(57-80)*	1.05(1.04-1.08)†	<0.001(<0.001)
Male	122(60.80)	49(57.65)	0.88(0.52-47)	0.62
MRSA	66(33.17)	53(62.35)	3.34(1.97-66)	**<0.001(=0.002)**
Chinese vs. Malay	114(56.28) vs48 (24.12)	63(74.12) vs. 14(16.47)	0.52(0.27-1.03)	0.06
Mode of acquisition				0.02‡
Community vs. Nosocomial	49(24.12) vs. 66(33.17)	11(12.94) vs. 42(49.41)	2.84(1.32-6.06)	0.007
Community vs. Healthcare associated	49(24.12) vs. 85(42.71)	11(12.94) vs. 32(37.65)	1.67(0.77-3.62)	0.188
Source of bacteremia				0.001‡
Unknown vs. Superficial skin	70(34.8) vs. 42(20.9)	34 (40) vs. 12(14.1)	0.58(0.27-1.25)	0.17(=0.022)
Unknown vs. Deep tissue source	70(34.8) vs. 30 (14.9)	34 (40) vs. 10(11.8)	0.68(0.30-1.56)	0.37
Unknown vs. Line source	70(34.8) vs. 55(26.4)	34 (40) vs. 17(20)	0.63(0.32-1.25)	0.19(=0.04)
Unknown vs. Other sources	70(34.8) vs. 4(2)	34 (40) vs. 12(14.1)	6.17(1.85-20.5)	0.003
IV drug abuse	27(13.07)	3(3.53)	0.24(0.07-0.83)	**0.02**
Diabetes	*86(43.2)*	*37(43.5)*	*1.03(0.61-1.71)*	**0.96**

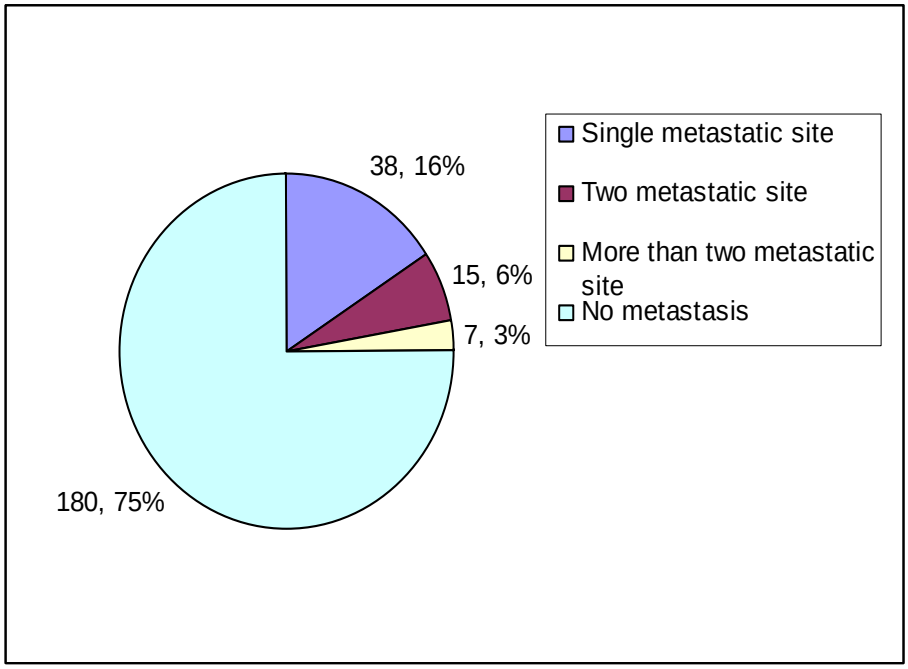
	Discharge (201) No. (%)	**Death (85) No.(%)**	**ODDS RATIO**	**P VALUE Uni ß(multi ɣ)**
Malignancy	25(12.56)	21(24.71)	2.28(1.20-4.36)	**0.01(=0.002)**
Liver disease	13(6.53)	5(5.88)	0.89(0.31-2.59)	0.84
Renal disease	71(35.68)	28(32.94)	0.89(0.52-1.52)	0.66
Pneumonia	20(9.95)	26(30.59)	3.98(2.07-7.66)	0.04
H/O skin disease	17(8.46)	20(23.53)	3.33(1.64-6.74)	0.001(<0.001)
Charlson index	4(2-6)	6(4-7.5)		<0.01
Apache Score	12(8-16)*	16(12-21)*	1.12(1.07-1.17)†	<0.01(0.008)
Persistent fever	37/179(20.67)	12/49(24.49)	1.24(0.59-2.62)	0.56
Persistent bacteremia	28/173(16.18)	15/43(34.88)	2.77(1.32-5.85)	**0.01**
Metastatic infections	42/181(23.2)	16/50(32)	1.55(0.78-3.09)	0.204
Appropriate empiric antibiotic	96/185(51.89)	28/68(41.18)	0.61(0.34-1.08)	0.13
Appropriate definitive antibiotic	114/179(63.96)	35/56(62.5)	0.91(0.48-1.74)	0.87
Surgical intervention following SAB	40(20.1)	11(12.9)	0.59(0.28-1.21)	0.150

*Denotes median and IQR for continuous variables, † Denotes the odd ratio for every unit change, for example, in this analysis a person 10 year older , would have an odds of 1.05^{10} times that of the younger, ‡ p value of global comparison using chi-square test ß :univariate p value , ɣ :multivariate p value

3.10 Metastatic infections

In patients with a length of stay (LOS) longer than 4 days (n=240), 61(25.4%) patients had one or more metastatic sites of infection. This included metastasis to a single site or multiple sites in 38 (16 %) and 23(9%) of patients respectively (Figure 11).

Figure 11: Metastatic infections in Staphylococcus aureus bacteremia (n=240)



Infective endocarditis was the commonest metastatic infection diagnosed in 35/ 240 patients (14.5%), followed by bony site (24/240,10%), visceral infection (23/240,9.5%) and soft tissue and muscle infection (n=9, 3.7%). The visceral metastatic sites included lung (14), kidney (7), Spleen (3), brain (3) and liver (2)(Figure 12).

Figure 13 shows the distribution of metastatic infections in MSSA and MRSA bacteremia. Of the 35 infective endocarditis cases, 28 were attributable to MSSA. While endocarditis and visceral metastatic infections were more common in MSSA(p value : 0.003 and 0.01 respectively) , bony metastatic infections were equally common in MSSA or MRSA bacteremia(p value =0.8).

Figure 12: Breakdown of the metastatic infection sites

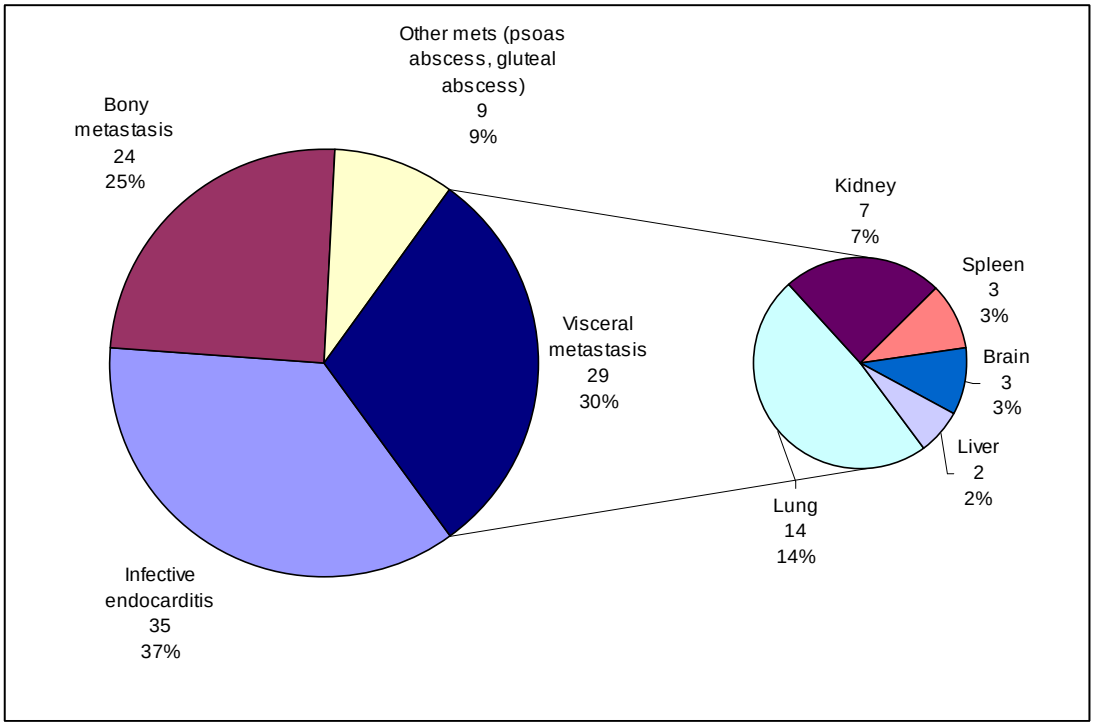
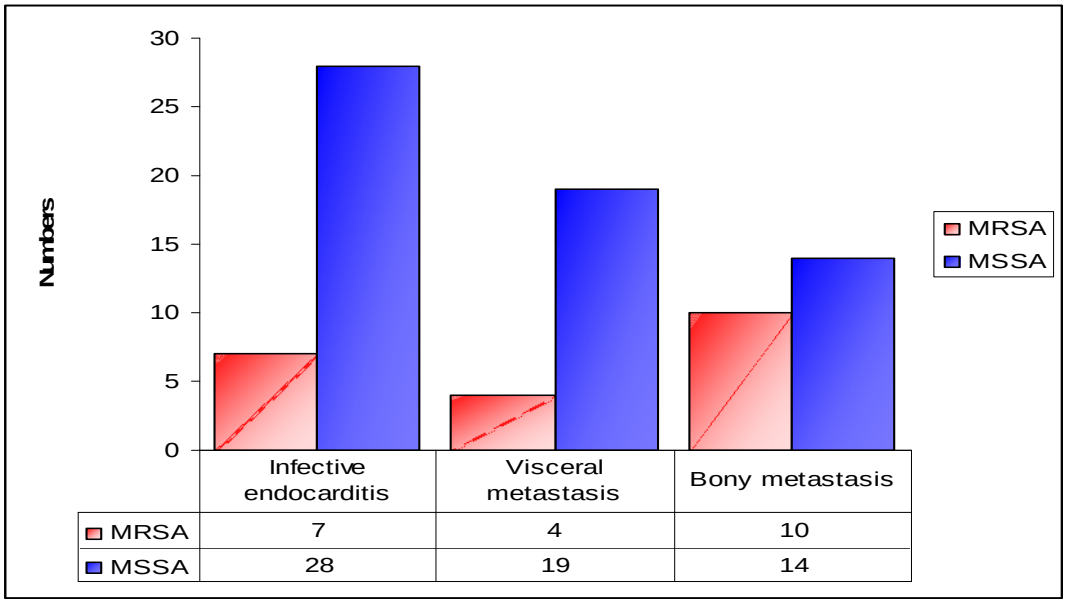


Figure 13: Metastatic infections in MRSA/MSSA bacteremia



The in hospital mortality was 20.5% among all patients with IE (7/35). This included 5 cases of MSSA IE and 2 of MRSA IE. TTE (Transthoracic echocardiography) detected

vegetations in 29 /33 IE patients who underwent this procedure. TEE (Transesophageal echocardiography) could detect vegetations (one on mitral valve and one on aortic) in 2 of the 4 TTE negative IE patients.

For analyzing the predictors of metastasis, 61 patients with metastasis were compared with 179 patients with non-metastatic bacteremia. (Table 9). On a Univariate analysis, younger age (Median: 47 vs. 60 p value=0.03), ethnicity (p value=0.017), MSSA infection (OR 2.55 CI_{95}:1.36- 4.81, p value=0.004), Community acquisition (p value: <0.001), IV drug abuse (OR: 37.12 CI_{95}: 12.21-112.83, p value: <0.001), Lower Charlson index (median 2 vs. 4, p value =0.001), unknown source, higher CRP (median 206 vs. 123 p value<0.001), persistent bacteremia (OR: 2.85 CI_{95}: 1.42-5.74 p value=0.003) and longer duration of symptoms before the detection of bacteremia (median 4 vs. 2, p value=0.022) were significant at PC 0.05. Renal disease (OR: 0.42 CI_{95}: 0.2-0.85, p value=0.01) and malignancies (OR=0.21CI_{95}: 0.06-0.69, p value=0.01) were less commonly associated with metastasis. For multiple logistic regression, all factors with a p value of less than 0.05 were included with the exception of CRP as the data was missing for many patients and age, duration of symptoms before presentation to hospital, renal disease and malignancy. The predictors of metastasis in this analysis were IVDU(<0.01) and persistent bacteremia(p=0.004).

3.11 Recurrence

Out of 201 discharges, recurrent bacteremia was encountered in 20 cases (9.9%). The time to recurrence varied from 16 to 460 days. In 5 cases, the recurrent strain was MRSA while the original bacteremia was due to MSSA.

Table 9 Analysis of predictors of metastatic infections

	Non Metastatic (179) No(%)	**Metastatic (61) No (%)**	**Odds ratio**	**P value Uni[ß] (Multi[ɣ])**
Age	60(48-72)*	47(36-66)*	0.98(0.96-0.99)†	**0.03**
Male	110(61.45)	39(63.93)	01.11(0.61-2.03)	0.73
MRSA	89(49.72)	17(27.87)	0.39(0.21-0.73)	**0.004**
Chinese vs. Malay	117(65.36) vs. 37(20.67)	31(50.82) vs. 17(27.87)	1.73(0.86-3.48)	0.122
Mode of acquisition				<0.001 ‡
Community vs. Nosocomial	26(14.61) vs. 79(44.38)	31(50.82) vs. 15(24.59)	0.159(0.074-0.34)	<0.01
Community vs. Healthcare associated	26(14.61) vs. 73(41.01)	31(50.82) vs. 15(24.59)	0.172(0.08-0.36)	<0.01
Source of bacteremia				0.001‡
Unknown vs. Superficial skin	45(25.14) vs. 45(25.14)	37(60.66) vs. 5(8.20)	0.13(0.05-0.37)	<0.001
Unknown vs. Deep tissue source	45(25.14) vs. 32 (17.88)	37(60.66) vs. 5(8.20)	0.19(0.067-0.53)	0.002
Unknown vs. Line source	45(25.14) vs. 50(27.93)	37(60.66) vs. 13(21.31)	0,31(0.15-0.67)	0.003
IVDU	4(2.23)	28(45.90)	37.12(12.21-112.83)	**<0.001(<0.001)**
Haemodialysis	40(22.35)	10(16.39)	0.68(0.32-1.46)	0.32
Malignancy	36(20.11)	3(4.92)	0.21(0.06-0.69)	**0.01**
Renal disease	62(34.64)	11(18.03)	0.42(0.20-0.85)	**0.01**
Liver disease	9(5.03)	7(11.48)	2.45(0.87-6.89)	0.08

	Non Metastatic (179) No(%)	**Metastatic (61) No (%)**	**Odds ratio**	**P value Uni ß (Multi ɣ)**
Apache score	12(8-17)*	11(7-14.5)*	0.95(0.91-1.00)†	0.10
Charlson index	4(2-6)*	2(0-5)*	0.82(0.73-0.92)†	**<0.001**
CRP	123(56-189)*	206(143-270)*	1.007(1.00-1.01)†	**<0.001**
Persistent fever	32/161(19.88)	16/571(28.07)	1.57(0.78-3.15)	0.20
Persistent bacteremia	23/151(15.23)	20/59(33.90)	2.85(1.42-5.74)	**=0.003(0.004)**
Duration of symptoms	2(1-4)*	4(2-7)*		0.02
In hospital Mortality	34/172(19.77)	16/57(28.07)	1.55(0.78-3.09)	0.19

*Denotes median and IQR for continuous variables, † Denotes the odd ratio for every unit change, for example, in this analysis a person 10 year younger, would have an odds of 0.98^{10} times that of the older patient, ‡ p value on a global comparison using chi square test, ß :univariate p value ɣ :multivariate significant p value

3.12 Outcomes and predictors of MRSA bacteremia

The outcomes of patients who died subsequent to the detection of MRSA bacteremia (n=53) were compared with those who survived (n=67) and were discharged after an improvement (Table 10). On a univariate analysis, mortality rate was higher in elderly age (OR: 1.04 $CI_{95:}$:1.02-1.07, p value <0.001), female sex (OR: 2.09 $CI_{95:}$:0.98-4.44 , p value=0.05), those with a higher APACHE II score (OR: 1.12 $CI_{95:}$:1.05-1.21, p value<0.001), higher Charlson index (OR: 1.26 $CI_{95:}$:1.09-1.45, p value<0.001) and having bony metastatic infection (OR: 4.99 $CI_{95:}$:1.20-20.69, p value =0.02). However, on a multivariate analysis, none of the above mentioned factors were significant.

3.13 Outcomes of higher vancomycin MIC among MRSA isolates

Out of 126 MRSA isolates, a vancomycin MIC of 2 or above was seen in 27(21.4% of all MRSA) isolates. Of these, only one isolate was a frank VISA with an MIC of 4. Twenty one isolates showed a rise in MIC from <1 to 2. A univariate analysis for outcomes and risk factors for a higher MIC (2 or above) (n= 27) as compared with an MIC of<=1 (n= 98) showed that patients with a higher vancomycin MIC were more likely to have a line source (OR: 8.90, p value: 0.009), deep tissue source (OR: 6.2, p value= 0.035), hemodialysed (OR: 3.25 $CI_{95:}$:1.25-8.46, p value=0.012) and more likely to encounter persistent bacteremia (OR 4.75 $CI_{95:}$:1.76-12.6, p =0.001), bony metastatic infections (OR: 6 $CI_{95:}$:1.53-23.4, p value: 0.03) and blood recurrences (OR: 5.34 $CI_{95:}$:1.32-21.5, p value=0.01). For analyzing the outcome further, bony metastatic infections, blood recurrences and persistent bacteremia were analysed by a multivariate logistic regression. In this model, only persistent bacteremia and bony metastasis were more likely in patients with a higher MIC (Table 11)

Table 10 Predictors of mortality of MRSA bacteremia

	MRSA SURVIVORS (n=67)	**MRSA DEATHS (n=53)**	**ODDS RATIO (CI_{95})**	**P VALUE Uni ß(multi γ)**
Age	61(50-70)*	68(57-79)*	1.04(1.02-1.07)†	<0.001
Male	47(70.1)	28(52.8)	0.48(0.22-1.01)	0.05
ICU stay	13(19.4)	16(30.2)	1.80(0.77-4.17)	0.17
Chinese vs. Malay	44(65.7) vs. 16(23.9)	40(75.5)vs. 9(17.0)	0.61(0.24-1.55)	0.31
Mode of acquisition				.48‡
Community vs. Nosocomial	2(3.0) vs. 39(59.1)	1(1.9) vs. 37(69.8)	1.89(0.17-21.8)	0.60
Community vs. Healthcare associated	2(3.0)vs. 25(37.9)	1(1.9) vs. 15(28.3)	1.2(0.1-14.3)	0.88
Source of bacteremia				0.25‡
Unknown vs. Superficial skin source	16(23.9) vs. 4(20.9)	15(28.3) vs. 8(15.1)	0.61(0.2-1.86)	0.38
Unknown vs. Deep tissue Source	16(23.9) vs14(20.9)	15(28.3) vs. 7(13.2)	0.53(0.16-1.68)	0.28
Unknown vs. Line source	16(23.9) vs20(29.9)	15(28.3) vs. 15(28.3)	0.8(0.3-2.1)	0.65
Unknown vs. Others	16(23.9) vs. 3(4.5)	15(28.3) vs. 8(15.1)	2.8(0.63-12.7)	0.17
Structural heart disease	12(17.9)	6(11.3)	0.59(0.20-1.68)	0.32

	MRSA SURVIVORS (n=67)	MRSA DEATHS (n=53)	ODDS RATIO (CI_{95})	P VALUE Uni ^{ß}(multi ^{γ})
Diabetes	28(41.8)	28(52.8)	1.56(0.76-3.22)	0.23
Hemodialysis	13(19.4)	12(22.6)	1.22(0.50-2.94)	0.66
Malignancy	12(17.9)	16(30.2)	1.98(0.84-4.67)	0.11
Lliver disease	3(4.5)	3(5.7)	1.28(0.25-6.62)	.77
Renal disease	20(29.9)	19(35.8)	1.31(0.61-2.83)	0.49
Pneumonia	9(13.4)	14(26.4)	2.31(0.91-5.87)	0.07
H/O skin disease	5(7.5)	8(15.1)	2.22(0.51-9.76)	0.18
Apache II score	12(8-15)*	16(12-21)*	1.12(1.05-1.21)†	<0.001
Charlson score	4(2-6)*	6(4-8)*	1.26(1.09-1.45)†	<0.001
Persistent fever	15(23.8)	8(25.0)	1.07(0.40-2.87)	0.90
Persistent bacteremia	16(26.2)	12(42.9)	2.11(0.82-5.41)	0.12
Metastatic infections	8(12.3)	8(22.2)	2.04(0.69-5.99)	0.19
Infective endocarditis	5(7.7)	2(5.6)	0.71(0.13-3.84)	.69

	MRSA SURVIVORS (n=67)	MRSA DEATHS (n=53)	ODDS RATIO (CI_{95})	P VALUE Uni ß(multi γ)
Visceral metastasis	2(3.1)	1(2.8)	0.90(0.08-10.28)	.93
Bony metastasis	3(4.6)	7(19.4)	4.99(1.20-20.69)	.02
Higher Vancomycin MIC (>2)	10(15.2)	15(28.3)	2.21(0.89-5.43)	0.08
Appropriate empiric antibiotic	29(48.3)	14(38.9)	0.77(0.34-1.72)	0.37
Appropriate definitive antibiotic	43(75.4)	20(64.5)	0.71(0.29-1.73)	0.28

*Denotes median and IQR for continuous variables, † Denotes the odd ratio for every unit change, for example, in this analysis a person 10 year older , would have an odds of 1.04^{10} times that of the younger, ‡ p value of global comparison using chi-square test ß :univariate p value , γ :multivariate p value

Table 11 Predictors and outcomes of a high vancomycin MIC value

	VANCOMYCIN MIC 2 OR ABOVE (n=27)	VANCOMYCIN MIC 1 OR BELOW (n=98)	ODDS RATIO	P VALUE Uni [ß](multi [γ])
Age	64(52-74)*	60(51-70)*	.99(.96-1.01)†	0.53
Male Sex	18(66.8)	60(61.2)	1.26(0.51-3.10)	0.61
ICU Stay	7(25.9)	22(22.45)	1.20(0.45-3.23)	0.71
Chinese vs Malay	19(70.37) vs 7(25.9)	69(70.4) vs 19(19.4)	1.33(0.49-3.6)	0.57
Healthcare associated infection	18(66.7)	63(64.5)		0.65[‡]
Nosocomial infection	9(33.5)	32(32.65)		0.65[‡]
Source of bacteremia				**0.009**[‡]
Unknown vs. Superficial skin source	2(7.4) vs. 3(11.1)	28(28.8) vs. 21(21.7)	2(0.3-13.06)	0.46
Unknown vs. Deep tissue source	2(7.4) vs. 7(25.9)	28(28.8) vs. 16(16.5)	6.12(1.13-33.1)	**0.035**
Unknown vs. Line infection	2(7.4) vs. 14(51.9)	28(28.8) vs. 22(22.7)	8.9(1.82-43.3)	**0.007**
Unknown vs Other source	2(7.4) vs. 1(3.7)	28(28.8) vs. 10(10.3)	1.4(0.12-17.2)	0.79
Diabetes	17(62.9)	42(43.3)	2.22(0.92-5.35)	0.07
Haemodialysis	10(37.04)	15(15.31)	3.25(1.25-8.46)	**0.012**

	VANCOMYCIN MIC 2 OR ABOVE (n=27)	VANCOMYCIN MIC 1 OR BELOW (n=98)	ODDS RATIO	P VALUE Uni ß(multi γ)
Malignancy	7(25.9)	23(23.7)	1.12(0.42-2.99)	0.812
Liver disease	1(3.7)	5(5.10)	0.71(0.08-6.39)	1
Renal disease	12(44.4)	28(28.57)	2(0.83-4.80)	0.117
Apache score	14(11-19)*	13(10-18)*	0.99(0.92-1.07)†	0.93
Charlson score	5.5(3-8)*	4(3-6)*	1.14(0.98-1.33)†	0.08
Persistent bacteremia	14(58.3)	16(28.6)	4.75(1.76-12.6)	**0.001(0.009)**
Persistent fever	7(30.4)	17(22.9)	1.46(0.51-4.15)	0.49
All metastatic site infection	7(28)	10(12.5)	2.72(0.90-8.14)	0.06
Infective endocarditis	2(8)	5(6.25)	1.30(0.23-7.17)	0.75
Visceral metastatic infection	1(4)	3(3.75)	1.06(0.10-10.7)	0.95
Bony metastatic infection	6(24)	4(5)	6(1.53-23.4)	**0.01(0.03)**
Overall in-hospital mortality	15(60)	38(40.5)	2.21(0.89-5.43)	0.08
Blood recurrences	5(18.5)	4(4.1)	5.34(1.32-21.5)	**0.01**

*Denotes median and IQR for continuous variables, † Denotes the odd ratio for every unit change, for example, in this analysis a person 10 year older , would have an odds of 1.05^{10} times that of the younger, ‡

3.14 Outcomes depending on the standard of care

The following standard of care was evaluated for our cohort of patients

- ID consultation
- Antibiotic appropriateness
- Follow up blood cultures
- Echocardiography following an episode of SAB
- Removal of source in particular line infection

3.14.1 ID Consultation details

Full details of whether an ID consultation took place before or after an episode of SAB was available for 264/300 (88%) cases. One hundred and eighty (180/264= 68%) patients had an ID consultation after the detection of SAB. Eighty four cases (84/264= 31.8%) never had an ID consult during the hospital stay after the detection of SAB.

An ID consult took place within the first 72 hours of notification in 163/180 cases (90.5%) and within 48 hours in 160/180 (88%) cases. The ID recommendations were adhered in 156/180 (87%) cases while the recommendations were not followed in 21/180 (11.6%) cases. The crude mortality was 23.7 % and 27.3% for patients who received and ID consultation within 72 hours and followed the recommendations versus those who did not receive an ID consultation respectively (OR: 0.82(0.45-1.51), p value: 0.63)

In order to understand the impact of an ID consultation, a randomized trial was undertaken, the interim results of which are elaborated in a later section.

3.14.2 Antibiotic details

Empirical antibiotic details were available in 263 cases. Appropriate empirical antibiotics were instituted in 48.6% of the cases. MRSA or MSSA bacteremia cases were equally likely to receive appropriate empirical antibiotics (44.4 vs. 51.6 %, p value =0.32). As per the criteria laid down, 63.6% of our patients received appropriate definitive antibiotics. MRSA bacteremia patients were more likely to receive appropriate definitive antibiotics than MSSA bacteremia (71.3 vs. 58.2%, p value=0.024). (Figure14)

Among patients not receiving appropriate empirical antibiotics (n=125), 12 patients did not receive any antibiotics at all (8%), 13 received oral antibiotics (10.4%) and the remaining patients received intravenous antibiotics such as ceftriaxone, augmentin, pipercillin/tazobactam, imipenem, meropenem, ciprofloxacin or ceftazidime.

In patients not receiving appropriate definitive antibiotics, 18 patients received an inappropriate class or route of administration, 41 received the correct class, however in an inadequate dosage and 29 patients received antibiotics for a duration less than that recommended for their bacteremia.

The outcome in terms of mortality was similar in patients who received appropriate empiric/definitive versus those who did not receive appropriate antibiotics. (Table 8)

3.14.3 Removal of line source

Data on the removal of lines was available from 54/61 line infection cases. The implicated line was removed in 49/54 cases

3.14.4 Echocardiography

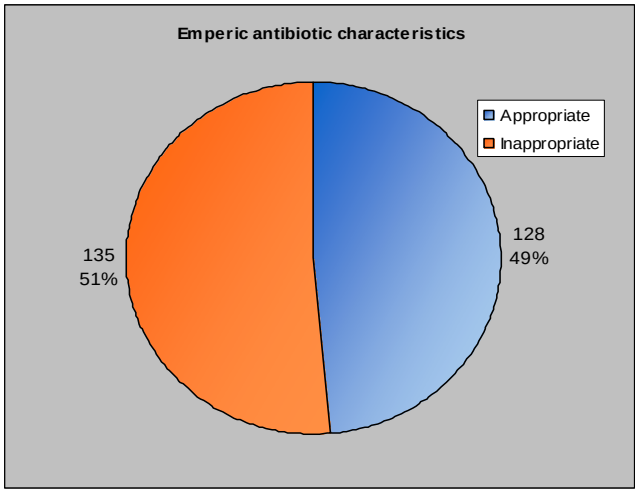
One hundred and eighty patients of SAB (60%) underwent an echocardiographic evaluation for presence of vegetations. All these patients had a Transthoracic echocardiography (TTE); however, 14 patients additionally (14/180) underwent a transesophageal echocardiography(TEE) later in the course of infection. In these patients, TEE was able to pick up 2 additional cases of endocarditis, which were negative by TTE.

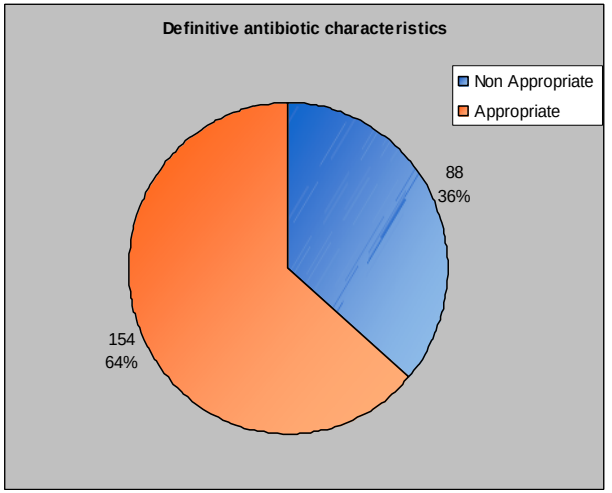
On a univariate analysis patients with nosocomial or healthcare associated infection (p=0.001), pneumonia (p=0.035), hemodialysis (p=0.003), malignancies (0.018), renal disease (p=0.016), shorter length of stay (mean 9.6 vs. 31.2, p =0.0002) and patients admitted under medical units (p=0.004) were less likely to have a follow up TTE. On a multivariate analysis, patients with a length of stay of less than 4 days ($p<0.01$) and nosocomial bacteremias (p value=0.025) were less likely to undergo a TTE.

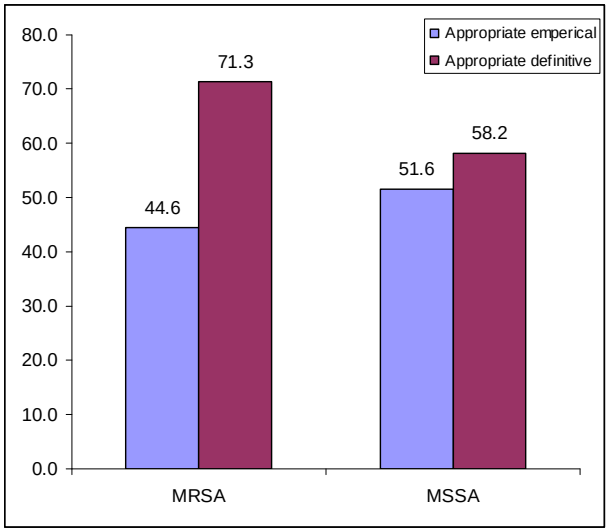
3.14.5 Follow up blood cultures

For those with an LOS >= 4 days, 68%(143/253) had a follow up blood culture 2-4 days following the bacteremia. Of the remaining 107 patients, 70 patients had a follow up blood culture early on (1 day after the bacteremia) or within 7 days of bacteremia or both.

Figure 14: Antibiotic characteristics







CHAPTER 4

RESULTS 2: A RANDOMISED CONTROL TRIAL OF ID CONSULT IN SAB

An IRB (Institution Review Board) approved trial recruiting patients with *Staphylococcus aureus* bacteremia began on October 12, 2007. The purpose of the trial was to determine if an ID consultation improved outcomes of patients with SAB.

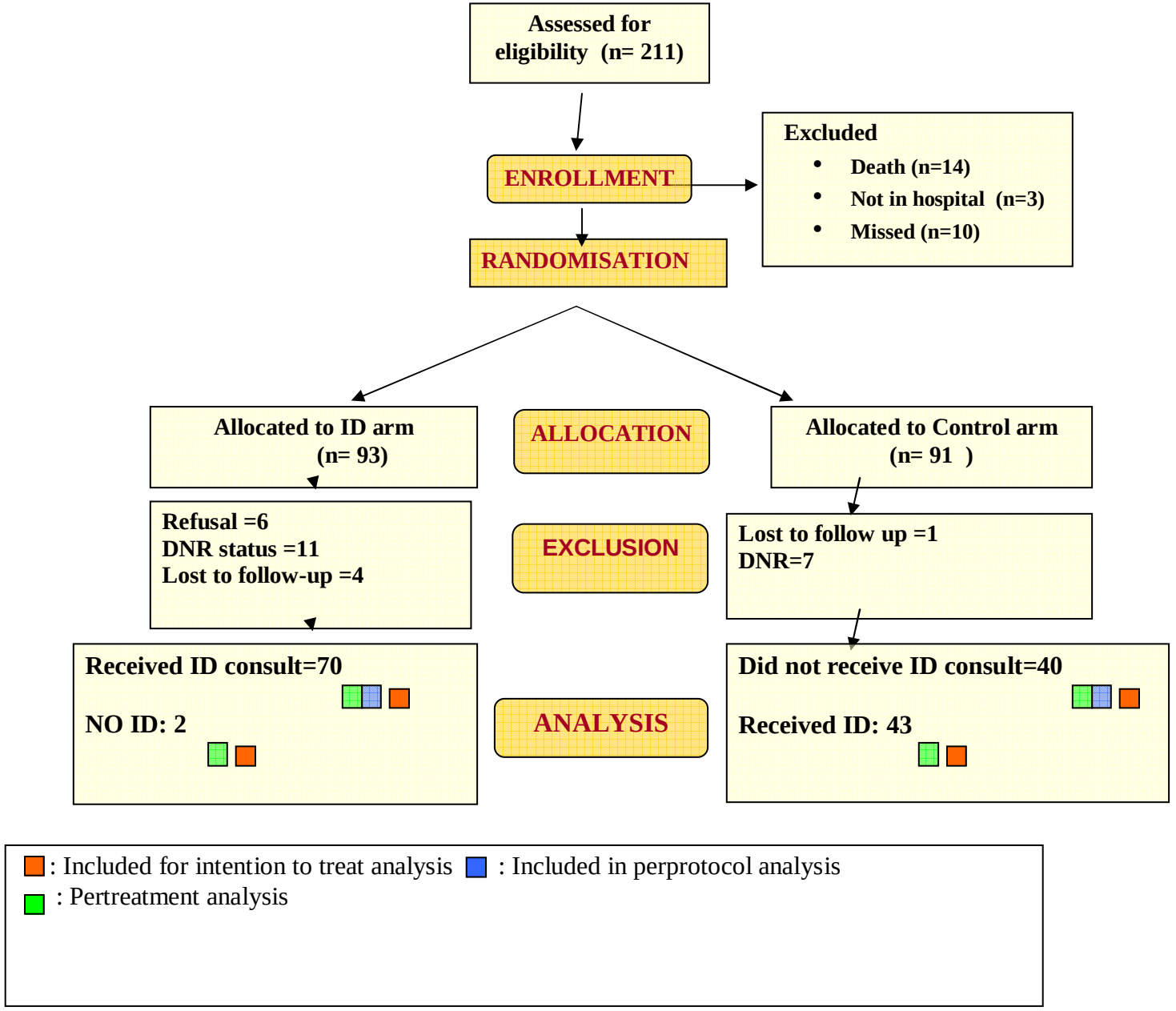
A total of 211 patients had SAB episodes from Oct 12, 2007 to Oct 30 2008. Of these 184 were recruited in the trial. Twenty seven patients were excluded because of death at the time of notification (n=14), patients were not in hospital (n=3) or patients were missed due to investigator factors in the trial (n=10).

The patients eligible for the trial were randomized to an ID consult or the control arm by stratified block randomization. Of the 184 patients eligible in this study, 93 were allocated to an ID consult while 91 were allocated to the control arm. Of those in the ID arm (n=93), the outcomes of 72 patients were finally analysed as others were lost to follow up (n=4) , were on supportive care (n=11) or the physician refused the patient to be included in the trial (n=6). Of the 91 patients in the control arm, 83 were finally analysed as 1 patient was lost to follow up and 7 were on supportive care (Figure 15).

We performed three types of trial analysis as mentioned below.

- **Per-Protocol analysis**: We compared patients in the ID arm who received the ID consult within 72 hours vs. patients in the control arm who did not receive the ID consult. Out of 83 patients in the control arm, 43 patients received ID consult later hence the remaining 40 patients were compared with 70 patients in the ID arm (2 patients in the ID arm did not receive an ID consult within 72 hours)

Figure 15: Details of randomized controlled trial



- **Intention to treat analysis**: We analysed the ID arm (n=72) and the control arm (n=83) irrespective of whether the patient had the ID consult
- **Pertreatment analysis**: We compared all patients who received an ID consult (n=113) vs. those who did not receive an ID consult (n=42). An ID consult was deemed to have taken place only if the patient had a consult in the first 72 hours of notification. Any consult after 72 hours was taken as NO ID Consult.

4.1 Per-protocol analysis

Patients in the control arm were more likely to have higher APACHE II score (median: 15 vs. 13,p value =0.01), hemodialysed (OR: 2.26, $CI_{95:}$:1.00-5.07 p value=0.05) and admitted under medical specialties (OR: 5.62 $CI_{95:}$: 1.2-25.9, p value=0.01) as compared to the ID arm. Standard of care varied in both arms. Patients in the ID arm were more likely to receive a transthoracic echocardiography (OR: 3.91 $CI_{95:}$: 1.71-8.92, p value<0.01), Appropriate definitive antibiotics (OR: 7.81 $CI_{95:}$:3.02-20.24, p value<0.01) and surgical intervention (OR: 15.6, $CI_{95:}$:2.01-121.37 p value=0.009) for SAB episode. However outcome did not vary appreciably. Overall mortality was 19 vs. 10%, blood recurrence 5.7 vs. 15% in the ID and control arm respectively (Table 12). On a multivariate analysis for the process measures, appropriate definitive antibiotics (<0.001) and a surgical intervention (p value=0.042) was different in both groups (Table 12).

4.2 Intention to treat analysis

The profile of patients was similar in the ID and control arms. The age, sex, mode of acquisition, source of infection, comorbidities and comorbidity scores were equally represented in both groups. The outcomes were also similar in both the groups. Crude in hospital mortality was 20 vs. 14%, blood recurrences 5.5 vs. 11% and persistent

bacteremia 20 vs 21% in the ID and control groups respectively. The standard of care was also similar in both the groups with the exception of surgical intervention. Patients in the ID arm had more surgical interventions (29% vs. 16%), however, this did not reach statistical significance (p=0.068)(Table 13)

4.3 Pertreatment analysis

On a univariate analysis, patients from surgical units (OR 0.2 $CI_{95:}$:0.06-0.71, p value =0.01), community acquired infection (p value=0.01) IV drug abusers (OR: 8.82 $CI_{95:}$:1.14-67.92, p value=0.02) and Infective endocarditis (OR: 3.36 $CI_{95:}$:0.95-11.85, p value=0.05) were more likely to have ID consults, while patients with higher Apache (median 15 vs. 12, OR: 0.92 $CI_{95:}$:0.87-0.98 p value=0.008) and lower Charlson score (median: 3 vs. 4.5, OR: 0.87 $CI_{95:}$:0.77-0.99 p value=0.047), hemodialysis (OR: 0.27 $CI_{95:}$:0.12-0.57, p value<0.01) and renal disease (OR: 0.31 $CI_{95:}$:0.15-0.66, p value<0.01) were less likely to have an ID consult. Transthoracic echocardiography (OR: 5.84 $CI_{95:}$:2.7-12.66, p value<0.01), Radiological imaging (OR: 2.8 $CI_{95:}$:1.01-7.77, p value=0.04), Surgical intervention (OR: 7.9 $CI_{95:}$:1.8-34.64, p value<0.01) and appropriate antibiotics (OR: 10.54 $CI_{95:}$:4.35-25.54, p value<0.01) were more likely follow up events in patients having an ID consultation. The outcomes did not differ remarkably in both groups. (Table 14)

Table 12 Perprotocol Analysis

	Control(40) No (%)	ID(70) No (%)	Odds ratio	P value Uni [ß](multi [γ])
Age	53.5(43.5-70)*	57(47-68)*	1.00(0.98-1.02)†	0.38
Male	21(52.50)	48(68.57)	1.97(0.89-4.39)	0.09
Ethnicity				0.98‡
Chinese vs. Malay	20(50.00) vs. 11(27.50)	38(54.29) vs. 18(25.71)	0.86(0.34-2.17)	0.75
Chinese vs. Indian	20(50.00) vs. 5(12.50)	38(54.29) vs. 8(11.43)	0.84(0.24-2.9)	0.78
Surgical department	2(5.00)	16(22.86)	0.18(0.04-0.82)	**0.01**
Mode of acquisition				0.22‡
Community vs. Nosocomial	6(15.00) vs. 10(25.00)	15(21.43) vs. 25(35.71)	1(0.30-3.31)	1.0
Community vs. Healthcare associated	6(15.00) vs. 24(60.00)	15(21.43) vs. 30(42.86)	0.5(0.16-1.48)	0.21
Source of bacteremia				0.41‡
Unknown vs. Superficial skin	12(30.00) vs. 6(15.00)	20(28.57) vs. 14(20.00)	1.4(0.42-4.6)	0.58
Unknown vs. Deep tissue source	12(30.00) vs. 4(10.00)	20(28.57) vs. 14(20.00)	2.1(0.56-7.8)	0.27
Unknown vs. Line source	12(30.00) vs. 18(45.00)	20(28.57) vs. 20(28.57)	0.66(0.255-1.73)	0.40
Apache score	15(12-20)*	13(8-18)*	0.92(0.85-0.98)†	**0.01**
Charlson score	4.5(2-6.5)*	4(2-6)*	0.93(0.81-1.07)†	0.35
IVDU	1(2.50)	9(12.86)	5.75(0.70-47.21)	0.07
Diabetes	20(50.00)	33(47.14)	0.89(0.41-1.94)	0.77

	Control(40) No (%)	ID(70) No (%)	Odds ratio	P value
Malignancy	6(15)	6(8.57)	0.53(0.15-1.77)	0.29
Haemodialysis	19(47.50)	20(28.57)	0.44(0.20-0.99)	**0.05**
Liver disease	2(5.00)	3(4.29)	0.85(0.14-5.32)	1.00
Renal disease	21(52.50)	26(37.14)	0.53(0.24-1.18)	0.12
Infective endocarditis	3(7.50)	11(15.94)	2.34(0.61-8.95)	0.25
Follow up blood c/s	23(57.50)	41(59.42)	1.08(0.49-2.38)	0.84
TTE	17(42.50)	52(74.29)	3.91(1.71-8.92)	**<0.01**
TEE	1(2.50)	4(5.71)	2.36(0.25-21.91)	0.65
Bone imaging	6(15.00)	17(24.29)	1.82(0.65-5.07)	0.25
Abdominal imaging	9(22.50)	19(27.14)	1.28(0.52-3.19)	0.59
Other imaging	5(12.50)	17(24.29)	2.25**(0.76**-6.64)	0.14
Surgical intervention	1(2.50)	20(28.57)	15.60(2.01-121.37)	**0.009(0.042)**
Appropriate empiric antibiotic	20/37(54.1)	39/69(56.82)	1.07(0.47-2.45)	0.80
Appropriate definitive antibiotic	10/37(24.3)	49/67(73.77)	7.81(3.02-20.24)	**<0.01(<0.001)**
In hospital mortality	4(10.26)	13(19.12)	2.07(0.62-6.85)	0.23
Blood recurrence	6(15.00)	4(5.71)	0.34(0.09-1.30)	0.10

*Denotes median and IQR for continuous variables, † Denotes the odd ratio for every unit change , for example, in this analysis a person 10 year older , would have an odds of 1.05^{10} times that of the younger, ‡

Table 13 Intention to treat analysis

	ID(n=72) No(%)	CONTROL(n=83) No (%)	Odds ratio	P value UNI ß
Age	53(36-68)*	57(47-67.5)*	1.07(0.99-1.02)†	0.16
Male	49(59.04)	50(69.44)	1.58(0.81-3.07)	0.18
Ethnicity				0.97‡
Chinese vs. Malay	48(57.83) vs. 19(22.89)	40(55.56) vs. 18(25.00)	1.2(0.41-3.48)	0.74
Chinese vs. Indian	48(57.83) vs. 8(9.64)	40(55.56) vs. 8(11.11)	0.9(0.288-2.8)	0.73
Medical department	17(20.48)	17(23.61)	0.83(0.39-1.78)	0.64
Mode of acquisition				‡
Community vs. Nosocomial	22(26.51) vs. 28(33.73)	15(20.83) vs. 25(34.72)	1.3(0.56-3.06)	0.62
Community vs. Healthcare associated	22(26.51) vs. 33(39.76)	15(20.83) vs. 32(44.44)	1.42(0.62-3.21)	0.85
Source of bacteremia				0.20‡
Unknown vs. Superficial skin source	32(38.55) vs. 13(15.66)	20(27.78) vs. 15(20.83)	1.84(0.72-4.67)	0.196
Unknown vs. Deep tissue source	32(38.55) vs. 9(10.84)	20(27.78) vs. 15(20.83)	2.66(0.98-7.2)	0.054
Unknown vs. Line source	32(38.55) vs. 29(34.94)	20(27.78) vs. 20(27.78)	1.1(0.496-2.5)	0.809
Apache	13(9-18)*	13(8-17.5)*	0.96(0.92-1.02)†	0.24
Charlson	3(1-5)*	4(2-6)*	1.05(0.94-1.18)†	0.36
IVDU	12.00(14.46)	9.00(12.50)	0.85(0.33-2.14)	0.72
Diabetes	31.00(37.35)	34.00(47.22)	1.50(0.79-2.85)	0.21

	ID(n=72) No(%)	CONTROL(n=83) No (%)	Odd ratio	P value
Haemodialysis	21.00(25.30)	21.00(29.17)	1.22(0.60-2.47)	0.59
Malignancy	15.00(18.07)	7.00(9.72)	0.49(0.19-1.27)	0.14
Liver disease	4.00(4.82)	3.00(4.17)	0.86(0.19-3.97)	0.85
Renal disease	24.00(28.92)	27.00(37.50)	1.48(0.75-2.89)	0.26
Infective endocarditis	15.00(18.07)	11.00(15.49)	0.83(0.35-1.95)	0.67
Follow up blood culture	51(61.45)	42(59.15)	0.91(0.48-1.74)	0.77
TEE	8(9.64)	4(5.56)	0.55(0.16-1.91)	0.38
TTE	57(68.67)	53(73.61)	1.27(0.63-2.56)	0.50
Bone imaging	19(22.89)	17(23.61)	1.04(0.49-2.20)	0.92
Abdominal imaging	21(25.30)	20(27.78)	1.14(0.56-2.32)	0.73
Other imaging	19(22.89)	17(23.61)	1.04(0.49-2.20)	0.92
Surgical intervention	14(16.9)	21(29.2)	2.02(0.942-4.37)	0.068
Appropriate empiric antibiotic	45/80(56.25)	40/71(56.34)	1.08(0.56-2.09)	0.99
Appropriate definitive antibiotic	47/79(59.42)	50/69(72.46)	1.85(0.89-3.85)	0.098
In hospital mortality	12(14.63)	14(20.00)	1.46(0.62-3.40)	0.38
Blood recurrences	9(10.84)	4(5.56)	0.48(0.14-1.64)	0.24

*Denotes median and IQR for continuous variables, † Denotes the odd ratio for every unit change, for example, in this analysis a person 10 year older , would have an odds of 1.05^{10} times that of the younger, ‡

Table 14 Pertreatment analysis

	No ID consult(42) No(%)	ID consult(113) No (%)	Odds ratio	P value
Age	56(46-69)*	56(39-67)*	1.00(0.98-1.02)†	0.78
Male	23(54.76)	76(67.26)	1.70(0.82-3.50)	0.15
Ethnicity				0.92‡
Chinese vs. Malay	22(52.38) vs. 11(26.19)	66(58.41) vs. 26(23.01)	0.78(0.335-1.85)	0.58
Chinese vs. Indian	22(52.38) vs. 5(11.90)	66(58.41) vs. 11(9.73)	0.73(0.22-2.3)	0.601
Surgical department	3(7.14)	31(27.43)	0.20(0.06-0.71)	**0.01**
Mode of acquisition				**0.01**‡
Community vs. Nosocomial	6(14.29) vs. 10(23.81)	31(27.43) vs. 43(38.05)	0.83(0.27-0.79)	0.74
Community vs. Healthcare associated	6(14.29) vs. 26(61.90)	31(27.43) vs. 39(34.51)	0.29(0.10-0.793)	**0.016**
Source of bacteremia				0.55‡
Unknown vs. Superficial skin source	12(28.57) vs. 7(16.67)	40(35.40) vs. 21(18.58)	0.9(0.3-2.63)	0.84
Unknown vs. Deep tissue source	12(28.57) vs. 5(11.90)	40(35.40) vs. 19(16.81)	1.14(0.35-3.7) ‡	0.83
Unknown vs. Line source	12(28.57) vs. 18(42.86)	40(35.40) vs. 31(27.43)	0.516(0.21-1.23) ‡	0.136
Apache	15(12-19)*	12(8-17)*	0.92(0.87-0.98)†	**0.008**
Charlson	4.5(2-7)*	3(1-6)*	0.87(0.77-0.99)†	**0.047**
IV drug abuse	1(2.38)	20(17.70)	8.82(1.14-67.92)	**0.02**
Diabetes	21(50.00)	44(38.94)	0.64(0.31-1.30)	0.21

	No ID consult(42) No(%)	ID consult(113) No (%)	Odds ratio	P value
Haemodialysis	20(47.62)	22(19.47)	0.27(0.12-0.57)	**<0.01**
Malignancy	7(16.67)	15(13.27)	0.77(0.29-	0.59
Liver disease	2(4.76)	5(4.42)	0.93(0.17-4.97)	1.00
Renal disease	22(52.38)	29(25.66)	0.31(0.15-0.66)	**<0.01**
Infective endocarditis	3(7.14)	23(20.54)	3.36(0.95-11.85)	**0.05**
Follow up blood c/s	24(57.14)	69(61.61)	1.20(0.59-2.47)	0.61
TTE	18(42.86)	92(81.42)	5.84(2.70-12.66)	**<0.01**
TEE	1(2.38)	11(9.73)	4.42(0.55-35.36)	0.18
Bone imaging	6(14.29)	30(26.55)	2.17(0.83-5.66)	0.11
Abdominal imaging	10(23.81)	31(27.43)	1.21(0.53-2.75)	0.65
Other imaging	5(11.90)	31(27.43)	2.80(1.01-7.77)	**0.04**
Surgical Intervention	2(4.76)	32(28.32)	7.90(1.80-34.64)	**<0.01**
Appropriate empiric antibiotic	21/39(53.76)	64/112(57.14)	1.05(0.49-2.23)	0.721
Appropriate definitive antibiotic	10/39(25.64)	87/109(79.81)	10.54(4.35-25.54)	**<0.01**
In hospital mortality	5/41(12.20)	21/111(18.92)	1.68(0.59-	0.33
Blood recurrences	6(14.29)	7(6.19)	0.40(0.12-1.26)	0.11

*Denotes median and IQR for continuous variables, † Denotes the odd ratio for every unit change , for example, in this analysis a person 10 year older , would have an odds of 1.05^{10} times that of the younger, ‡

CHAPTER 5

RESULTS 3: MOLECULAR EPIDEMIOLOGY OF SAB IN A TERTIARY CARE HOSPITAL

A representative subset of 130 strains first episode of SAB was subjected to Spa typing. The 130 strains were obtained from 128 patients, as two patients had a polymicrobial bacteremia with MRSA and MSSA. Overall, 72 MRSA and 58 MSSA were subjected to Spa typing. For each unique isolate, a Spa type was designated using an online database (www.ridom.net).

All the strains were typable. Table 15 shows the spa types in MRSA and MSSA. Overall, 38 spa types were obtained for 58 MSSA isolates and 9 spa types for 72 MRSA isolates. Sixty four of the MRSA isolates (89%) belonged to two spa types namely t032 and t037. One patient with polymicrobial infection had a different spa type for MRSA (t037) and MSSA (t189) while the other patient had the same spa type (t037).

Table 15 Spa types in MRSA and MSSA isolates

MSSA (n=58)								MRSA (n=72)	
Type	No	Type	No	Type	No	Type	No	Type	No
t008	1	t338	1	t4666	1	t1684	1	t037	49
t015	1	t346	2	t4667	1	t170	1	t032	15
t034	1	t3802	1	t548	1	t189	7	t1214	2
t037	2	t382	1	t622	1	t2119	1	t129	1
t084	4	t4209	1	t645	1	t213	2	t1566	1
t1182	1	t4662	1	t692	1	t2171	1	t202	1
t127	5	t4663	1	t693	1	t258	1	t291	1
t1509	1	t4664	1	t701	2	t304	1	t3555	1
t159	1	t4665	1	t731	1	t3155	1	t548	1
t164	4	t4666	1	t903	1				

Figure 16 shows the clustering analysis of MSSA and MRSA spa sequences respectively. As seen here, MSSA was more genetically diverse than MRSA. Maximum number of members within one clonal complex for MSSA was 10 as opposed to 49 (t037) for MRSA. Among MRSA there was no clustering seen at the level of wards.

Table 16 gives the antimicrobial profile of the various MRSA spa types isolated. All MRSA were resistant to Ciprofloxacin with the exception of t202 (n=1) and t548 (n=1). Of the resistant Ciprofloxacin strains, all t032 isolates were sensitive to cotrimoxazole and gentamicin, while all the t037 isolates were resistant to these antibiotics.

A univariate analysis was performed to compare the patient characteristics and outcome of MRSA patients with t032 spa type (n=15) with t037 (n=49). In this analysis, pneumonia and infective endocarditis were more common in patients with t032 spa type infection. However, patient outcomes did not vary significantly between the two groups. Mortality rate was 5 and 18 % for t032 and t037 MRSA bacteremia respectively (Table 17).

Table 16 Antimicrobial profile of MRSA spa types

Spa Type	Numbers	Cotrimoxazole	Ciprofloxacin	Clindamycin	Gentamicin
t032	15	S *	R†	S	S
t3555	1	S	R	S	S
t1214	2	S	R	S	S
t1566	1	R	R	S	S
t037	49	R	R	R	R
t129	1	S	R	S	R
t291	1	R	R	R	R
t202	1	S	S	S	S
t548	1	S	S	S	S

***: Denotes sensitivity †: Denotes resistance**

Figure 16: Clustering of MRSA and MSSA

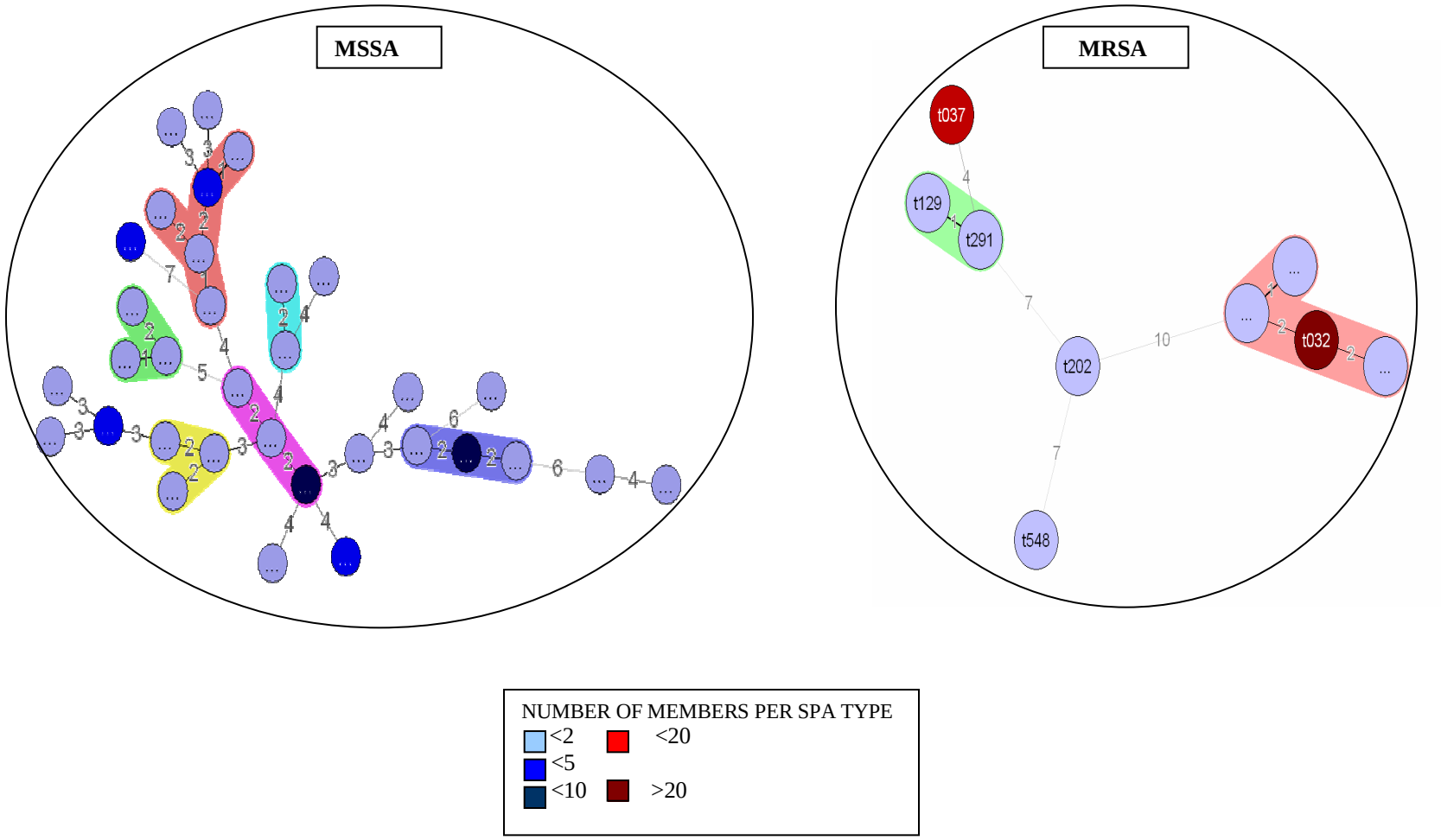


Table 17 Comparison of patient characteristics and outcomes of spa type t032 and t037 MRSA bacteremia

Pt Characteristics	**T037(n=49)**	**t032 (n=15)**	**Odds ratio**	**p value**
Age	63.5(51-69)*	68.5(54.5-75.5)*	0.98(0.94-1.02)†	0.40
Male sex	36(73.5)	8(53.33)	2.84(0.88-9.13)	0.14
Chinese vs. Malay	34(69.4) vs. 11(22.5)	12(80) vs. 3(20)	1.36(0.32-5.67)	1.36(0.32-5.67)
Source of bacteremia				0.46‡
Unknown source vs. Superficial Skin	8(16.3) vs. 11(22.5)	1(6.6) vs. 5(33.3)	0.24(0.024-2.48)	0.23
Unknown source vs. Deep tissue source	8(16.3) vs. 10(0.4)	1(6.6) vs. 4(26)	0.27(0.025-2.96)	0.28
Unknown source vs. Line infection	8(16.3) vs. 18(36.8)	1(6.6) vs. 3(20)	0.5(0.04-5.15)	0.56
Mode of acquisition				0.60‡
Healthcare associated infection	16(32.6)	5(33.3)		
Nosocomial infection	32(65.3)	9(60)		
Diabetes	25(51)	10(66.7)	0.6(0.18-1.90)	0.28
Pneumonia	3(6.1)	5(33.3)	0.14(0.03-0.67)	0.014
Renal Disease	15(30)	9(56.2)	0.55(1.73-1.75)	0.31
Malignancy	12(24.5)	0(0)	5.27(0.63-43.9)	0.054
Hemodialysis	10(20.4)	3(20)	1.08(0.25-4.54)	1
Apache Score	13(9-18)*	13(11-17)*	0.98(0.89-1.08)†	0.76
Charlson Score	5.5(3-7)*	4(3-6)*	0.95(0.76-1.18)†	0.67
Infective endocarditis	1(2.2)	3(23)	0.08(0.007-0.86)	0.032

Pt Characteristics	T037(n=49)	t032 (n=15)	Odds ratio	p value
Bony metastasis	6(13.3)	1(7.6)	1.95(0.21-17.7)	0.58
Visceral metastasis	2(4.4)	1(7.6)	0.59(0.05-7.04)	0.64
Persistent bacteremia	13(30.3)	5(45.5)32	0.58(0.16-2.19)	0.33
In hospital mortality	18(39.3)	5(33.3)	1.03(0.32-3.33)	0.7
Recurrences	6(12.2)	2(13.3)	0.95(0.17-5.27)	1

*Denotes median and IQR for continuous variables, † Denotes the odd ratio for every unit change, for example , in this analysis a person 10 year younger , would have an odds of 0.98^{10} times that of the older patient, ‡ p value on a global comparison usinf chi square test

CHAPTER 6

DISCUSSION

Staphylococcus aureus bacteremia (SAB) is a well-known and leading cause of community and hospital acquired bacteremia. In spite of the availability of effective antibiotics, the mortality rate due to SAB is still reported at 20-30%(3-7). In addition, SAB can be complicated by infective endocarditis, metastatic seeding especially to the bone and viscera and recurrence. There have been several reports on the SAB epidemiology, most of which are from the Western world. (3, 7-10), What appears in the last decade or so is that many countries have witnessed an increase in the incidence of SAB(3, 5) and the main epidemiological focus has been the growing problem of MRSA among SAB .The burden of MRSA bacteremia in terms of cost and resources is high. There have also been treatment related issues emerging in MRSA bacteremias with many strains showing a higher MIC to vancomycin, the most common antibiotic used for its treatment. In spite of this dynamic epidemiology, there have been scant reports that aim at defining the epidemiology and outcomes of SAB in the Asian Context. Hence, we conducted this study with the main aim to define the problem at a tertiary care center in Singapore. Our study is one of the largest cohorts of SAB patients to be studied prospectively in the Asian context. The main outcome measures of interest were the crude mortality rate, metastatic seeding and the recurrence with a special emphasis on the predictors of these adverse events. In addition, we also examined the effect of an ID consultation on the outcomes of SAB patients in a randomized trial, the first study of its kind to our knowledge. Previous studies aimed at evaluating the effect of ID consultation have been mainly observational or interventional, but in a non-randomized manner(131, 132). We also conducted molecular typing on a subset of our SAB isolates with the aim

to define clonality and to look at possible epidemiological associations among major clones of MRSA or MSSA.

6.1 Epidemiology

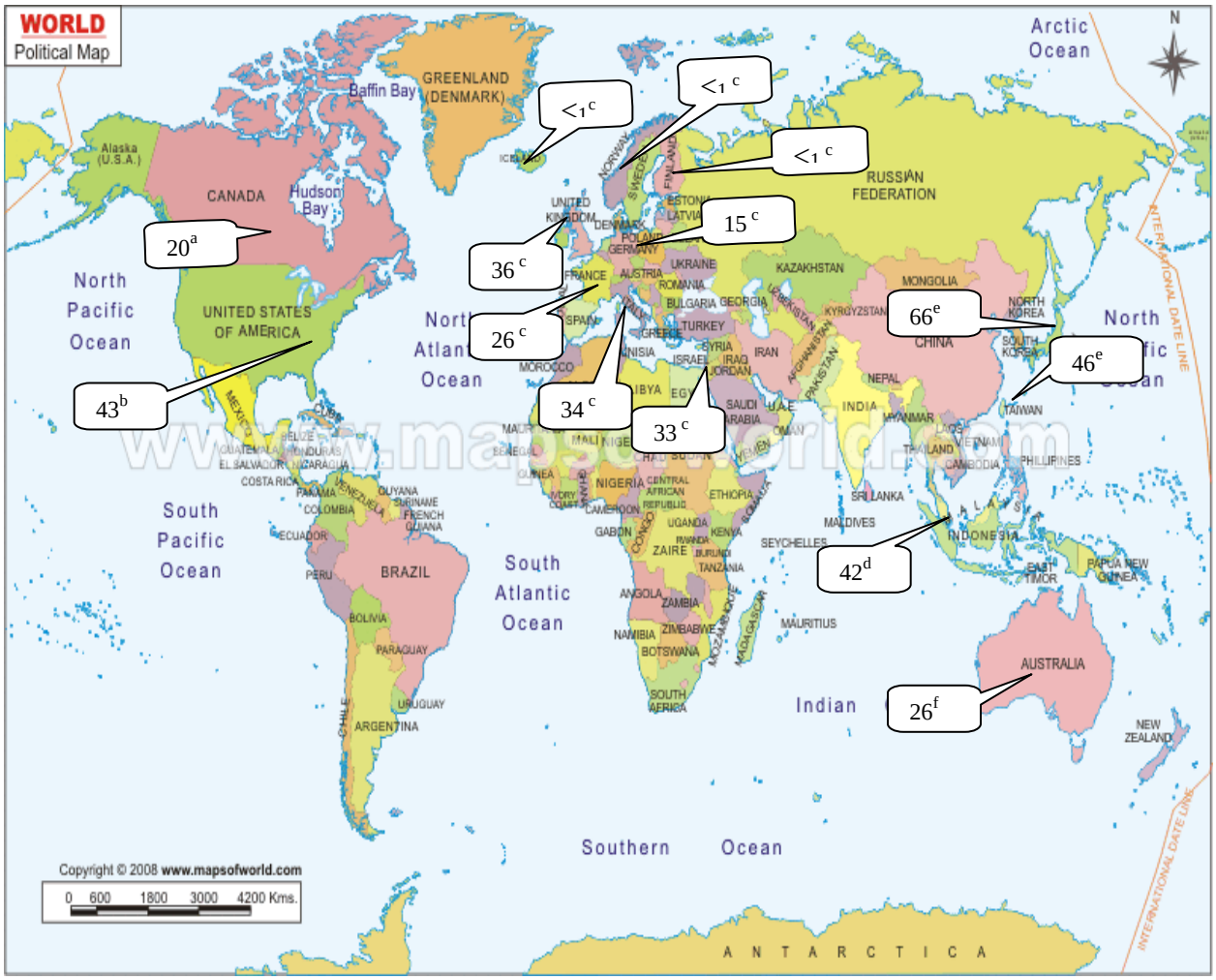
The overall SAB rate in our tertiary care center was 3.42/1000 discharges with the MRSA bacteremia rate of 1.44/1000 discharges. These rates were higher than the median SAB rate of 1.59/1000 admissions at principal referral metropolitan hospitals in Australia or 2.32/1000 discharges according to an US report(163). This high rate is possibly the result of the case mix at our center, a teaching hospital. Various countries including Singapore have reported a higher MRSA hospitalization rate among teaching as compared to non teaching hospitals. In Singapore, the MRSA rate /1000 discharges was 3.2 at the current center (National University Hospital) as compared to Changi General Hospital(164)(0.7, Table 18). These different rates might also be the result of different methods of detection of cases. In Australia SAB rate varied from 0.6-3.24 with referral metropolitan hospitals having higher rate than large metropolitan or private hospitals(10). In 1989, rates of nosocomial SAB rate was 1.13 per 1000 discharges among large teaching hospitals as compared to 0.44 per 1000 discharges among large non teaching hospitals in the US(13).

The epidemiology of SAB in our study was defined by a high percentage of MRSA (42%) and healthcare related infection (79-80%). The proportions of SAB cases attributable to MRSA at our center were comparable to countries such as the United Kingdom and USA and much higher than those reported in Scandinavian countries (Figure 17). As the majority of MRSA bacteremias are healthcare related and more difficult to treat, there is increasing need to beef up current infection control

Table 18 Incidence of MRSA infections in acute care hospitals per 1000 discharges and deaths (Adapted from MOH occasional paper 2007/23)

	2002	2003	2004	2005	2006
Alexandra Hospital	1.8	1.7	1.8	1.2	1.6
National University of Singapore	4.0	4.2	4.1	3.7	3.2
Tan Tock Seng Hospital	3.5	4.0	4.1	3.8	3.6
Changi General Hospital	0.7	0.5	0.7	0.5	0.7
Kandang Kerbau Hospital	0.3	0,1	0,2	0,3	0,3
Singapore General Hospital	5.9	5.2	4.0	4.5	4.0

Figure 17: Worldwide proportions of Staphylococcus infections due to MRSA



Footnotes: a(5) , b(163), c(165), d (current study) , e(166),f (10)

practices and set up adequate monitoring systems. Some of the measures implemented to tackle healthcare MRSA in various countries including Singapore include

- Active surveillance of high-risk patients such as ICU patients.
- Isolation, cohorting and management of colonized individuals
- Emphasis on hand hygiene
- Training of healthcare personnel

The impact of these measures is evident from the fact that countries such as Denmark, and Finland which have implemented an active "search and destroy (S&D)" policy have low rates of MRSA infections and bacteremias (167-170). It is possible that the S&D policy may also be effective in a healthcare setting where MRSA is endemic such as our tertiary care centre. In a simulation model proposed by Boostma and colleagues, a combined approach of screening high risk patients on admission plus screening of contacts when an index case is notified and a stepwise implementation could decrease a high endemicity to levels as low as <1% within 6-12 years(171).

6.2 Mortality

In our study, we noted an in-hospital mortality rate of 29% among SAB cases. Mylotte and colleagues reported a 30-day mortality of 23.2 % among 293 bacteremic episodes reported at a tertiary care center in USA from 1995-1999(52). In another large cohort of 724 patients in the US, the attributable mortality of SAB was 28%(75). Close to Singapore, in Thailand, a high attributable mortality was reported (48%) among SAB patients(63). Thus the mortality reported in our study was similar to the hospital reports from the Western World (52, 55, 57-59, 75).

In our study, the predictors of in hospital mortality in a multiple logistic regression analysis were MRSA infection, elderly age, malignancies, history of wide spread skin disease, and a higher Apache score. A localized skin source such as carbuncles or phlebitis was associated with a lower mortality rate as compared to an unknown source.

As a substantial proportion of patients with SAB have underlying comorbidities, various comorbidity scores have been used for adjusting the risk. Scores commonly in use are the Charlson Comorbidity index(64), APACHE II score(91), McCabe score(92) and Pitt bacteremic score(172). These scoring systems differ from each other in the parameters used. APACHE II score takes organ failure with the vitals and other laboratory parameters to generate a score, a higher score being associated with a worse prognosis. The score was primarily designed for use in ICU patients, however, has also been used in context of SAB infections. Various studies have also shown APACHE II to be an independent predictor of mortality in SAB patients (52, 69, 71). Some believe that the Delta APACHE score (the difference in APACHE score on the day of bacteremia and a day before the bacteremia) is more predictive of mortality than the APACHE II score alone taken on the day of bacteremia(173). The Charlson Comorbidity score is generated based on comorbidities and different weights are given to comorbidities such as Myocardial Infarction, Connective tissue disorder, malignancies, and chronic liver or renal disease. In an evaluation of 166 patients with SAB, a Charlson score of 3 or more was an independent predictor of attributable mortality (p value<0.001). Both, APACHE II and Charlson index are age-adjusted indices. McCabe score a simple scoring system classifies illness as rapidly fatal, ultimately fatal and non-fatal disease and was initially devised for the use of predicting fatality in patients with gram negative bacteremia. This

classification is based on physician's decision and hence subjective in nature. It has been mainly used for comparing the illness severity in different subgroup analysis(92). The Pitt bacteremic score takes into account few parameters such as temperature, events of hypotension, cardiac arrest and mechanical ventilation to generate a score. A score of 4 or more is more likely to be associated with critical illness(172). There are scant reports on the comparison of these scores in predicting mortality, especially in the context of SAB patients.

In our study, we used the Charlson and the APACHE II score on the date of withdrawal of blood culture to look for an association with mortality. Although Charlson and APACHE II score were significantly associated with mortality on a univariate analysis, only APACHE II score was significant on the multivariate analysis. Our findings are in line to those observed by Poses and colleagues who found in a comparative evaluation the overall APACHE score to be more predictive of in-hospital mortality in critically ill patients than the Charlson score(174). The better prediction of mortality is perhaps the result of the vitals and laboratory parameters taken into account in the APACHE score unlike the Charlson score and hence a better indicator of the severity of underlying sepsis. In addition, not all patients with serious comorbidities contribute to SAB mortalities. This is particularly true for patients with a removable focus of infection such as a hemodialysis catheter. As mentioned earlier, we found such patients to have a favorable outcome in terms of a decreased mortality rate. Previous studies have noted also noted the same(144). In spite of these findings, the APACHE score is not feasible to use routinely. Not all the laboratory information is available especially in patients not critically ill and those admitted in the general wards. Hence, Charlson might still be a

useful index for adjusting comorbidities especially in settings where the resources and laboratory information is limited. Our study did not look at the possibility of a combination of APACHE and Charlson parameters to predict mortality. In the above mentioned evaluation by Poses et al (174), the APACHE subscore based on the chronic organ insufficiency alone was not as predictive of mortality as the Charlson index . This is not surprising given the range of comorbidities covered by the Charlson index, which could hence supplement the information available by APACHE score. We are yet to evaluate the effect and comparative evaluation of other scores such as Pitt bacteremia score.

Some of the other factors predictive of SAB mortality in previous studies include elderly age(3-5, 7, 52, 54, 62, 64-66) , MRSA infection (67), a lung origin(4, 5, 7, 52, 62) , unknown source , septic shock(64, 65), endocarditis (4, 7), persistent bacteremia(62) and metastatic infection(62) , a greater severity of illness and comorbidities (4, 7, 62, 68-70) , a delay in institution of antibiotic (65) and nosocomial infection(54).

There are several reasons for the marked variation in the predictors noticed among various studies. Firstly, a high proportion of SAB patients have serious underlying comorbidities that may be independently responsible for causing mortalities. Secondly, studies vary in the definitions of mortality used. Different studies use attributable mortality, 30-day or in-hospital mortality. Although attributable mortality would be ideal, it is sometimes subjective. Thirdly, local epidemiology of SAB such as the mode of acquisition, MRSA percentage and population characteristics in particular the age profile and prevalence of comorbidities such as diabetes vary. Fourthly, most studies reflect the situation in individual tertiary care centers and the cohort studied are small numbers.

In spite of the heterogeneity, some mortality risk factors appear to be more commonly reported across studies such as age or MRSA infections. The impact of MRSA was also extensively studied in a meta-analysis including 31 studies concluding that MRSA was indeed associated with a higher mortality(67). The role of modifiable factors such as antibiotic treatment and source of infection is still unclear. In our study, we found that a skin source was associated with a lower mortality rate, presumably because it is more amenable to eradication. This finding is in line with the results of Kim et al (144).

We could not demonstrate a difference in mortality rates among patients receiving appropriate versus inappropriate antibiotics. In our study, around 60% of patients who died or survived were likely to receive appropriate definitive antibiotics (p value =0.87). Previous studies aimed at looking at the effect of appropriate antibiotic on mortality outcome have been conflicting. While some studies show that optimal antibiotic is associated with lower mortality (9, 73, 141, 142), other studies fail to find such a difference (31, 62, 89).

Previous studies have also shown that vancomycin is associated with higher mortality or recurrence among MSSA bacteremia(84, 134, 135, 140). In our SAB series, only 8 MSSA patients received vancomycin of which two had serious penicillin allergies. We also found that patients with MRSA bacteremia were more likely to receive appropriate antibiotics than MSSA patients, presumably because of our criteria of the antibiotic type (including cloxacillin and cefazolin while considering meropenem or augmentin as inappropriate) and high dosage of betalactams to define adequacy of antibiotics for MSSA bacteremia. Many patients with suboptimal antibiotics were receiving cloxacillin at a dose of 1-2gm /day. A previous study conducted by Jensen and colleagues stress the

importance of correct dosing of beta-lactams such as cloxacillin(65). They found a higher mortality rate among patients receiving less than 4 gm of cloxacillin /day. Although the cut–off set up by the Jensen study was 4gm/day, many clinicians still prefer administering a higher dose of cloxacillin (~8gm/day) so as to prevent recurrences and metastatic seeding of bacteria. This is mainly driven by the serious nature of the condition itself. More studies are required to determine the actual suboptimal dose at which poor outcomes such as recurrence, metastatic infection or mortality becomes more common. Without evidence, we believe it would be wise to stick on to advocating a high dose of cloxacillin in MSSA bacteremic patients.

We also noted in our study that a history of widespread skin disease was an independent predictor of mortality. There are scanty reports on this association, perhaps the strongest being the study conducted by Fowler and colleagues(75). They included 724 patients of SAB, 43% of whom had complicated bacteremias defined by attributable mortality, metastatic infection or recurrence. Skin findings suggestive of an acute systemic illness was a significant risk factor for complicated bacteremia in this cohort (OR: 2.04, p=0.002). However, it was not clear in this study whether the skin findings were suggestive of mortality as well or was related to other complications such as recurrence or metastatic infection. As many patients in our population had previous history of skin disease, in particular eczema, it is possible that underlying autoimmunity and use of immunosuppressives could have predisposed these patients to SAB septicemia. However, the mortality rate was not different in those who received steroid/immunosuppressive therapy versus others. It is also possible that many patients had Traditional Chinese Medicine (TCM), which is very popular in this region and thought to have

immunosuppressive effects. The role of TSST 1 toxin cannot be ruled out. In our study, we did not seek to detect the presence of this toxin. Pathophysiologically, *Staphylococcus aureus* can secrete a toxin called TSST 1 which is associated with skin lesions and shock and indeed shock has been noticed as an independent predictor of mortality.

6.3 Metastatic infection and Recurrence

As mentioned earlier, *Staphylococus aureus* bacteremia is frequently complicated by metastatic seeding to the endocardium, viscera and bones and other sites. The overall prevalence of complicated bacteremia varies from 11 to 53%(175, 176) and the prevalence of endocarditis ranges from 5-15%(4, 5, 7, 60, 65). The consequences of such dissemination are a prolonged course of antibiotics, higher mortality rate and chances of recurrence in the future. Hence, it is important to define the predictors of metastatic dissemination so that high-risk patients undergo extensive evaluation.

In our study, we reported the metastatic seeding of only those patients with an LOS of more than 4 days as the others could not undergo relevant investigations to ascertain the same. The prevalence of metastatic seeding and infective endocarditis was 25% and 14.5% respectively and hence in line with those reported elsewhere (see above). Predictors of metastatic dissemination were intravenous drug abuse and persistent bacteremia. We defined persistent bacteremia as a positive blood culture taken 72 hours after the initiation of an effective antibiotic class. Various studies conducted previously have used different definitions to define persistent bacteremia (53, 75). In spite of variations, most studies report an association between persistent bacteremia and metastatic dissemination(53)/infective endocarditis(76))/complicated bacteremia(75). In a prospective observational study of 245 SAB cases, Khatib and colleagues not only found

this association, but also were able to correlate the bacteremia duration to the complication rate(53). Based on this and another large cohort, a complicated outcome can be expected to be anywhere between 30-50% in patients with a persistent bacteremia of 3 days or more. In light of this information, many investigators have suggested a routine follow up blood culture be withdrawn 2-4 days after the onset of bacteremia. In our study, 68%(143/253) of patients with LOS>=4 days had a repeat blood culture within 4 days of bacteremia and an additional 16% of bacteremias within 7 days. Hence, although majority of patients had a repeat blood culture within 7 days, we feel there may a need to increase the coverage so that a repeat culture is available within 4 days of bacteremia

As an echocardiographic modality for diagnosing vegetations in IE, TTE and TEE are widely used. Although, TTE is a simpler non-invasive and cost effective method, it is less sensitive than TEE in the detection of vegetations. Previous studies have shown that TEE picks up more vegetations equally with a negative or indeterminate TTE result(77). With this and many other studies showing the advantage of TEE (177), many investigators have suggested that all patients with SAB be routinely evaluated with TEE, the major deterrent to this being the cost and invasive nature of the procedure. Recent studies have also shown that a TEE might not be as costly as perceived and indeed a TEE guided therapy could save more than $142 million healthcare expenditures while providing similar outcomes in SAB patients(178). On the other hand, the overuse of TTE has also been challenged especially in a clinical scenario where the pretest probability of the disease, in this case infective endocarditis is low (179). The primary aim of our study was not to compare the two modalities, however, we tried to determine if patients were adequately receiving TTE/TEE. Some findings require special attention. Firstly, in our

study, 180 of 300 patients had an echocardiographic evaluation for vegetations of which only 14 patients (14/300=4.6%) had a TEE an any point after the detection of SAB. In another study, around 20% of patients had a TEE(132). Hence, is it possible that we might be advocating TEE less often than required. Secondly, TTE was more commonly advocated when a patient had an ID consultation also suggesting a need to increase the use of a routine TTE following SAB.

Thirdly, among 33 clinical endocarditis patients, 29 showed vegetations with TTE. In two TTE negative patients, vegetations were detected with TEE. Although, on first impression, TTE appears to have performed well, it is tough to conclude the same in the absence of TEE being done in many patients. It is possible that more cases of endocarditis occur and these could be missed by TTE and picked up by TEE. Currently, at our centre a a sequential strategy appears to be in place, TEE mainly being reserved for those with a high index of suspicion and negative TTE. Whether such a strategy is adequate to identify all the endocarditis cases is not certain. In a simulation model, Heidenreich and colleagues(180) have shown that such a sequential strategy might be less cost effective and offer the same quality –adjusted life expectancy as compared to TEE as the initial diagnostic modality. In the same study, the investigators showed that the greatest benefit of an initial TEE was when the pretest probability of the disease was between 4-40% and precluded the additional utility of echocardiography in patients with a probability of less than 4% or more than 40%. In practice, it might not be easy to decide which patient would fall in the bracket of 4-40% especially in the absence of well-defined clinical parameters. Although the authors above provided a extensive pretest probability list for different clinical scenarios, this is based on limited previous reports

and not indeed convenient in an actual clinical scenario. Thus, more studies comparing the two modalities are required in the local context and in particular reference to SAB to determine the exact strategy for their use.

In our study, only 27% of the metastatic infections were from MRSA bacteremia and 20% of infective endocarditis was attributable to MRSA. Our proportion of metastatic infections and IE caused by MRSA, although slightly lower than that observed in the US (33-40%)(60, 74) , seem to reiterate the fact that metastatic dissemination can no longer be considered a sole entity of MSSA bacteremia among IVDU. Also, among MRSA patients, isolated bony infection appears to be as common as endocarditis (7 vs. 8 out of 126 MRSA cases respectively). In our study, we noticed an association between bony metastatic infections and a higher vancomycin MIC among MRSA. As previous studies have also shown an association with hVISA phenotype and development of osteomyelitis(124) and a higher mortality among patients with a higher vancomycin MIC(181), we believe that symptoms such a joint or back pain in patient with MRSA bacteremia especially when associated with persistent bacteremia and a higher vancomycin MIC should undergo further diagnostic imaging and managed aggressively. The rate of recurrence was 9.9% and comparable to other studies (9, 53-55, 83, 84). Due to the small numbers, we did not look for associations for recurrence. The time to recurrence ranged from 16-260 days. While some investigators believe that believe that relapses occur earlier than reinfection. (70, 86), we could not ascertain the same, as we are yet to perform the genotyping of the recurrent strain.

6.4 Infectious Disease Consultation

There are very few studies that address the issue of the impact of an ID consultation on the outcomes of patients with SAB. In the later half of 2007, we started recruiting patients in a randomized trial to look at the effect of an ID consultation. This design had several strengths. Essentially, we were comparing two arms (ID consult vs. Control), similar characteristics for who were achieved by randomization. We did not deny patients in the control arm an ID consultation which was left to the discretion of the primary physician. This design enabled us to perform three different forms of analysis to look for any differences in outcomes. To our knowledge, this is the first randomized controlled trial to look for the impact of an ID consultation in SAB. Although there is evidence from a previous study that patients with an ID consultation have improved outcomes, study design was flawed by the fact that patients characteristics in the group with or without ID consultation were not comparable(131).

Outcomes of 72 patients in the ID arm and 83 patients in the control arm were compared with an intention to treat, per protocol and pretreatment analysis. Notably, patients in the ID arm were more likely to have better process measures of receiving more appropriate antibiotics and surgical intervention in a per-protocol analysis. However, there was no difference in overall outcomes noted in spite of a better standard of care in patients with *Staphylococcus aureus* bacteremia. We believe that this is due to the small sample size or because we included the all-cause mortality instead of the attributable mortality. Our future aim would be to have an independent review of the cases for the attributable mortality and reanalyze our results. It is also possible that other factors driving mortality are stronger and may not be modifiable by management of SAB or an ID consultation.

Whilst mortality is one of our parameters and the strongest outcomes to advocate a mandatory ID consultation, we have yet to evaluate the effect of the ID consultation on other outcome parameters such as length of stay or hospital costs. By far the strongest outcome modified by an ID consultation was shown by Fowler and colleagues wherein patients for whom the physician's followed the ID recommendations were less likely to have a recurrence(131). However, in this study, patients who did not follow the recommendations were more likely to be hemodialysed, which could have thus been a confounder. In our study we found fewer recurrences in patients with an ID consultation, however, this was not statistically significant. Some of the reasons might be due to the small numbers or a short duration of follow up, which in some cases was only 8 weeks. Thus, it is premature to conclude based on our limited data that a mandatory ID consultation would not improve outcomes.

6.5 Problem of high Vancomycin MIC and recognition

The lack of sensitivity of standard microbiological methods in detecting higher vancomycin MIC and thus predicting VISA/hVISA is well known. In a study conducted in Israel, additional testing revealed that almost 75% of the hVISA isolates would have been missed without specific testing(123). Thus, there is a need of a simple diagnostic screening assay to routinely test MRSA isolates for hVISA/VISA. Population profile analysis (PAP) is considered the gold standard for diagnosis of hVISA, however, is cumbersome to perform(127). As per a CDC testing algorithm, all MRSA isolates should be screened for hVISA on a Vancomycin screen plate (BHI agar with 6ug/ml of vancomycin). However, such a high level of vancomycin might miss cases of VISA. A recent multicentric evaluation revealed that this method had a sensitivity of only

44%(182). In this study, MHA with 5ug/ml of vancomycin or teicoplanin and E-Test macromethod (ETM) were also evaluated. The best performance was obtained with ETM with a sensitivity and specificity of 99% and 93.3%. The ETM method differs from the routine E-Test MIC determination by employing a higher inoculum of the bacteria. This method is less cumbersome than PAP and thus becoming increasingly popular in Microbiology laboratories. The exact determination of the MIC is vital to guide the antimicrobial therapy especially in patients with persistent bacteremia occurring in spite of adequate vancomycin therapy. Currently, there is no consensus on whom should be screened for hVISA/VISA and thus the decision of a routine testing versus a clinical based depends on the individual centers.

In the absence of additional laboratory testing, we could not determine how many of our strains were hVISA. Based on standard detection method of VITEK, 21% of the MRSA isolates in our cohort had an MIC of 2 or above with only one frank VISA. There was no clonality observed among strains showing a higher MIC value. Such a high prevalence of MRSA with a vancomycin MIC >= 2 have also been noted by other investigators(120, 183, 184). In our study, MRSA strains with a higher MIC were associated with more persistent bacteremia, recurrence and bony metastatic infections. Although the mortality rate was higher in patients with a higher MIC, this was not statistically significant. This poor outcome among patients with higher MIC is in line with those observed by other investigators(118, 119).

6.6 Genotyping

We used spa typing to define the bacteremia isolates in our study. Previous studies have shown Spa Typing to be a useful genotyping tool. It is an easy typing method involving

the sequencing of a single genetic locus and results obtained can be compared with those obtained in other laboratories. Various studies have shown that this typing has a discriminatory index in between that of PFGE and MLST and hence, is a useful tool for tracking evolutionary trends or investigating an outbreak (147, 151, 153, 154). In our study, we used this typing method to define the clonality of our isolates and to establish associations with the epidemiological and microbiological characteristics.

Majority of the MRSA (89%) isolates belonged to two major clones, namely Spa type t032 and spa type t037. t032 has been mapped to the E-MRSA clone/ST22-SCCmecIV and t037 has been mapped to the ST-239-241 SCCmec III (Table 2). Both these clones are well known healthcare clones and differ mainly in their antimicrobial profile, t032 being sensitive to cotrimoxazole and gentamicin and t037 being resistant to both these antibiotics. Previous genotyping studies from Singapore have also shown the presence of these two circulating clones (156, 157). Hsu and colleagues at the Singapore General Hospital established a theory of progressive displacement of ST 239 by ST 22. They found a dramatic rise in the frequency of ST22 from 22% of all MRSA isolates (both bacteremic and non bacteremic) in 2003 to 33% in 2005(156). In our study, E-MRSA 15 constituted 21% of all blood isolates, thus the rate seems to be stable since 2005. There have been concerns about a worse outcome with E-MRSA 15. However, on our study, the outcomes of spa type t032 (E-MRSA) were similar to t037 in terms of mortality rates and recurrences. A previous study conducted at a different tertiary care center in Singapore have also noted the same results(73)

A cluster analysis of all spa types of MRSA showed a close association with the antimicrobial profile of these isolates. The spa types closely related to t032 (t3555 and

t1214) were sensitive to cotrimoxazole and gentamicin. T129 and t291 were closer to t037 than t032 and had a more resistant phenotype mirroring the profile of t037. And the two-ciprofloxacin sensitive strains of MRSA were distant to both these healthcare clones. C-MRSA appeared to be rare in our study with only documented case as defined by the clinical picture, antimicrobial profile (ciprofloxacin sensitive) and the spa type (t202). This finding is line with a previous report(20). C-MRSA has been previously documented in Singapore in mainly skin and soft tissue infection. Genotypically, majority are ST 30 and PVL positive(21). Currently, many other countries have witnessed a rise in community onset bacteremia. In a recent PFGE analysis of 864 invasive MRSA isolates, 82% of which were from blood, USA 300, the predominant C-MRSA clone in the USA was isolated from 66% of community cases and 22% of healthcare infections. Moreover USA 100 a predominant healthcare clone was seen in 23 % of community onset bacteremias(8). Thus, the distinction appears to be becoming blurred between healthcare and community clones and similar phenomenon has also been observed in Australia(18). There have been concerns of a poor outcome of C-MRSA infections as a result of carriage of the PVL toxin. However, several studies show that the outcomes and risk factors of USA 300 bacteremias appear to be similar to non USA 300 bacteremias or community MSSA infections (18, 155, 185). The problem of C-MRSA bacteremia is also recognized in certain Asian countries such as Taiwan where according to one report, 33 % of all community onset *Staphylococcus aureus* bacteremias were MRSA in nature(19). Majority of the strains encountered there were ST 59 and the PVL carriage was not uniform(185). Thus, with countries like Australia, USA and Taiwan witnessing a rise of community acquired MRSA infections, a constant vigil is warranted,

especially when an abnormal antibiogram or the common community phenotypes ST 30(spa types t138, t021, t019, t018, t012, t276, t318, t338, t391), ST 59(spa types t444, t216, t199) or ST 8 (spa type t008) are encountered.

Overall, we found Spa Typing to be an easy and cheap screening tool for typing *Staphylococcus aureus* strains. The data available can be compared with those in other countries, certain spa types can be mapped to MLST types and useful clustering and epidemiological associations can be drawn.

6.7 Limitations

1. Our study was conducted at a single tertiary care centre in Singapore, hence it is possible that the results here reflect the local situation and not what is prevalent in the entire country. Although we were able to observe the outcomes of 300 patients, much larger than what has been the case with other studies, we believe it is still not large enough particularly to look for associations for less common events such as VISA, persistent bacteremia and recurrence. Also, we did not evaluate the length of stay and the hospitalization costs of bacteremia.

2. The trial analysis, although randomized, was still not ideal as almost 50% of patients in the control arm received an ID consult. However, it was not ethical to deny patients in need of a consult in the control arm; hence the only way to look for differences in outcome would be a larger recruitment. We did not analyze the attributable mortality differences in both the trial arms.

3. We were not able to perform MLST or PFGE on our strains of SAB. However, we believe that this do not confound our findings as Spa typing, the genotyping method used

has been extensively validated previously by various investigators and the common spa types isolated in our study are well known clones with MLST mapping.

4. Most of the patients with a LOS shorter than 4 days could not be evaluated for metastatic infection due to the lack of imaging.

6.8 Conclusions

SAB appears to be a well-defined entity with a hospitalization rate, mortality and complications similar to reports from Western countries. The high proportions of infections caused by MRSA warrants an intensification of our current infection control practices. The high rate of comorbidities in patients with SAB has lead to the use of comorbidity scores for adjustments in analysis. While APACHE score appears to be more predictive of mortality, it is more cumbersome and may not be feasible in all situations. In such circumstances a Charlson score could be used and may also be used in conjunction to APACHE score to supplement the information. There is a need to monitor the development of complications more closely with the use of follow up blood cultures as a surrogate marker of persistent bacteremia and increased use of TEE is desirable. A high percentage of our MRSA strains had an MIC of 2 or above and was associated with a poor outcome. The genotyping using Spa typing revealed two major clones of MRSA (E-MRSA 15 / ST239-241). Amidst concerns of the poor outcome with the recent emergence of E-MRSA15, we found that the outcomes of such patients were similar to ST239-241. Spa typing was an effective and simple tool to define these clones. We are yet to demonstrate the impact of an ID consultation on the outcomes of SAB patients.

BIBLIOGRAPHY

1. "Mandell GL" BJ, "Dolin R". Principles and Practice of infectious Diseases. Fifth ed. FA W, editor: Churchill Livingstone; 2000.

2. Agency HP. Mandatory Surveillance of Healthcare associated infections 2006: Health Protection Agency2006.

3. Lyytikäinen O, Ruotsalainen E, Järvinen A, Valtonen V, Ruutu P. Trends and outcome of nosocomial and community-acquired bloodstream infections due to Staphylococcus aureus in Finland, 1995-2001. Eur J Clin Microbiol Infect Dis. 2005 Jun;24(6):399-404.

4. Benfield T, Espersen F, Frimodt-Møller N, Jensen A, Larsen A, Pallesen L, et al. Increasing incidence but decreasing in-hospital mortality of adult Staphylococcus aureus bacteraemia between 1981 and 2000. Clin Microbiol Infect. 2007 Mar;13(3):257-63.

5. Laupland K, Ross T, Gregson D. Staphylococcus aureus bloodstream infections: risk factors, outcomes, and the influence of methicillin resistance in Calgary, Canada, 2000-2006. J Infect Dis. 2008 Aug;198(3):336-43.

6. Morin C, Hadler J. Population-based incidence and characteristics of community-onset Staphylococcus aureus infections with bacteremia in 4 metropolitan Connecticut areas, 1998. J Infect Dis. 2001 Oct;184(8):1029-34.

7. Allard C, Carignan A, Bergevin M, Boulais I, Tremblay V, Robichaud P, et al. Secular changes in incidence and mortality associated with Staphylococcus aureus bacteraemia in Quebec, Canada, 1991-2005. Clin Microbiol Infect. 2008 May;14(5):421-8.

8. Klevens R, Morrison M, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA. 2007 Oct;298(15):1763-71.

9. Das I, O'Connell N, Lambert P. Epidemiology, clinical and laboratory characteristics of Staphylococcus aureus bacteraemia in a university hospital in UK. J Hosp Infect. 2007 Feb;65(2):117-23.

10. Collignon P, Nimmo G, Gottlieb T, Gosbell I. Staphylococcus aureus bacteremia, Australia. Emerg Infect Dis. 2005 Apr;11(4):554-61.

11. Laupland K, Gregson D, Flemons W, Hawkins D, Ross T, Church D. Burden of community-onset bloodstream infection: a population-based assessment. Epidemiol Infect. 2007 Aug;135(6):1037-42.

12. Wisplinghoff H, Bischoff T, Tallent S, Seifert H, Wenzel R, Edmond M. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004 Aug;39(3):309-17.

13. Banerjee S, Emori T, Culver D, Gaynes R, Jarvis W, Horan T, et al. Secular trends in nosocomial primary bloodstream infections in the United States, 1980-1989. National Nosocomial Infections Surveillance System. Am J Med. 1991 Sep;91(3B):86S-9S.

14. Garner J, Jarvis W, Emori T, Horan T, Hughes J. CDC definitions for nosocomial infections, 1988. Am J Infect Control. 1988 Jun;16(3):128-40.

15. Lesens O, Hansmann Y, Brannigan E, Hopkins S, Meyer P, O'Connel B, et al. Healthcare-associated Staphylococcus aureus bacteremia and the risk for methicillin resistance: is the Centers for Disease Control and Prevention definition for community-

acquired bacteremia still appropriate? Infect Control Hosp Epidemiol. 2005 Feb;26(2):204-9.

16. Tambyah P, Habib A, Ng T, Goh H, Kumarasinghe G. Community-acquired methicillin-resistant Staphylococcus aureus infection in Singapore is usually "healthcare associated". Infect Control Hosp Epidemiol. 2003 Jun;24(6):436-8.

17. Friedman N, Kaye K, Stout J, McGarry S, Trivette S, Briggs J, et al. Health care--associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med. 2002 Nov;137(10):791-7.

18. Robinson J, Pearson J, Christiansen K, Coombs G, Murray R. Community-associated versus healthcare-associated methicillin-resistant Staphylococcus aureus bacteraemia: a 10-year retrospective review. Eur J Clin Microbiol Infect Dis. 2008 Oct.

19. Chi C, Wong W, Fung C, Yu K, Liu C. Epidemiology of community-acquired Staphylococcus aureus bacteremia. J Microbiol Immunol Infect. 2004 Feb;37(1):16-23.

20. Wijaya L, Hsu L, Kurup A. Community-associated methicillin-resistant Staphylococcus aureus: overview and local situation. Ann Acad Med Singapore. 2006 Jul;35(7):479-86.

21. Hsu L, Koh Y, Chlebicka N, Tan T, Krishnan P, Lin R, et al. Establishment of ST30 as the predominant clonal type among community-associated methicillin-resistant Staphylococcus aureus isolates in Singapore. J Clin Microbiol. 2006 Mar;44(3):1090-3.

22. Huang Y, Su L, Wu T, Lin T. Changing molecular epidemiology of methicillin-resistant Staphylococcus aureus bloodstream isolates from a teaching hospital in Northern Taiwan. J Clin Microbiol. 2006 Jun;44(6):2268-70.

23. Hsueh P, Chen W, Teng L, Luh K. Nosocomial infections due to methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci at a university hospital in Taiwan from 1991 to 2003: resistance trends, antibiotic usage and in vitro activities of newer antimicrobial agents. Int J Antimicrob Agents. 2005 Jul;26(1):43-9.

24. Wang F, Chen Y, Chen T, Liu C. Risk factors and mortality in patients with nosocomial Staphylococcus aureus bacteremia. Am J Infect Control. 2008 Mar;36(2):118-22.

25. Chia J, Hsu L, Chai L, Tambyah P. Epidemiology and outcomes of community-onset methicillin-susceptible Staphylococcus aureus bacteraemia in a university hospital in Singapore. BMC Infect Dis. 2008;8:14.

26. Hsu L, Tan T, Jureen R, Koh T, Krishnan P, Tzer-Pin Lin R, et al. Antimicrobial drug resistance in Singapore hospitals. Emerg Infect Dis. 2007 Dec;13(12):1944-7.

27. "Fowler V, "Sexton D. Epidemiology and risk factors for Staphylococcus aureus bacteremia in adults. 2007.

28. Carnicer-Pont D, Bailey K, Mason B, Walker A, Evans M, Salmon R. Risk factors for hospital-acquired methicillin-resistant Staphylococcus aureus bacteraemia: a case-control study. Epidemiol Infect. 2006 Dec;134(6):1167-73.

29. Pujol M, Peña C, Pallares R, Ayats J, Ariza J, Gudiol F. Risk factors for nosocomial bacteremia due to methicillin-resistant Staphylococcus aureus. Eur J Clin Microbiol Infect Dis. 1994 Jan;13(1):96-102.

30. Libert M, Elkholti M, Massaut J, Karmali R, Mascart G, Cherifi S. Risk factors for meticillin resistance and outcome of Staphylococcus aureus bloodstream infection in a Belgian university hospital. J Hosp Infect. 2008 Jan;68(1):17-24.

31. Roghmann M. Predicting methicillin resistance and the effect of inadequate empiric therapy on survival in patients with Staphylococcus aureus bacteremia. Arch Intern Med. 2000 Apr;160(7):1001-4.

32. Lodise TJ, McKinnon P, Rybak M. Prediction model to identify patients with Staphylococcus aureus bacteremia at risk for methicillin resistance. Infect Control Hosp Epidemiol. 2003 Sep;24(9):655-61.

33. Lentino J, Baddour L, Wray M, Wong E, Yu V. Staphylococcus aureus and other bacteremias in hemodialysis patients: antibiotic therapy and surgical removal of access site. Infection.28(6):355-60.

34. Marr K, Kong L, Fowler V, Gopal A, Sexton D, Conlon P, et al. Incidence and outcome of Staphylococcus aureus bacteremia in hemodialysis patients. Kidney Int. 1998 Nov;54(5):1684-9.

35. Dobkin J, Miller M, Steigbigel N. Septicemia in patients on chronic hemodialysis. Ann Intern Med. 1978 Jan;88(1):28-33.

36. Kessler M, Hoen B, Mayeux D, Hestin D, Fontenaille C. Bacteremia in patients on chronic hemodialysis. A multicenter prospective survey. Nephron. 1993;64(1):95-100.

37. Hoen B, Paul-Dauphin A, Hestin D, Kessler M. EPIBACDIAL: a multicenter prospective study of risk factors for bacteremia in chronic hemodialysis patients. J Am Soc Nephrol. 1998 May;9(5):869-76.

38. Goldman M, Vanherweghem J. Bacterial infections in chronic hemodialysis patients: epidemiologic and pathophysiologic aspects. Adv Nephrol Necker Hosp. 1990;19:315-32.

39. Boelaert J, Daneels R, Schurgers M, Matthys E, Gordts B, Van Landuyt H. Iron overload in haemodialysis patients increases the risk of bacteraemia: a prospective study. Nephrol Dial Transplant. 1990;5(2):130-4.

40. Taylor G, McKenzie M, Buchanan-Chell M, Caballo L, Chui L, Kowalewska-Grochowska K. Central venous catheters as a source of hemodialysis-related bacteremia. Infect Control Hosp Epidemiol. 1998 Sep;19(9):643-6.

41. Hoen B, Kessler M, Hestin D, Mayeux D. Risk factors for bacterial infections in chronic haemodialysis adult patients: a multicentre prospective survey. Nephrol Dial Transplant. 1995;10(3):377-81.

42. Powe N, Jaar B, Furth S, Hermann J, Briggs W. Septicemia in dialysis patients: incidence, risk factors, and prognosis. Kidney Int. 1999 Mar;55(3):1081-90.

43. Bonomo R, Rice D, Whalen C, Linn D, Eckstein E, Shlaes D. Risk factors associated with permanent access-site infections in chronic hemodialysis patients. Infect Control Hosp Epidemiol. 1997 Nov;18(11):757-61.

44. Levine D, Crane L, Zervos M. Bacteremia in narcotic addicts at the Detroit Medical Center. II. Infectious endocarditis: a prospective comparative study. Rev Infect Dis.8(3):374-96.

45. Crane L, Levine D, Zervos M, Cummings G. Bacteremia in narcotic addicts at the Detroit Medical Center. I. Microbiology, epidemiology, risk factors, and empiric therapy. Rev Infect Dis.8(3):364-73.

46. Bassetti S, Battegay M. Staphylococcus aureus infections in injection drug users: risk factors and prevention strategies. Infection. 2004 Jun;32(3):163-9.

47. Ruotsalainen E, Sammalkorpi K, Laine J, Huotari K, Sarna S, Valtonen V, et al. Clinical manifestations and outcome in Staphylococcus aureus endocarditis among injection drug users and nonaddicts: a prospective study of 74 patients. BMC Infect Dis. 2006;6:137.

48. Wertheim H, Vos M, Ott A, van Belkum A, Voss A, Kluytmans J, et al. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. Lancet.364(9435):703-5.

49. Pujol M, Peña C, Pallares R, Ariza J, Ayats J, Dominguez M, et al. Nosocomial Staphylococcus aureus bacteremia among nasal carriers of methicillin-resistant and methicillin-susceptible strains. Am J Med. 1996 May;100(5):509-16.

50. Walker S, Peto T, O'Connor L, Crook D, Wyllie D. Are there better methods of monitoring MRSA control than bacteraemia surveillance? An observational database study. PLoS ONE. 2008;3(6):e2378.

51. Khatib R, Riederer K, Saeed S, Johnson L, Fakih M, Sharma M, et al. Time to positivity in Staphylococcus aureus bacteremia: possible correlation with the source and outcome of infection. Clin Infect Dis. 2005 Sep;41(5):594-8.

52. Mylotte J, Tayara A. Staphylococcus aureus bacteremia: predictors of 30-day mortality in a large cohort. Clin Infect Dis. 2000 Nov;31(5):1170-4.

53. Khatib R, Johnson L, Fakih M, Riederer K, Khosrovaneh A, Shamse Tabriz M, et al. Persistence in Staphylococcus aureus bacteremia: incidence, characteristics of patients and outcome. Scand J Infect Dis. 2006;38(1):7-14.

54. Johnson L, Almoujahed M, Ilg K, Maolood L, Khatib R. Staphylococcus aureus bacteremia: compliance with standard treatment, long-term outcome and predictors of relapse. Scand J Infect Dis. 2003;35(11-12):782-9.

55. Cassettari V, Strabelli T, Medeiros E. Staphylococcus aureus bacteremia: what is the impact of oxacillin resistance on mortality? Braz J Infect Dis. 2005 Feb;9(1):70-6.

56. Khatib R, Johnson L, Sharma M, Fakih M, Ganga R, Riederer K. Persistent Staphylococcus aureus bacteremia: incidence and outcome trends over time. Scand J Infect Dis. 2009;41(1):4-9.

57. Cosgrove S, Qi Y, Kaye K, Harbarth S, Karchmer A, Carmeli Y. The impact of methicillin resistance in Staphylococcus aureus bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect Control Hosp Epidemiol. 2005 Feb;26(2):166-74.

58. Fätkenheuer G, Preuss M, Salzberger B, Schmeisser N, Cornely O, Wisplinghoff H, et al. Long-term outcome and quality of care of patients with Staphylococcus aureus bacteremia. Eur J Clin Microbiol Infect Dis. 2004 Mar;23(3):157-62.

59. Harbarth S, Rutschmann O, Sudre P, Pittet D. Impact of methicillin resistance on the outcome of patients with bacteremia caused by Staphylococcus aureus. Arch Intern Med. 1998 Jan;158(2):182-9.

60. Chang F, MacDonald B, Peacock JJ, Musher D, Triplett P, Mylotte J, et al. A prospective multicenter study of Staphylococcus aureus bacteremia: incidence of endocarditis, risk factors for mortality, and clinical impact of methicillin resistance. Medicine (Baltimore). 2003 Sep;82(5):322-32.

61. Shurland S, Zhan M, Bradham D, Roghmann M. Comparison of mortality risk associated with bacteremia due to methicillin-resistant and methicillin-susceptible Staphylococcus aureus. Infect Control Hosp Epidemiol. 2007 Mar;28(3):273-9.

62. Liao C, Chen S, Huang Y, Hsueh P. Outcome of patients with meticillin-resistant Staphylococcus aureus bacteraemia at an Emergency Department of a medical centre in Taiwan. Int J Antimicrob Agents. 2008 Oct;32(4):326-32.

63. Nickerson E, Wuthiekanun V, Day N, Chaowagul W, Peacock S. Meticillin-resistant Staphylococcus aureus in rural Asia. Lancet Infect Dis. 2006 Feb;6(2):70-1.

64. Charlson M, Pompei P, Ales K, MacKenzie C. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5):373-83.

65. Jensen A, Wachmann C, Espersen F, Scheibel J, Skinhøj P, Frimodt-Møller N. Treatment and outcome of Staphylococcus aureus bacteremia: a prospective study of 278 cases. Arch Intern Med. 2002 Jan;162(1):25-32.

66. McClelland R, Fowler VJ, Sanders L, Gottlieb G, Kong L, Sexton D, et al. Staphylococcus aureus bacteremia among elderly vs younger adult patients: comparison of clinical features and mortality. Arch Intern Med. 1999 Jun;159(11):1244-7.

67. Cosgrove S, Sakoulas G, Perencevich E, Schwaber M, Karchmer A, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis. 2003 Jan;36(1):53-9.

68. Bader M. Staphylococcus aureus bacteremia in older adults: predictors of 7-day mortality and infection with a methicillin-resistant strain. Infect Control Hosp Epidemiol. 2006 Nov;27(11):1219-25.

69. Mylotte J, Aeschlimann J, Rotella D. Staphylococcus aureus bacteremia: factors predicting hospital mortality. Infect Control Hosp Epidemiol. 1996 Mar;17(3):165-8.

70. Chang F. Staphylococcus aureus bacteremia and endocarditis. J Microbiol Immunol Infect. 2000 Jun;33(2):63-8.

71. Lodise T, McKinnon P, Swiderski L, Rybak M. Outcomes analysis of delayed antibiotic treatment for hospital-acquired Staphylococcus aureus bacteremia. Clin Infect Dis. 2003 Jun;36(11):1418-23.

72. Lodise T, McKinnon P. Clinical and economic impact of methicillin resistance in patients with Staphylococcus aureus bacteremia. Diagn Microbiol Infect Dis. 2005 Jun;52(2):113-22.

73. "Hsu LY" CN, "Koh TH", "Kang ML", "Tan BH", "Tambyah PA". EMRSA-15 Bacteremia is not Associated with a Worse Outcome Compared with Bacteremia Caused by Multidrug-Resistant MRSA. International Journal of Biomedical Science2007.

74. Fowler VJ, Miro J, Hoen B, Cabell C, Abrutyn E, Rubinstein E, et al. Staphylococcus aureus endocarditis: a consequence of medical progress. JAMA. 2005 Jun;293(24):3012-21.

75. Fowler VJ, Olsen M, Corey G, Woods C, Cabell C, Reller L, et al. Clinical identifiers of complicated Staphylococcus aureus bacteremia. Arch Intern Med. 2003 Sep;163(17):2066-72.

76. Hill E, Vanderschueren S, Verhaegen J, Herijgers P, Claus P, Herregods M, et al. Risk factors for infective endocarditis and outcome of patients with Staphylococcus aureus bacteremia. Mayo Clin Proc. 2007 Oct;82(10):1165-9.

77. Fowler VJ, Li J, Corey G, Boley J, Marr K, Gopal A, et al. Role of echocardiography in evaluation of patients with Staphylococcus aureus bacteremia: experience in 103 patients. J Am Coll Cardiol. 1997 Oct;30(4):1072-8.

78. Complications of Staphylococcus aureus bacteremia [database on the Internet] [cited 16-june-2009]. Available from: http://www.uptodateonline.com.

79. Mylotte J, McDermott C, Spooner J. Prospective study of 114 consecutive episodes of Staphylococcus aureus bacteremia. Rev Infect Dis.9(5):891-907.

80. Jernigan J, Farr B. Short-course therapy of catheter-related Staphylococcus aureus bacteremia: a meta-analysis. Ann Intern Med. 1993 Aug;119(4):304-11.

81. Sanabria T, Alpert J, Goldberg R, Pape L, Cheeseman S. Increasing frequency of staphylococcal infective endocarditis. Experience at a university hospital, 1981 through 1988. Arch Intern Med. 1990 Jun;150(6):1305-9.

82. Libman H, Arbeit R. Complications associated with Staphylococcus aureus bacteremia. Arch Intern Med. 1984 Mar;144(3):541-5.

83. Ehni W, Reller L. Short-course therapy for catheter-associated Staphylococcus aureus bacteremia. Arch Intern Med. 1989 Mar;149(3):533-6.

84. Chang F, Peacock JJ, Musher D, Triplett P, MacDonald B, Mylotte J, et al. Staphylococcus aureus bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. Medicine (Baltimore). 2003 Sep;82(5):333-9.

85. Fowler VJ, Kong L, Corey G, Gottlieb G, McClelland R, Sexton D, et al. Recurrent Staphylococcus aureus bacteremia: pulsed-field gel electrophoresis findings in 29 patients. J Infect Dis. 1999 May;179(5):1157-61.

86. Hartstein A, Mulligan M, Morthland V, Kwok R. Recurrent Staphylococcus aureus bacteremia. J Clin Microbiol. 1992 Mar;30(3):670-4.

87. Selvey L, Whitby M, Johnson B. Nosocomial methicillin-resistant Staphylococcus aureus bacteremia: is it any worse than nosocomial methicillin-sensitive Staphylococcus aureus bacteremia? Infect Control Hosp Epidemiol. 2000 Oct;21(10):645-8.

88. Melzer M, Eykyn S, Gransden W, Chinn S. Is methicillin-resistant Staphylococcus aureus more virulent than methicillin-susceptible S. aureus? A comparative cohort study of British patients with nosocomial infection and bacteremia. Clin Infect Dis. 2003 Dec;37(11):1453-60.

89. Talon D, Woronoff-Lemsi M, Limat S, Bertrand X, Chatillon M, Gil H, et al. The impact of resistance to methicillin in Staphylococcus aureus bacteremia on mortality. Eur J Intern Med. 2002 Feb;13(1):31-6.

90. Blot S, Vandewoude K, Hoste E, Colardyn F. Outcome and attributable mortality in critically Ill patients with bacteremia involving methicillin-susceptible and methicillin-resistant Staphylococcus aureus. Arch Intern Med. 2002 Oct;162(19):2229-35.

91. Knaus W, Draper E, Wagner D, Zimmerman J. APACHE II: a severity of disease classification system. Crit Care Med. 1985 Oct;13(10):818-29.

92. "McCabe W" JG. Gram-negative bacteremia. Archives of Internal Medicine1962. p. 847-55.

93. Collopy B, Dalton M, Wright C, Mullany C. Comparison of the clinical significance of methicillin-resistant and methicillin-sensitive Staphylococcus aureus isolations. Med J Aust. 1984 Feb;140(4):211-4.

94. Asensio A, Guerrero A, Quereda C, Lizán M, Martinez-Ferrer M. Colonization and infection with methicillin-resistant Staphylococcus aureus: associated factors and eradication. Infect Control Hosp Epidemiol. 1996 Jan;17(1):20-8.

95. Engemann J, Carmeli Y, Cosgrove S, Fowler V, Bronstein M, Trivette S, et al. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with Staphylococcus aureus surgical site infection. Clin Infect Dis. 2003 Mar;36(5):592-8.

96. Graffunder E, Venezia R. Risk factors associated with nosocomial methicillin-resistant Staphylococcus aureus (MRSA) infection including previous use of antimicrobials. J Antimicrob Chemother. 2002 Jun;49(6):999-1005.

97. Rubin R, Harrington C, Poon A, Dietrich K, Greene J, Moiduddin A. The economic impact of Staphylococcus aureus infection in New York City hospitals. Emerg Infect Dis.5(1):9-17.

98. Wakefield D, Helms C, Massanari R, Mori M, Pfaller M. Cost of nosocomial infection: relative contributions of laboratory, antibiotic, and per diem costs in serious Staphylococcus aureus infections. Am J Infect Control. 1988 Oct;16(5):185-92.

99. Abramson M, Sexton D. Nosocomial methicillin-resistant and methicillin-susceptible Staphylococcus aureus primary bacteremia: at what costs? Infect Control Hosp Epidemiol. 1999 Jun;20(6):408-11.

100. Fridkin S, Hageman J, McDougal L, Mohammed J, Jarvis W, Perl T, et al. Epidemiological and microbiological characterization of infections caused by Staphylococcus aureus with reduced susceptibility to vancomycin, United States, 1997-2001. Clin Infect Dis. 2003 Feb;36(4):429-39.

101. Institute/NCCLS CaLS. Performance standards for antimicrobial susceptibility testing. Sixteenth Information Supplement M100-S16 ed: Wayne PA, CLSI; 2006.

102. Reduced susceptibility of Staphylococcus aureus to vancomycin--Japan, 1996. MMWR Morb Mortal Wkly Rep. 1997 Jul;46(27):624-6.

103. Song J, Hiramatsu K, Suh J, Ko K, Ito T, Kapi M, et al. Emergence in Asian countries of Staphylococcus aureus with reduced susceptibility to vancomycin. Antimicrob Agents Chemother. 2004 Dec;48(12):4926-8.

104. Bierbaum G, Fuchs K, Lenz W, Szekat C, Sahl H. Presence of Staphylococcus aureus with reduced susceptibility to vancomycin in Germany. Eur J Clin Microbiol Infect Dis. 1999 Oct;18(10):691-6.

105. Kantzanou M, Tassios P, Tseleni-Kotsovili A, Legakis N, Vatopoulos A. Reduced susceptibility to vancomycin of nosocomial isolates of methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother. 1999 May;43(5):729-31.

106. Reverdy M, Jarraud S, Bobin-Dubreux S, Burel E, Girardo P, Lina G, et al. Incidence of Staphylococcus aureus with reduced susceptibility to glycopeptides in two French hospitals. Clin Microbiol Infect. 2001 May;7(5):267-72.

107. Pina P, Marliere C, Vandenesch F, Bedos J, Etienne J, Allouch P. An outbreak of Staphylococcus aureus strains with reduced susceptibility to glycopeptides in a French general hospital. Clin Infect Dis. 2000 Nov;31(5):1306-8.

108. Guerin F, Buu-Hoï A, Mainardi J, Kac G, Colardelle N, Vaupré S, et al. Outbreak of methicillin-resistant Staphylococcus aureus with reduced susceptibility to glycopeptides in a Parisian hospital. J Clin Microbiol. 2000 Aug;38(8):2985-8.

109. Sng L, Koh T, Wang G, Hsu L, Kapi M, Hiramatsu K. Heterogeneous vancomycin-resistant Staphylococcus aureus (hetero-VISA) in Singapore. Int J Antimicrob Agents. 2005 Feb;25(2):177-9.

110. Staphylococcus aureus with reduced susceptibility to vancomycin--United States, 1997. MMWR Morb Mortal Wkly Rep. 1997 Aug;46(33):765-6.

111. Ward P, Johnson P, Grabsch E, Mayall B, Grayson M. Treatment failure due to methicillin-resistant Staphylococcus aureus (MRSA) with reduced susceptibility to vancomycin. Med J Aust. 2001 Nov;175(9):480-3.

112. Sievert D, Rudrik J, Patel J, McDonald L, Wilkins M, Hageman J. Vancomycin-resistant Staphylococcus aureus in the United States, 2002-2006. Clin Infect Dis. 2008 Mar;46(5):668-74.

113. Tiwari H, Sen M. Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infect Dis. 2006;6:156.

114. Emaneini M, Aligholi M, Hashemi F, Jabalameli F, Shahsavan S, Dabiri H, et al. Isolation of vancomycin-resistant Staphylococcus aureus in a teaching hospital in Tehran. J Hosp Infect. 2007 May;66(1):92-3.

115. Moise P, Smyth D, El-Fawal N, Robinson D, Holden P, Forrest A, et al. Microbiological effects of prior vancomycin use in patients with methicillin-resistant Staphylococcus aureus bacteraemia. J Antimicrob Chemother. 2008 Jan;61(1):85-90.

116. Sakoulas G, Eliopoulos G, Moellering RJ, Wennersten C, Venkataraman L, Novick R, et al. Accessory gene regulator (agr) locus in geographically diverse

Staphylococcus aureus isolates with reduced susceptibility to vancomycin. Antimicrob Agents Chemother. 2002 May;46(5):1492-502.

117. Sakoulas G, Moellering RJ. Increasing antibiotic resistance among methicillin-resistant Staphylococcus aureus strains. Clin Infect Dis. 2008 Jun;46 Suppl 5:S360-7.

118. Sakoulas G, Moise-Broder P, Schentag J, Forrest A, Moellering RJ, Eliopoulos G. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant Staphylococcus aureus bacteremia. J Clin Microbiol. 2004 Jun;42(6):2398-402.

119. Maclayton D, Suda K, Coval K, York C, Garey K. Case-control study of the relationship between MRSA bacteremia with a vancomycin MIC of 2 microg/mL and risk factors, costs, and outcomes in inpatients undergoing hemodialysis. Clin Ther. 2006 Aug;28(8):1208-16.

120. Lodise T, Miller C, Graves J, Evans A, Graffunder E, Helmecke M, et al. Predictors of high vancomycin MIC values among patients with methicillin-resistant Staphylococcus aureus bacteraemia. J Antimicrob Chemother. 2008 Nov;62(5):1138-41.

121. Neoh H, Hori S, Komatsu M, Oguri T, Takeuchi F, Cui L, et al. Impact of reduced vancomycin susceptibility on the therapeutic outcome of MRSA bloodstream infections. Ann Clin Microbiol Antimicrob. 2007;6:13.

122. Charles P, Ward P, Johnson P, Howden B, Grayson M. Clinical features associated with bacteremia due to heterogeneous vancomycin-intermediate Staphylococcus aureus. Clin Infect Dis. 2004 Feb;38(3):448-51.

123. Maor Y, Rahav G, Belausov N, Ben-David D, Smollan G, Keller N. Prevalence and characteristics of heteroresistant vancomycin-intermediate Staphylococcus aureus bacteremia in a tertiary care center. J Clin Microbiol. 2007 May;45(5):1511-4.

124. Maor Y, Hagin M, Belausov N, Keller N, Ben-David D, Rahav G. Clinical features of heteroresistant vancomycin-intermediate Staphylococcus aureus bacteremia versus those of methicillin-resistant S. aureus bacteremia. J Infect Dis. 2009 Mar;199(5):619-24.

125. Khosrovaneh A, Riederer K, Saeed S, Tabriz M, Shah A, Hanna M, et al. Frequency of reduced vancomycin susceptibility and heterogeneous subpopulation in persistent or recurrent methicillin-resistant Staphylococcus aureus bacteremia. Clin Infect Dis. 2004 May;38(9):1328-30.

126. Rybak M, Leonard S, Rossi K, Cheung C, Sader H, Sadar H, et al. Characterization of vancomycin-heteroresistant Staphylococcus aureus from the metropolitan area of Detroit, Michigan, over a 22-year period (1986 to 2007). J Clin Microbiol. 2008 Sep;46(9):2950-4.

127. Walsh T, Bolmström A, Qwärnström A, Ho P, Wootton M, Howe R, et al. Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. J Clin Microbiol. 2001 Jul;39(7):2439-44.

128. Leonard S, Rybak M. Evaluation of vancomycin and daptomycin against methicillin-resistant Staphylococcus aureus and heterogeneously vancomycin-intermediate S. aureus in an in vitro pharmacokinetic/pharmacodynamic model with simulated endocardial vegetations. J Antimicrob Chemother. 2009 Jan;63(1):155-60.

129. Turner B, McKee L, Fanning T, Markson L. AIDS specialist versus generalist ambulatory care for advanced HIV infection and impact on hospital use. Med Care. 1994 Sep;32(9):902-16.

130. Jollis J, DeLong E, Peterson E, Muhlbaier L, Fortin D, Califf R, et al. Outcome of acute myocardial infarction according to the specialty of the admitting physician. N Engl J Med. 1996 Dec;335(25):1880-7.

131. Fowler VJ, Sanders L, Sexton D, Kong L, Marr K, Gopal A, et al. Outcome of Staphylococcus aureus bacteremia according to compliance with recommendations of infectious diseases specialists: experience with 244 patients. Clin Infect Dis. 1998 Sep;27(3):478-86.

132. Jenkins T, Price C, Sabel A, Mehler P, Burman W. Impact of routine infectious diseases service consultation on the evaluation, management, and outcomes of Staphylococcus aureus bacteremia. Clin Infect Dis. 2008 Apr;46(7):1000-8.

133. Kim S, Kim K, Kim H, Kim N, Kim E, Oh M, et al. Outcome of vancomycin treatment in patients with methicillin-susceptible Staphylococcus aureus bacteremia. Antimicrob Agents Chemother. 2008 Jan;52(1):192-7.

134. Lodise TJ, McKinnon P, Levine D, Rybak M. Impact of empirical-therapy selection on outcomes of intravenous drug users with infective endocarditis caused by methicillin-susceptible Staphylococcus aureus. Antimicrob Agents Chemother. 2007 Oct;51(10):3731-3.

135. Khatib R, Saeed S, Sharma M, Riederer K, Fakih M, Johnson L. Impact of initial antibiotic choice and delayed appropriate treatment on the outcome of Staphylococcus aureus bacteremia. Eur J Clin Microbiol Infect Dis. 2006 Mar;25(3):181-5.

136. Stevens D, Bisno A, Chambers H, Everett E, Dellinger P, Goldstein E, et al. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clin Infect Dis. 2005 Nov;41(10):1373-406.

137. "Fowler VG " SD. Treatment of Staphylococcus aureus bacteremia in adults. www.uptodate.com2008.

138. Raad I, Sabbagh M. Optimal duration of therapy for catheter-related Staphylococcus aureus bacteremia: a study of 55 cases and review. Clin Infect Dis. 1992 Jan;14(1):75-82.

139. Naber C, Baddour L, Giamarellos-Bourboulis E, Gould I, Herrmann M, Hoen B, et al. Clinical Consensus Conference: survey on Gram-Positive Bloodstream Infections with a focus on Staphylococcus aureus. Clin Infect Dis. 2009 May;48 Suppl 4:S260-70.

140. Kim S, Park W, Lee K, Kang C, Bang J, Kim H, et al. Outcome of inappropriate initial antimicrobial treatment in patients with methicillin-resistant Staphylococcus aureus bacteraemia. J Antimicrob Chemother. 2004 Aug;54(2):489-97.

141. Romero-Vivas J, Rubio M, Fernandez C, Picazo J. Mortality associated with nosocomial bacteremia due to methicillin-resistant Staphylococcus aureus. Clin Infect Dis. 1995 Dec;21(6):1417-23.

142. Soriano A, Martínez J, Mensa J, Marco F, Almela M, Moreno-Martínez A, et al. Pathogenic significance of methicillin resistance for patients with Staphylococcus aureus bacteremia. Clin Infect Dis. 2000 Feb;30(2):368-73.

143. Marr K, Sexton D, Conlon P, Corey G, Schwab S, Kirkland K. Catheter-related bacteremia and outcome of attempted catheter salvage in patients undergoing hemodialysis. Ann Intern Med. 1997 Aug;127(4):275-80.

144. Kim S, Park W, Lee K, Kang C, Kim H, Oh M, et al. Outcome of Staphylococcus aureus bacteremia in patients with eradicable foci versus noneradicable foci. Clin Infect Dis. 2003 Sep;37(6):794-9.

145. Watanakunakorn C, Baird I. Staphylococcus aureus bacteremia and endocarditis associated with a removable infected intravenous device. Am J Med. 1977 Aug;63(2):253-6.

146. Deurenberg R, Vink C, Kalenic S, Friedrich A, Bruggeman C, Stobberingh E. The molecular evolution of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect. 2007 Mar;13(3):222-35.

147. Cookson B, Robinson D, Monk A, Murchan S, Deplano A, de Ryck R, et al. Evaluation of molecular typing methods in characterizing a European collection of epidemic methicillin-resistant Staphylococcus aureus strains: the HARMONY collection. J Clin Microbiol. 2007 Jun;45(6):1830-7.

148. "Koneman EW" AS, "Janda WM", "Schreckenberger PC","Winn WC". Color Atlas and Diagnostic Microbiology. Fifth ed: Lippincott Williams and Wilkins; 1997.

149. Enright M, Day N, Davies C, Peacock S, Spratt B. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol. 2000 Mar;38(3):1008-15.

150. Shopsin B, Gomez M, Montgomery S, Smith D, Waddington M, Dodge D, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol. 1999 Nov;37(11):3556-63.

151. Strommenger B, Kettlitz C, Weniger T, Harmsen D, Friedrich A, Witte W. Assignment of Staphylococcus isolates to groups by spa typing, SmaI macrorestriction analysis, and multilocus sequence typing. J Clin Microbiol. 2006 Jul;44(7):2533-40.

152. Malachowa N, Sabat A, Gniadkowski M, Krzyszton-Russjan J, Empel J, Miedzobrodzki J, et al. Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, spa typing, and multilocus sequence typing for clonal characterization of Staphylococcus aureus isolates. J Clin Microbiol. 2005 Jul;43(7):3095-100.

153. Hallin M, Deplano A, Denis O, De Mendonça R, De Ryck R, Struelens M. Validation of pulsed-field gel electrophoresis and spa typing for long-term, nationwide epidemiological surveillance studies of Staphylococcus aureus infections. J Clin Microbiol. 2007 Jan;45(1):127-33.

154. Koreen L, Ramaswamy S, Graviss E, Naidich S, Musser J, Kreiswirth B. spa typing method for discriminating among Staphylococcus aureus isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol. 2004 Feb;42(2):792-9.

155. Popovich K, Weinstein R, Hota B. Are community-associated methicillin-resistant Staphylococcus aureus (MRSA) strains replacing traditional nosocomial MRSA strains? Clin Infect Dis. 2008 Mar;46(6):787-94.

156. Hsu L, Koh T, Singh K, Kang M, Kurup A, Tan B. Dissemination of multisusceptible methicillin-resistant Staphylococcus aureus in Singapore. J Clin Microbiol. 2005 Jun;43(6):2923-5.

157. Hsu L, Loomba-Chlebicka N, Koh Y, Tan T, Krishnan P, Lin R, et al. Evolving EMRSA-15 epidemic in Singapore hospitals. J Med Microbiol. 2007 Mar;56(Pt 3):376-9.

158. Lalani T, Federspiel J, Boucher H, Rude T, Bae I, Rybak M, et al. Associations between the genotypes of Staphylococcus aureus bloodstream isolates and clinical characteristics and outcomes of bacteremic patients. J Clin Microbiol. 2008 Sep;46(9):2890-6.

159. Feil E, Cooper J, Grundmann H, Robinson D, Enright M, Berendt T, et al. How clonal is Staphylococcus aureus? J Bacteriol. 2003 Jun;185(11):3307-16.

160. Fowler VJ, Nelson C, McIntyre L, Kreiswirth B, Monk A, Archer G, et al. Potential associations between hematogenous complications and bacterial genotype in Staphylococcus aureus infection. J Infect Dis. 2007 Sep;196(5):738-47.

161. Melles D, Gorkink R, Boelens H, Snijders S, Peeters J, Moorhouse M, et al. Natural population dynamics and expansion of pathogenic clones of Staphylococcus aureus. J Clin Invest. 2004 Dec;114(12):1732-40.

162. van Belkum A, Melles D, Snijders S, van Leeuwen W, Wertheim H, Nouwen J, et al. Clonal distribution and differential occurrence of the enterotoxin gene cluster, egc, in carriage- versus bacteremia-associated isolates of Staphylococcus aureus. J Clin Microbiol. 2006 Apr;44(4):1555-7.

163. Kuehnert M, Hill H, Kupronis B, Tokars J, Solomon S, Jernigan D. Methicillin-resistant-Staphylococcus aureus hospitalizations, United States. Emerg Infect Dis. 2005 Jun;11(6):868-72.

164. G H. Healthcare-Associated Methicillin-Resistant Staphylococcus Aureus(MRSA) Infections in Singapore. 2007 [cited 2009 7, July]; Available from: http://www.moh.gov.sg/mohcorp/publicationsinfopapers.aspx?id=17810.

165. European Antimicrobial Resistance Surveillance System (EARSS). 2007.

166. Bell J, Turnidge J. High prevalence of oxacillin-resistant Staphylococcus aureus isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY antimicrobial surveillance program, 1998-1999. Antimicrob Agents Chemother. 2002 Mar;46(3):879-81.

167. Kotilainen P, Routamaa M, Peltonen R, Oksi J, Rintala E, Meurman O, et al. Elimination of epidemic methicillin-resistant Staphylococcus aureus from a university hospital and district institutions, Finland. Emerg Infect Dis. 2003 Feb;9(2):169-75.

168. Faria N, Oliveira D, Westh H, Monnet D, Larsen A, Skov R, et al. Epidemiology of emerging methicillin-resistant Staphylococcus aureus (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. J Clin Microbiol. 2005 Apr;43(4):1836-42.

169. Bartels M, Kristoffersen K, Boye K, Westh H. Rise and subsequent decline of community-associated methicillin resistant Staphylococcus aureus ST30-IVc in Copenhagen, Denmark through an effective search and destroy policy. Clin Microbiol Infect. 2010 Jan;16(1):78-83.

170. van Trijp M, Melles D, Hendriks W, Parlevliet G, Gommans M, Ott A. Successful control of widespread methicillin-resistant Staphylococcus aureus colonization and infection in a large teaching hospital in the Netherlands. Infect Control Hosp Epidemiol. 2007 Aug;28(8):970-5.

171. Bootsma M, Diekmann O, Bonten M. Controlling methicillin-resistant Staphylococcus aureus: quantifying the effects of interventions and rapid diagnostic testing. Proc Natl Acad Sci U S A. 2006 Apr;103(14):5620-5.

172. Chow J, Yu V. Combination antibiotic therapy versus monotherapy for gram-negative bacteraemia: a commentary. Int J Antimicrob Agents. 1999 Jan;11(1):7-12.

173. Yzerman E, Boelens H, Tjhie J, Kluytmans J, Mouton J, Verbrugh H. Delta APACHE II for predicting course and outcome of nosocomial Staphylococcus aureus bacteremia and its relation to host defense. J Infect Dis. 1996 Apr;173(4):914-9.

174. Poses R, McClish D, Smith W, Bekes C, Scott W. Prediction of survival of critically ill patients by admission comorbidity. J Clin Epidemiol. 1996 Jul;49(7):743-7.

175. Lowy F. Staphylococcus aureus infections. N Engl J Med. 1998 Aug;339(8):520-32.

176. Fowler VJ, Justice A, Moore C, Benjamin DJ, Woods C, Campbell S, et al. Risk factors for hematogenous complications of intravascular catheter-associated Staphylococcus aureus bacteremia. Clin Infect Dis. 2005 Mar;40(5):695-703.

177. Sullenberger A, Avedissian L, Kent S. Importance of transesophageal echocardiography in the evaluation of Staphylococcus aureus bacteremia. J Heart Valve Dis. 2005 Jan;14(1):23-8.

178. Rosen A, Fowler VJ, Corey G, Downs S, Biddle A, Li J, et al. Cost-effectiveness of transesophageal echocardiography to determine the duration of therapy for intravascular catheter-associated Staphylococcus aureus bacteremia. Ann Intern Med. 1999 May;130(10):810-20.

179. Kuruppu J, Corretti M, Mackowiak P, Roghmann M. Overuse of transthoracic echocardiography in the diagnosis of native valve endocarditis. Arch Intern Med.162(15):1715-20.

180. Heidenreich P, Masoudi F, Maini B, Chou T, Foster E, Schiller N, et al. Echocardiography in patients with suspected endocarditis: a cost-effectiveness analysis. Am J Med. 1999 Sep;107(3):198-208.

181. Lodise T, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro B, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant Staphylococcus aureus bacteremia treated with vancomycin. Antimicrob Agents Chemother. 2008 Sep;52(9):3315-20.

182. Voss A, Mouton J, van Elzakker E, Hendrix R, Goessens W, Kluytmans J, et al. A multi-center blinded study on the efficiency of phenotypic screening methods to detect glycopeptide intermediately susceptible Staphylococcus aureus (GISA) and heterogeneous GISA (h-GISA). Ann Clin Microbiol Antimicrob. 2007;6:9.

183. Tenover F, Moellering RJ. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for Staphylococcus aureus. Clin Infect Dis. 2007 May;44(9):1208-15.

184. Mohr J, Murray B. Point: Vancomycin is not obsolete for the treatment of infection caused by methicillin-resistant Staphylococcus aureus. Clin Infect Dis. 2007 Jun;44(12):1536-42.

185. Wang J, Chen S, Wang J, Wu G, Chiang W, Hsueh P, et al. Comparison of both clinical features and mortality risk associated with bacteremia due to community-

acquired methicillin-resistant Staphylococcus aureus and methicillin-susceptible S. aureus. Clin Infect Dis. 2008 Mar;46(6):799-806.

Appendix 1: DNA extraction protocol

Materials required

1M Tris-Cl pH 8.0 solution
0.5 M Sodium EDTA solution
Triton® X-100
Sterile water
Lysozyme (10mg/ml) solution
Lysostaphin (1mg/ml) solution
QIAGEN DNeasy® Blood and tissue Kit
1.5-2 ml microcentrifuge tube
Heating block(at 56^{O}C)
Water bath (at 37^{O} C)
Centrifuge

Preparation of enzymatic lysis buffer

Enzymatic lysis buffer (stock) was prepared in the proportion given below (Table 1). The final working solution was prepared fresh by adding lysozyme and lysostaphin toi the stock in the proportions given in Table 2

Table 1: Constitution of the enzymatic lysis buffer stock

Constituents	For 10 ml lysis solution (in µl)	For 50 ml lysis solution (in µl)
Tris Cl (1M) pH 8.0	200	1000
Sodium EDTA(0.5M)	40	200
Triton® X-100	120	600

Table 2: Final working solution of enzymatic lysis buffer

No of samples	Amount of lysis buffer	Lysostaphin (1mg/ml) (in µl)	Lysozyme (10mg/ml) (in µl)
5	1000	30	30
10	2000	60	60
15	3000	90	90
20	4000	120	120
25	5000	150	150
30	6000	180	180

Procedure

1. 8-10 colonies of *Staphylococcus aureus* from a blood agar plate (subcultured the previous day) was suspended in 180ul of lysis buffer in a microcentrifuge tube
2. The mixture was vortexed
3. Incubate for 30 min at $37^{O}C$
4. Add 25µl of Proteinase K and 200µl of Buffer AL (without ethanol). Mix by vortexing
5. Incubate at $56^{o}C$ for 30 minutes
6. Add 200µl of ethanol to the samples and mix thoroughly by vortexing
7. Place the mixture into a DNeasy Minispin column placed in a 2ml collection tube (provided in the kit). Centrifuge at 8000rpm for 1min. Discard the flow-though and collection tube
8. Place the DNeasy Minispin column in a new collection tube. Add 500µl of Buffer AW1 and centrifuge at 8000rpm for 1min. Discard the flow-though and collection tube
9. Place the DNeasy Minispin column in a new collection tube. Add 500µl of Buffer AW2 and centrifuge at 14000rpm for 3min. Discard the flow-though and collection tube
10. Place the DNeasy Minispin column in a clean 1.5-2 ml microcentrifuge tube and pipet 200µl of Buffer AE(elution buffer) directly on the DNeasy membrane. Incubate at room temperature for 1 min and then centrifuge at 8000rpm for 1 min
11. Discard the flow through and store the DNA in microcentrifuge tube at $-20^{o}C$
12. The quality of DNA was measured with a spectrophotometer before use.

Appendix 2: PCR Purification

The protocol used was based on the number of bands visualised on a gel. If a single band was present for each sample then the *QIAquick PCR Purification Kit* was used(Protocol 1). If more than one bands was present , then the sample was rerun with 20ul of PCR products and each individual band was cut and DNA extracted using the *QIAquick gel extraction* kit (Protocol 2).

PROTOCOL 1 (PCR PURIFICATION)

1. Add 5 volumes of buffer PBI to 1 volume of the PCR sample into the 1.5 ml microcentrifuge tube and mix.
2. Place a QIAquick spin column in a provided 2 ml collection tube
3. Transfer the sample mixture from the 1.5 ml microcentrifuge tube to the QIAquick spin column with the collection tube.
4. To bind DNA, centrifuge the QIAquick column for 30 – 60 s.
5. Discard the flow-through in the collection tube. Replace the collection tube to the same spin column.
6. To wash, add 0.75 ml Buffer PE to the QIAquick column and leave it to stand for 5 min.
7. Centrifuge the QIAquick column for 30 – 60 s.
8. Discard the flow-through in the collection tube and reuse it. Centrifuge the column for an additional 1 min.
9. Discard the collection tube and place the QIAquick column in a new, labelled 1.5 ml microcentrifuge tube.
10. To elute the DNA, add 50 µl Buffer EB to the center of the QIAquick membrane and leave it to stand for 1 min. Then centrifuge the column for 1 min.
11. Discard the QIAquick column and keep the purified DNA collected in the 1.5 ml microcentrifuge tube.

PROTOCOL 2 (GEL EXTRACTION)

1. Excise the DNA fragment from the agarsoe gel using a clean scalpel
2. Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100mg~100 µl)
3. Incubate at 50^{o}C for 10 min. Vortex intermittently every 2-3 min
4. After the gel slice has dissolved completely, check that the mixture is yeloow in color.
5. Add 1 gel volume of isopropanol to the sample ans mix
6. Place a QIAquick spin column in a 2 ml collection tube (provided in the kit)
7. To bind DNA, add the sample to the QIAquick column and centrifuge for 1min
8. Discard the flow through and place the QIAquick column in the same collection tube.
9. Add 0.5 ml of Buffer QG to QIAquick and centrifuge for 1min.
10. To wash, add 0.75 ml Buffer PE to the QIAquick column and leave it to stand for 5 min.
11. Centrifuge the QIAquick column for 30 – 60 s.
12. Discard the flow-through in the collection tube and reuse it. Centrifuge the column for an additional 1 min.

13. Discard the collection tube and place the QIAquick column in a new, labelled 1.5 ml microcentrifuge tube.
14. To elute the DNA, add 50 µl Buffer EB to the center of the QIAquick membrane and leave it to stand for 1 min. Then centrifuge the column for 1 min.
15. Discard the QIAquick column and keep the purified DNA collected in the 1.5 ml microcentrifuge tube.