

**PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*
(MRSA) AMONG PAEDIATRIC PATIENTS ADMITTED IN INTENSIVE CARE
UNIT AND NEONATAL INTENSIVE CARE UNIT AT KENYATTA NATIONAL
HOSPITAL-NAIROBI, KENYA.**

**A dissertation in partial fulfillment for the Degree of Masters of Medicine (M.MED)
in Paediatrics and Child Health, University of Nairobi (UON).**

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DECLARATION

I, Samuel Rutare, hereby declare that this is my original work and has not been presented for the award of a degree in any other university. I also declare that the intellectual content of this thesis is the product of my own work, although I have received invaluable assistance from my supervisors and others which I dully acknowledge.

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DEDICATION

To God the Almighty, who is the provider of everything.

To my late Mother, who couldn't live long to witness this work.

To my great loving father, beloved sisters and brothers.

To my M.Med friends as well, without them life during this program would probably have been miserable

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ABBREVIATIONS

CA-MRSA	Community Acquired Methicillin-resistant <i>Staphylococcus aureus</i> .
DNA	Deoxyribonucleic acid
HA-MRSA	Hospital Acquired Methicillin-resistant <i>Staphylococcus aureus</i>
ICU	Intensive Care Unit.
IQR	Inter-quartile range
KEMRI	Kenya Medical Research Institute.
KNH	Kenyatta National Hospital
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NBU	Newborn Unit
NICU	Neonatal Intensive Care Unit
NUR	National University of Rwanda
PCR	Polymerase Chain Reaction
SPSS:	Statistical Package for Social Sciences
WHO:	World Health Organization.

ABSTRACT

Back ground: Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a public health problem globally. MRSA infection increases morbidity, risk of mortality, increased financial burden and loss of productivity. MRSA on average accounts for 57% of *S.aureus* isolates causing nosocomial infection in intensive care units (ICUs) and are increasingly reported from many other countries worldwide. This was the first study to be carried out in Kenyatta National Hospital (KNH), the national teaching and referral hospital in Kenya to find out the prevalence of MRSA.

Justification: There has not been any study done to establish the prevalence of MRSA in Kenyatta National Hospital NICU and ICU. The findings from this study will facilitate rational planning, protocol and guidelines formulation in both ICU and NICU.

Objective: To determine the prevalence of methicillin resistant *Staphylococcus aureus* among paediatric patients admitted in neonatal intensive care unit (NICU) and ICU of KNH.

Methods: This was a cross sectional descriptive study carried out over a period of five months in NICU and ICU. Children admitted in these units were recruited into the study after getting a written informed consent from their parents or guardians. Nasal swabs and tracheal aspirates were collected and taken to Kenya Medical Research Institute (KEMRI)-Microbiology laboratory where conventional culture techniques, characterization of *S. aureus*, determination of the *mecA* gene for MRSA using PCR techniques and antibiotics susceptibility were performed. SPSS version 17.0 was used for data analysis.

Results: One hundred and fifty patients were recruited into the study. Of these 99 were males and 51 females. Sixty seven patients were from NICU and 83 were from ICU. A total of 218 samples (155 nasal swabs and 63 tracheal aspirates) were collected from these patients and *S.aureus* was isolated from 71 samples (32.6%). Of the 71 *S.aureus* isolated 33 (46.5%) were methicillin resistant. *S.aureus* showed highest sensitivity to vancomycin and linezolid, followed by amikacin and highly resistance to most of the commonly used antibiotics here at KNH.

Conclusion: *Staphylococcus aureus* was isolated from one third of the nasal and tracheal aspirates of patients in the NICU and ICU. MRSA is highly prevalent (46.5%) among the *S.aureus* isolates. MRSA isolates were highly sensitive to vancomycin, linezolid and amikacin.

Recommendations: Continuous surveillance of antimicrobial susceptibility to inform policy and practice, and a study to establish levels of nasal carriage among health workers in these units should be done. Our second line antibiotics in NICU and ICU are not effective against MRSA and empiric antibiotic should be vancomycin, amikacin or linezolid.

1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of *staphylococcal* bacteria that is resistant to beta-lactams. These antibiotics include methicillin and other more commonly used antibiotics such as oxacillin, penicillin and amoxicillin. MRSA infections in the community are mainly skin infections. The most severe form of MRSA infections occur among patients in hospitals. Three out of ten people are colonized in the nose with *Staphylococcus aureus* and about 2% are colonized with MRSA.¹

MRSA was first described in 1961 and since then it has become an important human pathogen.² The first reports on MRSA infection were from Boston City Hospital in 1968.³ The National Nosocomial Infections Surveillance System of the Centers for Disease Control and Prevention showed in August 2003 that, on average, six out of ten *S.aureus* isolates from ICUs are methicillin resistant and this was higher than the reported prevalence of about 40% between 1995-1999.⁴ MRSA colonization or infection varies from one region to the other, type of health care facility, and the specific population being studied. *Staphylococcus aureus* is an important cause of community and hospital-acquired infections. MRSA infections are mainly hospital acquired and are increasingly reported from different regions of the world.^{4, 5, 6} MRSA is resistant to many different classes of antibiotics and this is a growing concern.⁷

ACQUISITION OF *STAPHYLOCOCCUS AUREUS* RESISTANCE TO METHICILLIN

The resistance of *S.aureus* against methicillin is caused by expression of Penicillin binding protein 2A (PBP2A) encoded by the *mecA* gene. This protein which has low affinity for beta-lactam antibiotics such as amoxicillin, methicillin and oxacillin, renders these antibiotics ineffective treating infections caused by *Staphylococcus aureus*. The origin of *mecA* gene is not known, but evidence supports horizontal transfer between different staphylococcal species and other gram positive genera. The *mecA* gene in *Staphylococcus aureus* is located on the genetic element *staphylococcal* cassette chromosome (SCC).^{8,9}

2. LITERATURE REVIEW

MRSA PREVALENCE

MRSA infections are mainly hospital acquired and are as a result of health-care related procedures.⁶ Since the first description of MRSA in 1961,² its prevalence has been increasing as shown by the data from continuing surveillance initiatives such as the National Nosocomial Infection Surveillance System and European Antimicrobial Resistance Surveillance System show. The prevalence of MRSA infection has been low and fairly stable for many years in the Scandinavian countries but it has begun to rise. Currently it's the most commonly identified antibiotic-resistant bacteria in many countries.^{4,6}

Figure 1 depicting the MRSA prevalence by country.⁶

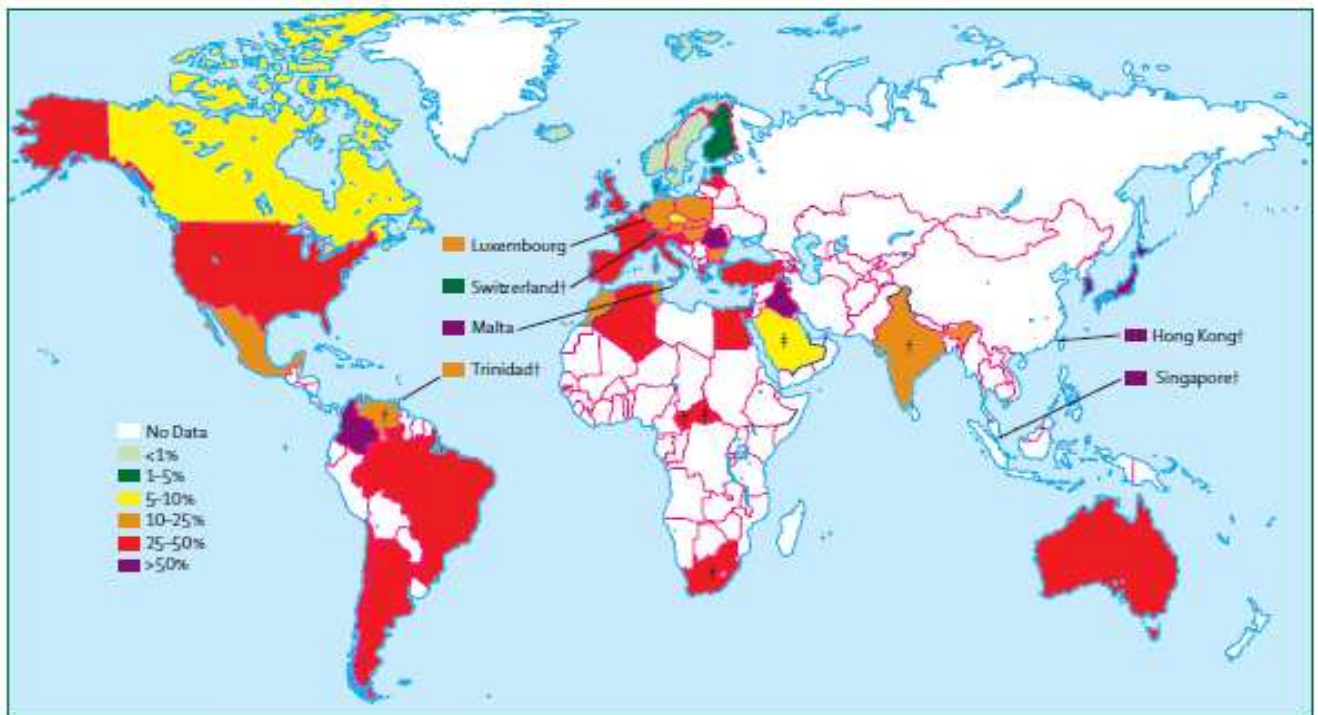


Figure 1: Worldwide prevalence of MRSA displayed by country*

*All presented MRSA proportions are from peer-reviewed studies undertaken since 1998.^{20,44} Prevalence estimates for Morocco, Algeria, Tunisia, Egypt, Jordan, Lebanon, and Turkey are from the antimicrobial resistance in the Mediterranean region website²⁰ at www.sih.gov.mt/armed/earss.asp. Studies providing most recent estimate of the MRSA proportion taken into account. If more than one study reported over same period, study including different types of clinical isolates was preferred over studies including only one specific type of specimen. †-Prevalence estimates are based on a study that included only one hospital. ‡-Prevalence estimates are based on studies between 1993 and 1997.

Figure 1. Worldwide prevalence of MRSA displayed by country

Despite the few routine surveillance systems, many countries have MRSA prevalence data. Different studies have different study designs, different inclusion criteria for health-care institutions, different antibiotic testing, and different selection of clinical and surveillance specimens. All these differences make comparison of international data difficult. There is limited data in Africa especially on patterns of sensitivity of MRSA to different antibiotics. In 2003, Kesah et al carried out MRSA prevalence study in eight African hospitals and Malta. They found out that the prevalence of MRSA in Nigeria, Kenya and Cameroon ranged from 21-30% and that all MRSA isolates were 100% sensitive to vancomycin.¹⁰ Despite the scarcity of data for many developing countries especially those in Africa and Asia, it appears that MRSA pandemic is beginning to emerge.^{6, 10, 11}

IMPACT OF MRSA INFECTION

There is evidence that MRSA infection increases the risk of mortality, morbidity, medical care costs and loss of productivity.^{6, 12} The increased medical care costs accrue directly as expenses caused by extension of hospital stay, additional diagnostic or therapeutic procedures, and additional antibiotic use while loss of productivity is due to absence from work during hospitalization.^{6, 12}

CONTROL OF MRSA IN HEALTH CARE FACILITIES

Screening of patients

Patients are important reservoir of MRSA in health-care facilities. These patients do not have signs or symptoms of the infection but are carriers of MRSA and can serve as bacterial source of transmission to other people. Many of these carriers of MRSA are not detected by the routine cultures that are ordered by doctors. Screening of patients by culture of the anterior nares swabs alone will identify 80% and screening from other body sites will increase the sensitivity to over 92%.^{6, 13, 14}

Many researchers have reported on different occasions that combined measures to control and prevent MRSA transmission in health facilities are effective. These measures include: screening of high-risk patients in intensive care units, contact precautions, rational use of antibiotics, mupirocin treatment of nasal carriers of MRSA, appropriate hand hygiene and staff education

can reduce MRSA transmission even in facilities where it is highly prevalent. Screening of carriers of MRSA is also done among those admitted to selected non-intensive-care wards, those thought to be at high risk of MRSA at the time of admission, roommates of patients with MRSA infection and elderly patients.^{13, 14, 15, 16, 17}

Different studies have evaluated the cost-effectiveness of active surveillance cultures and contact/droplet precautions for MRSA control in hospitals. In a study, Papia et al showed that a policy of screening high-risk patients for MRSA colonization on admission to hospital is cost-effective and should be implemented for infection control in hospitals.¹⁸ In another study, Karchmer et al also concluded that the combination of surveillance cultures and barrier precautions result in cost savings for hospitals.¹⁹

Screening of staff

Health-care workers are not as important reservoirs as patients who are colonised or infected with MRSA. However, health care personnel who are nasal carriers of MRSA can also transmit MRSA. Nasal decolonisation using mupirocin cream is indicated for health-care workers who are MRSA nasal carriers.²⁰

Isolation and barrier nursing

Patients colonised or infected with MRSA should be isolated and placed in a private room, or housed with other patients who have MRSA. There is no randomized controlled trial to support this practice in interrupting MRSA transmission.^{6, 16} Guidelines recommend wearing of gowns and gloves when caring for MRSA-positive patients and this is supported by epidemiological studies. The effectiveness of the use of gloves and gowns to care for patients with MRSA has not been established from any randomised trials.^{13, 16, 21, 22}

Hand hygiene

Poor hand-hygiene has been documented on many occasions and is widely believed to be the predominant method by which MRSA is transmitted to patients.^{6, 13, 22} People with skin lesions on hands are colonized with MRSA and are likely to be sources of infection transmission to other people. Several studies have shown that improvement in hand-hygiene practices, when coupled with surveillance cultures and contact precautions, greatly reduce the transmission of MRSA. Guidelines have uniformly recommended that health-care workers clean their hands, preferably with an alcohol-based hand rub or an antimicrobial soap and water, after caring for patients with MRSA for control of the infections.^{13, 16, 21, 22}

Environmental cleaning

Contaminated environmental surfaces are important reservoirs for MRSA. Documented frequency of contaminated environmental surfaces has varied from a few percent in most studies to as high as 64-74% in others.²³ The US Centers for Disease Control and Prevention isolation guidelines recommend that hospitals have adequate procedures for routine care, cleaning, and disinfection of environmental surfaces, beds, bedrails, bedside equipment, and other frequently touched surfaces for control of MRSA infection.^{21, 24}

Korn et al conducted a study to evaluate the Colonization of hospitalized patients with MRSA in Intensive Care Units in Brazil and found out that 46% were already colonized at the time of admission. Fifty two percent of patients negative for MRSA at admission were colonized while in the ICU and no risk factor (age, previous hospitalization, prior surgery) was associated with acquiring MRSA.²⁵

The findings of the Canadian prospective surveillance study assessing antimicrobial resistance in patients in ICUs in Canada showed that MRSA prevalence was 22.3% of all *S. aureus* isolates. Most of these isolates were hospital acquired and less than 10% were community-acquired. Other bacteria isolated in the study were *E.coli*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *coagulase negative Staphylococci*, *Enterococcus*, *Streptococcus pneumoniae*, *Klebsiella-pneumoniae*, and *Enterobacter cloacae*.²⁶

In England, screening of patients at admission in the paediatric intensive care unit conducted between October 2008 and November 2009 found an MRSA prevalence of 1.6%. During the screening period, there were 20 MRSA culture-positive patients. One patient was bacteraemic with MRSA on admission while the remaining 19 had asymptomatic colonization and no MRSA infection cases arising de novo in PICU were identified during the entire screening period.²⁷

In Johns Hopkins Children's Center, a study showed that those patients colonized but not sick with the methicillin resistant *S.aureus* (MRSA) are at increased risk for developing full-blown infections. Of 3,140 children admitted to the Hopkins Children's pediatric intensive care unit (PICU) between 2007 and 2010, 153 arrived at the hospital already colonized with MRSA while 15 children acquired the bacterium while in the unit. Seven of the 15 children who became colonized with MRSA in PICU went on to develop full-blown infections and these findings highlight the risk of MRSA spread among PICU patients.²⁸

Al-Talib et al conducted a study on MRSA nosocomial infection trends in Hospital Universiti Sains Malaysia during 2002-2007 and showed that; The MRSA infection rate was 1 per 100 hospital admissions. Duration of hospitalization, previous antibiotic use, and bedside invasive procedures were associated with acquiring MRSA. The prevalence of MRSA was highest in the orthopedic wards at 25.3%, followed by surgical wards at 18.2% and intensive care units (ICUs) at 16.4%. The resistance of MRSA isolates to different antibiotics was erythromycin at 98.0%, co-trimoxazole at 94.0%, gentamycin at 92.0% and clindamycin at 6% resistance. All MRSA isolates sensitive to vancomycin.²⁹

In the Egyptian study of incidence and risk factors for MRSA infection among Alexandria University Pediatric Intensive Care Admissions showed that the prevalence of *S.aureus* infection in the studied patients was 15% (4.2% were methicillin-sensitive *S.aureus*, 7.5 % community acquired-MRSA and 3.3% were hospital acquired-MRSA). All the risk factors studied were highly associated with nosocomial MRSA acquisition (referred from wards, hospitalized within the last 12 months, having surgery within last 12 months, having an outpatient visit within last 12 months, having a chronic disease, previous use of injectable drugs).The mortality rate was higher in patients with MRSA infection compared to methicillin sensitive *S.aureus* (MSSA) patients.³⁰

Another study conducted in Johns Hopkins Hospital PICU from March 2007 to May 2008 showed that the median age was 5 years (IQR 1–12 years), and 45% of patients were female. Screening cultures were performed on nasal swabs from 1,210 children (72%) obtained at the time of PICU admission. Screened patients were more likely to have been hospitalized in the previous one year compared to those that were not screened at admission (29% vs. 22%, $p < 0.01$). There were no other significant differences in demographic or clinical characteristics between those patients screened for MRSA colonization and those not screened. At the time of admission to PICU, 6 % (72/1210) of the screened patients were colonized with MRSA.³¹

Several studies conducted in India have shown that MRSA infection is alarming. A study done in central India showed that the prevalence of MRSA isolates was 51.8% of all *S.aureus* isolated from different clinical specimens and all MRSA strains were resistant to penicillin. The resistance to ciprofloxacin was at 84%, erythromycin at 74.5%, pristinamycin at 66.2% mupirocin at 11% and rifampicin resistance was at 16.6%. All MRSA strains were sensitive to vancomycin and linezolid.³² In another study carried out in India by Verma S et al demonstrated that the prevalence of MRSA had increased rapidly from 12% in 1992 to 80.89% in 1999 and MRSA strains showed high resistance to several antibiotics. The prevalence of MRSA isolates in Tata hospital in Mumbai was high at 87% in 1995 and decreased to 64% in 1996. All MRSA were sensitive to vancomycin and teicoplanin.³³ The study carried out by Kavitha Prabhu, Sevitha Bhat et al between August 2009 and March 2010 in the Department of Microbiology, Yenepoya Medical College, Mangalore-India showed that MRSA isolates were 29% (12/ 41) of *S.aureus*.³⁴

A study done by Nwankwo et al in Nigeria showed that the age and sex distribution of patients with *S. aureus* infection in Kano were; Males (62.0%) had higher infection rate than females. The highest frequency of isolates of *S.aureus* occurred in the age group (0-10) yrs while the least was in adults above 50 years and the difference was statistically significant ($p < 0.0001$). The highest number of isolates was from wound infections 46(30.7%) followed by Ear swab 32(21.3%). The least were from pleural aspirate and skin swab 1(0.07%) each. The antibiotic sensitivity pattern of MRSA was ciprofloxacin at 56.3% while levofloxacin had 93.7% and ofloxacin had 68.7%. All isolates were sensitive to vancomycin.³⁵ Baddour et al in their hospital based study in Saudi Arabia showed that the prevalence of MRSA ranged from 12% to 49.4%.

Mean age was 44 years with males constituting 64.4% of patients with MRSA infection. Approximately 41.5% of the isolates came from patients in the extreme age groups. The overall susceptibility of MRSA to the various antibiotics tested was: fusidic acid 4.3%, sulfamethoxazole/trimethoprim 33.8%, gentamicin 39.6%, mupirocin 77.0%, gatifloxacin 78.9%, chloramphenicol 80.7%, linezolid 95.1%, quinupristin/dalfopristin 100%. Some differences were noted in the resistance of isolates among the participating hospitals reflecting antibiotic usage.³⁶

Ojulong et al conducted a study in Kampala, Uganda, and found that the prevalence of MRSA was 31.5% of the 54 *S. aureus* isolates. The most effective antibiotic against MRSA was vancomycin and resistance to trimethoprim/sulfamethoxazole, chloramphenicol and erythromycin were highest at 88.2%. Resistance to ciproflaxacin and gentamycin was 70.6% and 58.8%, respectively. All strains were 100% resistant to penicillin and clindamycin sensitivity was found to be 100%.³⁷

Omari et al. conducted a study at Kenyatta National Hospital in 1997 and showed that the most commonly isolated organisms were *E.coli*, Klebsiella and *S.aureus*. These bacteria had multiple resistance to the commonly used antimicrobials namely, penicillins, tetracyclines, trimethoprim/sulphamethoxazole and gentamycin. The resistance pattern was high among both gram negative and positive bacteria isolates. Prevalence of MRSA was 40% and showed multiple antibiotic resistance. The study also showed that *S.aureus* accounted for 57% of the gram positive isolates.³⁸

Naik et al carried out a study in Eritrea and found the following results; High resistance was observed against ampicillin (85%), penicillin (77%) and tetracycline (78%). Low resistance was observed against amoxicillin-c (8%), amikacin (7%) and ciprofloxacin (5%). 32% of the isolates were resistant to chloramphenicol and gentamycin. About 23 % of the *S.aureus* isolates were resistant to erythromycin. Of the 278 isolates 26 (9%) isolates were MRSA.³⁹

Marais E, Aithma N, Perovic O et al conducted a study; Antimicrobial susceptibility of methicillin-resistant *S.aureus* isolates from South Africa and got the following results; 248 *mecA*-positive MRSA isolates were available for antibiotic susceptibility. There were 101

females (42.2%) and 137 males (57.6%) gender was not recorded for 10 patients. The average age was 38.7 years, 22 patients were <1 year old, 46 patients <18 years old, 190 patients \geq 18 years old, and in 12 isolates the patient age was missing.⁴⁰

MRSA resistance against erythromycin, trimethoprim/sulfamethoxazole, tetracycline, gentamicin and ciprofloxacin ranged between 55% and 78%. All isolates were sensitive to vancomycin, teicoplanin, linezolid, quinopristin/dalfopristin and fusidic acid.⁴⁰

Smolinski et al showed that drug options for treatment of infections are becoming increasingly limited, largely as a result of growing antimicrobial resistance and the development of new antibiotics has been severely curtailed. In the past three decades, only two new classes of antibiotics were developed and resistance to one class emerged even before the drugs entered the commercial market. In the event of a natural or intentionally introduced microbial threat, antimicrobials may be the only available first line of response. A readily available supply, therefore, should be a priority in the preparedness plans.⁴¹

Abera et al in North West Ethiopia reported that the isolation rates of MRSA and MRCoNS of 55% and 78% respectively. MRSA and MRCoNS showed higher rates of multi-drug resistance against the commonly prescribed antibiotics such as penicillin G 100%, ceftriaxon 99.5%, tetracycline 90%, erythromycin 77.5%, ciprofloxacin 75.3% and gentamicin 71%.⁴²

Downie et al in systematic review and met analysis of nineteen studies from 13 countries, with over 4000 blood culture isolates, Downie et al found that among neonates, *S.aureus*, *Klebsiella* spp. and *E.coli* accounted for 55% (39–70%) of culture positive sepsis. In infants outside the neonatal period, the most prevalent pathogens were *S.aureus*, *E.coli*, *Klebsiella* spp., *Streptococcus pneumoniae* and *Salmonella* spp. which accounted for 59% (26–92%) of culture positive sepsis. For neonates, penicillin/gentamicin had comparable in vitro coverage to third-generation cephalosporins (57% vs 56%). In older infants (1–12 months), in vitro susceptibility to penicillin/gentamicin, chloramphenicol/penicillin and third-generation cephalosporins was 63%, 47% and 64%, respectively.⁴³

In Kenya Ouko et al reported MRSA prevalence of 26.3% with majority infecting the HIV positive patients (p=0.046). More Staphylococcal infections were common in HIV patients

($p < 0.001$). *S.aureus* susceptibility to different antibiotics tested in both groups was as follows; In HIV positive; Oxacillin (68.0%), cefotaxime (66.7%), vancomycin (93.2%), augumentin (75.7%), Trim/sulphamethoxazole (49.3%), erythromycin (43.5%), chloramphenicol (71.6%), tetracyclin (56.2%) and gentamycin (62.5%). In HIV negative patients, the susceptibility of *S.aureus* to different antibiotics tested was as follows: vancomycin, oxacillin at 74.4%, cefotaxime at 76.7%, augumentin at 79.1% tetracyclin at 52.4% trim/sulphamethoxazole at 31%, erythromycin at 37.2%, chloramphenicol at 76.7%, and gentamycin at 57.1%. The difference in sensitivity of *S.aureus* to different antibiotics in both HIV positive and HIV negative was not statistically significant.⁴⁴

3. PROBLEM STATEMENT

After seven decades of antibiotic use, forms of *S.aureus* have evolved that are resistant to most common antibiotics. These *S.aureus* which are resistant to methicillin are given the name "methicillin-resistant *Staphylococcus aureus*" (MRSA).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has increasingly become a more important human pathogen since its initial description in 1961 and the first outbreak of infection in 1968.^{2,3} The data from the National Nosocomial Infections Surveillance System of the Centers for Disease Control and Prevention showed in August 2003 that MRSA on average accounts for 57% of *S. aureus* isolates causing nosocomial infection in intensive care units (ICUs).^{4,5}

There is evidence that hospital-acquired MRSA infection increases morbidity, risk of mortality, medical care costs and loss of productivity.^{6, 12} At the start of this research, there was no local data on the magnitude of MRSA infection burden among children in our ICU and NICU-Kenyatta National Hospital.

4. STUDY RATIONALE AND JUSTIFICATION

Previously there has not been any study done to establish the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in the Kenyatta National Hospital NICU and ICU. However there have been a number of cultures positive MRSA in different specimens and this has not been quantified. This lack prompted me carry out this study. The findings from this study will facilitate rational planning, protocol and guidelines formulation in both ICU and NICU so that appropriate antibiotic therapy is given in time for better outcome and short stay in these units.

5. STUDY UTILITY

The findings from this study will

- Inform the clinicians on the burden of MRSA in the main ICU and NICU amongst pediatric admitted patients hence strengthening infection control systems.
- This information will be useful for treatment and care of patients and development of protocols and treatment guidelines.
- These findings from the study may form the basis for planning of screening all patients on admission to ICU and NICU for MRSA, regular screening of health personnel in the units and other infection control measures.

6. HYPOTHESIS

MRSA is a common nosocomial infection among paediatric patients admitted to Kenyatta National Hospital ICU and NICU.

7. OBJECTIVES

7.1 Primary objective

To determine the prevalence of MRSA amongst paediatric patients admitted in ICU and NICU- Kenyatta National hospital-Kenya.

7.2 Secondary objectives

1. To determine the antibiotic susceptibility of MRSA isolated from paediatric patients admitted in ICU and NICU at Kenyatta National hospital-Kenya.
2. To assess the proportion of hospital acquired and of community acquired MRSA among all the identified MRSAs.
3. To establish the spectrum of bacteria isolates in the samples collected from paediatric patients admitted in ICU and NICU at Kenyatta National hospital-Kenya.

8. METHODOLOGY

8.1 Study design

This is a cross sectional, descriptive study.

8.2 The study site

The study was conducted in ICU and NICU at Kenyatta National Hospital, a level 6 National Referral and Teaching Hospital. NICU receives patients directly from labour ward, those that deteriorate and develop NICU needs while in NBU or general paediatric wards plus referral from peripheral hospitals and private clinics in the country. The Neonatal Intensive care unit (NICU) is a small unit of 4 beds equipped with four Babylog 8000 plus ventilators capable of giving different modes of ventilator support to neonates in need. NICU is within the newborn unit. Main ICU has a capacity of 21 beds. It receives adult patients and paediatric patients above one month of age both females and males. ICU and NICU are run by consultants, doctors doing anesthesiology and paediatric post graduate residents plus a team of registered nurses (RN), some of whom have undergone specialized paediatric nursing training.

8.3 Study population

All consecutive pediatric admissions in NICU and ICU (i.e. from birth to eighteen years of age), except those whose parents declined to consent.

8.4 Inclusion criteria

All pediatric patients admitted to ICU and NICU (i.e. from birth up to eighteen years of age) and whose parents or guardian(s) provided a written consent.

8.5 Exclusion Criteria

All patients admitted to ICU and NICU whose parents or guardian(s) declined to consent.

8.6 Sample size

All pediatric patients admitted to ICU and NICU during the study period of five months except those who declined the consent were recruited into the study.

Sample size was calculated as per Fisher's formula for calculating sample size using precision around a proportion which provided minimum sample required.

$$n = \frac{z^2 p (1-p)}{d^2}$$

n= minimal sample size required for the study.

z= 1.96 (normal deviate corresponding to 95% confidence interval)

d= 0.05 (degree of precision around the mean)

P= 10.8% (represents prevalence of (MRSA) infection among Alexandria University Pediatric Intensive Care Admissions in Egypt.³⁰

$$\text{Thus } n = \frac{1.96^2 \times 0.108 \times 0.892}{0.05^2}$$

The minimum sample size n=148.

8.7 Recruitment procedure, data collection and sampling technique

Data was collected during the five months study period (from mid June to mid November 2012). The investigator visited NICU and ICU every day in the morning at 8am and in the afternoon to recruit study participant(s). Informed consent was obtained from the parents or guardians after a full explanation of everything concerning the study as detailed in the consent form. After obtaining the consent, the investigator took nasal swabs from anterior nares, as routine septic screen at ICU or NICU admission. Tracheal aspirates were also taken for those that were intubated.

These samples were taken on the first day of admission and then on fourth day while in the unit for determining whether the MRSA was community or hospital acquired.

Tracheal aspirates were collected in universal bottles and nasal swabs were collected and put in Bijou bottles which contained Stuart transport media. The collected samples were then taken to KEMRI-microbiology laboratory where they were worked on (culture, drug susceptibility and PCR for the *mecA* gene).

Using a questionnaire, socio-demographic information was obtained from parents/guardians. Clinical data was obtained from patient's files and from results of the laboratory tests. Data link log was made in such a way that the study number appeared on the questionnaire without any identification of the patient. A sheet of paper containing identifications of the patient (i.e. names, age, sex, in patient number, and study number) was kept confidentially by the investigator himself under key and lock.

The investigator was assisted by a research assistant who received training on data collection using the questionnaire as shown in the appendix. A formal request was also made to the nurse on duty to inform the investigator of a new admission and whenever a parent or guardian of a newly admitted patient to ICU or NICU was available for obtaining informed consent. The investigator visited the mothers who were admitted into the maternity ward when they were stable enough to provide informed consent.

Culture and drug sensitivity

The specimens were cultured on mannitol salt and sheep blood agar media (SBA) and incubated aerobically at 37⁰C for 18 to 24 hours. The colonies which appeared yellowish in mannitol medium with β -haemolysis on SBA underwent catalase test and the ones that were catalase positive were then gram stained and subjected to coagulase test. Confirmation of *S. aureus* was done using analytical profile index (API Staph, Biomerieux).⁴⁵ The confirmed *S. aureus* isolates were subjected to the following antimicrobial susceptibility testing against oxacillin (OX)=1 μ g, vancomycin (VA)=30 μ g, gentamycin (GN)=10 μ g, amoxicillin clavulanic acid (AMC)=30 μ g, chloramphenicol (C)=30 μ g, erythromycin (E)=15 μ g, tetracycline (T)=30 μ g, cefotaxime(CTX)=30 μ g,Ampicillin(AMP)=10 μ g,Amikacin(AK)=30 μ g),Clindamycin(DA)=2 μ g, Meropenem(MEM)=10 μ g,Linezolid(LZD)=30 μ g,Mupirocin(MUP)=5 μ g) and sulfamethoxazole/trimethoprim (SXT)=25 μ g using disk diffusion.^{45,46}

Determination of *mecA* gene by PCR

DNA Extraction (boiling method): *S.aureus* was grown on Brain Heart Infusion broth (BHI) overnight and then centrifuged at 10,000 rpm for 5 minutes at room temperature. The supernatant was discarded and the sediment cells re-suspended in 1 ml of TE buffer and vortexed. After this, 200 μ l was transferred to a new sterile tube and boiled for 30 minutes to release the DNA. The

suspension was centrifuged at 15,000 rpm for 10 minutes and the supernatant used as template DNA for PCR.

PCR mix: PuRe Taq Ready-To- Go PCR beads (Amersham biosciences) with a total reaction volume of 25 μ l, was used in the PCR run using the following primer set: F- `GGTGGTTACAACGTTACAAG-3 = 0.2 μ l; R- 5`GCATTGTAGCTAGCCATTCC-3` = 0.2 μ l; the template DNA = 1.0 μ l; with sterile distilled water of 23.6 μ l.

PCR products identification and conditions: Initial denaturation step of 3 minute at 94⁰C followed by a further 30-second of denaturation at 94⁰C; annealing step at 55⁰C for 30-seconds and extension at 72⁰C for 30-seconds for 35 cycles. The PCR products identification was by gel electrophoresis in 1.5% agarose (TAKARA) and visualized under ultraviolet light against a standard molecular base pair (1kb) ladder.^{45, 46}

8.7 Data management

The data collected included demographic data, clinical presentation at admission, diagnosis on admission, risk factors, and isolated microbes including MRSA plus drug susceptibility for each antibiotic which was classified as sensitive, resistant and intermediate depending on diffusion distance. The data collected was recorded into a worksheet (appendix) and then entered into personal computer for analysis using SPSS.

8.8 Data analysis

Data was entered into Epidata sheet and then exported to SPSS version 17.0 statistical package for analysis. Frequencies, means and proportions were calculated. To compare the means, student t-test was used or its non parametric equivalent if data was not normally distributed. Statistical significance was taken at the level $p < 0.05$ and results were presented in form of frequency tables, bar graphs, linear graphs or charts as appropriate.

8.9 Dissemination of results

The result of the study will be strictly disseminated for educational purposes; copies of the study findings will be submitted to the department of paediatrics and child health, Kenyatta National Hospital, University library and submitted to paediatric scientific journals for publication.

The results will also be presented in scientific conferences.

8.10 Ethical consideration

An approval to carry out this study was sought from the department paediatrics and child health-University of Nairobi and Kenyatta National Hospital Ethical Review Committee. Objectives and procedures of the study were fully explained to parents and a written informed consent was obtained from parents prior to enrolling their children into the study.

The information collected from patients or from their parents will always be kept confidential and used only for the purposes of achieving the objectives. Data recording and storing was done by the investigator and carefully stored under key and lock. Parents or guardians were clearly explained that the study primarily intends to provide valuable information that would be used to improve the care of patients admitted to NICU and PICU. During the data collection emergency care and resuscitation was always given a priority to the study.

9. RESULTS

9.1 Characteristics of recruited participants

During the study period, 150 patients were recruited into the study. As shown in table 1, Ninety-nine (66%) were males. Of the 150 patients, 83 (55%) were from ICU while 66 (44%) were from NICU. Over 80% of these patients were admitted to ICU or NICU for more than 72 hrs after being in other wards. Most of our patients (58%) were urban dwellers. The age of the participants was not normally distributed.

Table 1. Characteristics of recruited participants (n=150)

Characteristics	n	%
Age in days(Median(IQR))	180(1827)	
Sex		
Male	99	66
Female	51	34
Unit		
ICU	83	55.3
NICU	67	44.7
Duration of hospitalization at the time of collecting samples		
<48 hrs	29	19.3
>72 hrs	121	80.7
Residence		
Urban	87	58
Rural	63	42

9.2 Pattern of bacterial isolates from nasal and tracheal aspirates

From the 150 patients, a total of 218 specimens were obtained and processed for bacterial culture. Of these 155 (71%) were nasal swabs and 63 (29%) were tracheal aspirates. Overall there were 152 isolates. Among these 152 bacterial isolates, *Staphylococci* were the most prevalent with 47% being *S.aureus* and 28% Coagulase negative *staphylococci*. Others were *Klebsiella* at 15%, *E.coli* at 5% and enterococcus was 1% as shown in figure 2 below.

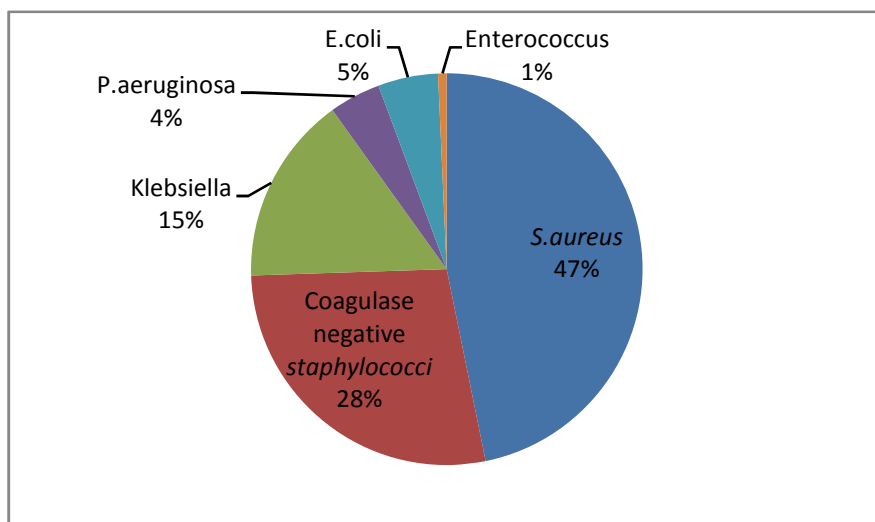
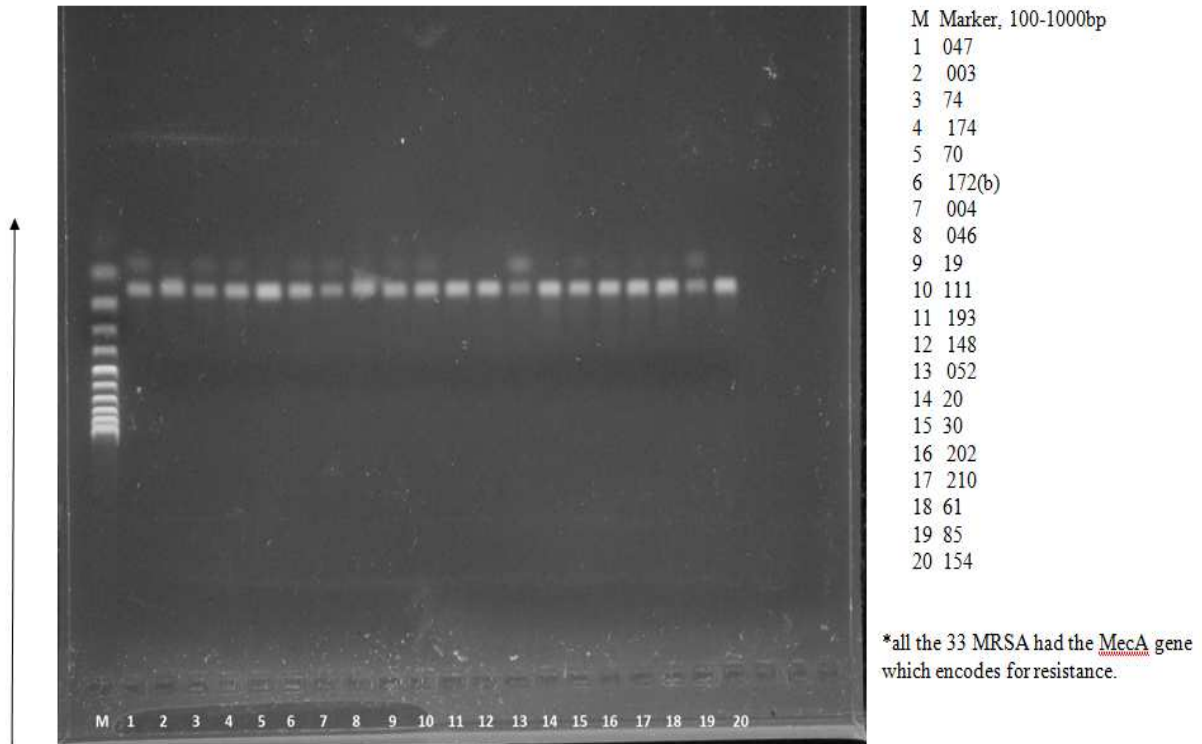


Figure 2. Spectrum of bacteria isolated in the nasal swab and tracheal aspirate cultures

9.3 Gel photomicrograph of *mecA* gene which encodes for resistance in *S.aureus*

Figure 3 shows gel photomicrograph of *mecA* gene PCR product from *S.aureus*, showing well M with 1000 bp ladder; 1, 2, 3, 4, 5,6,7,8,9,10 and 11-20 are positive clinical isolates for *mecA* gene. Clinical isolate 210 (well 17) was coagulase negative *staphylococcus* that was used as a negative control but it was also positive for *mecA* gene. All the 33 methicillin/oxacillin resistant *S.aureus* had the *mecA* gene which encodes for the resistance to methicillin.



In the pictures, our target gene, MecA(148bp) is located between 100 and 200bp.

Figure 3. Gel photomicrograph of *mecA* gene which encodes for resistance in *S.aureus*

9.4 Prevalence of MRSA in ICU and NICU

As shown in Table 2 below, out of 33 patients who were MRSA positive, 21 (64%) were from ICU and 12 (36%) were from NICU. Prevalence of MRSA among patients in ICU was 25% and that among patients in NICU was 18%. The odds of MRSA isolation in the ICU compared to the NICU was OR=1.55 (95%CI 0.65-3.7) p=0.2. Thus there was a 50% higher tendency of isolating MRSA in the ICU compared to isolating it in the NICU but the difference was not statistically significant.

Table 2. Prevalence of MRSA in ICU and NICU

Unit.	MRSA positive (N%)	MRSA negative (N%)	Odds	OR(95% CI)
ICU	21(25%)	62(75%)	0.34	1.55(0.65-3.7)
NICU	12(18%)	55(82%)	0.22	1.00

9.5 Proportion of MRSA isolated in samples taken within 48 hours of admission and the samples taken above 72 hours of admission

Patients admitted in NICU or ICU were referrals from other wards where they had been admitted for various durations. Twenty nine (19.33%) of the 150 patients were screened after being in the hospital for ≤ 48 hours and 121(80.67%) patients after hospital stay above 72 hours.

From table 3 below, it is observed that 29 patients were screened within 48 hours of admission of whom 4(13.8%) were MRSA positive while 25(86.2%) were MRSA negative. It is also observed that 121 patients were screened after 72 hours of admission of whom 29(24%) were MRSA positive and 92(76%) were MRSA negative.

The odds of MRSA isolation from samples taken within 48 Hours of admission compared to the odds of isolation after 72 hours of admission into the units was OR= 0.45 (95% CI 0.11-1.48) $p=0.17$. There was no difference in the rate of MRSA isolation from samples taken within 48 hours of admission and from samples taken more than 72 hours of admission.

It was not possible to determine whether the MRSA's isolated were hospital or community acquired because most of our patients were admitted into the ICU or NICU after more than 48hours of admission into different other wards.

Table 3. The proportion of MRSA isolated from samples taken within 48hours of admission and the samples taken above 72 hours of admission

MRSA Isolation	MRSA positive	MRSA negative
Within first 48 hours of admission.	4(13.79 %)	25 (86.2%)
Above 72 hours of admission	29(24 %)	92 (76 %)

9.6 *S.aureus* isolated from nasal swab and from tracheal aspirate cultures

As shown in table 4 below, 61 (85.9%) of the 71 *S.aureus* were cultured from nasal swab specimens and 10 (14.1%) were cultured from tracheal aspirate specimens.

Table 4. Percentage of *S.aureus* isolated in nasal swab and in tracheal aspirate cultures

Type of sample cultured.	Number of <i>S.aureus</i> isolated	Percentage
Nasal swab	61	85.9%
Tracheal aspirate	10	14.1%

9.7 Frequency of finding MRSA in nasal swab and in tracheal aspirate

Thirty three (33) MRSA were isolated from 155 nasal swabs and 63 tracheal aspirate cultures. Twenty nine (29) of the 33 MRSA were from nasal swab cultures and 4 from tracheal aspirate cultures as shown in table 5 below. Therefore the frequency of getting MRSA from a nasal swab was $29/155=18.7\%$, and the frequency of getting MRSA from the tracheal aspirate was $4/63=6.3\%$. The frequency of getting a MRSA positive in the nasal swab is three times higher than the frequency of getting MRSA positive in the tracheal aspirate, OR=3.39 [(95% CI 1.07-11.96) p=0.02].

Table 5. Frequency of finding MRSA in nasal swab and in tracheal aspirate

Type of sample cultured.	MRSA positive	MRSA negative	Odds	OR(95% CI)
Nasal swab	29	126	0.23	3.39(1.07-11.96)
Tracheal aspirate	4	59	0.07	1.00

9.8 MRSA in different age groups

Table 6 below, shows the distribution of MRSA isolation in different age groups. Children aged more than five years had the highest level of MRSA isolation rate 13(34.2%), while the age group 2-5 years had the lowest rates 2(12.5%).

Table 6. MRSA in different age groups

Age group	Yes(MRSA) N (%)	No(MRSA) N (%)
≤7 days	7(16.3%)	36(83.7%)
8days-28days	3(17.6%)	14(82.4%)
29days-2years	8(22.2%)	28(77.8%)
>2years-5years	2(12.5%)	14(87.5%)
>5 years	13(34.2%)	25(65.8%)

9.9 MRSA with Gender

As shown in table 7 below, the rate of isolation of MRSA in males was 22.2% compared to 21.6% in females (p=0.855).

Table 7. MRSA with Gender

Sex	MRSA positive	MRSA negative.	Total
Male	22 (22.2%)	77(77.8%)	99(100%)
Female	11 (21.6%)	40(78.4%)	51(100%)

9.10 MRSA and Mortality

From table 8 below, we observe that 52 of the 150 patients died, giving an overall mortality rate of 34.7%. Among the 33 patients who were MRSA positive, 11 died giving a mortality rate of 33.3% compared to 35.0% death rate among the MRSA negatives. This shows that mortality rate was almost the same in both groups, suggesting that having MRSA positive was not associated with a higher mortality in this study, OR=0.93(95% CI 0.37-2.23), p=0.855.

Table 8. MRSA and Mortality.

Outcome	MRSA positive	MRSA negative.	Total
Alive	22 (67%)	76(65%)	98(65.3%)
Died	11 (33%)	41(35%)	52(34.7%)
Total	33 (100%)	117(100%)	150(100%)

9.11 Antibiotics used in both ICU and NICU for the 150 patients

This figure 4 shows that ceftriaxone is the most commonly used antibiotic (46.7%) i.e. 70 patients of 150 used it. Gentamycin is the second most commonly used antibiotic (43.3%), followed by crystalline penicillin at 40% and vancomycin is the least commonly used antibiotic at 2%. Others included; Metronidazole, chloramphenicol, zinnat, augmentin, levofloxacin, erythromycin, septrin plus anti Tuberculosis drugs.

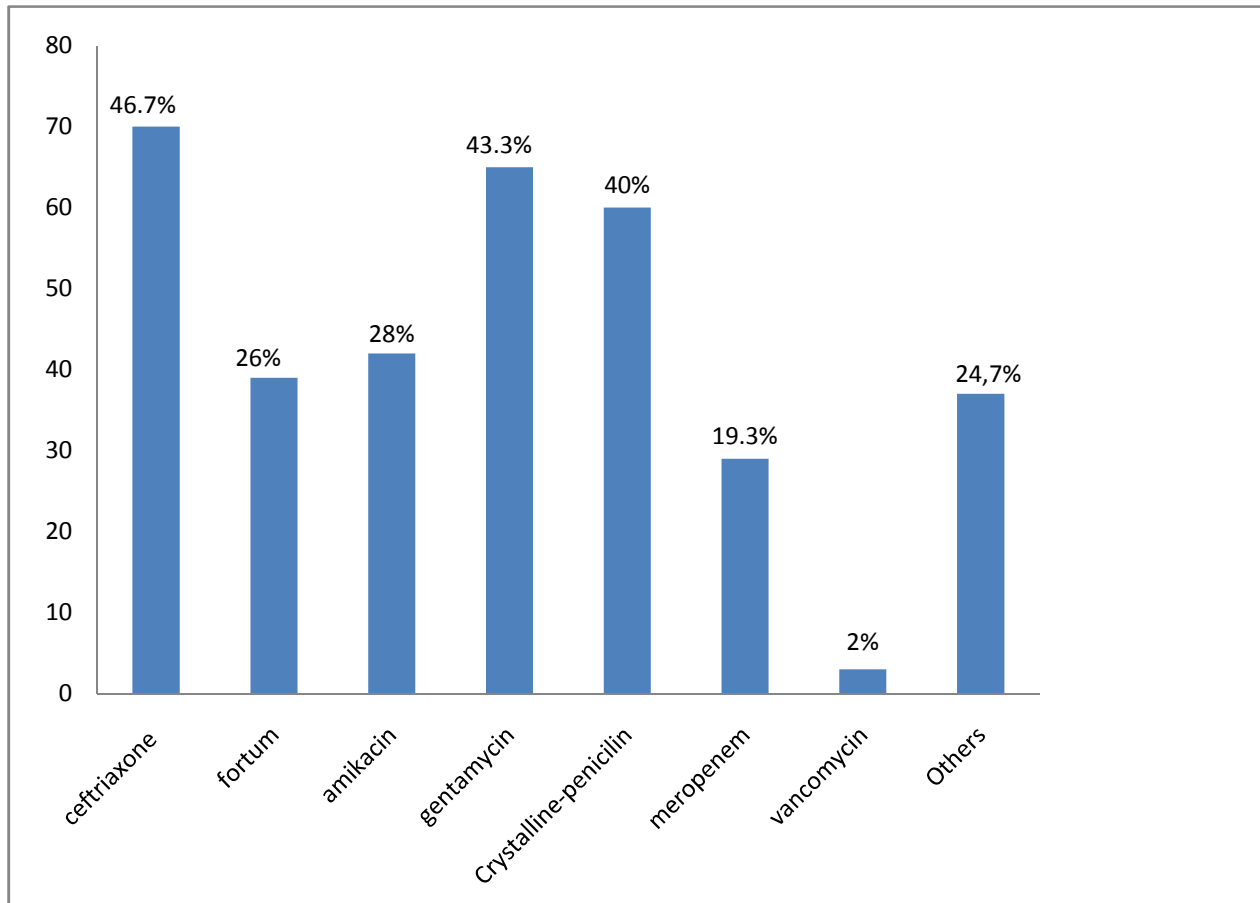


Figure 4. Antibiotics used in both ICU and NICU for the 150 patients

9.12 Susceptibility of *S.aureus* isolates to different antibiotics

From Table 9 shown below, the sensitivity of *S.Aureus* to both vancomycin and linezolid was the highest at 98.6% followed by amikacin at 94.4%. ampicillin showed the highest resistance (94.4%), followed by erythromycin (47.9%). The resistance of *S.aureus* to SMX-TMP was at 45.5%, gentamycin at 38%, amoxicillin-clavulanic acid (38%), Cefotaxime (38%). It should be noted that none of the tested antibiotics had 100% sensitivity.

Table 9. Susceptibility of *S.aureus* isolates to different antibiotics

Antibiotic tested	Resistance	Sensitivity	Intermediate
Ampicillin	67(94.4%)	4(5.6%)	0%
Vancomycin	1(1.4%)	70(98.6%)	0%
Gentamycin	27(38%)	39(54.9%)	5(7.1%)
Amikacin	3(4.2%)	67(94.4%)	1(1.4%)
Amoxicillin clavulanic acid	27(38%)	44(62%)	0%
Oxacillin	33(46.5%)	35 (49.3%)	3(4.2%)
Cefotaxime	27(38%)	32(45.1)	12(16.9%)
SMX-TMP	33(45.5%)	37(52.1%)	1(1.4%)
Clindamycin	6(8.5%)	62(87.3%)	3(4.2%)
Chloramphenicol	9(12.7%)	62(87.3%)	0%
Erythromycin	34(47.9%)	32(45.1%)	5(7%)
Tetracyclin	21(29.6%)	49(69%)	1(1.4%)
Meropenem	25(35.2%)	46(64.8%)	0%
Linezolid	1(1.4%)	70(98.6%)	0%
Mupirocin	6(8.5%)	65(91.5%)	0%
Ciprofloxacin	26(36.6%)	43(60.6%)	2.8% %

9.13 Susceptibility of MRSA to different antibiotics

As demonstrated in Table 10 below, the sensitivity of MRSA to vancomycin and linezolid was the highest at (97%), followed by amikacin at 87.9%. MRSA was 100% resistant to ampicillin, followed by amoxicillin clavulanic acid and cefotaxime at (81.8%). The resistance of MRSA to SMX-TMP is at 78.8%, both erythromycin and meropenem at 75.8% and gentamycin resistance is at 69.7%.

Table 10. Susceptibility of MRSA to different antibiotics.

Antibiotic	Resistant	Sensitive	Intermediate
Ampicillin	33(100%)	0(0%)	0(0%)
Amoxicillin/clavulanic acid	27(81.8%)	6(18.2%)	0(0%)
Cefotaxime	27(81.8%)	0(0%)	6(18.2%)
SMX-TMP	26(78.8%)	7(21.2%)	0(0%)
Erythromycin	25(75.8%)	3(9.1%)	5(15.2%)
Meropenem	25(75.8%)	8(24.2%)	0(0%)
Gentamicin	23(69.7%)	8(24.2%)	2(6.1%)
Ciprofloxacin	21(63.6%)	11(33.3%)	1(3%)
Tetracyclin	10(30.3%)	22(66.7%)	1(3%)
Clindamycin	6(18.2%)	24(72.7%)	3(9.1%)
Chloramphenicol	6(18.2%)	27(81.8%)	0(0%)
Mupirocin	6(18.2%)	27(81.8%)	0(0%)
Amikacin	3(9.1%)	29(87.9%)	1(3%)
Vancomycin	1(3%)	32(97%)	0(0%)
Linezolid	1(3%)	32(97%)	0(0%)

9.14 Susceptibility of coagulase negative *Staphylococci* to different antibiotics

Table 11 below shows that the sensitivity of coagulase negative *Staphylococci* to both vancomycin and linezolid was the highest at 100%, followed by amikacin at 89.7%. The resistance of coagulase negative *Staphylococci* to ampicillin was 100%, SMX-TMP at 76.9%, erythromycin at 74.4%, gentamycin and oxacillin resistance at 64.1%.

Table 11. Susceptibility of coagulase negative *Staphylococci* to different antibiotics.

Antibiotic	Resistant.	Sensitive.	Intermediate.
Ampicillin	39(100%)	0(0%)	0(0%)
Vancomycin	0(0%)	39(100%)	0(0%)
Gentamicin	25(64.1%)	12(30.8%)	2(5.1%)
Amikacin	3(7.7%)	35(89.7%)	1(2.6%)
Amoxicillin clavulanic acid	13(33.3%)	26(66.7%)	0(0%)
Oxacillin	25(64.1%)	11(28.2%)	3(7.7%)
Cefotaxim	18(46.2%)	10(25.6%)	11(28.2%)
SMX-TMP	30(76.9%)	9(23.1%)	0(0%)
Clindamycin	7(17.9%)	32(82.1%)	0(0%)
Chloramphenicol	11(28.2%)	28(71.8%)	0(0%)
Erythromycin	29(74.4%)	7(17.9%)	3(7.7%)
Tetracyclin	17(43.6%)	22(56.4%)	0(0%)
Meropenem	14(35.9%)	24(61.5%)	1(2.6%)
Linezolid	0(0%)	39(100%)	0(0%)
Mupirocin	4(10.3%)	35(89.7%)	0(0%)
Ciprofloxacin	14(35.9%)	21(53.8%)	4(10.3%)

9.16 Factors associated with isolation of MRSA

The factors associated with isolation of MRSA are shown in table 12. Only Surgery performed to the patient in the last 12 months was significantly associated with having MRSA positive as shown in table 12. The odds of MRSA isolation in the patients who underwent Surgery in the last 12 months compared to those who did not have surgery performed on them was OR=2.4 (95% CI 1.03-5.72) $p=0.04$.

Eighteen (18.8%) of patients below two years were MRSA positive while 15(27.8%) of patients above two years were MRSA positive ($p=0.2$). The association of MRSA and age below two years was not statistically significant.

Fifteen (21.7%) of the patients who had had previous admission in the last one year were MRSA positive compared to 18(22.2%) MRSA positive patients among patients without previous admissions in the preceding one year ($p=0.943$). There was no association of having MRSA and previous admission in one year.

There was no association between prolonged hospitalization longer than 14 days and having MRSA positive ($p=0.176$).

Eight (24%) of 33 MRSA cases had chronic medical conditions while 26 (22%) of MRSA negative cases had chronic medical conditions. The association of a chronic medical condition in acquiring MRSA was not statistically significant $p=0.807$.

Table 12. Factors associated with isolation of MRSA

Risk factors	MRSA		P- Value
	Positive N=33	Negative N= 117	
Age less than 2 yrs	18(55%)	78(67%)	0.2
Previous admission in one year	15(45%)	54(46%)	0.943
Prolonged hospitalization >14 days.	14(42%)	35(30%)	0.176
Surgery in the last 12 months	11(33%)	20(17%)	*0.04
Invasive procedures, e.g. Indwelling lines or catheters.	32(97%)	110(94%)	0.505
Chronic medical condition	8(24%)	26(22%)	0.807
Frequent antibiotic use	10(30%)	24(21%)	0.235
Skin condition e.g. eczema, impetigo, abscess	1(3%)	2(2%)	0.632

10. DISCUSSION

In this study that investigated the prevalence of MRSA among paediatric patients admitted in ICU and NICU at Kenyatta National Hospital, the key findings are; MRSA prevalence of 46.5% among *S.aureus* isolates and the most sensitive antibiotics against MRSA were vancomycin, linezolid and amikacin. One in three of the 218 specimens cultured grew *S.aureus*. *Staphylococcus aureus* were 47% of all bacterial isolates.

The MRSA prevalence of 46.5% among *S.aureus* in this present study was slightly higher than a 40% prevalence of MRSA reported by Omari et al in a study of pattern of bacterial infections and antimicrobial susceptibility at Kenyatta National Hospital, Nairobi, Kenya in 1997. In this same study *S.aureus* accounted for 56.9% of the gram positive isolates.³⁸ This slight increase may be due to focusing the study on samples collected from areas of highest risk for nosocomial infections. In addition the selection of specimens for analysis may skew the results. In our study we collected tracheal aspirates and nasal swabs the later contain alot of *S.aureus* compared to blood, urine, and CSF cultures. Another possibility is that MRSA is on the rise due to use, abuse, availability and consumption of antibiotics over the years or due to inadequate infection control mechanisms.

A study conducted by Ojulong et al in Mulago Hospital, Kampala, Uganda reported a 31.5% prevalence of MRSA of all *S.aureus* isolates³⁷ and this was lower than the 46.5% prevalence of MRSA in our study. In our study we took nasal swabs which carry more *S.aureus*. Another reason explaining this slight difference in MRSA prevalence could be better infection control mechanisms.

In a study conducted by Abera et al found out that the isolation rate of methicillin-resistant *staphylococci* (MRSA) was 55%⁴² which was similar to our isolation rate.

A study done by Korn et al found out that 46% of patients were colonized with MRSA at admission to ICU²⁵ and this was higher than the 22% (33/150) prevalence of MRSA among patients in our study . The difference is probably due to abuse, availability and consumption of antibiotics or poor infection control systems.

A study done by Verma et al. in India showed that the prevalence of MRSA increased rapidly since 1993 from 12% to 80.89% in 1999. MRSA prevalence was very high in Tata hospital in Mumbai where, it reached to 87% in 1995 and tapered to 64% in 1996. All the *S.aureus* were sensitive to vancomycin and taicoplanin.³³ In this study the prevalence of MRSA of all *S.aureus* remained in the range of 64 to 81% and this was higher than 46.5% prevalence of MRSA among *S.aureus* isolates in our present study. This difference was most likely due to abuse, availability and consumption of antibiotics or poor infection control systems in the two regions.

Naik et al in Eritrea showed MRSA prevalence of 9%³⁹ and this was lower than 46.5% MRSA prevalence in our study. This again could be explained by differences in availability and consumption of antibiotics or poor infection control systems. It should be noted that in the study carried out by Naik et al, nasal swabs were not taken and these have a better yield of *S.aureus*.

In the Egyptian study at Alexandria University Pediatric Intensive Care Unit, *S.aureus* infection prevalence was 15% of admissions (4.2% were methicillin-sensitive *S.aureus*, 7.5 % community acquired-MRSA and 3.3% were hospital acquired-MRSA)³⁰ and this is similar to 22% (33/150) prevalence of MRSA among admissions in our study.

Manal M Baddour et al showed that the prevalence of MRSA in the study hospitals ranged from 12% to 49.4%³⁵ and this was similar in our present study.

In our study, the prevalence of MRSA among admissions was 22%. In England study, screening on a paediatric intensive care unit admissions showed that MRSA prevalence was 1.6%²⁷. A Johns Hopkins Children's Center study of 3,140 children admitted to the Hopkins Children's pediatric intensive care unit (PICU) between 2007 and 2010 with routine screening at admission showed that 153(4.9%) arrived at the hospital already colonized with MRSA. A tiny subset of children 15 in all came to the hospital MRSA-free but acquired the bacterium while in the PICU.²⁸ This difference was most likely due to availability and use of antibiotics or poor infection control systems in the regions with higher prevalence of MRSA.

In the Canadian prospective surveillance study assessing antimicrobial resistance in ICU patients in Canada showed that MRSA prevalence was 22.3% of all *S.aureus* isolates, most of them were health care-associated and less than 10% were community-acquired.²⁶ This was lower than the 46.5% prevalence of MRSA among *S.aureus* isolates in our study and this can be explained by better policies and guidelines of antibiotic use or better infection control systems in Canada.

The resistance patterns of MRSA to the commonly used antibiotics (oxacillin, ampicillin gentamycin, amoxicillin clavulanic acid, erythromycin, cefotaxime, and sulfamethoxazole/trimethoprim) ranged from 69.7% for gentamycin to 100% for ampicillin and cloxacillin and these resistance patterns were similar to those reported in other studies that tested similar antibiotics.^{29, 32, 36, 37, 40, 42} In our study, high resistance patterns were observed against the commonly prescribed antibiotics and this was evident when we compared the resistance to two aminoglycoside group antibiotics gentamycin (69.7%) which is widely used in our setting than amikacin (9.1%).

In our study the sensitivity patterns of MRSA against vancomycin, linezolid and amikacin were above 90% and this was similar to other studies that tested the same drugs.^{32, 33, 35, 36, 37, 39 40, 44}

In our present study, the ratio of male to female patients was approximately 2:1, but the rate of MRSA isolation was not associated with gender. A study done by Nwankwo et al.³⁵ in Nigeria showed that sex distribution of patients with *S.aureus* infection in Kano was; Males (62.0%) had higher infection rate than females (38.0%). Baddour et al³⁶ study showed that males constituted 64.4% of patients with MRSA infection.

In our study 55% (18/33) MRSAs were observed in less than 2 years age group. However this was not statistically significant because even in other age groups were MRSAs and this was in line with the study done by Nwankwo et al in Nigeria which showed that the highest frequency of isolates of *Staphylococcus aureus* occurred in the age group less than 10 years.³⁵

11. STUDY STRENGTH

All the 33 MRSA (46.5%) of *S.aureus* isolates were confirmed MRSA with PCR showing presence of the *mecA* gene which encodes for resistance in *S.aureus*, therefore these results are reliable and reflect the true prevalence of MRSA in the studied units.

12. STUDY LIMITATIONS

The study findings are not generalizable of MRSA prevalence in all paediatric admissions, or in other wards, this strictly reflect only ICU and NICU admissions-Kenyatta National Hospital.

We were not able to answer the second specific objective of our study; to assess the proportion of hospital acquired and community acquired MRSA among all the identified MRSAs, because most of the study participants were admitted to ICU or NICU when they are above 72 hours of admission in different other wards.

The ideal time would be to cover a much longer period (e.g. whole year) of data collection but for time limit to comply with training program time frame; this study covered five months. Longer period of data collection would have increased number of study participants and hence answering secondary objective number two. However, we are confident that results have achieved the main objective of the study.

13. CONCLUSION

1. MRSA is highly (46.5%) prevalent among the *s.aureus* isolates from nasal and tracheal aspirates in NICU and ICU paediatric patients at Kenyatta National Hospital.
2. MRSA isolates were highly sensitive to both vancomycin and linezolid, followed by amikacin. MRSA isolates were highly resistant to most of the commonly used antibiotics e.g; ampicillin, erythromycin, SMX-TMP, gentamycin, augumentin, cefotaxime, meropenem and mupirocin and ciprofloxacin.

14. RECOMMENDATIONS

1. Our standard second line antibiotics in NICU and ICU are not effective against MRSA and therefore empiric antibiotics should be vancomycin, amikacin or linezolid.
2. Our research findings have also indicated a need for continuous surveillance of antimicrobial susceptibility and adjustments in antimicrobial drug policy should be done for better management of the patients.
3. Nasal screening for MRSA in every patient at admission and in the units should be mandatory for effective infection control and prevention.
4. A prevalence study of nasal carriage of MRSA among health care providers in these units should be conducted to find out if they are not sources of MRSA transmission.

COMPETING INTERESTS: I declare that I have no competing interests.

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16. APPENDICES

16.1 PARENT/GUARDIAN INFORMED CONSENT FORM.

Study Title: The prevalence of methicillin resistant *staphylococcus aureus* (MRSA) among pediatric patients admitted in NICU and ICU- Kenyatta National Hospital (KNH).

Investigator: Dr. RUTARE Samuel (MB ChB), Paediatric Resident, University of Nairobi.

Supervisors:

1. Prof. Ruth NDUATI (MB ChB), M.Med (paeds), MPH, Associate professor of pediatrics, Department of Pediatrics and Child Health, University of Nairobi.
2. Prof. Francis ONYANGO (MB ChB), M.Med (paed), MPH, Associate professor, Department of Paediatrics and Child Health, University of Nairobi.
3. Dr. Samuel KARIUKI (BVM, MSC, PHD) Chief Research Officer and Director Centre for microbiology Research. KEMRI-Kenya.

Introduction:

Antibiotics are drugs that are used to treat diseases caused by infections. The bacteria we wish to study is called *staphylococcal aureus*, it is often found naturally on the skin but when it enters into the bloodstream it causes disease. The antibiotics that are used to treat infections from these bacteria are in a class called beta-lactam. Some of the common antibiotics in this class are amoxil, penicillin and methicillin. Sometimes patients are infected with *staphylococcal aureus* that is resistant to an antibiotic called methicillin. This study is seeking to understand how often this occurs in children admitted at Kenyatta National Hospital.

What is the purpose of this study?

I am conducting this study as research for the degree of masters of medicine (m.med) in paediatrics and child health. We shall determine the burden of bacteria that does not respond to antibiotics among children admitted to KNH intensive care unit (ICU) and new born intensive care unit (NICU). Please read this information or have it read to you so that you understand what we are asking you to do. Feel free to ask question so that you understand fully

Why is this study important?

Studies done in other countries indicate that many patients admitted in ICU and NICU have this bacteria (*staphylococcal aureus*) that is resistant to commonly used antibiotics. This study is important because we do not have the information about the resistance patterns of staphylococcal aureus in our hospital ICU and NICU. This information will help the hospital be equipped with the alternative medications in case your child is found to have staphylococcal aureus resistant to methicillin.

Who is in this study?

We will enroll approximately 150 children who will have been admitted to the intensive care unit (ICU) and new born intensive care unit (NICU) - Kenyatta National Hospital and this research will run for a period of five months.

Why participate in the study?

We would like to include your child in the study because your child has been admitted in ICU/NICU. He /she is eligible to participate in the study and we want to give all paediatric patients admitted in ICU or NICU an equal chance to participate in this study so that we know the exact burden of the problem.

What will be done to my child if I agree?

If you agree for your child to participate in the study, we will ask you a few questions about your child, and then we will do a nasal swab because this is where the bacteria (*staphylococcal aureus*) mainly lives. If your child is breathing with the assistance of a machine, a sample of secretions from the windpipe will also be taken for evaluation.

Are there any risks to my child?

The study carries no extra risk or cost to your child because these form part of the procedures carried out to look for infection in a patient in the intensive care unit.

Are there any benefits if my child participates?

If you agree to take part in this study there is no direct benefit except that when your child is found to have this bacteria (MRSA), he/she will be treated accordingly. The results from this study will provide information on the burden of these bacteria in ICU and NICU of this hospital and hence will be useful in the day to day care of our patients.

What happens if I refuse to participate?

Participation is voluntary. You are free to decide if you want your child to participate. If you agree you can still change your mind at any time and withdraw from the study. This will not affect your child's care now and in the future.

Who will have information about my child in this study?

Information will be shared amongst doctors. The information will be kept confidentially and securely without your child's name on it.

Who has allowed this study to take place?

The ethics and research committees of University of Nairobi/Kenyatta National Hospital have studied the proposed study carefully and given permission for it to be done.

What if I have questions to ask about this study?

Feel free to ask me any questions now and at any other time. You can contact me for any further clarifications.

, **Dr Samuel RUTARE**, Tel. (+254)0720560778, e-mail; rutasamuel@yahoo.com.

If you have any questions on your rights as a research participant you can contact the **Kenyatta National Hospital Ethics and Research Committee (KNH- ESRC)** by calling **2726300 Ext. 44355**. E-mail: KNHplan@ken.Healthnet.org

CERTIFICATE OF CONSENT.

I , being the parent/ guardian ofhave understood the information in above on what the study entails. I have had a chance to ask questions and they have been answered satisfactorily. I understand that I can withdraw from the study at any stage and that this will not affect me/ my child in any way.

I hereby consent to my child’s participation in this study.

Parent/guardian’s signature: Date:

Parent/ guardian’s name: Time:

Doctor’s Signature: Date:

Doctor’s Name: Time:

FOMU YA RIDHAA KUSHIRIKI KWENYE UTAFITI (Consent form in Swahili)

Kichwa cha habari: Kuchunguza kiwango cha maambuki ya bacteria sugu wa *Staphylococcal aureus* dhidi ya dawa ya methicillin kwa wagonjwa chini ya miaka kumi na nane wanaolazwa wodi ya mahututi (ICU/NICU) Hospitali ya Taifa ya Kenyatta.

.Mtafiti: Dr. RUTARE Samuel (MB ChB), Paediatric Resident, University of Nairobi.

Wasimamizi:

1. Prof. Ruth NDUATI (MB ChB), M.Med (paeds), MPH, Associate Professor of Pediatrics, Department of Pediatrics and Child Health, University of Nairobi.
2. Prof. Francis ONYANGO (MB ChB), M.Med (paed), MPH, Associate Professor, Department of Paediatrics and Child Health, University of Nairobi.
3. Dr. Samuel KARIUKI (BVM, MSC, PHD) Chief Research Officer and Director Centre for microbiology Research. KEMRI-Kenya.

Utangulizi: Wadudu watakaochunguzwa katika utafiti huu ni *staphylococcal aureus* amabao kwa kawaida upatikana kwenye ngozi ya binadamu bila kusababisha matatizo. lakini wakati mwingine wadudu hawa wanaweza kuingia kwenye damu na kusababisha magonjwa. Dawa amabazo utumika kutibu wadudu hawa ni amoxil, penicillin and methicillin, lakini wakati mwingine wadudu hawa uwa sugu na hivyo kushindwa kutibiwa na dawa zilizotajwa hapo juu. hivyo ni nia ya utafiti huu kujua kwa kiasi gani tatizo lipo kwa watoto wanaolazwa wodi ya mahututi (ICU na NICU) hapa KNH.

Nia ya utafiti: Kuchunguza kiwango cha maambuki ya bacteria sugu wa *staphylococcal aureus* dhidi ya dawa ya methicillin kwa wagonjwa wanaolazwa wodi ya mahututi (ICU/NICU) Kenyatta National hospital. Pia nafanya utafiti huu kama sehemu ya masomo yangu ya masters kwa watoto hapa chuoni.

Umuhimu wa utafiti huu: Utafiti kama huu umefanyika katika nchi nyingine na kuonesha kuwa kuna wadudu wa *staphylococcal aureus* amabao ni sugu kwa madawa ya amoxly, methicillin na penicillin kwa watoto wanaolazwa wodi ya mahututi (ICU & NICU). Takwimu kama hizi hazipo hapa hospitali ya Kenyatta, hivyo takwimu zitakazopatikana kwenye utafiti huu zitasaidia hospital kuwa na dawa mbadara kwa matibabu ya watoto watakao patikana na wadudu sugu.

Walengwa wa utafiti huu: Natarajia kuchunguza watoto mia moja watakao lazwa wodi ya mahututi (ICU na NICU) kwa muda wa miezi tanu hapa hospitali ya taifa Kenyatta .

Jinsi ya kushiriki: Kushiriki katika utafiti huu ni ihari yako unaweza kukubali au kukataa,na hata ukikataa bado mtoto atapata huduma zinazotolewa hospitalini hapa kama kawaida .

Ushiriki wako: Kama utakubali kushiriki utaulizwa maswali machache na pia mwisho mtoto atatolewa vipimo puani na kooni na ghrama ya vipimo hivyo haitakuwa juu yako.

Faida za kushiki: Hakuna faida ya moja kwa moja kwa wewe kushiriki katika utafiti huu ,labda vipimo vikionesha wadudu sugu waliotajwa hapo juu mtoto atatibiwa .

Utunzaji wa siri: Taarifa utakazozitoa hapa kuhusu wewe au mtoto zitatunzwa vizuri bila kushirikisha wale wasiostahili

Ruhusa ya kufanya utafiti: Ruhusa ya kufanya utafiti huu imetolewa na idara ya utafiti ya hospitali ya taifa kenyatta na idara ya watoto chuo kikuu cha Nairobi

Mawasiliano: Iwapo kama una swali au unahitaji maelezo kuhusu utafiti huu tuwasiliane kupitia;

Dr Samuel RUTARE, Tel. (+254)0720560778, e-mail; rutasamuel@yahoo.com,

Kenyatta National Hospital Ethics and Research Committee (KNH- ESRC)-2726300

Ext. 44355. EMAIL: KNHplan@ken.Healthnet.org

CHETI CHA RIDHAA:

Mimi mzazi/mlezi....., nimeelezwa na nimesoma maelezo haya.
Na nimeuliza Maswali yangu na yamejibiwa vizuri.

Nimekubali mimi na mwanangu kushiriki kwenye utafiti huu.

Sahihi ya mzazi/mleziTarehe.....

sahihi ya daktari.....Tarehe.....

**PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*
(MRSA) AMONG PAEDIATRIC PATIENTS ADMITTED IN ICU AND NICU AT
KENYATTA NATIONAL HOSPITAL-KENYA.**

**16.2 DATA COLLECTION SHEET:
PATIENT DATA**

Date of enrollment.....Study number

Qn1. Age of the participant(days).

- a) ≤ 7 days.....
- b) 8-28 days.....
- c) ≥ 28 days-2 years.....
- d) 2years-5years.....
- e) Above 5 years ≤ 18 years

Qn2.Sex a) Female.....
b) Male.....

Qn3. Place of delivery

- a) Home delivery
- b) Hospital delivery.....
- c) On the way to hospital.....

Qn4. Area of residence (yes, no)

- a) Town
- b) Village.....

Qn5. Temperature the day of taking the sample (i.e. 1st or 4th day of admission).

Temperature on taking the sample	1 st day	4 th day
a) $\leq 34.5^{\circ}\text{c}$		
b) $34.6 - 35.0^{\circ}\text{c}$		
c) $35.1 - 36.0^{\circ}\text{c}$		
d) $36.1 - 36.5^{\circ}\text{c}$		
e) $36.6 - 37.0^{\circ}\text{c}$		
f) $37.1 - 37.5^{\circ}\text{c}$		
g) $\geq 37.5^{\circ}\text{c}$		

Qn6. Diagnosis on admission to NICU or ICU (yes, no).

- a) Pneumonia
- b) Asthma.....
- c) Meningitis.....
- d) Cerebral palsy on vent. Support.....
- e) Shock.....
- f) RDS.....
- g) Asphyxia

- h) Neonatal Sepsis.....

- I) Others.....

Qn7. HIV status

- a) Positive.....
- b) Negative.....
- c) Not done.....

Qn8. Risk factors (yes, no).

- a) Age less than 2 years.....
- b) Previous admission in one year.....
- c) Prolonged hospitalization >14 days.....
- d) Surgery performed on patient in the last 12 months.....
- e) Indwelling lines and or catheters.....
- f) Invasive procedures.....
- e) Chronic medical condition (specify).....
- g) Crowding in house if >5people
- h) Frequent antibiotic use
- i) Skin condition (e.g. eczema, impetigo, abscess).....

Qn 9. Referred from (yes, no).

- a) A ward in KNH
- b) From another hospital.....
- c) Direct from home

Qn10. Duration of hospitalization at time of taking sample (yes, no).

- a) 1-2 days of hospitalization
- b) ≥ 3 days of hospitalization

Qn11. Specimen taken (yes, no).

- a) Nasal swab on 1st day of admission
- b) Nasal swab on 4th day of admission
- c) Tracheal aspirate on 1st day of admission
- d) Tracheal aspirate on 4th day of admission

Qn12. Antimicrobial(s) used before MRSA diagnosed (yes, no)

- a) Ceftriaxon.....
- b) Fortum.....
- c) Amikacin.....
- d) Gentamycin.....
- e) X-Pen.....
- f) Meropenem.....

- g) Vancomycin.....

- i) Others.....

Qn13. *Staphylococcal aureus* isolated (yes, no)

- a) Nasal swabbing on 1st day of admission.....
- b) Nasal swabbing on 4th day of admission.....
- c) Tracheal aspirate on 1st day of admission.....
- d) Tracheal aspirate on 4th day of admission.....

Qn14. Bacteria isolated

- a) *Staphylococcal aureus*.....
- b) *Klebsiella*.....
- c) *Acinetobactor*.....
- d) *Pseudomonas*.....
- e) *E.coli*.....
- f) Others.....

Qn15. Drug sensitivity to *Staphylococcus aureus* and other bacteria isolated.

- a) Oxacilline.....
- b) Ampicilline.....
- c) Vancomycin
- d) Gentamycin
- e) Amikacin.....
- f) Augumentin/Amoxicillin Clavulunic acid
- g) Chloramphenicol
- h) Erythromycin
- i) Tetracycline.....
- j) Cefotaxime.....
- k) Sulfamethoxazole/trimethoprim.....
- l) Clindamycin.....
- m) Meropenem.....
- n) Linezolid.....
- o) Mupirocin.....
- P) Ciprofloxacin.....

Qn16. *MecA* gene identified on PCR (yes, no).

- a) Nasal swabbing on 1st day of admission.....
- b) Nasal swabbing on 4th day of admission.....
- c) Tracheal aspirate on 1st day of admission.....
- d) Tracheal aspirate on 4th day of admission.....

16.3 TIME FRAME:

The following was a proposed time-frame of the study process:

Number	Activity	Estimated Time
1	Proposal Development and Presentation	November 2010 to 5th- Jan 2012
2	Proposal Submission to the department for marking	January 2012
3	Submission of proposal for ethical approval	February 2012
4	Pretesting	March 2012
5	Data Collection	April to September 2012
6	Data Analysis	October 2012
7	Dissertation writing	November to December 2012
8	Dissertation submission	January 2013
9	Poster Presentation	March 2013

Total sample size is 150 cases. The study was done over a period of five months because of the patient turnover in the intensive care unit (ICU) and new born intensive care unit (NICU).

16.4 STUDY BUDGET

Category	Remarks	Units	Unit Cost (KShs)	Total (KShs)
Proposal Development	Printing drafts	1000 pages	5	5,000
	Proposal Copies	8copies	500	4,000
	Literature review via internet (40 hours)			5,000
Laboratory Investigations.	Transport media	500gm tin.	8000	8,000
	Nasal swabs (culture and sensitivity).	300	500	150,000
	Tracheal aspirates (culture and sensitivity).	300	500	150,000
	PCR analysis(staphy positive cultures)	Approx. 50	500	25,000
Data Collection	Stationery (pens, papers, etc).	15	100	1,500
	Research assistant 1 for 5 months	1 Assistant	15000 each month	75,000
Data Analysis	Statistician	1	20,000	20,000
Thesis Write Up	Printing drafts	1000 pages	5	5,000
	Printing Thesis	10 copies	500	5,000
Contingencies 5%				22,675
Grand total				476,175 Ksh.

Maximum sample size was 150 cases and we were taking 2 nasal swabs, 2 tracheal aspirates for each patient (i.e. first sample on 1st day of admission and second sample on 4th day while still in the unit so that we can know if it is hospital or community acquired MRSA).