RISK FACTORS FOR METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTION AT QUEEN SIRIKIT NATIONAL INSTITUTE OF CHILD HEALTH (EMPHASIS IN INSTRUMENTAL PROCEDURE)

SUSITH RANJAN WADIKAWAGE

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……...
Mr. Susith Ranjan Wadikawage
Candidate

……...
Assoc. Prof. Pornthep Chanthavanich,
M.D., M.Sc. (M.C.H), D.T.C.H
Co-Advisor

……...
Asst. Prof. Kriengsak Limkittikul,
Dip. Thai Board of Pediatrics
Major-Advisor

……...
Special Assoc. Prof. Tawee
Chotpitayasunondh,
Dip. Thai Board of Pediatrics
Co-Advisor

……...
Assoc. Prof. Krisana Pengsaa,
Dip. Thai Board of Pediatrics
Co-Advisor

……...
Mr. Porntep Suandork,
Dip. Thai Board of Pediatrics,
Cert. Pediatric Infectious Diseases
Co-Advisor

……...
Assoc. Prof. Chukiat Sirivichayakul,
Dip. Thai Board of Pediatrics
Co-Advisor

……...
Assoc. Prof. Rassmidara Hoonsawat,
Ph.D.
Dean
Faculty of Graduate Studies

……...
Assoc. Prof. Pornthep Chanthavanich,
M.D., M.Sc. (M.C.H.), D.T.C.H.
Chair
Master of Clinical Tropical Medicine
(Tropical Pediatrics)
Faculty of Tropical Medicine

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March 15, 2005

……...
Mr. Susith Ranjan Wadikawage
Candidate

……...
Asst. Prof. Kriengsak Limkittikul,
Dip. Thai Board of Pediatrics
Chair

……...	……...
Assoc. Prof. Pornthep Chanthavanich, M.D., M.Sc. (M.C.H), D.T.C.H Member	Assoc. Prof. Krisana Pengsaa, Dip. Thai Board of Pediatrics Member
……...	……...
Special Assoc. Prof. Tawee Chotpitayasunondh, Dip. Thai Board of Pediatrics Member	Assoc. Prof. Chukiat Sirivichayakul, Dip. Thai Board of Pediatrics Member
……...	……...
Mr. Porntep Suandork, Dip. Thai Board of Pediatrics, Cert. Pediatric Infectious Diseases Member	Prof. Usa Thisyakorn Dip. Thai Board of Pediatrics, Cert. Pediatric Infectious Diseases Cert.of Proficiency Board in Pediatric Infectious Diseases Member
……...	……...
Assoc. Prof. Rassmidara Hoonsawat, Ph.D. Dean Faculty of Graduate Studies Mahidol University	Assoc. Prof. Pratap Singhasivanon, M.B.B.S., D.T.M.&H. (Bangkok), M.P.M., Dr.P.H. (Epidemiology) Dean Faculty of Tropical Medicine Mahidol University

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Susith Ranjan Wadikawage

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SUSITH RANJAN WADIKAWAGE 4738680 TMCT/M

M.C.T.M. (Trop. Ped.)

THEMATIC PAPER ADVISORS: KRIENGSAK LIMKITTIKUL, M.D., DIP. THAI BOARD OF PEDIATRICS, KRISANA PENGSAA, M.D., DIP. THAI BOARD OF PEDIATRICS, D.T.M.&H., CHUKIAT SIRIVICHAYAKUL, M.D., DIP. THAI BOARD OF PEDIATRICS, D.T.M.&H., PORNTHEP CHANTHAVANICH, M.D., D.T.M.&H., M.Sc. (M.C.H.), D.T.C.H., TAWEE CHOTPITASUNONDH, M.D., DIP. THAI BOARD OF PEDIATRICS, D.T.M.&H., PORNTEP SUANDORK, M.D., DIP. THAI BOARD OF PEDIATRICS.

ABSTRACT

This retrospective study was conducted at Queen Sirikit National Institute of Child Health by reviewing 274 medical records of pediatric patients from whom *Staphylococcus aureus* (*S. aureus)* was isolated from January 2002 to December 2003, to identify the ratio of MRSA and MSSA and the risk factors for methicillin-resistant *S. aureus* (MRSA) infection in children. The susceptibility patterns to the commonly used antibiotics were also reviewed.

General information of the patients as well as information about clinical specimens, site of infection, antibiotic sensitivity pattern, instrumentation procedures and surgical interventions were collected by pre-formed questionnaire.

It was found that age less than 1 year, presence of chronic or underlying disease, having instrumentation procedures and undergoing surgical interventions were significant risk factors for MRSA. The instrumentation procedures caused highest risk for MRSA isolation, with an odd ratio of 11 (95% CI 5.2-23.3). Nearly half of the patients with *S. aureus* infection (43.8%) had received instrumentation procedures.

In the hospital-acquired infections, MRSA was significantly associated with endotracheal intubation and vascular catheterization, with odds ratios of 3.3 (95% CI 1.0-10.9) and 8.3 (95%CI 9.0-36.5), respectively.

Both MRSA and MSSA were highly susceptible to vancomycin. Co-trimoxazole, erythromycin and gentamicin were effective only against MSSA. Both strains of *S. aureus* were resistant to penicillin G, implying that this drug has no place in the treatment of *S. aureus* infection.

KEY WORDS: *STAPHYLOCOCCUS AUREUS/* MRSA/ MSSA/ RISK FACTORS / CHILDREN/HOSPITAL-ACQUIRED/COMMUNITY ACQUIRED.
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LIST OF ABBREVIATIONS

Abbreviation/Symbols	Terms
ATB	Antibiotics
CA	Community Acquired
CI	Confidence Interval
cm	Centimeter
CoNS	Coagulase Negative Staphylococcus
CSF	Cerebrospinal Fluid
DD/MM/YY	Day/Month/Year
°C	Degree Celsius
e.g.	Example
ENT	Ear/Nose/Throat
et al	An others
Gr.	Grade
HA	Hospital Acquired
ICU/NICU	Intensive Care Unit/ Neonatal Intensive Care Unit
kg	Kilogram
L	Liter
LTCFs	Long Term Care Facilities
Mg	Milligram
µg	Microgram
MICs	Minimal Inhibition Concentrations
ml	Milliliter
mm	Millimeter
mo	Month
mol	Mole
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Sensitive *Staphylococcus aureus*
NA	Not Applicable

LIST OF ABBREVIATIONS (CONT)

Abbreviation/Symbols	Terms
NaCl	Sodium Chloride
NCCLS	National Committee for Clinical Laboratory Standards
No.	Number
OPD	Out Patient Department
p	Probability
PBP 2a	Penicillin Binding Protein 2a
QSNICH	Queen Sirikit National Institute of Child Health
S. aureus	*Staphylococcus aureus*
SCC mec	Staphylococcal Chromosome Cassette *mec*
TSS	Toxic Shock Syndrome
U S	United State
USD	US Dollar
VISA	Vancomycin Intermediate Resistant *Staphylococcus aureus*
VRSA	Vancomycin Resistant *Staphylococcus aureus*
W/v	Weight Per Volume
%	Percent
β	Beta
<	Less Than
>	More Than
≥	More Than and Equal
≤	Less Than and Equal
χ^2	Chi-Square

CHAPTER I
INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a ubiquitous environmental organism, with a predilection to skin, particularly of the face, nose and hands, and routinely found in one third of adults as normal flora. Nasal carriage is mediated by teichoic acid and as many as 50% of population being intermittent carriers.

Since its description in 1880 and 1882 by Ogston, *S. aureus* has remained a versatile and dangerous pathogen in human.

S. aureus is a significant cause of morbidity and mortality in children. It is one of the most commonly isolated pathogen in neonatal sepsis.

The frequencies of both community acquired and hospital-acquired staphylococcal infections have increased steadily, with little change in overall mortality. Treatment of these infections has become more difficult because of the emergence of multi-drug resistant strains. *S. aureus* resistant to commonly used antibiotics, such as methicillin and oxacillin are called methicillin resistant *S. aureus* (MRSA). Strains of MRSA have become a major problem in neonatal intensive care unit (Haley et al., 1995). It is also a common cause of pneumonia, bacteremia, endocarditis, osteomyelitis, empyema, toxic shock syndrome, food poisoning, burn and wound infections (Locksley et al., 1982; Haddadin et al., 2002).

Like other strains of *S. aureus*, the body site most commonly colonized with MRSA is the anterior nares. Other body sites that may be colonized with MRSA include open wounds, the respiratory tract, perineum, upper extremities, umbilicus (in infants), urinary tract, and axilla. Some patients are colonized for only a few weeks and then become culture-negative without any specific therapy. However, patients with serious underlying diseases that require repeated hospitalization may be colonized for more than 3 years.

MRSA infections are usually mild, superficial infections of the skin that can be treated successfully with proper skin care and antibiotics. However, MRSA can be

difficult to treat and can progress to life-threatening blood or bone infections because there are fewer effective antibiotics available for treatment.

Since the first case report of MRSA infection in the United States in 1968 (Barret et al., 1968), MRSA has become an increasingly significant problem, now accounting for about 50% of nosocomial *S. aureus* infection isolates in the United States (Lowy, 1998).

Subsequently the view of MRSA as a nosocomial pathogen in patients with well-described risk factors has been challenged with recognition of community acquired MRSA (CA-MRSA).

Many other investigators have identified and reported MRSA strains leading to serious clinical problems (Stroch et al., 1986; Crossley et al., 1979).

CA-MRSA is an increasingly common pathogen in pediatric population (Sattler et al., 2002; Herold et al., 1998; Fergie, 2001).

Whether MRSA is more virulent than methicillin susceptible *S. aureus* (MSSA) is a controversial issue. Some investigators have demonstrated higher mortality associated with MRSA bacteremia in analysis that controlled other factors (Romero-vivas et al., 1995; Conterno et al., 1998; Blot et al., 2002), while others have demonstrated that inappropriate antimicrobial therapy, co- morbid condition, and advanced patient age rather than MRSA accounted for increased mortality associated with MRSA bacteremia (Soriano et al., 2000; McClelland et al., 1999; Harbarth et al., 1998).

One study (Boyce, 1998) showed that patients with serious MRSA infections stayed in the hospital for an average of 12 days longer, and had the average hospital costs of USD 5,100 greater than comparable patients with MSSA infections. However, fatality rates among patients with MRSA infections are not significantly higher than those observed among patients with infection caused by MSSA.

In conclusion, MRSA infection is important because of the following reasons:

1. MRSA is pathogenic, transmissible and is a common cause of hospital-acquired infections. MRSA outbreak can occur when one strain is transmitted to other patients.

2. Limited treatment options. Glycopeptides group (vancomycin) often is the only drug of choice for treatment of severe MRSA infections, although some strains remain susceptible to fluoroquinolones, trimethoprim/sulfamethoxazole, gentamicin or rifampin. Because of the rapid emergence of rifampin resistance, this drug should never be used as a single agent to treat MRSA infections.

3. These infections are associated with prolonged hospital stay and increased hospital costs, and few therapeutic options are available to treat affected patients.

According to the above mentioned reasons, it is interesting to study the risk factors for MRSA infection in children and to compare the risk factors for MRSA and MSSA. The data from this study may be helpful for further control and proper management of infection caused by *S. aureus*.

CHAPTER II
OBJECTIVES

HYPOTHESIS

The risk factors for HA- MRSA and CA-MRSA are different.

OBJECTIVES OF STUDY:

General Objectives

1. To estimate the ratio of MRSA and MSSA in pediatric patients with *S. aureus* infection.
2. To evaluate the risk factors of MRSA comparing with MSSA in pediatric patients.

Specific Objectives

1. To study risk factors of MRSA comparing with MSSA infection.
2. To study risk factors of hospital comparing with community acquired *S. aureus* infection.
3. To compare the antibiogram pattern of MRSA and MSSA.
4. To study risk factors of MRSA infection in association with instrumentation.

CHAPTER III
REVIEW OF LITERATURE

Microbiology and Genome

S. aureus is a member of the Family Micrococcaceae . On microscopical examination the organisms appear as gram-positive cocci in clusters. It is easily identified and distinguished from other staphylococcal species by their tendency to produce classical golden pigmented colonies, positive coagulase, mannitol fermentation, deoxyribonuclease tests and appearance of clustered, grape-like gram positive cocci on gram staining.

Other features of this organism are the production of a variety of biologically active components include enzymes, hemolysins, leukocidins, toxins and the cell surface proteins and cell wall components. These biological active components including enterotoxins A-E which are associated with food poisoning and toxic shock syndrome (TSS), the epidermolytic toxins A and B which are implicated in cases of scalded skin syndrome and TSS-1 which is associated with the most cases of TSS.

The staphylococcal genome consists of a circular chromosome with prophages, plasmids, and transposons. Genes governing virulence and resistance to antibiotics are found on the chromosome, as well as the extra chromosomal elements. These genes are transferred between staphylococcal strains, species, or other gram-positive bacterial species through the extra chromosomal elements.

In *S. aureus*, resistance to methicillin and related β-lactam antibiotics is encoded by the *mec*A gene, which is carried on a mobile genetic element termed the staphylococcal chromosome cassette *mec* (SCCmec). The transfer of this element is mediated by two site-specific recombinases, *CcrA* and *CcrB*, which catalyse precise excision of SCCmec and its orientation in specific integration into the chromosome of recipient cells.

Staphylococcus resistance to oxacillin/methicillin occurs when an isolate carries an altered penicillin-binding protein 2a (PBP2a), which is encoded by the

*mec*A gene. This alteration does not allow the drug to bind well to the bacterial cell, causing resistance to β -lactam antimicrobial agents.

Mechanism of Resistance to Antimicrobial Agents

Penicillin is inactivated by β-lactamase, a serine protease that hydrolyzes the β-lactam ring. Methicillin is β-lactamase resistant penicillin. Resistance to methicillin confers resistance to all penicillin and cephalosporin. This high level of resistance requires the presence of the *mec* gene that encodes PBP2a (Chambers, 1997). Resistance to vancomycin has been reported in clinical isolates of *S. haemolyticus* (Schwalbe et al., 1987), a coagulase-negative species.

It is expected that *S. aureus* may acquire this resistance property and vancomycin resistance strains are likely to pose a major therapeutic challenge in the future.

Confirmation of sensitivity by the broth-dilution method is recommended to declare the antibiotic resistance (Tenover et al., 1998).

Pathogenesis

The pathogenesis of *S. aureus* infection depends on bacterial factors: virulence determinant factors of the bacteria, and host defense mechanism. Several factors contribute to the increased susceptibility to infection; these include the presence of foreign material, intravenous catheter, long-term indwelling catheter, and breach of skin and mucus membrane.

The cellular events leading to septic shock in staphylococcal infection are similar to infection with gram negative bacteria.

Staphylococcal bacteremia may be complicated by endocarditis, metastatic infection, or septic syndrome. The typical pathological finding of staphylococcal disease is abscess formation. Leukocytes are the primary host defense against *S. aureus* infection (Verdrengh and Tarkowski, 1997).

Epidemiology

Human are a natural reservoir of *S. aureus*. About 20- 30% of healthy people carry *S.aureus* bacteria in their noses at various times without illness. Most of people

begin to have staphylococcus growing harmlessly on their bodies before the age of one week. Their fingers can carry staphylococcus bacteria from one area of the body to another to cause infections in wounds or broken skin. Both MSSA and MRSA isolates are persistent colonizers (Casewell et al., 1986; Sanford et al., 1994). Persons colonized with *S. aureus* are at increased risk for subsequent infections (Wenzel et al., 1995).

The prevalence of MRSA in hospitals varies considerably from one region to another and among hospitals in the same city and from country to country.

The main reservoir of MRSA in hospitals is patients colonized or infected with MRSA. Although colonized patients have no signs or symptoms of infection, they can still serve as a source from which transmission may occur. Colonized personnel and contaminated environmental surfaces can also serve as reservoirs, but are not as important as affected patients. Presumably, MRSA reservoirs in long-term care facilities (LTCFs) are similar to those in hospitals.

S. aureus including MRSA can be spread among people having close contact with infected people. MRSA is almost always spread by direct physical contact and not through the air. Spread may also occur through indirect contact by touching objects (e.g., towels, sheets, wound dressings, clothes, workout areas, or sport equipment's contaminated by the infected skin of a person with *S. aureus* or MRSA.

MRSA infections commonly occur among persons in hospitals and healthcare facilities. However, MRSA can cause illness in persons outside the hospitals and healthcare facilities as well. Cases of MRSA infection in the community have been associated with recent antibiotic use, sharing contaminated items, having recurrent skin diseases, and living in crowded settings.

The number of both community-acquired and hospital-acquired staphylococcal infection have increased in the past twenty years. This trend either parallels increased use of intravascular device (Banerjee et al., 1991; Steinberg et al., 1996) or resulting in part from selective antibiotics pressure (Panlilio et al., 1992; Speller et al., 1997).

At the beginning, these MRSA cases were restricted to the patients residing in LTCs, intravenous drug users and those who were recently hospitalized or who underwent surgery, but in 1980, the first community acquired (CA) MRSA infection

in the US was reported. Several other investigators (Herold et al., 1998; Frank et al., 1999; Hussain et al., 2000) found a high rate of community acquired MRSA among hospitalized children without risk factors.

Many investigators have studied the epidemiology of MRSA; some of them have reported increased prevalence of MRSA infection in nursery, community, and in children with or without risk factors; others have studied and compared risk factors for CA-MRSA and hospital acquired (HA) MRSA, and tried to find out whether CA-MRSA is due to the spread of nosocomial infection into community or not.

Study in Queen Sirikit National Institute of Child Health (QSNICH) (Napaporn, 2003) has shown increased prevalence of MRSA, and that most of MRSA cases occurred in children less than one year of age. Similarly, Endo et al. (1996), reported increased prevalence of MRSA, and that MRSA has become the most frequent pathogen causing sepsis and/or meningitis in the nursery.

A review from five Canadian university hospitals, from 1990 to 1992 (Embil et al., 1994), has shown that 63% of MRSA isolates were identified within 72 hours of admission. This finding indicated increasing cases of CA-MRSA; also a study in the United State (US) hospital (Buckingham et al., 2004), reported that CA-MRSA has emerged as a potentially invasive pathogen among children in Memphis area, and that CA-MRSA strains were not nosocomially spread.

In the US, several other reports (Boyce et al., 1982;Herold et al., 1998) from their hospitals indicated that MRSA infection was no longer confined to hospital environment in patient with well-described risk factors, and found that MRSA infection occurred in children without identifiable risk factors.

Some investigators have traced the origin of CA-MRSA strain; their objective was to find out the proportion of nosocomial spread of CA-MRSA strains. A study in San Francisco (Charlebios et al., 2004), indicated that a large proportion of CA-MRSA are feral descendent of hospital endemic colonies, while the study in Memphis area, the US (Buckingham et al., 2004), found that CA-MRSA and HA-MRSA are completely independent strains.

Several other investigators have studied the risk factors for MRSA, CA-MRSA, and HA-MRSA. Some of them (Thompson et al., 1982; Locksley et al., 1982), reported that the risk factors for HA-MRSA infection included, prolonged or

recurrent antibiotic exposure, prolonged hospitalization and hospitalization in intensive care unit; while others (Levin et al., 1982; Saravolatz et al., 1982; Strausbaugh et al., 1991), found that outpatients with MRSA infection generally had been chronically ill, and many have history of nursing home residence, recent admission to acute or chronic health care facility, prior receipt of antibiotics or intravenous drug abuse and exposure to self administered prophylactic antibiotics.

By comparative studying of risk factors for CA-MRSA and CA-MSSA, no significant difference in the exposure between the two groups was found (Sattler et al., 2002).

Clinical Laboratory Diagnosis for MRSA

The National Committee for Clinical Laboratory Standards (NCCLS) has recommended "Screening Test for Oxacillin-resistant *S. aureus*" using an agar plate containing 6 μg/ml of oxacillin and Mueller-Hinton agar supplemented with NaCl (4% w/v; 0.68 mol/L).

Accurate detection of oxacillin/methicillin resistance can be difficult due to the presence of two subpopulations (one susceptible and the other resistant) that may coexist within a culture (Kloos and Bannerman, 1999). All cells in a culture may carry the genetic information for resistance but only a small number can express the resistance in vitro. This phenomenon is termed heteroresistance and occurs in staphylococci resistant to penicillinase-stable penicillins, such as oxacillin.

Heteroresistance is a problem for clinical laboratory personnel because cells expressing resistance may grow slower than the susceptible population. This is why NCCLS recommends incubating isolates being tested against oxacillin, methicillin, or nafcillin at 35 º C for a full 24 hours before reading (NCCLS, 1999).

Treatment of *S. aureus* Infection

Penicillin remains the drug of choice if the isolate is sensitive to it. Semisynthetic penicillin (nafcillin or oxacillin) is indicated for β-lactamase-producing strains.In patients allergic to penicillin, a cephalosporin such as cefazolin or cephalotin is an acceptable alternative.

Glycopeptides (vancomycin) is the drug of choice for methicillin-resistant isolates. Patients unable to tolerate vancomycin can be treated with fluoroquinolones, trimethoprim-sulfamethoxazole, clindamycin, or minocycline. However they are not as effective as vancomycin.

CHAPTER IV
MATERIALS AND METHODS

STUDY DESIGN AND STUDY SITE

This was a retrospective study, under the management of Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University. It was conducted at Queen Sirikit National Institute of Child Health (QSNICH), Bangkok, Thailand. Data collection was carried out from the first week of November 2004 to the first week of January 2005.

DATA COLLECTION

In this retrospective study, children from whom *S.aureus* was isolated from any body site by microbiology laboratory at QSNICH were identified.

Two hundred and seventy-four medical records and susceptibility results of clinical specimens collected since January 2002 to December 2003 were reviewed.

Demographic data of patients as well as the information about clinical specimens, site of infections, the antibiotic sensitivity pattern, instrumentation, surgical interventions and past illnesses were collected by using pre-formed questionnaires.

In addition to laboratory data, hospitalization and outcome details were extracted from the medical record of the patients.

SAMPLE SIZE

The sample size was calculated by using the following formula:

$$N = \frac{Z^2 \alpha/2 \, (p) \, (q)}{\delta^2}$$

Where N=number of sample. q = 1-p

Z α/2 =the standard normal number deviate for two sided α Where (1-α) is the confidence level (since α=0.05 for a 95% confidence level, Z α/2 = 1.96)

p= prevalence of MRSA from the previous study, p=0.2(data from QSNICH).

δ= the effect size, is the difference of estimate that we wish to detect

$$N = \frac{(1.96)^2 * (0.2)*(0.8)}{(0.05)^2}$$

= 246 patients

OPERATIONAL DEFINITION

Immunosuppressive drug

Immunosuppression would be concerned when its effect was considered during *S. aureus* infection onset; e.g. If the patient was treated with prednisolone, a dose of 2 mg/kg/day more than 2 weeks, the immunosuppression effect should exist until 2 weeks after stopping.

Staphylococcal Resistant Pattern

In this retrospective study, identified *S.aureus* infections were stratified into MRSA and MSSA on the basis of the property of methicillin resistance or methicillin sensitive, respectively.

The MRSA was classified into CA-MRSA or HA-MRSA on the basis of preset criteria.

The 1999 NCCLS breakpoints for *S. aureus* were taken as reference for definition of MRSA, MSSA. The minimal inhibitory concentration and the zone size used for defferentiation are shown in the following table. The criteria are different from those for coagulase-negative Staphylococci (CoNS).

Minimal Inhibitory Concentration (MICs)	Oxacillin Susceptible	Oxacillin Intermediate	Oxacillin Resistant
S. aureus	≤ 2 μg/ml	no intermediate MIC	≥ 4 μg/ml
CoNS	≤ 0.25 μg/ml	no intermediate MIC	≥ 0.5 μg/ml

Zone Sizes	Oxacillin Susceptible	Oxacillin Intermediate	Oxacillin Resistant
S. aureus	≥13 mm	11-12 mm	≤10 mm
CoNS	≥18 mm	no intermediate zone	≤17 mm

Oxacillin is tested instead of methicillin and isolates are called MRSA instead of ORSA, this is because:

1. Oxacillin is more resistant to degradation in storage and is more likely to detect most heteroresistant strains.

In addition, methicillin is no longer commercially available in the United States. Antimicrobials like oxacillin and nafcillin now are used for treatment of *S. aureus* infections.

2. When resistance was first described in 1968, methicillin was used to test and treat infections caused by *S. aureus*. Now, methicillin is no longer the agent of choice for testing or treatment of susceptible staphylococcal infections. However, the acronym MRSA is still used by many to describe these isolates because of its historic role.

On the basis of above information, MRSA and MSSA were defined according to NCCLS MICs breakpoints of ≥ 4 μg/ml and ≤ 2 μg/ml respectively.

Additionally, MRSA and MSSA will be defined if a clear zone of inhibition is ≤10 mm and ≥13 mm, respectively.

Hospital Acquired (HA)-*Staphylococcus aureus* Infection

1. Any isolate of *S. aureus* infection occurred > 48 hours after hospitalization.
2. Any new infection or super infection occurred 48 hours after hospitalization.

Community Acquired (CA)-*Staphylococcus aureus* Infection

1. Any isolate from outpatients.
2. Any isolation within 48 hrs of hospitalization for in-patients.
3. Any isolate from patients with abscess or cellulitis on admission regardless the isolation time after hospitalization.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

1. Age less than or equal 15 years infected with *S. aureus* confirmed by culture and had the susceptibility result according to NCCLS recommendation.
2. The patient medical record was available for reviewing.

Exclusion Criteria

The infection was assumed as contamination, which defined by in charge physician or laboratory unit.

DATA ANALYSIS

Descriptive statistics were used to describe the demographic characteristics, clinical symptoms and signs and laboratory tests of subjects. The data was analyzed by utilizing statistical test with a preset confidence interval (CI) 95 % ($p < 0.05$) and χ^2-tests for categorical variables was used for comparison. The statistical software package of SPSS was used.

CHAPTER V
RESULTS

DEMOGRAPHIC DATA OF THE SUBJECTS AND CHARACTERISTIC OF SPECIMENS

In this study, 274 medical records that met the inclusion and exclusion criteria were reviewed. There were 154 male (56.2%) and 120 female (43.8%) patients. Their age ranged from neonate to 15 years with average age 25.3 months. Their mean (SD) weight and height were 9.0 (8.5) kilograms and 75.6 (24.7) centimeters, respectively. Eleven percent of subjects had a history of school attendance and 91.2 % had no information of daycare attendance. Sixteen percent of children had a history of previous hospitalization, 50.7 % of children had chronic and underlying diseases, 4.0 % of the children took immunosuppressive medications and 2.2% had prior cutaneous infections. According to 78 children, which the number of family members was available, the mean (SD) of number of family member was 4.3(1.4) (Table 1).

Table 1. The Characteristics of Patients

Characteristic	Number of patients with available data	Mean (SD)	Number (%)
Gender Male: Female	274		154 (56.2):120 (43.8)
Age (month)	274	25.3 (39.6)	
Weight (kg)	268	9.0 (8.5)	
Height (cm)	120	75.6 (24.7)	
School attendance	255		28 (11.0)
Previous hospitalization	236		44 (16.1)
Chronic & underlying diseases	268		136 (50.7)
Immunosuppressive drug	262		11 (4.0)
Prior cutaneous infection	246		6 (2.2)
Number of family member	78	4.27 (1.4)	

Most of the patients from whom *S. aureus* was isolated, were from pediatric ward (54.4%) followed by neonatal ward (20.4%) and surgical ward (10.6%). About eleven percent of isolates were from the patient in critical care units (ICU and NICU). Only 3.2% of the isolated specimens were from the outpatient department (Table 2).

Table 2. Distribution of *S. aureus* Infection according to the Admitted Wards

Admitted ward	Number (N= 274;%)
Pediatric ward	149 (54.4)
Neonatal ward	56 (20.4)
Surgical ward	29 (10.6)
Pediatric ICU	16 (5.8)
NICU	13 (4.8)
Pediatric OPD	5 (1.8)
ENT/EYE OPD	2 (0.7)
Surgical OPD	2 (0.7)
ENT/EYE ward	2 (0.7)

According to the site of *S. aureus* infection, skin and soft tissues (32.5%) was the most common site followed by systemic (more than 1 organs) (28.1%) and respiratory tract (15.7%). The least common site was gastrointestinal tract (2.1%), including pus from double lumen catheters, fluids from mouth in palatoplasty, fluid from peritoneal cavity, pus from exploratory laporatomy and pus from colostomy.

Sixty-four percent of patients did not get surgical procedure. In those who underwent surgical procedures the most common one was abdominal surgery (14.2%) followed by soft tissue surgery (6.9%) and a surgery on musculoskeleton system (6.2%) (Table 3).

Table 3. Site of infection and Surgical Intervention Presented in Number of Isolation of *Staphylococcus aureus* Specimens

	Site of infection Number (%)	**Surgical procedure Number (%)**
Skin & soft tissues	89 (32.5)	19 (6.9)
Genitourinary tract	8 (2.9)	5 (1.2)
Musculoskeleton	8 (2.9)	17 (6.2)
Gastrointestinal tract	6(2.1)	39 (14.2)
Multi organ system (>1 organs)	77 (28.1)	1 (0.4)
Respiratory tract	43 (15.7)	8 (2.9)
Eye	22 (8.1)	1 (0.4)
ENT	7 (2.6)	3 (1.1)
Others*	14 (5.1)	8 (2.9)
No surgical intervention	-	174 (63.5)
Total	274	

Other*:sites of infection: stoma wound, oral cavity, mandible, bleb of toe.

surgical procedures: palatoplasty, excision of bleb of toe, drainage of mandibular abscess.

Nearly half of the patients with *S. aureus* infection had received instrumentation procedure and 18% of the patients had received more than one instrumental procedure. Forty-one percent of the procedures were endotracheal intubation and 27% were central vascular catheterizations (Table 4).

Table 4. Instrumental Procedures in *S. aureus* Infected Patients

Instrumental Procedures	Number of patients (%)
No procedure	150 (56.2)
Procedures (at least 1)	117 (43.8)
Procedures (at least 2)	48 (18.0)
Procedures (at least 3)	11 (4.1)

Procedures	Number (%)
Endotracheal intubation	72 (40.9)
Vascular catheterization	48 (27.3)
Urinary catheterization	29 (16.5)
Endoscopy	1 (0.6)
Bronchoscopy	3 (1.7)
Intercostal drainage	4 (2.3)
Other	19 (10.8)

PERCENTAGE OF MRSA AND MSSA IN *S. AUREUS* ISOLATED SPECIMENS

In this study, the ratio of MRSA: MSSA was 1:2.9. The skin and soft tissues (36.5%) was the most common source of specimen for isolation of *S. aureus*. On the other hand, ear discharge (1.8%) was the least common specimen being submitted. The blood (30.3%) was the most common source of sterile specimen collected followed by tracheal secretion (12.0%). (Table5)

From the total 274 specimens, there were 71 (25.9%) and 203 (74.1%) isolated MRSA and MSSA, respectively. Isolation of MRSA from blood and sputum were more common than MSSA (Table 5).

Table 5. Source of Specimen for *S. aureus* Isolation

Source of specimen	MRSA (N=71)	MSSA (N=203)	Total Number (N=274)(%)
Skin & soft tissues	10	90	100 (36.5)
Blood	39	44	83 (30.3)
Tracheal suction	8	25	33 (12.0)
Eye discharge	2	22	24 (8.8)
Urine	2	5	7 (2.6)
Sputum	4	2	6 (2.2)
Ear discharge	0	5	5 (1.8)
Other	6	10	16 (5.8)

RISK FACTORS FOR *S. AUREUS* INFECTION

In comparison between the hospital-acquired and community-acquired *S. aureus* infection, age (less than 1 year), having chronic and underlying disease, surgical intervention and having instrumentation were significantly related to the hospital-acquired *S. aureus* infection. The instrumentation procedure had the highest relationship with hospital-acquired infection (odd ratio 11.9; 95% CI 16-23). Gender, school attendant, previous hospitalization, immunosuppressive condition and prior cutaneous infection were not different between hospital- and community-acquired infection (Table6).

Table 6. Risk Factors for Hospital- and Community-Acquired *S. aureus* Infection

Risk factors	HA (%)	CA (%)	Odd ratio (95%CI)	p value
Age				
<1y	80 (75.5)	80 (47.6)	3.4	<0.001
1-15y	26 (24.5)	88 (52.4)	(1.9-6.0)	
Gender				
Male	64 (60.4)	90 (53.6)	1.3	0.33
Female	42 (39.6)	78 (46.4)	(0.8-2.2)	
School attendant				
Yes	7 (7.0)	21(13.5)	0.5	0.15
No	13 (93.0)	134 (86.5)	(0.2-1.3)	
Previous hospitalization				
Yes	19 (21.1)	25 (17.1)	1.3	0.55
No	71 (78.9)	121 (82.9)	(0.6-2.7)	
Chronic & underlying disease				
Yes	79 (75.9)	57 (34.8)	5.9	<0.001
No	25 (24.1)	107 (65.2)	(3.3-10.8)	
Immunosuppression				
Yes	5 (5.1)	6 (3.7)	3.6	0.75*
No	94 (94.9)	157 (96.3)	(0.9-14.7)	
Prior cutaneous infections				
Yes	2 (2.1)	4 (2.6)	0.8	1.00*
No	93 (97.9)	147 (97.4)	(0.1-5.1)	
Surgical intervention				
Yes	53 (51.0)	43 (25.9)	2.9	<0.001
No	51 (49.0)	123 (74.1)	(1.7-5.2)	
Instrumentation procedure				
Yes	80 (77.7)	37 (22.6)	11.9	<0.001
No	23 (22.3)	127 (77.4)	(16.4-22.6)	

Fishers exact test*

Considering the strains of *S. aureus* infection, age less than 1 year, having chronic and underlying disease, having instrumentation intervention and surgical intervention were the significant risk factor for MRSA infection. The instrumentation intervention had highest risk for MRSA infection with odd ratio 11 (95%CI 5-23). In our study, school attendant was found as a protective risk for MRSA isolation (Table 7). Gender, previous hospitalization, immunosuppressive condition and prior cutaneous infection were not different between MRSA and MSSA infections.

Table 7. Risk Factors for *S. aureus* Infection in the MRSA and MSSA

Risk factors	MRSA	MSSA	Odd ratio (95% CI)	p value
Age				
<1y	57 (83.9)	103 (51.8)	4.8	<0.001
1-15y	11 (16.1)	96 (48.2)	(2.3-10.4)	
Gender				
Male	45 (66.1)	109 (52.9)	1.7	0.076
Female	23 (33.9)	97 (47.1)	(1.0-3.2)	
School attendant				
Yes	2 (2.9)	26(13.9)	0.2	0.024
No	66 (97.1)	161(86.1)	(0.03-0.9)	
Previous hospitalization				
Yes	17 (27.4)	27 (15.5)	2.1	0.06
No	45 (72.6)	147 (84.5)	(1.0-4.3)	
Chronic & underlying disease				
Yes	59 (83.1)	77 (39.1)	7.7	<0.001
No	12 (16.9)	120 (60.9)	(3.7-16.3)	
Immunosuppression				
Yes	1 (1.5)	10 (5.1)	0.3	0.29*
No	66 (98.5)	185 (94.9)	(0.01-2.1)	
Prior cutaneous infections				
Yes	1 (1.5)	5 (2.8)	0.6	1.00*
No	63 (98.5)	177 (97.2)	(0.02-5.1)	
Surgical intervention				
Yes	39 (57.4)	57 (28.2)	3.4	<0.001
No	29 (42.6)	145 (71.8)	(1.9-6.3)	
Instrumentation procedure				
Yes	57 (82.6)	60 (30.3)	10.9	<0.001
No	12 (17.4)	138 (69.7)	(5.2-23.3)	

Fishers exact test*

ANTIBIOGRAM OF *S. AURUES*

Both MRSA and MSSA were susceptible to vancomycin. Most of MSSA was still susceptible to co-trimoxazole, erythromycin and gentamicin, which were not benefit for MRSA treatment. Almost all of both strains of *S. aureus* resisted to penicillin G, which implied that this drug should not be used for *S. aureus* infection (Table 8).

Table 8. The Antibiotic Susceptibility Pattern of MRSA and MSSA

Antibiotics	MRSA (N=71)		MSSA (N=203)	
	Susceptible(%)	Resistant(%)	Susceptible(%)	Resistant(%)
Co-trimoxazole	3 (4.2)	68 (95.8)	203 (100)	0
Erythromycin	1 (1.4)	70 (98.6)	116 (95.9)	5 (4.1)
Gentamicin	0	71 (100)	202 (99.5)	1 (0.5)
Penicillin G	0	71 (100)	6 (3.0)	197 (97.0)
Vancomycin	71 (100)	0	203 (100)	0

When the susceptibility pattern of MRSA was compared between hospital- and community-acquired organisms, we found that both strains had similar pattern of resistance to co-trimoxazole, erythromycin, gentamicin, penicillin G and vancomycin (Table9).

Table 9. The Antibiotic Susceptibility Pattern of MRSA according to the Hospital- and Community-Acquired Infections

Antibiotics	***MRSA-HA (N=52)***		***MRSA-CA (N=19)***		**p value**
	Susceptible (%)	**Resistant (%)**	**Susceptible (%)**	**Resistant (%)**	
Co-trimoxazole	1 (1.9)	51 (98.1)	2 (10.5)	17 (89.5)	0.17*
Erythromycin	1 (1.9)	51 (98.1)	0	19 (100)	0.7*
Gentamicin	0	52 (100)	0	19 (100)	NA
Penicillin G	0	52 (100)	0	19 (100)	NA
Vancomycin	52 (100)	0	19 (100)	0	NA

* Fischer Exact test

When the susceptibility pattern of MSSA was compared between hospital- and community-acquired, we found that both strains also had the similar pattern of resistance to co-trimoxazole, erythromycin, gentamicin, penicillin G and vancomycin (Table10).

Table10. The Antibiotic Susceptibility Pattern of MSSA in Hospital- and Community-Acquired Infections

Antibiotics	**MSSA-HA (N=54)**		**MSSA-CA (N=149)**		**p value**
	Susceptible (%)	**Resistant (%)**	**Susceptible (%)**	**Resistant (%)**	
Co-trimoxazole	54 (100)	0	149 (100)	0	NA
Erythromycin	32 (59.3)	22 (40.7)	84 (56.4)	65 (43.6)	0.71
Gentamicin	53 (98.2)	1 (1.8)	149 (100)	0	0.26*
Penicillin G	0	54 (100)	6 (4.1)	143 (95.9)	0.15
Vancomycin	54 (100)	0	149 (100)	0	NA

* Fischer Exact test

RELATION OF DIAGNOSIS, STRAIN OF *S. AUREUS* AND SOURCE OF INFECTION

In the hospital-acquired infection, we found that there was no significant difference between both MRSA and MSSA in systemic and soft tissue infections, however in case of systemic infection, the percentage of MRSA was slight higher than those of MSSA.

Table 11. Comparison of Diagnosis and Strains of *S. aureus* in Hospital-Acquired Infection

Diagnosis	HA (N=106)			
	MRSA (%)	**MSSA (%)**	**Odd ratio (95%CI)**	**p value**
Skin & soft tissues infection				
Yes	5 (9.6)	10 (20.4)	0.4	0.174
No	47 (90.4)	43 (79.6)	(0.1-1.4)	
Systemic infection				
Yes	30 (55.8)	20 (37.0)	2.2	0.06
No	23 (44.2)	34 (63.0)	(1.0-5.2)	

In the community-acquired infection, we found that the MSSA was higher percentage than MRSA in skin and soft tissue infections ; on the other hand, the MRSA was significantly higher than MSSA in systemic infection.

Table 12. Comparison of Diagnosis and Strains of *S. aureus* in Community-Acquired Infection

Diagnosis	CA (N=168)			
	MRSA (%)	**MSSA (%)**	**Odd ratio (95%CI)**	**p value**
Skin & soft tissues infection				
Yes	3 (15.8)	72 (48.3)	0.02	0.01*
No	16 (84.2)	77 (51.7)	(0.04-0.77)	
Systemic infection				
Yes	8 (36.8)	21 (14.8)	3.87	0.01*
No	12 (63.2)	122 (85.2)	(1.26-11.81)	

* Fischer Exact test

RELATIONSHIP OF MRSA INFECTION AND INSTRUMENTAL PROCEDURE

In the hospital-acquired infection, we found that the MRSA infection was more common in patients who had received endotracheal intubation and central vascular catheterization comparing to those who had not received those procedures. There was no significant association between strain of *S. aureus* infection and urinary catheterization (Table 13).

Table 13. Relationship of Instrumentation and Hospital-Acquired MRSA Infection

Instrumental Procedure	HA (N=164)			
	MRSA (%)	MSSA (%)	Odd ratio (95%CI)	p value
Endotracheal intubation				
Yes	6 (31.6)	18 (12.4)	3.3	0.04
No	13 (68.4)	127 (87.6)	(1.0-10.9)	
Vascular catheterization				
Yes	5 (26.3)	6 (4.1)	8.3	0.003
No	14 (73.7)	139 (95.9)	(1.9-36.5)	
Urinary catheterization				
Yes	1 (5.3)	10 (6.9)	0.8	0.79
No	18 (94.7)	135 (93.1)	(0.02-5.9)	

CHAPTER VI
DISCUSSION

The pediatric patients who are commonly found to be infected with *S. aureus* infection was the focus of this study. Previous studies showed even neonates were at risk of *S. aureus* infection (Endo et al., 1996). There were numbers of risk factors contributing for the emergence and spreading of MRSA infections (Sattler et al., 2002; Herold et al., 1998; Fergie and Percell, 2001).

In our study, it was found that most *S. aureus* infections had been isolated from children in pediatrics and neonatal wards. The finding that large proportion of *S. aureus* had been isolated from blood stream gives an impression that this organism can easily invade the blood stream and can cause serious consequences. Gastrointestinal surgery was the most common procedure among the surgical intervention that associated with *S. aureus* infection. Skin and soft tissue was the most common site of infection, however it may not represent the real pathogen, because it might be only colonization.

From this study, it is noted that half of *S. aureus* isolated patients had undergone instrumentation procedures. The three most common procedures were endotracheal intubation, vascular catheterization and urinary catheterization. It may be because these three procedures were necessary for general intensive care.

Identifying risk factors and taking appropriate measures to control those are very important in order to control the spreading of the organism within the community. MRSA infection was more common in children whose aged less than 1 year, which confirmed the previous study by Napaporn (2003). Moreover having chronic or underlying disease as risk factors supports the study by Layton (1995), and undergoing instrumentation were the risk factors for MRSA infection. The finding from Benerjee et al (1991) and Steinberg et al (1996) showed that vascular device was a factor increasing the *S. aureus* infection and selective antibiotic pressure may increase the resistance strains, this confirm the findings by Panlilio (1992). School attendance in this study was shown to be a protective factor for MRSA infection,

which was a confounding factor with older age, confirmed by multivariate analysis. (data not shown).

When susceptibility to antibiotics pattern was concerned, glycopeptides was very effective against both MRSA and MSSA infection. co-trimoxazole, gentamicin and erythromycin were still effective against MSSA infection. Penicillin G had no place for the treatment of both MRSA and MSSA infection. This finding suggests that co-trimoxazole, erythromycin and gentamicin might be the alternative drugs for MSSA infection in case of cloxacillin allergy. This study does not find any significant difference between MRSA acquired from community and hospital, and between MSSA acquired from community and hospital, which implied that the source of the organism might not be helpful for choosing antibiotics.

In hospital-acquired *S. aureus* infection, 60% of systemic infection was caused by MRSA; therefore glycopeptides should be considered when the patients had nosocomial systemic infection.

The community-acquired skin and soft tissue infection was commonly caused by MSSA strain. It implied that the first line antibiotics used for the skin and soft tissue infection occurred in community should be cloxacillin.

This study also showed the relationship between instrumentation procedure and strain of pathogen in hospital-acquired *S. aureus* infection. The vascular catheterization in hospital-acquired infections was related to MRSA strain. Endotracheal intubation was also a risk factor for MRSA infection.

CHAPTER VII
CONCLUSION

Proper management of *S. aureus* infection in pediatrics patients is becoming more and more important as it may lead to emergence of resistant strains, serious clinical manifestations.

Age less than 1 year, presence of chronic or underlying diseases, exposure to instrumentation and undergoing surgical procedures were the most important risk factors for emergence of hospital-acquired MRSA strains.

Skin and soft tissue infections were more common than systemic (involving more than one system) infection.

Gender, previous hospitalization, presence of immunosuppressive conditions and prior cutaneous infection were not significant risk factors for MRSA infection both in hospital- and community-acquired.

Almost all of both community- and hospital-acquired MRSA strains were resistant to co–trimoxazole, erythromycin, gentamicin, and penicillin G but sensitive to vancomycin.

Penicillin G is no longer effective against both MRSA and MSSA strains. Most of the MSSA were still susceptible to co–trimoxazole, erythromycin, and gentamicin. Vancomycin was effective against both MRSA and MSSA isolations. It is worth to preserve glycopeptides for the resistant staphylococcal infections.

Identification of potential risk factors of *S. aureus* infection and judicious use of antimicrobials are mandatory in order to see the better outcome.

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APPENDIX

PATIENT'S CASE RECORD FORM Subject No___ ___ ___

Hospital Number ___ ___ ___ ___ ___ ___/___ ___

Admission Number ___ ___ ___ ___ ___/___ ___

Date of Admission __ __ /__ __/__ __ Date of Discharge __ __ /__ __/__ __ (DD/MM/YY)

1. Patient Profile

1.1 Birth Date __ __ /__ __/__ __ (DD/MM/YY) or Age __ __ years __ __ mo

1.2 Gender ____ Male=1/Female=2

1.3 Weight ___ ___. ___ kg
☐ no data

1.4 Height or Length ___ ___ ___ cm
☐ no data

1.5 School attendant:
☐ no school attendant
☐ kindergarten
☐ elementary school (Gr. I-VI)
☐ primary school (Gr. VII-IX)
☐ secondary school (Gr. X-XII)
☐ no data

1.6 Is there any day-care attendance in the last six months?
☐ No ☐ Yes, if yes, how long?_____ months ☐ no data

1.7 Is there any previous hospitalization within 1 year?
☐ No ☐ Yes, if yes, how many times? _____ ☐ no data
The latest admission date: __ __/__ __/__ __ (DD/MM/YY)
Duration of hospitalization: _____ days(last admission)
Diagnosis: _____

1.8 Does the patient have any chronic disease?
☐ No ☐ Yes, if yes, define_____ ☐ no data

1.9 Does the patient take any immunosuppressive drug?
☐ No ☐ Yes, if yes, define_____ ☐ no data

1.10 Is there any history of prior cutaneous infection within 6 months?
☐ No ☐ Yes, if yes, define_____ ☐ no data
When did the latest occur? ____/____/____(DD/MM/YY)

1.11 Number of persons in the household: _____ (include the patient) ☐ no data

2. The clinical features

2.1 System of *S. aureus* infection:

☐ skin and soft tissue ☐ musculoskeleton ☐ respiratory tract
☐ cardiovascular system ☐ gastrointestinal system ☐ hepatobiliary system
☐ genitourinary tract ☐ systemic (>1 organ) ☐ nervous system
☐ other, define_____

2.2 System of surgical intervention (can be >1)

☐ skin and soft tissue ☐ musculoskeleton ☐ respiratory tract
☐ cardiovascular system ☐ gastrointestinal system ☐ hepatobiliary system
☐ genitourinary tract ☐ systemic ☐ nervous system
☐ other, define_____ ☐ no any intervention ☐ no data

2.3 Instrumental procedure (can be >1)

☐ endotracheal tube ☐ urinary catheter ☐ endoscope
☐ vascular catheter ☐ no any instrument ☐ no data
☐ other, define _____

2.4 Admitted ward

☐ pediatric ward ☐ newborn ward ☐ surgical ward ☐ ENT or eye ward
☐ ICU pediatric ☐ NICU ☐ ICU surgery ☐ OPD ENT or eye
☐ OPD pediatric ☐ OPD well baby ☐ OPD surgery ☐ other, define_____

2.5 Type of infection:

☐ community acquired ☐ hospital acquired

2.6 Antibiotic treatments and their outcome:

Before the result of culture:

_____dose _____mg/kg/day start date ___/___/___ stop date ___/___/___
_____dose _____mg/kg/day start date ___/___/___ stop date ___/___/___
_____dose _____mg/kg/day start date ___/___/___ stop date ___/___/___

After isolation:

☐ No change Outcome ☐ cure ☐ improve ☐ not improve ☐ dead

☐ Change(1) ATB: name _____, dose ____ mg/kg/day, duration __ days
ATB: name _____, dose ____ mg/kg/day, duration __ days
Outcome after change(1) ☐ cure ☐ improve ☐ not improve ☐ dead

☐ Change(2) ATB: name _____, dose ____ mg/kg/day, duration __ days
ATB: name _____, dose ____ mg/kg/day, duration __ days
Outcome after change(2) ☐ cure ☐ improve ☐ not improve ☐ dead

☐ Change(3) ATB: name _____, dose ____ mg/kg/day, duration __ days
ATB: name _____, dose ____ mg/kg/day, duration __ days
Outcome after change(3) ☐ cure ☐ improve ☐ not improve ☐ dead

3. Pathogen

3.1 Lab specimen No. ___ ___ ___ ___ ___/__ ___

3.2 Date of specimen collection: ____/____/____

3.3 Source of specimen:

☐ blood ☐ CSF ☐ pleural fluid ☐ pericardial fluid
☐ ear discharge ☐ sputum ☐ tip of catheter ☐ skin and soft tissue
☐ urine ☐ other, define_____

3.4 Resistance of *Staphylococcus aureus*:

☐ MSSA ☐ MRSA ☐ VISA ☐ VRSA

3.5 Susceptibility pattern:

Antibiotic	Susceptible	Intermediate	Resistant	Method	MIC
Amikacin					
Ampicillin					
Cefotaxime					
Ceftriaxone					
Ceftazidime					
Chloramphenicol					
Ciprofloxacin					
Co-trimoxazole					
Erythromycin					
Gentamicin					
Imipenam					
Oxacillin					
Meropenam					
Nalidixic acid					
Netilmicin					
Nitrofurantoin					
Norfloxacin					
Penicillin G					
Tetracycline					
Vancomycin					

BIOGRAPHY

NAME:	Susith Ranjan Wadikawage
DATE OF BIRTH:	28 September 1968
PLACE OF BIRTH:	Walasmulla, Sri Lanka.
INTITUTIONS ATTENTED:	M.B.B.S, (1996) Faculty of Medicine, University of Ruhuna, Sri Lanka. National Institute of Health Services (NIHS) Kalutara, Sri Lanka (2003). D.T.M.&H, (2004) Faculty of Tropical Medicine, Mahidol University, Thailand.
OFFICIAL ADDRESS:	District Medical Officer, Base Hospital, Tangalle, Sri Lanka (2002-up to now). TP-0094 472240261.
HOME ADDRESS:	No 31, Indipokunugoda Road, Tangalle, Sri Lanka. TP-0094 472242044. -0094 777 250568.
E –MAIL:	susithranjan@yahoo.com