SURGICAL SITE INFECTIONS AMONG PATIENTS UNDERWENT CLEAN AND CLEAN-CONTAMINATED SURGERY IN HOSPITAL UNIVERSITI SAINS MALAYSIA: RISK FACTORS, MICROBIOLOGICAL AND *Staphylococcus aureus* MOLECULAR PROFILE

By

WONG JUN LEONG

Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

DECEMBER 2015

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisor Associate Professor Dr. Siti Asma' binti Hassan for allowing me to join her research group and also for her continuous encouragement, patience, and guidance over the years. She has enlightened me how to conduct research experiments and write research papers. In addition, I am grateful to my co-supervisor, Professor Dr. Habsah binti Hasan, Professor Dr. Mohamad Ziyadi bin Hj Ghazali and Dr. Zaidi bin Zakaria, and biostatistician Dr. Siti Azrin binti Ab Hamid for their constant advice and problem solving assistance that making me able to complete my study well.

I want to thank my colleagues Chan Shiao Ee, Engku Ibrahim Syubli bin Engku Safruddin, Engku Nur Syafirah binti Engku Abd Rahman, Fitrien binti Husin, Low Kim Fatt, Mohd Fazli bin Ismail, Muhammad Azharuddin bin Azali, Muhammad Lukman bin Yahya, Nik Zuraina binti Nik Mohd Noor, Nur Adila binti Zakaria, Nur Adlina binti Zainuddin, Nur Amalina binti Khazani, Nur Izzah Farakhin binti Ayub, Nur Izzati binti Hamdan, Nurul Najian binti Aminuddin Baki, Siti Nurain binti Osman, Tengku Ahmad Akram bin Tengku Mohd Ariffin, Yasmin Kahirani binti Muhammad Ismadi and others for their support, enthusiasm, and friendship that have helped me through many failed experiments. I also gratefully acknowledge all lecturers, administrative officers, Amanina binti Aminuddin, Fadzilah binti Hj Ahmad as well as other Medical Lab Technologists, from the Department of Medical Microbiology and Parasitology, Universiti Sains Malaysia (USM) for their dedication in helping me and answering all of my numerous questions.

I would also like to thank the research funding support received in the form of a Short Term Grant (304.PPSP.61312112) from USM. In addition, support from Pahang State Foundation in the form of an education loan is gratefully acknowledged.

Last but not least, my deep appreciation goes to my parents (Wong Chai Sing, Lau Poh Chian), siblings (Jun Yen, Jin Yee and Jun Chau), Lim Zhe Xin and family who have influenced me the most and were always to back up and encourage me. They have been my source of strength. I dedicate this thesis to them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

°C …… Degree Celcius

BLAST …… Basic Local Alignment Search Tool

BMI …… Body Mass Index

CABG …… Coronary Artery Bypass Graft

CDC …… Centers for Disease Control and Prevention

cna …… Collagen adhesins

dATP …… Deoxyadenosine triphosphate

dCTP …… Deoxycytidine triphosphate

dGTP …… Deoxyguanosine triphosphate

DM …… Diabetes Mellitus

DNA …… Deoxyribonucleic acid

dTTP …… Deoxythymidine triphosphate

ESBL …… Extended spectrum beta-lactamase

HCAI …… Healthcare associated infection

hlg …… Gamma haemolysin

IBM …… International Business Machines Corporation

ica …… Polysaccharide intercellular adhesins

ILTKP …… Infeksi Luka di Tapak Kawasan Pembedahan

MIC ……. Minimal inhibitory concentration

MRSA …… Methicillin-resistance *Staphylococcus aureus*

MSSA …… Methicillin-sensitive *Staphylococcus aureus*

NCBI …… National Center for Biotechnology Information

NICE …….. The National Institute for Health and Care Excellence

PCR …… Polymerase Chain Reaction

PVL …….. Panton-Valentine leukocidin

S. aureus ……. *Staphylococcus aureus*

SdrE …….. Putative adhesins

SPSS …… Software Package used for Statistical Analysis

SSI …….. Surgical Site Infection

TBE …….. Tris-Biorate-EDTA

TSS …… Toxic Shock Syndrome

TSST-1 …….. Toxic Shock Syndrome Toxin 1

USM …….. Universiti Sains Malaysia

JANGKITAN PADA KAWASAN TAPAK PEMBEDAHAN DALAM KALANGAN PESAKIT YANG MENJALANI PEMBEDAHAN BERSIH DAN PEMBEDAHAN BERSIH TERCEMAR DI HOSPITAL UNIVERSITI SAINS MALAYSIA: FAKTOR RISIKO, PROFIL *Staphylococcus aureus* DAN PROFIL MIKROBIOLOGI

ABSTRAK

Infeksi luka di tapak kawasan pembedahan (ILTKP) adalah diantara masalah kesihatan yang kerap dialami oleh pesakit yang menjalani pembedahan di hospital. Kejadian ini dipantau secara rutin kerana ia melibatkan peningkatan kadar mortaliti, morbiditi, dan juga kos rawatan. Jangkitan ini biasanya disebabkan oleh mikroorganisma seperti *Staphylococcus aureus*, *Streptococcus* spp, *Enterococcus* spp, dan *Pseudomonas aeruginosa*. Kenalpasti jenis mikroorganisma penyebab serta corak kepekaan antimikrobial amat membantu dalam pelan rawatan. Oleh itu, tujuan kajian ini adalah untuk menentukan kadar dan faktor risiko ILTKP serta mengenal pasti agen penyebab dan corak kepekaan antimikrobialnya.

Prospektif kajian kohort ini dijalankan dari Jun 2013 hingga Julai 2014 di Hospital Universiti Sains Malaysia. Tujuh puluh dua orang pesakit yang menjalani pembedahan bersih dan bersih tercemar telah bersetuju untuk menyertai kajian ini dan mereka dipantau sama ada terdapat sebarang tanda-tanda ILTKP selama 30 hari selepas pembedahan. Pemeriksaan calitan hidung untuk mengesan *Staphylococcus aureus* dan Methicillin-resistance *Staphylococcus aureus* juga telah dijalankan sebelum pembedahan. Sampel tisu atau swab luka telah diambil

dari luka pesakit yang dijangkiti untuk mengenal pasti mikroorganisma yang menyebabkan jangkitan serta corak kepekaan antimikrobialnya. Bagi mikroorganisma *Staphylococcus aureus* analisis molekular telah dijalankan untuk mengesan kewujudan gen virulen seperti *TSST, PVL*, *cna, hlg, icaA*, dan *SdrE*.

Prevalen ILTKP untuk pembedahan bersih dan bersih tercemar adalah masing-masing 20% dan 11.8%. Analisis regresis logistik ringkas menunjukkan bahawa risiko berkaitan ILTKP ialah pesakit yang dimasukkan dua hari dan lebih sebelum pembedahan (OR 12.67; 95% CI, 2.02 to 79.53), menjalani pembedahan CABG (OR 10.20; 95% CI, 2.66 to 39.08), mempunyai penyakit yang tersirat (OR 9.46; 95% CI, 1.15 to 77.50), sejarah kencing manis sebelum pembedahan (OR 9.40; 95% CI, 2.36 to 37.39), jumlah tempoh dimasukkan ke hospital lebih daripada tujuh hari (OR 7.84; 95% CI, 2.12 to 29.0), tempoh pembedahan melebihi empat jam (OR 7.08; 95% CI, 1.84 to 27.27), dan keluar dari hospital tiga hari atau lebih selepas pembedahan (OR 5.13; 95% CI, 1.39 to 18.84).

Analisis regresis logistik berganda pula menunjukkan bahawa pesakit yang mempunyai sejarah kencing manis (OR 6.97; 95% CI, 1.49 to 32.71) dan pesakit yang menjalani pembedahan CABG (OR 5.54; 95% CI, 1.22 to 25.03) mempunyai risiko ILTKP yang tinggi. Gram negatif mikroorganisma adalah penyebab utama ILTKP. icaA gen adalah gen virulen yang paling biasa dikesan pada *Staphylococcus aureus*.

Kesimpulannya, ILTKP pada pembedahan bersih dan bersih tercemar adalah tinggi di hospital USM. Pesakit yang menghidap kencing manis dan pesakit yang menjalani pembedahan CABG adalah berisiko tinggi untuk

mendapat ILTKP. Mikroorganisma Gram negatif adalah lebih kerap menjangkiti pesakit berbanding Gram positif dan semua mikroorganisma ini adalah dalam kumpulan yang peka kepada antibiotik.

SURGICAL SITE INFECTIONS AMONG PATIENTS UNDERWENT CLEAN AND CLEAN-CONTAMINATED SURGERY IN HOSPITAL UNIVERSITI SAINS MALAYSIA: RISK FACTORS, MICROBIOLOGICAL AND *Staphylococcus aureus* MOLECULAR PROFILE

ABSTRACT

Surgical site infections (SSI) are among the most commonly encountered healthcare associated infection. The incidence were closely been monitored as it is associated with considerable morbidity and mortality. The common aetiological agents responsible for the infection include *Staphylococcus aureus*, *Streptococcus* spp, *Enterococcus* spp, and *Pseudomonas aeruginosa*. The identification of the causative agents as well as their antimicrobial sensitivity pattern helps in the treatment plan. Therefore the aims of this study were to determine the incidence and risk factors of SSI as well as to identify the causative microorganisms and their sensitivity profile.

This prospective cohort study was conducted from June 2013 until July 2014 at Hospital Universiti Sains Malaysia. Seventy-two patients underwent clean and clean-contaminated surgeries were consented preoperatively and strictly followed up for any signs of SSI for duration of 30 days post operation. Nasal screening for *Staphylococcus aureus* and Methicillin-resistance *Staphylococcus aureus* was carried out preoperatively. Tissue samples or wound swab from infected patients were taken for microbial identification and its sensitivity pattern. *Staphylococcus aureus* strain isolated were proceed to

polymerase chain reaction analysis to detect the virulence genes (*TSST, PVL, cna, hlg, icaA*, and *SdrE*).

The overall incidence rate of SSI was 18.1% specifically for clean and clean-contaminated surgeries are 20% and 11.8%, respectively. Significant risk associated with SSI by simple logistic regression analysis included patients admitted two days or more prior to surgery (OR 12.67; 95% CI, 2.02 to 79.53), underwent CABG surgery (OR 10.20; 95% CI, 2.66 to 39.08), underlying diseases (OR 9.46; 95% CI, 1.15 to 77.50), history of diabetes mellitus (DM) prior to the surgery (OR 9.40; 95% CI, 2.36 to 37.39), total hospitalization period more than seven days (OR 7.84; 95% CI, 2.12 to 29.0), duration of surgery more than four hours (OR 7.08; 95% CI, 1.84 to 27.27), and discharged home three days or longer after surgery (OR 5.13; 95% CI, 1.39 to 18.84).

Multiple logistic regression method demonstrated that the patients who have history of DM (OR 6.97; 95% CI, 1.49 to 32.71) and underwent CABG surgery (OR 5.54; 95% CI, 1.22 to 25.03) had significant risks of SSI. Gram negative microorganism was the leading causative microorganism and in *Staphylococcus aureus* strains, icaA gene was the most common virulence gene detected.

In conclusion, SSI among clean and clean-contaminated surgeries are high in our setting. DM and patients underwent CABG operation are at high risk to get SSI. Gram negative microorganisms are common as compare to Gram positive, however they are all sensitive strains.

CHAPTER 1

INTRODUCTION

Surgery is one of the major and important branches of medicine that perform a surgical procedure to the human body for diagnostic, treatment, prevention as well as palliative purpose. Microorganisms have an opportunity to invade when a part of the body is operated during surgical procedure and causing a post-operative infection. Such infection occurred post-operatively and was referred as surgical site infection (SSI).

The previous term 'Surgical Wound Infection' was replaced by the term 'SSI' in year 1992 (Horan *et al.*, 1992). SSI is defined as an infection that occurs at site of incision, or any anatomical parts that was either opened or manipulated during the procedure that occurs within 30 days after surgery, or within one year if involve implantation (Horan *et al.*, 1992). However, based on the Centers for Disease Control and Prevention (CDC) criteria, stitch abscess was not considered as SSI because stitch abscess is considered the minimal inflammation and discharge limited only to the point of suture penetration (Horan *et al.*, 1992).

SSI remain as the most common Healthcare-associated infection (HCAI) especially in patients who underwent surgery (Dionigi *et al.*, 2001). According to a surveillance done in the United States acute hospital in year 2011, SSI made up of 21.8% of inpatient infections (Magill *et al.*, 2014).

Studies reported that prevalence rate of SSI were range between 1.8% and 20.3%, depends on the types of surgery (Dimick *et al.*, 2004; Khan *et al.*, 2006; Emmanuel *et al.*, 2012). Epidemiology studies reported that prevalence of SSI were 1.9% in United States, 0.6% in Scotland, 5-10% in Australia and Japan, and 12.9% in University of Malaya Medical Centre (Hughes *et al.*, 2005; Coleman *et al.*, 2010; Scottish Surveillance of Healthcare Associated Infection Programme., 2010; Tan *et al.*, 2010; Mu *et al.*, 2011).

Microorganisms responsible for the development of SSI varies. *Staphylococcus aureus* (*S. aureus*) was reported as one of the leading causative agent for SSI (A. report from the NNIS System., 2004; Magill *et al.*, 2014). But in the past decade, Methicillin-resistance *Staphylococcus aureus* (MRSA) was responsible for most of the SSI and it becomes a challenge for medical practitioner due to emergence and spread of resistance to a wide range of antibiotics (Klein *et al.*, 2007). *Streptococcus sp.* is the second most common pathogen for SSI, followed by *Enterococcus sp.* and *Pseudomonas aeruginosa* (Weigelt *et al.*, 2009).

SSI often led to substantial morbidity and mortality in some patients. Several studies reported that mortality rate of SSI were between 3% and 25.3% (Kathryn B. Kirkland *et al.*, 1999; Awad, 2012; Horasan *et al.*, 2013). A study done in Duke University Medical Center and Durham Regional Hospital had reported mortality rate of SSI patient with MSSA was 6.7% and MRSA was 20.7%, MRSA infected patients are 3.4 times at higher risk to die in 90-day post operation (Engemann *et al.*, 2003).

Besides that, the duration of hospital stay of SSI patient are also longer than patients that did not develop any complication by two to 36 days, depending on types of surgical intervention (Kathryn B. Kirkland *et al.*, 1999; Dimick *et al.*, 2004; Broex *et al.*, 2009; Jenks *et al.*, 2014). Prolonged hospital stay will require more resources, included additional diagnostic tests, and therapeutic use of antibiotics. It also reduces the availability of beds for other patients as well as human resources. As a consequences, this not only increase the financial burden of patients' family but also burden the healthcare system and economic.

A study done in the year 2009 has found that treating a SSI patient required as high as 10,523 British pound (Tanner *et al.*), which include extended hospital charges, medicine as well as additional diagnostic tests. Many factors can affect the postoperative outcome of the patients such as patient-related (age, underlying disease, tobacco use, diabetic mellitus status, obesity) and procedure-related (method of skin preparation, selection of prophylactic antibiotics, duration of operation, surgeon experience) (Arabshahi and Koohpayezade, 2006; Utsumi *et al.*, 2010; Hafez *et al.*, 2012). Identification of modifiable risk factors for SSI helps in developing the prevention strategies on reducing SSI, especially in limited resources healthcare facilities.

SSI is a preventable surgical complication. Many SSI can be prevented if SSI care bundle has been applied properly. The National Institute for Health and Care Excellence (NICE) had published a guideline on prevention and treatment of SSI in year 2008 (Leaper *et al.*, 2008). This guideline gives details of the methods on how to prevent SSI in pre-, intra- and postoperative phase.

Most of the time, our local data consist only those who acquired the infection during hospitalization period, but SSI can develop within 30 days after surgery. Lack of close monitoring system which failed to capture the SSI cases from the patients that seek treatment from private clinical or other healthcare institutional lead to under reporting the incidence rate. Knowing the incidence is crucial to develop infection control policy for SSI. Furthermore, a cohort prospective study will enable us to do elaborate profiles of the patient who develop SSI and identify the problems with regards to preoperative preparation, during operation, post-operative wound care and co morbid illness associated with SSI.

Peacock *et al.* (2002) demonstrated virulent genes play a role in determining invasive disease of *S. aureus* as well as its effect was accumulative and increase the risk of disease. Among the S. aureus virulence genes, collagen adhesins (*cna*), polysaccharide intercellular adhesins (*ica*), gamma haemolysin (*hlg*), and putative adhesins (*SdrE*) are the most common isolated virulence gene in invasive isolates (Peacock *et al.*, 2002). By studying the associated virulence genes in *S. aureus* can help in demonstrating their involvement in the occurrence of SSI.

Therefore this study set up to determine the incidence and quantify the risk factors of SSI. This is an essential step in order to develop the preventive strategy to reduce the occurrence of SSI. In addition, identify the causative agent, especially *S. aureus* and MRSA strain by using molecular method is not necessary for treatment in SSI but can determine the evolution of the properties of *S. aureus* and MRSA as well as provides useful information for characterization of S. aureus and MRSA strain.

CHAPTER 2

LITERATURE REVIEW

2.1 Surgical Site Infections (SSI)

SSI is an infection that occurs at site of incision or any anatomical parts that was either opened or manipulated during surgical procedure. It can occur within 30 days after surgical procedure or within one year if involve implantation. Complication of a SSI may range from spontaneously wound pus discharge and inflammation to life-threatening, such as a sternal wound infection after CABG surgery.

SSI may caused by contamination of an incision with microorganisms either from the instruments, environment or patient's own body during surgery. However, SSI caused by microorganisms from an outside source following surgery is less common. SSI can badly effect on patient’s quality of life and associated with considerable morbidity and extended hospital stay.

In addition, prolonged hospital stay will require more resources, included additional diagnostic tests, and therapeutic use of antibiotics. As a consequences, this not only increase the financial burden of patients’ family but also burden the healthcare system and economic. Besides that, patients in primary care are allowed to discharge home earlier on the day or following day case as well as fast-track surgery are associated in increased numbers of infections.

2.1.1 Signs of SSI

According to Leaper *et al*. (2008), the common signs and symptoms presence in surgical site infections are:

1. Moderate to high grade fever (a low grade fever on the first two days is common due to physiological respond following surgery).
2. Foul smelling drainage or pus from the wound. It can be bloody, greenish, whitish, yellowish or mixed colours. The drainage may be foamy or thick.
3. Swelling of the wound, sometimes can feel hardening as the tissue underneath are inflamed.
4. Warm feeling on the skin around the wound.
5. Redness of the surrounding skin around the wound, sometimes may even feel warm.
6. Pain or tenderness around the wound. Normally following operation, the pain is steadily and slowly diminished during healing process, but if the pain increases for no reason, probably there is an infection developing in the wound.

The above symptoms present on the first 48-72 hours are usually normal physiological response following surgery due to the healing process, but if it becomes severe and worsen or delayed healing, then infection should be suspected. A more specific clinical diagnostic criteria based on signs and symptoms is used to define an SSI according to site of infection can be found on Table 2.1 (Horan *et al.*, 1992).

2.1.2 Sites of SSI

Based on the site of infection, SSI can be classified into three categories, which are superficial, deep, and organ or space. Superficial SSI occurred at the epidermis, dermis and subcutaneous fat tissue layer; deep SSI occurred at the fascia and muscle under the subcutaneous fat tissue layer; and organ or space SSI occurred at the organ or space within the body (Figure 2.1).

Any infections that involve more than two layers will be classified into the deeper site. For example infection that involved superficial and deep layer is classified as deep SSI.

Table 2.1 Criteria for defining a SSI (Horan *et al.*, 1992)

Superficial Incisional SSI

Infection occurs within 30 days after
the operation *and*
Infection involves only skin or subcutaneous tissue
of the incision *and* at least *one* of the following:

1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.
3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat *and* superficial incision is deliberately opened by surgeon, *unless* incision is culture-negative.
4. Diagnosis of superficial incisional SSI by the surgeon or attending physician.

Do *not* report the following conditions as SSI:

1. Stitch abscess (minimal inflammation and discharge confined to the points of suture penetration).
2. Infection of an episiotomy or newborn circumcision site.
3. Infected burn wound.
4. Incisional SSI that extends into the fascial and muscle layers (see deep incisional SSI).

Note: Specific criteria are used for identifying infected episiotomy and circumcision sites and burn wounds.

Deep Incisional SSI

Infection occurs within 30 days after the operation if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operation
and
Infection involves deep soft tissues (e.g., fascial and muscle
layers) of the incision *and* at least *one* of the following:

1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site.
2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localized pain, or tenderness, unless site is culture-negative.
3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
4. Diagnosis of a deep incisional SSI by a surgeon or attending physician.

Notes:

1. Report infection that involves both superficial and deep incision sites as deep incisional SSI.
2. Report an organ/space SSI that drains through the incision as a deep incisional SSI.

Organ/Space SSI

Infection occurs within 30 days after the operation if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operation
and
Infection involves any part of the anatomy (e.g., organs or spaces), other than the incision, which was opened or manipulated during an operation *and* at least *one* of the following:

1. Purulent drainage from a drain that is placed through a stab wound into the organ/space.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space.
3. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
4. Diagnosis of an organ/space SSI by a surgeon or attending physician.

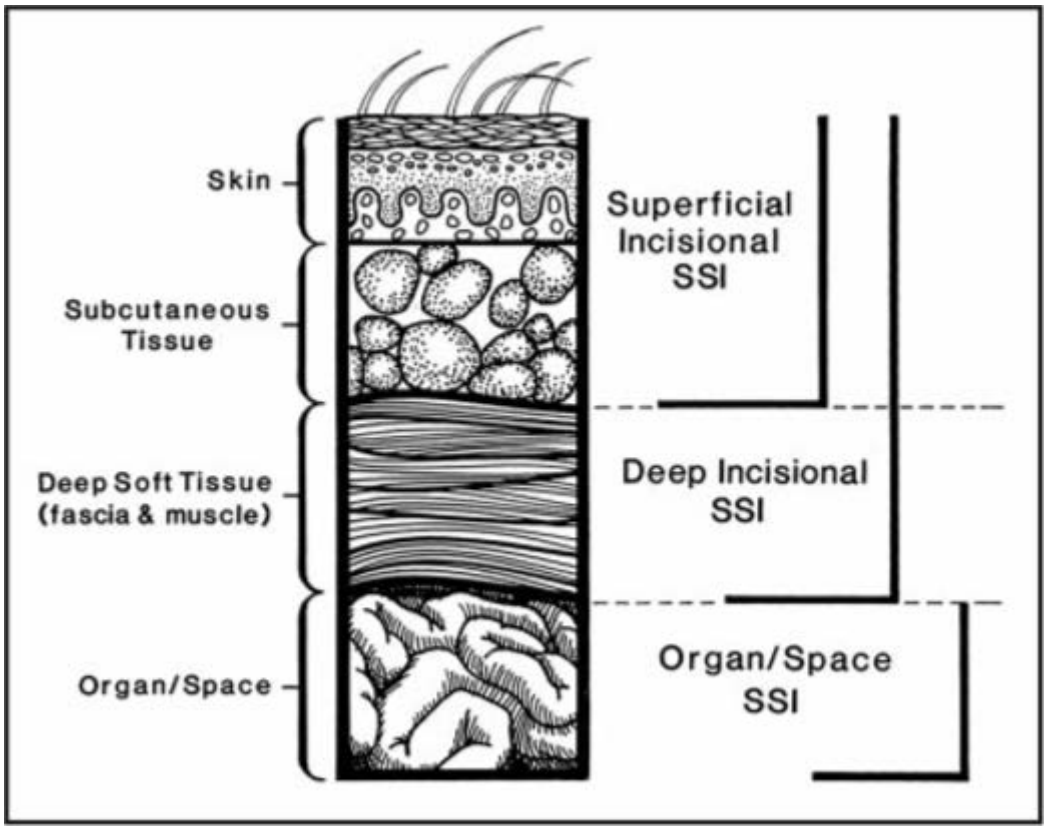

Figure 2.1 Cross-section of abdominal wall according to the CDC classifications of SSI. (Adapted from Horan *et al.* (1992))

2.1.3 Class of SSI

Besides the classification based on to the site of infection, SSI also can be categorized according to the types of surgical wounds (Table 2.2).

Table 2.2 The CDC criteria were used to classify and define the types of surgical wounds (Mangram *et al.*, 1999).

I. Class I or Clean: An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage.

II. Class II or Clean-Contaminated: An operative wound in which the respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions and without unusual contamination.

III. Class III or Contaminated: Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique or gross spillage from the gastrointestinal tract, and incisions in which acute, non-purulent inflammation are encountered.

IV. Class IV or Dirty-Infected: Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera.

2.1.4 Risk Factors for SSI

Development of SSI influenced by various factors, such as smoking, advanced age as well as procedure-related factors, including surgeon experience, and inappropriate antimicrobial prophylaxis. By eliminating or minimize these risk factors, the risk of SSI can be reduced or prevented (Savage and Anderson, 2013). Risk factors can be divided into patient-related and procedure-related. Among these, some risks are modifiable while some are not (Table 2.3).

Table 2.3 Risk factors that affect the outcome of surgery (Mangram *et al.*, 1999; Phillips *et al.*, 2014).

	Patient-related risk factors	**Procedure-related risk factors**
Modifiable	Nutritional status	Length of preoperative hospitalization
	Uncontrolled blood sugar level	Duration of surgery
	Smoking	Preoperative skin preparation
	Immunosuppression	Preoperative hair removal
	Alcoholism	Preoperative antimicrobial prophylaxis
	Colonization with microorganisms	Sterilization of instruments
	Anaemia	Patient's body temperature during intraoperative period
	Co-existent infection at other part of the body	Operating room ventilation
Non-modifiable	Age	Foreign material in the surgical site
	Renal failure	Surgical drains
	Jaundice	Surgeon experience
	Malnutrition	Surgeon technique
	Ascities	Length of postoperative hospitalization
	Obesity	
	Cancer	

Modifiable risk factors such as patient's nutritional status, blood sugar level, anaemia, and smoking status can be modified and eliminated. For example patient with hyperglycaemia can be treated and normalize the blood sugar level prior to operation. While non-modifiable risk factors such as age, renal failure and malnutrition cannot be restored to normal.

Although not all risk factors are modifiable, but optimizing modifiable risk factors before surgery can reduce the risk of SSIs (Savage and Anderson, 2013). However, this only applied on the elective surgery. For emergency operation, due to the urgency and short of time to optimize the modifiable risk factors, not all modifiable risk cannot be treated prior the operation, and emergency operation itself also as a risk factor that contribute to SSI (Neumayer *et al.*, 2007).

2.1.5 Prevention Bundles

SSI is a preventable surgical complication. Strictly follow the infection control measures during pre-, intra- as well as post-operation were able to reduce and prevent the occurrence of SSI. One of the prevention strategies is the concept of care bundles. Majority of SSIs can be prevented by implement care bundles (Herruzo Cabrera, 2010; Miyahara *et al.*, 2014; Phillips *et al.*, 2014). According to the NICE guideline, care bundles are divided into three phases, which are preoperative, intraoperative and postoperative phases (Leaper *et al.*, 2008). Each phase consists of several measures to minimize the risk of SSI, and all of this known as care bundle.

2.1.5.1 Preoperative Care Bundles

2.1.5.1 (i) Optimize patient's risk factors

Risk factors such as gender, age, duration of surgery, anaemia, cigarette smoking, diabetes mellitus and obesity were evaluated. Their association and influence on occurrence of SSI was studied as well. However, risk factors such as gender and age are not modifiable. Therefore focusing on modifiable risk factors (Table 2.3) such as smoking cessation, weight reduction, diabetic control, reduce or stop alcoholic consumption, and coexisting infection are essential and they should be optimized and treated prior to any elective operation (Neumayer *et al.*, 2007; Ministry of Health Malaysia., 2010).

2.1.5.1 (ii) Nasal Screening for Methicillin-resistance *Staphylococcus aureus*

Many previous studies revealed that *S. aureus* was the commonest isolated pathogen involved in SSI, it accounts up to 46% of Methicillin-sensitive and Methicillin-resistance *Staphylococcus aureus* (MRSA) (Owens and Stoessel, 2008; Weigelt *et al.*, 2009; Kang *et al.*, 2012; Takesue *et al.*, 2012). Epidemiologic studies have shown that majority of SSI are originated from patients itself, such as endogenous flora and nasal colonization.

S. aureus is a pathogenic microorganism, however it colonized in human skin flora as transient and resident flora in 20% of normal healthy adult (Kluytmans *et al.*, 1997). It commonly colonized on skin, axilla, perineal area, groin, and anterior nares (Friedrich *et al.*, 2006). NICE guidelines (2008)

recommends nasal decolonization should only target to patients who are nasally colonised with MRSA instead of all *S. aureus*. However, a few studies have shown that nasal decolonization for both Methicillin-sensitive *S. aureus* (MSSA) and MRSA significantly reduce the incidence of SSI (Bode *et al.*, 2010; Rao *et al.*, 2011).

MRSA decolonization protocol are very depends on the health care institutions and hospitals. Based on the latest MRSA decolonization protocol from Hospital USM, MRSA nasal carriage should be treated with either Mupirocin 2% ointment three times a daily for five days or Chlorhexidine 1% cream three times daily for seven days (Hospital Universiti Sains Malaysia., 2012).

2.1.5.1 (iii) Antimicrobial Prophylaxis

Antimicrobial prophylaxis is the use of antibiotics in order to prevent the occurrence of infection (Wendy, 2005). Antimicrobial agent used for prophylaxis should be actively against and cover the most common and most likely cause of infection during and after the procedure (Ministry of Health Malaysia., 2010). The selection of antibiotics to be used as prophylaxis must ideally based on the antibiogram pattern of the particular institution or hospital, therefore the choice of antimicrobial agent would not be same in every hospital, nation and region.

NICE guidelines recommends a single dose of intravenous antimicrobial prophylaxis to be given to clean surgery that involved implant or prosthetic placement; clean-contaminated surgery; contaminated surgery and dirty-infected operation within 60 minutes prior to the incision. Repeat dose should be given when the duration of operation is longer than the half-life of the antimicrobial

agent (Leaper *et al.*, 2008; Phillips *et al.*, 2014). Benefit and efficacy on antimicrobial prophylaxis is well established and a study conducted by Mazaki (2014) also found that there were significant reduction of incidence of SSI when patients were given antimicrobial prophylaxis.

According to the National Antibiotic Guideline (2008) published by Ministry of Health, Malaysia, it recommends antimicrobial prophylaxis to be given as soon as the patient has been stabilized after induction (Table 2.4). Continuing antimicrobial prophylactic until removal of surgical drain is not recommended.

Table 2.4 Antimicrobial prophylaxis agent recommended by Ministry of Health, Malaysia (Ministry of Health Malaysia., 2008).

Type of operation	**Preferred chemoprophylaxis agent**	**Alternative chemoprophylaxis agent**
Coronary artery bypass graft	β-lactam/β-lactamase inhibitors, e.g. Amoxycillin/Clavulanate 1.2g IV **OR** Ampicillin/Sulbactam 1.5g IV **OR** Cefazolin 1g IV **OR** Cloxacillin 1g IV	Cefuroxime 1.5g IV
Laparoscopic cholecystectomy	Cefuroxime 1.5g IV **OR** 3rd gen. Cephalosporins, e.g. Cefoperazone 1g IV	β-lactam/β-lactamase inhibitors, e.g. Ampicillin/Sulbactam 1.5g IV **OR** Amoxycillin/Clavulanate 1.2g IV
Hernioplasty	Cloxacillin 1g IV	β-lactam/β-lactamase inhibitors, e.g. Amoxycillin/Clavulanate 1.2g IV **OR** Ampicillin/Sulbactam 1.5g IV

2.1.5.1 (iv) Hair Removal

Removal of hair during routine operation is not recommended, unless that area interfere the incision site (National Institute for Health and Care Excellence., 2008). Hair removal can be done in such case, if it's necessary, and it's preferable to use electronic clippers instead of razor or shaving to reduce the injury on skin that can lead to colonization or infection (National Institute for Health and Care Excellence., 2008; Phillips *et al.*, 2014). Infection control guideline published by Minister of Health, Malaysia (2010) recommend hair removal should be done just before operation.

2.1.5.1 (v) Other preoperative measures

Besides that, NICE guideline also recommends patient to shower or bath preoperatively, wear antiseptic impregnated clothes and to avoid routine mechanical bowel preparation to reduce SSI (Leaper *et al.*, 2008; Savage and Anderson, 2013).

2.1.5.2 Intraoperative Care Bundles

2.1.5.2 (i) Operating Room Environment

Operating room must be clean from any soil or contamination of any body fluids, such as blood. Air entered operating room must be filtered and maintained at least 15 to 20 air changes hourly. Operating room must also under positive pressure, temperature optimized at around 21°C and humidity should be maintained around 40-60% (Owens and Stoessel, 2008; Ministry of Health Malaysia., 2010; Phillips *et al.*, 2014).

2.1.5.2 (ii) Skin Preparation

Skin should be sterilized just before incision by using antiseptics. Common antiseptics used for operation is either povidone-iodine or chlorhexidine alcohol aqueous. Studies compared the efficacy of povidone-iodine or chlorhexidine alcohol to sterilize the skin shown that chlorhexidine alcohol offered significant protection and more advance than povidone-iodine (Macias *et al.*; Darouiche *et al.*, 2010; Noorani *et al.*, 2010; Banjong *et al.*, 2011).

2.1.5.2 (iii) Maintaining Patient's Body Temperature and Homeostasis

Maintaining patient's body temperature, oxygenation, perfusion and homeostasis during intraoperative period are important to reduce the chances of SSI. Patient who was hypothermia during intraoperative period required longer time in wound healing and increase occurrence of SSI (Kurz *et al.*, 1996). Studies also found that hypothermia patients stayed longer during postoperative hospitalization period (Kurz *et al.*, 1996; Harper *et al.*, 2003).

2.1.5.2 (iv) Other intraoperative measures

Surgeons, anaesthesiologists, nurses as well as other operating team members that involves in operation must strictly follow WHO (2009) Hand Hygiene Guideline when decontaminate their hands. Operating team members must also wear sterile gowns, gloves, facemasks, and caps during the operation to minimize the SSI due to transmission of potential pathogens (Leaper *et al.*, 2008; Owens and Stoessel, 2008). Besides that, surgeon experience and skill also greatly affect the outcome (Phillips *et al.*, 2014).

2.1.5.3 Postoperative Care Bundles

2.1.5.3 (i) Wound Care

Unless dressing soaked, otherwise sterilize dressing on primarily closed wound should be protected 24 to 48 hours postoperatively. If inspection, changing, or removing of dressing are necessary during first 48 hours, use an aseptic technique and strictly compiled to WHO Hand Hygiene Guideline (Leaper *et al.*, 2008; Owens and Stoessel, 2008; World Health Organization., 2009; Ministry of Health Malaysia., 2010; Phillips *et al.*, 2014).

2.1.5.4 Treatment of SSI

Treatment of SSI is basically based on the site of infection. Once SSI was diagnosed by surgeon or physician, early empiric antimicrobial agent is important to eliminate the pathogen. For superficial SSI and pus discharge, wound cleansing is the first step to remove the pus and disinfect the surrounding tissue.

In deep SSI, the pus may locate beneath the superficial tissue. In such condition, drainage of pus and fluid from the infected site is adequate while in severe case, debridement of the wound and an affected tissue is necessary. Occasionally, patient is required to be hospitalized and re-operate the wound.

Organ or space SSIs are often diagnosed by signs of infection and instrumental examination. Drainage of pus are done under the guidance of ultrasonography or computed tomography examination. If it’s not possible, re-operate would be the only choice of treatment.

Debrided tissue, aspirated pus or pus swab should be quickly transport to laboratory for identification of pathogenic microorganism as well as antimicrobial sensitivity test to find out the most suitable antimicrobial agent.

2.2 *Staphylococcus aureus*

Staphylococcus was first identified by a Scottish surgeon, Sir Alexander Ogston, in pus from a surgical abscess in a knee joint in Aberdeen, United Kingdom in year 1880. He proposed the name '*Staphylococci*' based on its shape and morphology (Humphreys, 2012). *Staphylococci* are resistant to high salt concentration as well as dry conditions, therefore it can survive for long periods in the environment such as the skin and upper respiratory tract of human and animals.

Staphylococcus aureus (*S. aureus*) is the major pathogen in the genius *Staphylococcus*, due to its ability to infect human and animals. It is differentiated from other species by its ability to clot blood plasma via the action of enzyme *coagulase* (Humphreys, 2012).

List below is the hierarchy taxonomic for *S. aureus* (UniProt., 2014).

Domain: Bacteria;

Phylum: Firmicutes;

Class: Bacilli;

Order: Bacillales;

Family: *Staphylococcaceae*;

Genus: *Staphylococcus*;

Species: *Staphylococcus aureus*

S. aureus are grouped under genus *Staphylococcus* within family *Staphylococcaceae*. The microorganism is Gram-positive, round shape, arranged in grape-like cluster, about 1µm in diameter, non-sporing, non-motile, usually non-capsulated, produce enzyme *coagulase*, and ferment mannitol (Chapman, 1945; Humphreys, 2012). Therefore it produces positive reaction in mannitol salt agar and changes the colour from red/pink to yellow. This microorganism can grow on most types of media, including Mueller Hinton agar, blood agar, DNase agar, Tryptic Soy Agar, and Mannitol Salt agar (MSA). Its colonies are circular, smooth and shiny surface, and often pigmented.

It differs from other microorganism by mean of the characteristic appearance on Mannitol Salt agar and DNase agar. On Mannitol Salt agar, its yellow appearance due to the ability to ferment mannitol while on DNase agar, a clear zone surround the colonies can be seen when added a few drop of hydrochloride acid. *S. aureus* was then confirmed by tube coagulase test to differ from other coagulase-negative Staphylococci.

2.2.1 Pathogenicity

S. aureus is present in the nose of 21.5 - 30% of healthy individual and may be found on the skin as well (Fishbain *et al.*, 2003; Davis *et al.*, 2004; Alex, 2007; Humphreys, 2012).

It is an opportunistic pathogen that can cause a numerous diseases, from minor skin infections to life threatening diseases, such as impetigo, scalded skin syndrome, abscesses, osteomyelitis, pneumonia, meningitis, endocarditis, toxic

shock syndrome (TSS), sepsis as well as surgical site infection. It also one of the major causative organisms in surgical site infection.

Our body consists of three defence mechanism to protect us from *S. aureus* invasion. Primary and the most frontier barrier consists of our skin layer, and physiological factors; secondary barrier is our cellular factors and inflammatory response, which consist of phagocytes, neutrophils, basophils, interleukin and other inflammatory markers; tertiary which is the final barrier comprises active immune response provided by lymphocytes, such as immunoglobulin (Hattie, 2009).

By the help of virulent factors, *S. aureus* can invade and against our defence barrier and cause infections.

2.2.2 Virulent factors

S. aureus contain numbers of cell-associated and extracellular factors to assist it to overcome the human defend mechanism, and to colonized, invade as well as survive in the human body (Table 2.5). Although not all the role of every factors are well studied, but it's likely that they are responsible for enabling the *S. aureus* to bind and to resist and survive from the intracellular killing by phagocytes and bactericidal activities from humoral factors (Humphreys, 2012).

Table 2.5 List of some virulent factors of *S. aureus* (Peacock *et al.*, 2002; Mark *et al.*, 2009; Humphreys, 2012).

Virulent gene	Function
Adhesins	
FnbA & *FnbB*	Adhesin for fibronectin
ClfA, ClfB & *Efb*	Adhesins for fibrinogen
cna	Adhesin for collagen
Protein A	Binds Fc domain of immunoglobulin and von Willibrand factor
SdrC, SdrD & *SdrE*	putative adhesins
Bbp	Adhesin for bone sialoprotein
EbpS	Adhesin for elastin
Map/Eap	Major histocompatibility complex class II analogue protein
Toxins	
Toxic Shock Syndrome Toxin 1. (*TSST-1*)	Exotoxin with superantigen activity
Enterotoxins A, B, C, D, E, G, H, I & J	Exotoxins with superantigen activity
Exfoliative toxins A & B	Exotoxins with superantigen activity
Alpha-toxin	Cytolytic pore-forming toxin
Beta-toxin	Sphingomyelinase
Delta-toxin	Cytolytic toxin
Panton-Valentine leukocidin (*PVL*)	Bicomponent leukocidin
Gamma-toxin	Bicomponent leukocidin
Others	
ica locus	Biofilm formation
Coagulase	Binds prothrombin, activating conversion of fibrinogen to fibrin
V8 protease	Serine protease
agr subgroup	Global regulator
Lipase	Degrades lipid
Staphylokinase	Degrades fibrin
Deoxyribonuclease	Degrades DNA
Epidermolytic toxins	Cause blistering of skin
Cell wall polymers	
Peptidoglycan	Inhibits inflammatory response; endotoxin-like activity
Teichoic acid	Phage adsorption; reservoir of bound divalent cations