T. T. Ouko, MSc, Microbiologist, Centre for Microbiology Research, Kenya Medical Research Institute, P. O. Box 54840, Nairobi, Kenya, J. N. Ngeranwa, BVM, MSc, PhD, G. O. Orinda, BVM, MSc, PhD, Department of Biochemistry, Kenyatta University, P. O. Box 54840, Nairobi, Kenya, C. C. Bii, BSc, MSc, PhD, Principal Researcher, Centre for Microbiology Research, Kenya Medical Research Institute, P. O. Box 54840, Nairobi, Kenya, E. Amukoye, MMed, Director, M. Lucy, BSc, Microbiologist, Centre for Respiratory Research, Kenya Medical Research Institute, P. O. Box 54840, Nairobi, Kenya and C. N. Wamae, BSc, MSPH, PhD, IDD(K), Acting Dean and Director of Research and Development, Kenya Methodist University, P. O. Box 267 - 06200, Nairobi, Kenya

Request for reprints to: Mr. T. T. Ouko, Centre for Microbiology Research, Kenya Medical Research Institute, P. O. Box 54840, Nairobi, Kenya

OXACILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG HIV INFECTED AND NON-INFECTED KENYAN PATIENTS

T.T. OUKO, J.N. NGERANWA, G.O. ORINDA, C. C. BII, E. AMUKOYE, M. LUCY and C. N. WAMAE

ABSTRACT

Background: Infections due to methicillin resistant *S. aureus* (MRSA) present global challenges to clinicians since therapeutic options are limited and suboptimal dosing contributes to heightened mortality and increased length of hospital stay particularly among the HIV infected patients.

Objectives: To assess the prevalence and relative risk of MRSA infections in HIV infected patients.

Design: Cross sectional analytical study.

Setting: Kenya Medical Research Institute, Opportunistic Infection Laboratories in Nairobi.

Subjects: Four hundred and thirty six male and female patients aged one to 65 years, of whom 220 were HIV-infected and 216 were non-infected.

Results: There was 436 male (57.1%) and female (42.9%) respondents. The prevalence of MRSA was 26.3% with majority infecting the HIV infected patients (P=0.046). Likewise, the overall *Staphylococcal* infections were more common in HIV patients (P <0.001). The common test for MRSA oxacillin disk diffusion had a sensitivity and specificity of 100% and 92%.

Conclusion: HIV is a predisposing factor to *Staphylococcal* infection and there are indications that treatment with β-lactam antibiotics may no longer be relied on as sole empiric therapy for several ill HIV patients whose infections may be of MRSA in origin. There is need for an informed choice in administration of appropriate antibiotics in order to minimise treatment failures due to the multidrug resistance and Vanvomycin intermediate *S. aureus* (VISA) strains. Molecular epidemiology of MRSA strains in understanding new and emerging trends is recommended.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were first detected in the early 1960s, shortly after methicillin came into clinical use (1) and since then, there has been an increase in prevalence in many geographic locations throughout the world (2). Freestanding nursing homes and other long-term healthcare facilities, have reported MRSA rates of 10 to 15% (3). In Africa, studies done at Muhimbili Medical Hospital in Tanzania, Kenyatta National Hospital in Kenya and in South Africa found prevalence rates of MRSA at 0.4, 39.8 and 26.9% respectively (4-6).

These prevalence variations are as a result of different geographical locations with varying MRSA strains of different virulence or colonisation properties, or it may reflect differences in antimicrobial utilisation and hospital infection control practices. Molecular epidemiology studies have shown that a limited number of MRSA strains have spread by clonal dissemination between different hospitals, cities, countries, and even continents and are now the major cause of hospital infections worldwide (7).

HIV-infected patients have an exceptional vulnerability to invasive bacterial infections in comparison to immuno-competent and HIV non-

infected patients (8). Multiple resistance to the commonly used antibiotics exhibited by bacterial strains such as MRSA, have continued to increase globally and present a considerable dilemma to clinicians, since therapeutic options are limited and sub optimal dosing contributes to heightened mortality and increased length of hospital stay particularly among the HIV infected patients (9). In Kenya, the antimicrobial regimens used in treatment of *staphylococcal* infections in HIV-infected and non-infected individuals are usually administered without informed choice from the laboratory. This practice poses serious challenges in the management of MRSA infections in HIV patients because of the reported high prevalence rate levels of HIV and MRSA (4,10).

The transitional change of MSSA to MRSA is mainly the result of MecA gene which encodes an extra penicillin-binding protein (PBP) 2a or PBP2' that has decreased affinity for β-lactam antibiotics, thus allowing cell wall synthesis to continue despite inactivation of native PBPs (11) and is virtually resistant to all β-lactam antibiotics including penicillin, cephalosporin, monobactams and carbapenems (1,7). In the past, vancomycin has been considered the only agent to which MRSA have not developed resistance to. However, due to overuse of glycopeptide antibiotics, MRSA with reduced susceptibility to vancomycin have emerged as well (12). Reports on emergence of VISA in the USA have been noted which suggest that S. *aureus* strains are constantly evolving and full vancomycin resistant S. *aureus* (VRSA) may soon evolve leaving no treatment options (13).

Frequencies, patterns and distributions of resistant bacteria vary significantly with geographic regions. In Kenya, there is no proper epidemiological data on the scope and extent of MRSA infections in HIV infected individuals. Given the potential severity of infections caused by MRSA, their recent emergence in settings outside the hospital environment and the challenges in treatment and control measures, it is important that epidemiological studies are encouraged to provide baseline data on the prevalence and relative risk of MRSA infections among patients with and without HIV infection. Such data, as presented in this study, may be used to guide implementation of appropriate control measures to minimize spread of MRSA.

MATERIALS AND METHODS

This was a cross sectional study conducted at four different sites: Mbagathi District Hospital and Alupe sub-District hospital admitting both HIV infected and non-infected patients, two institutions: Cottolengo and Thomas Barnardos in Nairobi that provide homes for both HIV infected and non-infected children

respectively. The respondents were of known HIV status and were from either those admitted to hospital for a period of more than three months or drawn from confined institutions offering medical care with history of hospital admission of less than one week. The participants were recruited based on clinical signs of suspected bacterial infections. Specimens for culture were obtained from various sources depending on the symptoms and included superficial lesions (wound swabs), blood for suspected systemic deep seated infections such as septicaemia, sputum for suspected pneumonia and lung aspirates for those with severe pneumonia, CSF from suspected meningitis and urine for suspected cases of urinary tract infections.

Culture of the samples for the isolation of S. *aureus*, drug susceptibility and PCR for *mecA* gene was conducted at KEMRI/JICA Opportunistic Infection (OI) Laboratory in Nairobi.

Culture and drug sensitivity: Culture of the specimens was performed on mannitol salt and sheep blood agar media (SBA) and incubated aerobically at 35°C for 18 to 24 hours. The colonies appearing yellowish in mannitol medium with β-haemolysis on SBA was gram stained and subjected to coagulase test. Confirmation of S. *aureus* was done using analytical profile index (API Staph, Biomerieux) (14). The confirmed S. *aureus* isolates were subjected to antimicrobial susceptibility testing against oxacillin (OX), vancomycin (VAN), gentamycin (GEN), amoxicillin clavulanic acid (AMC), chloramphenicol (CHL), erythromycin (ERY), tetracycline (TET), cefotaxime (CTX), and sulfamethoxazole/trimethoprim (SXT) using disk diffusion for immediate patient treatment and management before being pooled for minimum inhibitory concentration (MIC) testing using E-test and interpreted as per the Clinical and Laboratory Standards Institute (15). The resistance rate was calculated as the total number of isolates minus those sensitive. Multi-drug resistance was defined as resistance to oxacillin and or cefotaxime plus three or more of the drugs (16,17).

Determination of mecA gene by PCR: The test organism was grown on Brain Heart Infusion broth (BHI) overnight then centrifuged at 10,000 rpm for five minutes at room temperature. The supernatant was discarded and the sediment cells re-suspended in 1 ml of TE buffer and vortexed. After this, 200μl 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 ten minutes and the supernatant used as template DNA for PCR. PuRe Taq Ready-To- Go PCR beads (Amersham biosciences) with a total reaction volume of 25μl, was

used in the PCR run using the following primer set: F'GGTGGTTACAACGTTACAAG-3''=02μl; R-5'GCA TTGTAGCTAGCCATTCC3'=0.2μl; the template DNA = 1.0μl; with sterile distilled water of 23.6μl. The PCR conditions were set as follows: initial denaturation step of three minute at 94°C followed by a further 30-second of denaturation at 94°C; annealing step at 55°C for 30-seconds and extension at 71°C for 30-seconds for 35 cycles. The PCR products were separated by gel electrophoresis in 1.5% agarose (TAKARA) and visualised under ultraviolet light against a standard molecular base pair (1kb) ladder (18).

Data analysis: Demographic characteristics of the respondents, prevalence of *S. aureus* and antibiotic responses were analysed using EPI INFO 2000 (CDC, ATLANTA). Analysis of variance on multiple proportions was performed using Chi-square test.

RESULTS

Demographic characteristics of respondents: A total of 436 patients, 57.1% males and 42.9% females clinically suspected to have a bacterial infection were recruited from four study sites: Mbagathi 150 (34.4%), Alupe sub District hospital 50 (11.5%), Cottolengo 76 (17.4%) and Thomas Benardos 160 (36.7%). Out of the respondents, 220 (50.5%) were HIV-infected while 216 (49.5%) were HIV non-infected (Table 1). The number of HIV infected and non-infected children were 76 and 160 respectively verses 144 and 56 adults respectively. The age of the patients studied ranged from one to sixty five years with majority of the patients (70%) falling below 30 years.

Prevalence of Staphylococcus aureus: The prevalence of staphylococcal infections among the respondents was 118/436 (27.1%). The MRSA prevalence accounted for 31/118 (26.3%) with majority of the strains being recovered from cases of superficial lesions 18/118 (15.3%) and systemic deep seated infections 118/13 (11%). The HIV-infected group were more likely to be infected with staphylococcal compared to the HIV non-infected P<0.001 with an MRSA rate among the HIV of 9.6% as compared to the HIV non-infected 4.6%, P=0.046 (Table 1).

The MRSA from males and females was 3.9% and 3.2% respectively with a prevalence variation ranging from 4/50 (8%) in Alupe to 10/76 (13.2%) in Cottolengo in the HIV positive group and 3.6% in Mbagathi to 5% in Thomas Benardos in the HIV negative group. Five strains from HIV positive patients exhibited vancomycin intermediate *S. aureus* resistance (VISA) from Alupe sub-District and Mbagathi District hospitals (Table 2).

Table 1
Staphylococcus aureus strains isolation by HIV status

	HIV positive (n=220)		HIV- Negative (n=216)		P-value
	No.	(%)	No.	(%)	
S. aureus isolates	75	34.1	43	19.4	P<0.001
MRSA	21	9.6	10	4.6	P=0.046

Table 2
Number of S. aureus isolates by HIV and institutions

Institution	No. of respondents	No. of MSSA isolates	No. of MRSA isolates	HIV serostatus
Cottolengo	76	24	10	HIV positive children
Alupe sub-District hospital	50	13	***4	HIV positive adults
Mbagathi District hospital	94	17	**7	HIV positive adults
Mbagathi District hospital	56	11	2	HIV negative adults
Thomas Benardos	160	22	8	HIV negative children
Total	436	87	31	

***Three of the strains were vancomycin intermediate *S. aureus* (VISA)
** Two of the strains were VISA

MRSA detection: Of the 118 isolates tested for *mecA* gene, 31 were positive. The correlation between phenotypic test results and the *mecA* gene showed 38 MRSA by oxacillin disk diffusion and 40 MRSA by cefotaxime disk diffusion as the only common two tests used for the detection of MRSA.

Antibiotic susceptibility of the isolates: The *S. aureus* isolates were found to be highly sensitive to vancomycin with 4.2% of the 118 *S. aureus* isolates exhibiting intermediate resistance having an MIC of >8 µg/ml. The second most effective drug was AMC with a sensitivity of 76.9% followed by CHL at 73.5%. The sensitivity rate to OX and CTX were both at 70.3% with GEN and TET at 60.5% and 54.8% respectively. Low sensitivity rates of the isolates were seen in SXT 42.7% and ERY at 41.1% (Table 3). It was noted that 23 (19.5%) of the isolates were resistant to OX plus three or more of the drugs tested of which 21 were MRSA. Two of the strains were *mecA* gene negative and were resistant to four of the drugs including OX. Of the 21 MRSA, six strains were resistant to seven drugs, six to six drugs, six to five drugs and three to four drugs while the five strains which exhibited vancomycin intermediate resistance were from the 74 strains from HIV 6.8% (Table 3) with an MIC of 8 µg/ml. The drug susceptibility test profile was compared and found to have no statistical difference in the profile (Table 3).

Table 3
Statistical comparison of antibiogram by HIV status

Drug	HIV positive		HIV negative		X^2 - test
	No.	% sensitivity	No.	% sensitivity	P-value
Oxacillin	75	68.0	43	74.4	0.46
Cefotaxime	75	66.7	43	76.7	0.26
Vancomycin	74	93.2	43	100	0.08
Amoxicillin-clavulanic acid	74	75.7	43	79.1	0.68
Trimethoprin-Sulphamethoxazole	75	49.3	42	31.0	0.055
Erythromycin	69	43.5	43	37.2	0.51
Chloramphenicol	74	71.6	43	76.7	0.55
Tetracycline	73	56.2	42	52.4	0.69
Gentamycin	72	62.5	42	57.1	0.59

Figure 1
Gel photomicrograph of mecA gene PCR product showing well number 1 with 1000 bp ladder; 2, 3, 7-10 and 12 are negative clinical isolates for mecA; 4 is a positive mecA (S. aureus A TCC 33592); 5, 6 and 11 are positive clinical isolates for mecA.; well 12 negative control for mecA gene (S. aureus ATCC 25923)
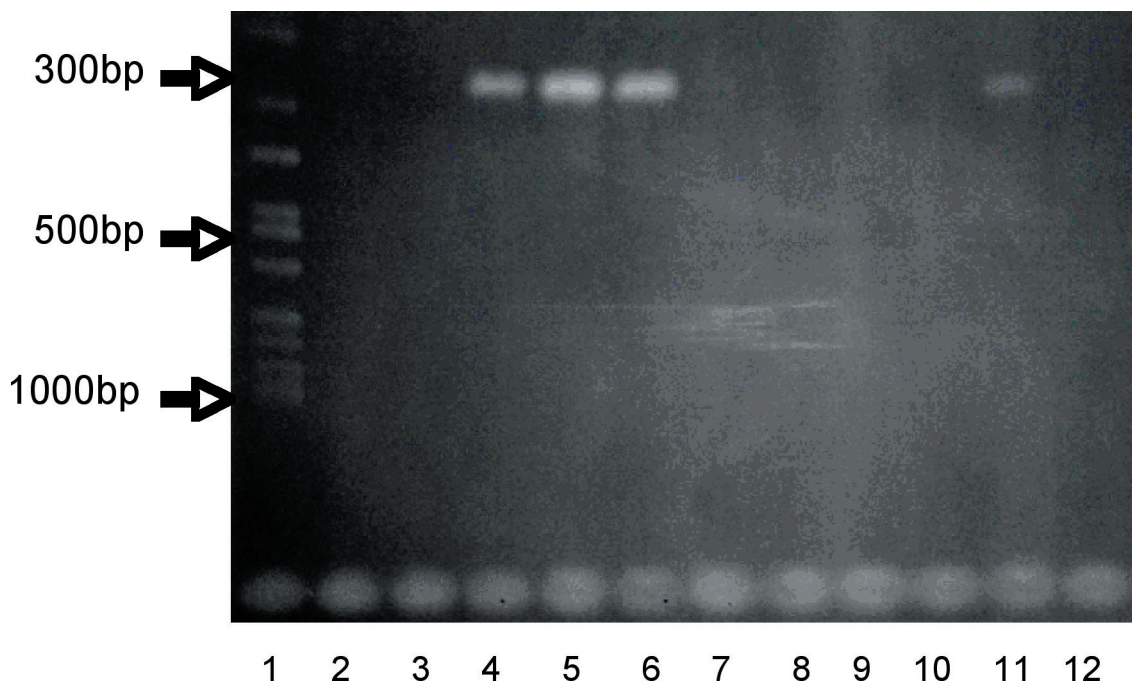
DISCUSSION

This study showed a prevalence rate of MRSA at 26.3%, which from an epidemiological point of view is considered high since it has been shown that MRSA prevalence rate of greater than 25% to be high and indicates poor national infection control practices (19). The isolates in this study are considered hospital based (HA-MRSA) because all the respondents were either admission cases for a period of more than three months or drawn from confined institutions offering medical care with history of hospital admission of less than one week. This differs from community acquired strains type (CA-MRSA) where the source has to be from outside healthcare settings with no prior history of hospitalisation within the preceding two years (20).

Although the MRSA prevalence finding compares well with studies observed in South Africa 26.9% (6) and 29% quoted by the National Nosocomial Infection Surveillance System (NNISS) in USA (21), higher prevalence rate (39.8%) was reported in Kenya, in by Omar *at al* (4) which could have probably been attributed to high sensitivity of oxacillin disk diffusion test system used by the author for MRSA detection, thereby picking and accounting for strains of *S. aureus* which were simply over-expressing β-lactamase enzymes and were not necessarily MRSA as have been argued in other studies (6,22,23).

Several resistance surveillance studies have been conducted which provide comparable and validated results on the status of MRSA. In Europe, surveillance studies including 27 countries across Europe found an overall 20% MRSA prevalence with the highest proportion in Southern and parts of Western Europe and lowest proportion (5%) in Northern Europe (24). Reports from Nigeria, Kenya and Cameroon, have quoted rates between 21% to 30%, and below 10% in Tunisia, Malta, Tanzania and Algeria (5,25). This variability, besides the use of different test systems for MRSA detection, could as well have been attributed to geographic variation of MRSA strains with different virulence properties and or may reflect differences in antimicrobial utilisation and infection control practices.

One of the resistance mechanisms of *S. aureus* is as a result of chromosomally localised *MecA* gene which codes for an additional penicillin binding protein PBP 2' or PBP2a that has low affinity for beta lactam antibiotics and substitutes functions of the native PBP during cell wall synthesis. The transcription of the gene is usually induced through exposure to beta lactam drugs (14) and since HIV positive patients are more exposed to such drugs during opportunistic infection management, it may perhaps explain why the chances of contracting *staphylococcal* infections of MRSA origin in HIV is twice higher (OR = 2.174) as compared to the HIV negative group.

Antimicrobial resistance among bacterial pathogens is a significant problem worldwide with consequences including increased hospital stay, medical costs, morbidity and mortality of patients (26). The observed 19.5% multi drug resistant strains and the 6.8% VISA found in the HIV positive cases is worrying and are more likely to have serious implications on the treatment and healthcare management of HIV patients. The multi-drug resistance level shown in this study and the expected high cost and delay involved in offering appropriate treatment for diseases of multi-drug resistance origin (27) reflect an increase in mortality particularly among the 11% patients with deep seated and systemic cases. Besides, there is a likelihood danger of spread of these strains (HA-MRSA) to the community and an increase in morbidity due to lack of proper infection control measures in most public hospitals which control the largest hospital attendance in Kenya.

Several risk factors associated with MRSA infections have been described (28). The study showed a higher isolation rate and a significant difference in the prevalence of *staphylococcal* infections in the HIV infected group (p< 0.001) and those strains of MRSA in origin (p= 0.046). A study done in San Diego, California in 2005, showed an annual incidence of *staphylococcal* infections among the HIV as eight-fold higher than in HIV non-infected (29). The difference may probably be due to the vulnerability of HIV patients to this opportunistic pathogen (MRSA) which normally resides on human skin and picks up when the right opportunity such as cancer drugs and diseases which lowers immunity strikes. Besides, it could also be related to the low level of infection control practices by healthcare management in these institutions.

Bacterial resistance is a threat to antimicrobial treatment of infections in the 21st century, (30). *S. aureus* has always been difficult to treat for the numerous infections that are caused by this organism at community and hospital environments. The introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of this pathogen (31,32). As such, surveillance on the antimicrobial susceptibility patterns of *S. aureus* is of utmost importance in understanding new and emerging resistance trends and more particularly, in the management of opportunistic infections in the HIV infected patients.

The high MRSA prevalence in the study indicates that there may be very few treatment options left for *Staphylococcal* infections since all beta lactam drugs as first line treatment can no longer be used effectively. This finding agrees with the 26.9% reported in South Africa (6).

Although there was no significance drug resistance pattern difference among the two groups, the 6.8% vancomycin intermediate resistant in

HIV positive adults suggests serious public health implications in treatment of these patients whose immunity is low and may not afford the cost of the limited treatment options available.

Antimicrobial resistance among bacterial pathogens is a significant problem in many countries with severe consequences including increased medical costs, morbidity and mortality of patients (26). Since the emergence of *S. aureus* strains with resistance to penicillin and methicillin (1,33) it has become a well-known aetiologic agent with a wide variety of infections that are difficult to treat. The resistance mechanism to *S. aureus* exhibits mainly in three forms, the one mediated by blaZ, the gene that encodes β-lactamase. This predominantly extra cellular enzyme, synthesised when *staphylococci* are exposed to β-lactam antibiotics, hydrolyzes the β-lactam ring, rendering the β-lactam inactive. blaZ is under the control of two adjacent regulatory genes, the antirepressor blaR1 and the repressor blaI (34). Recent studies have demonstrated that the signalling pathway responsible for β-lactamase synthesis requires sequential cleavage of the regulatory proteins BlaR1 and BlaI.

Following exposure to β-lactams, BlaR1, a transmembrane sensor-transducer, cleaves. It had been hypothesised that the cleaved protein functions as a protease that cleaves the repressor BlaI, directly or indirectly and allows blaZ to synthesize enzyme itself (35,36).

Another resistance mechanism is the presence of the chromosomally localised *mecA* gene which codes for an additional PBP, termed PBP2' or PBP2a that has low affinity for β-lactam antibiotics and substitutes for the native PBPs during cell wall synthesis when they are inhibited by β-lactams (14). Transcription of the *mecA* gene is induced in some isolates by β-lactams, and such induction is regulated by MecI and MecR1, a repressor and a signal-transducing protein, respectively (14). The mecI and mecR1 genes, when present, are carried beside mecA on the SCCmec element (37-39). Cross-regulation by BlaI and BlaR1 of mecA transcription also occurs, encoded by blaI and blaR1 genes carried on the β-lactamase plasmid along with blaZ (14).

Until now, the background PBPs in *S. aureus* have been assumed to be constitutively expressed. Recently, constitutive pbpB transcription has been studied (40), though the factors that control or induce pbpB gene expression have not yet been explored. Lastly, there is the borderline resistance, which is a low-level type of resistance to methicillin exhibited by strains which neither produce mecA gene nor over express β-lactamase enzyme.

Besides the high level of oxacillin resistance attained through acquisition of the mecA gene, another resistance mechanism, such as over expression of betalactamase, may have accounted for

the 7/118 (6%) oxacillin resistance in S. *aureus* which were mecA gene negative as have been indicated elsewhere (23).

The mechanism(s) responsible for mecA transfer is not known, but evidence supports horizontal transfer of mec DNA between *staphylococcal* species and of the mecA gene between different gram-positive genera (41).

In conclusion, HIV is a predisposing factor to MRSA infections and the multi-drug resistant MRSA and VISA is threatening to return us to the era that preceded the development of antibiotics. Even though there was no antibiogram pattern difference among the isolates from both HIV and non-HIV positive groups, there are indications that treatment with β-lactam antibiotics may no longer be relied on as sole empiric therapy for several ill HIV patients whose infections may be of *staphylococcal* and more so MRSA in origin MRSA prevalence is best predicted by use of PCR as the gold standard for mecA gene in epidemiological studies, however, oxacillin is more suitable for immediate treatment and management of *Staphylococcal* diseases. The high MRSA prevalence, the multi-drug resistance and the emerging strains of VISA call for an informed choice in administration of appropriate antibiotics. Molecular epidemiology of MRSA strains in understanding new and emerging trends is recommended.

ACKNOWLEDGEMENTS

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