Prevalence of Methicillin resistant Staphylococcus aureus (MRSA) among isolates from surgical site infections in Mulago hospital- Kampala, Uganda.

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Citation

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Abstract

Background. Methicillin-resistant Staphylococcus aureus (MRSA) isolates are resistant to penicillins and all other -lactam antibiotics. Nosocomial MRSA are also resistant to a variety of other antibiotic classes. MRSA infections are associated with a high morbidity and mortality in Uganda. There is limited data on the magnitude of MRSA in surgical site infections. The objective of this study was to determine the prevalence of MRSA among S. aureus isolates from surgical site infections in Mulago Hospital, Kampala, Uganda. Methods One hundred eighty eight pus swabs were collected from patients with surgical site infections. Swabs were inoculated for culture at the Microbiology laboratory Faculty of Medicine, Makerere University. S. aurues was identified biochemically. All S. aureus isolates were subjected to oxacillin agar screen and then tested with a polymerase chain reaction (PCR) assay for detection of the mec
A gene which codes for oxacillin resistance. Results Out of the 188 specimen cultured, 54 (28.7%) grew S. aureus. Seventeen (31.5%) of the 54 isolates were confirmed as MRSA by PCR. Conclusion.
S. aureus is highly (28.7%) prevalent in surgical site infections in Mulago National Referral Hospital, Kampala Uganda. MRSA is highly (31.5%) prevalent among populations of S. aureus isolated from surgical site infections in Mulago National Referral Hospital, Kampala Uganda

INTRODUCTION

Staphylococcus aureus continues to be a major cause of both nosocomial and community-acquired infections. Methicillin was first introduced in 1960 for the treatment of penicillinase-resistant microbial infections and a year later, MRSA isolates resistant to all - lactam antibiotics were isolated (Chambers, 2001). Oxacillin resistance (presence of the mecA gene responsible for oxacillin resistance) is a specific predictor of resistance to all - lactam antibiotics including carbapenems (Chambers, 2001). Methicillin-resistant Staphylococcus aureus (MRSA) has become one of the most important pathogens that cause postoperative infections and it accounts for up to 40% of nosocomial Staphylococcus aureus infections in large Hospitals and 25%-30% in smaller hospitals in the USA (Murray et al, 2003, Ajali, 1999 & Rodriguez et al 1974).

In Europe, MRSA prevalence ranges from over 50% in Portugal and Italy to below 2% in Switzerland and the Netherlands, where infection control measures have been shown to work (Verhoef et al, 1999). In Asia, the prevalence lies around 50%, with extremely high rates in Hong Kong (75%) and Japan (72%) (Diekema et al, 2000). In other studies comprising of many African Hospitals the prevalence of MRSA was put at 15 % with Kenya and Nigeria having the highest prevalence of 21-30 % (Gorwitz et al,2006). In Uganda, about 10% of the surgical procedures become septic which account for an increasing morbidity and mortality with the commonest organism isolated being S. aureus, though no MRSA had been isolated by 1999.(Ojikan-Odeke.,1978, Mugisa D.B,1998 & Olaro .C, 1999)

MRSA infections are of special concern because these infections are associated with prolonged hospital stay, increased hospital costs, and have a few therapeutic options for affected patients (Saxen et al 2003). There is limited

information on surgical site infections caused by MRSA in Uganda. This study was designed to determine the prevalence of MRSA in a population of Staphylococcus aureus isolated from patients with surgical site infections (SSI) at Mulago Hospital, Kampala, Uganda. The ultimate goal of this study is to contribute to reduction of overall surgical site infections in Uganda to barest minimum especially in this era of HIVAIDS pandemic.

MATERIALS AND METHODS

Study setting. The present study was carried out at Mulago National referral Hospital,

Kampala, Uganda, between February and May 2007. All patients (n = 188) who

underwent elective and emergency surgery during the study period were enrolled. Each of them had a pus swab taken from active site or on a purulent pus discharge. This was done on a patient who developed SSI after surgery as defined by Centers for Disease Control and Prevention criteria (Horan et al, 1992).

IDENTIFICATION OF

Sterile dry cotton swabs with Amies transport media (DELTALAB Rubi, Spain) were used to collect staphylococci from active part of infected surgical wounds or pus discharge. The swab was rolled gently but firmly on the base of the wound while applying an even pressure. The swab was replaced in its sheath which has transport medium and then transported to the laboratory where it was inoculated directly on phenol red mannitol salt agar (Liofilchem, Italy) and incubated at 37°C for 24 hrs. S. aureus colonies were selected on the basis of their yellow mannitol fermenting colonies, Gram reaction and morphological characteristics, catalase, DNase and coagulase tests. The S. aureus isolates were then sub-cultured onto 5% blood agar to show -hemolysis. S. aureus isolates positively identified by above mentioned tests were transported at room temperature as monocultures in brain heart infusion broth to the Department of Medical Microbiology and Molecular biology, Makerere University Kampala, Uganda, for mecA gene identification using polymerase chain reaction method.

According to previous report (NCCLS, 2003), Oxacillin salt agar (containing Mueller-Hinton agar (SIGMA) plates with 4% NaCl and 6 µg of oxacillin per ml) screening of Staphylococcus aureus isolates yielded positive results. All isolated identified Staphylococcus strains were frozen at -20°C until all isolates were obtained and ready for molecular analysis.

MOLECULAR DETECTION OF A GENE

The mecA gene from Staphylococcus was obtained using methods previously reported by Sakoulas et al (2001), with slight modifications. Briefly, bacterial suspensions from the frozen isolates were thawed and then subcultured on 5% blood agar plates. Plates were then incubated at 37°C for 18-24hrs, 2-3 bacteria colonies from fresh growth isolates were suspended in 100µl distilled water in eppendorf tubes. The tubes were then boiled for 2 minutes and centrifuged at 13,000 rpm for 2 minutes. The supernatant containing the DNA extract was collected and kept at 4°C. The extracted DNA was loaded and ran on the Agarose gel (120 V) to check for its quality prior to PCR amplification. The Primers [Forward P4: 5' TCCAGATTACAACTTCACCAGG3'and Reverse P7: 5'CCACTTCATATCTTGTAACG-3': MWG – Biotech- Germany] were designed to amplify a 162bp segment of the mecA. The primer concentration for each of primers P4 and P7 was optimized at 100L (i.e. 50ng) for each and were added to the PCR reaction mix consisting of RNAse-free water 8L, master mix 1L, and Taq polymerase 0.1L.The total reaction volume was 10.1L. 0.5L of S. aureus cultured was then inoculated in the mix to serve as genomic DNA source.

The PCR mixture contained PCR buffer (ABgene, UK) supplemented with $MgCl_2$ (25mM) and primers, 200 mM dTTP, dATP, dCTP, and dGTP. The contents were mixed in thin walled PCR tubes and the reaction mixture was placed in a DNA thermal cycler (Peltier PTC-200) to be amplified in 31 repeating cycles, each cycle having three basic steps: 1-min denaturation at 94°C followed by annealing for 30 s at 55°C and elongation for 1 min at 72°C. The samples were held in the thermal cycler at 4°C until they were loaded onto a 1.5% agarose gel and were electrophoresed for 1hr at 120 V, stained with ethidium bromide, and viewed with UV light. The presence of a 162-bp band was considered a positive result. The marker used was 100kbp (New England-Biotech)

QUALITY CONTROL STRAINS

The following control strains were used for both oxacillin agar screen and PCR method; S. aureus ATCC 29213 as an oxacillin susceptible strain. S. aureus ATCC 43300 as an oxacillin resistant strain.

DATA COLLECTION AND MANAGEMENT

The principle investigator, assisted by research assistants collected data. Informed consent was obtained and a standardized questionnaire was administered. The data collected included age, sex, date and time of collection and patient number. Other data collection was the presence or absence of MRSA after oxacillin agar screen and PCR.

The data was then analyzed using STATA statistical software. Dichotomous variables were compared using a chi square, and continuous variables were compared using multivariate statistic, with the help of a statistician.

RESULTS

Of the 188 patients enrolled, 62.9% of these patients were males and 37.1% were females. The average age of the patients was 31years with a range of 13 - 87 years. Fifty four (28.7%) patients had S.aureus, of the 54 isolates of S. aureus from these patients, 17 (31.5%) were MRSA by PCR assay as shown in the tables 1 and 2 below. The males were twice as much more likely to get MRSA as compared to females in this study (Table. 1). Oxacillin agar screen had a sensitivity of 70.59% and a specificity of 64.86% (Table.2). These results showed that this method could correctly classify 66.67% isolates of S.aureus as being MRSA.

{image:1}

{image:2}

DISCUSSION

Staphylococcus aureus is an important pathogen in human infections and is implicated in a wide variety of infections, from mild skin infections to more serious and invasive infections, including septicemia, pneumonia, endocarditis, deep-seated abscesses, and toxinoses including food poisoning and toxic shock syndrome (Tenover and Gaynes 2000; Holmes et al., 2005). Surgical site infections (SSI) by Staphylococcus aureus are known predominant cause of nosocomial infection, resulting in considerable morbidity and mortality in tropical Africa. Data on MRSA infection especially for SSI are highly limited in Uganda and other tropical African countries (Kesah et al, 2003; Adebayo et al, 2006).

The 28.7% Staphylococcus aureus observed in this study is higher that 8.8% prevalence in deep-seated recurrent genital ulcers in Nigeria (Agwu et al., 2007) and 10.7% prevalence in wound infections in Iran (Rastegar et al 2005). The 28.7% Staphylococcus aureus reported in the present study may: depict the level of Staphylococcus aureus carriage in this locality; be attributed to the level of contamination arising from the habit of some of the volunteers to treat their wound aseptically before seeking appropriate medical attention and may also be due to low personal hygiene and poor health education which still persists in Uganda compared to Nigeria and Iran.

In our study and out of 17 isolates positive for MSRA, 13 (76.5%) male patients were infected with MRSA more than 4 (23.5%) females. This confirms earlier report (Agwu et al., 2006) that most tropical bacteria infections are more common in males than females because predisposing sites for the infections such as overpopulation, prisons, military camps, construction sties and factories are predominated by males.

In this study, the prevalence of MRSA among S. aureus isolates in SSI was found to be 31.5%. This is in similar to the 26.9%-29.6% prevalence reported in the USA, Middle East and other selected African hospitals (Kesah et al, 2003, Boyce, 1998, Bell et al, 2002 & Baddour et al., 2006). This may show that the level of awareness and control measure for MRSA prevalence in Uganda is comparable to the standard control elsewhere. Emergence of documented multi-drug resistance bacteria pathogens (Agwu et al., 2004) due to indiscriminate use of antibiotics (Agwu et al., 2005) may explain the observed 31.5% prevalence of MRSA in SSI at the Mulago National Referral Hospital Kampala, Uganda.

According to a review by Swierzewski, J.S. (2008), the population at increased risk of health-care associated MRSA infection includes: patients who have recently been hospitalized (within the past year) and patients in long-term health care facilities, including nursing homes and dialysis centers. Medical conditions that weaken the immune system (e.g., HIV/AIDS, cancer), recent invasive medical procedures (e.g., surgery, catheterization, dialysis), and recent use of antibiotics also increases the risk for HA-MRSA. Health care workers (e.g., doctors, nurses, physician assistants) and people who are in close contact with health care workers are at increased risk for developing staph infections, including MRSA. Children also have a higher risk for infection, possibly because their immune systems are not fully developed.

It appears there is a decline in the overall ability of different

healthcare settings to stop or reduce the spread of MRSA to the barest minimum. In developing countries, it has always been contended that the inappropriate use of antibiotics for community infections may increase the prevalence of resistant bacteria infection (Agwu et al., 2005) including MRSA. Therefore, the use of two mechanisms to control the spread of MRSA could reduce the burden of SSIs in this region. First, the number of surgical patients who are colonized before surgery would be reduced, decreasing the likelihood of an inadequate antimicrobial prophylaxis choice in those settings where preoperative screening for MRSA colonization is not done (e.g.using a - lactam agent for a patient with unrecognized MRSA colonization). Second, for those patients who are not colonized with MRSA prior to surgery, successful control of transmission would reduce the risk of acquiring it from an external source during or after surgery. It has been reported that 60% of the S. aureus SSIs appeared not to have originated from nasal carriage of S. aureus by the patient, suggesting an exogenous source (Farr, 2002 & Perl et al, 2002).

In conclusion, S. aureus is highly (28.7%) prevalent in surgical site infections in Mulago National Referral Hospital, Kampala Uganda. MRSA is highly (31.5%) prevalent among populations of S. aureus isolated from surgical site infections in Mulago National Referral Hospital, Kampala Uganda.

This study has opened a broad research horizon that will enable future researchers to investigate: the source of MRSA; the link between MRSA acquisition and various factors like age, sex, occupation, ethnicity, geographical location, hospitalization, antibiotic usage, surgery and distinction between community-acquired MRSA and hospital acquired MRSA.

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