CO-INFECTIONS: A THREAT IN THE TREATMENT OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM TOPICAL WOUNDS OF BOTH HIV POSITIVE AND NEGATIVE PATIENTS

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**ABSTRACT**

*Background:* *Staphylococcus aureus* (*S. aureus*) is documented as an opportunistic pathogen in HIV/AIDS infection. Increasing prevalence of resistance by *S. aureus* to commonly prescribed antimicrobials pose a threat in its treatment, resulting to increased morbidity and mortality. The importance of each pathogen in resistance of *S. aureus* to antimicrobials has not been established.

*Objectives:* To identify, characterised and determine bacteria associated with wound infection. To determine the relationship between HIV and infectors of wounds and the correlation of co-infection in *S. aureus* and drug susceptibility.

*Design:* A cross-sectional study conducted for over five years from 2003-2007.

*Setting:* Centre for infections and parasitic disease Control Research, Kenya Medical Research Institute Clinic.

*Subject:* Outpatient who were 18 years and above presenting with topical wounds at CIPDCR- Busia; Kenya Medical Research Institute (KEMRI) clinic.

*Results:* A total of 175 isolates were obtained. One hundred and four (59.4%) were *S. aureus*, 28(16.0%) *Proteus* spp, 25(14.3%) *E. coli* and 18(10.3) *Pseudomonas* spp. Wounds had one bacteria, 58(48.3%) had more than one and three (2.5%) had none. Out of the 70 HIV + patients, 38(54.3%) had one bacteria and 32(45.7%) had more than one bacteria. Out of the 50 HIV - patients, 21(42.0%) had one bacteria and 26(52.0%) had more than one bacteria. Resistances of *S. aureus* from co-infected (with more than one bacteria) wounds were higher than those *S. aureus* from singly infected wounds that is, MRSA in single infection and co-infections showed significance differences as follows: [P values = 0.045, odds =5.5 (1-29.2) for *S. aureus* versus *S. aureus* and *E. coli*], [P values = 0.032, odds =9.0 (1.0-8.0); *S. aureus* versus *S. aureus* and *Proteus*], [P values = 0.046, odds = 8.0 (0.88-72.1); *S. aureus* versus *S. aureus* and *Pseudomonas*]. Significant drug susceptibility difference between HIV+ and HIV- patients was shown by chloramphenicol with P =0.027, odds= 5.7(1.3-24.1). Thus HIV + patients are five fold likely to develop resistance to chloramphenicol as compared to HIV – patients.

*Conclusion:* More antibiotic susceptibility studies need to be done to elucidate the pathogenic mechanisms of *S. aureus* that is isolated from co-infected wounds.

**INTRODUCTION**

*Staphylococcus aureus* is one of the most significant human indigenous flora(1). In normal circumstances, it exists naturally as an innocuous organism. However, this organism has been documented as human opportunistic pathogen which may cause disease depending on whether it is ingested, inhaled to the lungs or breaks the skin and mucous membranes and enters underlying tissues; thus resulting into infections which may cause morbidity and mortality (2,3). In conditions of impaired body defense mechanism such as HIV infection, diabetes mellitus
and hyperalimentation (4,5). *S. aureus* become very virulent resulting in a broad spectrum of infections ranging from mild to life threatening impetigo, boils, septicemia, meningitis and pneumonia(3,6,7,8).

It has been observed that there is increasing frequency in resistance of *S. aureus* to commonly prescribed antimicrobials world wide (7,9). Initially, *S. aureus* was resistant to penicillin, but later to other antibiotics including penicillinase stable antibiotic (3,10). In the USA, MRSA prevalence which was 15-20% in 1997 rose to 32% in 2001 to 2002 (15) and 37% in 2007 Crum et al (13). A review study on prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in eight African hospitals between 1996-1997, showed that, the prevalence of MRSA in Kenya was 21-30%(11). In the year 2001, a study at Kenyatta National Hospital- Kenya, MRSA prevalence had increased to 33% (Kariuki et al; unpublished data). This increasing resistance of *S. aureus* to commonly prescribed antimicrobials (4,5,8,14-16) presents a major threat and concern in its management and treatment.

In particular, Methicillin Resistant *Staphylococcus aureus* (MRSA) which was initially a nosocomial pathogen, has recently spread in the communities and emerged as an important frequent opportunistic pathogen causing skin and soft tissue infections in HIV/AIDS individuals (9). Therefore, there was a need to carry out this study to determine the effects of co-infections in the antibiotic resistance of *S. aureus* isolated from HIV positive individual and come up with an effective regimen of antibiotics in the management of *S. aureus*. This is because, many patients present at CIPDCR - clinic with skin conditions. Due to other logistics CIPDCR was preferred because there are no similar studies done in Western part of Kenya.

**MATERIALS AND METHODS**

The study population included patients who presented at the Centre for infectious and Parasitic Diseases Control Research (CIPDCR) - (KEMRI)- Busia clinic. Qualified Clinicians recruited patients who were 18 years and above showing signs and symptoms of HIV/AIDS and had wounds. Patients were referred to VCT by clinicians. In the VCT, they were counselled and tested by trained VCT counsellors. They were again referred back to the clinicians who referred them to the laboratory for further investigations. In the laboratory, the wounds were cleaned using sterile saline. Specimens were collected using sterile swabs and placed into normal saline. Thereafter, specimen were cultured on 5% sheep blood and MacConkey agars and incubated at 37°C for 18-24 hours. Smears were also made from the original swabs for gram stain to provide relevant information on bacteria on the wounds. Colonies that were small and lacto-fermenters on MacConkey or golden yellow and haemolytic on sheep blood agar were identified by standard microbiological techniques (22). These included, gram stain reaction; bacteria cell arrangement, catalase and coagulase among others for identification of *S. aureus*. Other isolates were also identified appropriately using standard microbiological procedures. Sensitivity was done by the Kirby-Bauer disk diffusion technique using Muller hinton agar (17). Drugs tested were Penicillin 10 units, Ceftriaxone 30µg, Ampicillin 10µg, Amikacin 30µg, Ciprofloxacin 5µg, Erythromycin 15µg, Oxacillin 1µg, Gentamicin 10µg, Cefotaxime 30µg, Chloramphenicol 30µg and Cefuroxime 30µg from BBL Company. Incubation was done at 37°C for 18-24 hours. Zones of inhibition were measured and interpreted based on the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (3,6,17). *Staphylococcus aureus* ATCC 25923 was used as a control. Patients were referred back to the clinicians for management.

Detection of Methicillin Resistance *Staphylococcus Aureus* (MRSA): Pure colonies of *S. aureus* from overnight cultures were inoculated onto Mueller Hinton agar containing 2% sodium chloride. Oxacillin disk was used to detect MRSA. The plates were incubated at 35°C for 24 hours. Those isolates, which showed zone diameters of ≤ 10mm, were considered as MRSA. Known MRSA and MSSA strains were used as controls.


RESULTS

Table 1
Spectrum and isolation rate of pathogenic organisms (isolates) from wounds. n=175

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Total</th>
<th>HIV positive</th>
<th>HIV negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>104(59.4)</td>
<td>62(35.4)</td>
<td>42(24.0)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>28(16.0)</td>
<td>14(8.0)</td>
<td>14(8.0)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25(14.3)</td>
<td>15(8.4)</td>
<td>10(5.7)</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>18(10.3)</td>
<td>11(6.3)</td>
<td>7(4.0)</td>
</tr>
</tbody>
</table>

S. aureus was the most common isolate, followed by Proteus spp, E. coli and Pseudomonas spp. In HIV +, E. coli was as common as Proteus spp. In HIV -, Proteus was more common than E. coli. Proteus was as prevalent in HIV + as in HIV -. S. aureus, E. coli and Pseudomonas spp. were more likely to be found in HIV + than in HIV.

Table 2
Number of pus swabs and isolates from both single and co-infected wounds (n=120)

<table>
<thead>
<tr>
<th>Total No. of patients/ Pus swabs processed</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV positive</td>
</tr>
<tr>
<td>S. aureus</td>
</tr>
<tr>
<td>Proteus spp.</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>E. coli</td>
</tr>
</tbody>
</table>

S. aureus was a constant pathogen in all co-infections. Some wounds of HIV – patients did not have E. coli as single infection.

Table 3
Susceptibility test of S. aureus based on resistance rate (%) n=104

<table>
<thead>
<tr>
<th>Isolates</th>
<th>S. aureus alone</th>
<th>S. aureus and E. coli</th>
<th>S. aureus and Proteus spp.</th>
<th>S. aureus and Pseudomonas spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column a</td>
<td>Column b</td>
<td>Column c</td>
<td>Column d</td>
<td>Column e</td>
</tr>
<tr>
<td>HIV status</td>
<td>HIV+</td>
<td>HIV-</td>
<td>HIV+</td>
<td>HIV-</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>n=30</td>
<td>n=16</td>
<td>n=13</td>
<td>n=10</td>
</tr>
<tr>
<td>Pencillin</td>
<td>17(57)</td>
<td>9(56)</td>
<td>10(79)</td>
<td>7(70)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6(20)</td>
<td>4(13)</td>
<td>7(54)</td>
<td>3(30)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>18(60)</td>
<td>8(30)</td>
<td>9(69)</td>
<td>3(30)</td>
</tr>
<tr>
<td>Oxacin</td>
<td>15(50)</td>
<td>7(24)</td>
<td>11(85)</td>
<td>7(70)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>7(23)</td>
<td>3(10)</td>
<td>6(49)</td>
<td>4(40)</td>
</tr>
<tr>
<td>Cefitoxime</td>
<td>12(40)</td>
<td>6(20)</td>
<td>7(54)</td>
<td>3(30)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>17(57)</td>
<td>3(10)</td>
<td>9(69)</td>
<td>3(30)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>4(13)</td>
<td>2(7)</td>
<td>4(31)</td>
<td>2(30)</td>
</tr>
</tbody>
</table>

Note: Only S. aureus were tested
Generally, the resistances of S. aureus to all these antibiotics tested were higher in co-infections than in single infections. Figure 1 below with p-values = < 0.05 as an example.
There were statistical significance differences in MRSA between single infection and co-infections as follows: [P-values = 0.045, Odds=5.5 (1-29.2); S. aureus vursus S. aureus and E. coli], [P-values = 0.032, Odds=9.0 (1.0-8.0); S. aureus vursus S. aureus and Proteus], [P-values =0.046, Odds= 8.0 (0.88-72.1); S. aureus vursus S. aureus and Pseudomonas].

Resistance of S. aureus to chloramphenicol is showing statistical significant differences between HIV + and HIV – patients (P= 0.027), odds 5.7(1.3-24.1) figure 2.
DISCUSSION

Infections caused by S. aureus are a common problem worldwide (7,9). The emergence of antibiotic resistance by this pathogen is perturbing. Both nosocomial and community acquired infections due to this pathogen has also been reported. In this study, S. aureus was the most frequently isolated pathogen from pus swabs obtained from both HIV positive 62(35.4%) and HIV negative 42(24.0%) (Table 1) and it was the most common pathogen in both single and co-infections (Table 2). The fact that S. aureus exist naturally as harmless organisms, its colonisation of nasal cavities (18) and on healthy skin of human might have given it a greater opportunity as an opportunist pathogen in HIV infected patients thus entering ruptured skin of these immunosuppressed individuals causing secondary infections.

This rate of isolation has been reported in many other studies (8,18-20) which did not specify isolation rate in relation to HIV status. Other organisms (Proteus spp, E. coli, Pseudomonas sp.) were isolated in small numbers (4.0-8.4%) (Table1). This isolation rate is different from Ohene’s and Thanni’s studies, where pseudomonas spp was the most frequent isolate, followed by S. aureus then proteus spp and E. coli the least (19). Co-infections were observed in a total of 32 (45.7%) of 70 HIV positive and 26 (52.0%) of 50 HIV negative patients as follows; S. aureus and E. coli 13(19%) HIV+ and 10(20%) HIV-, S. aureus and Pseudomonas spp 9(13%) HIV+ and 5(10%) HIV-, S. aureus and Proteus spp 10(14%) HIV+ and 11(22%) HIV- (Table 2). Some wounds were infected with only one pathogen such as S. aureus; 30(43%) HIV+ and 16(23%) HIV-, Proteus; 4(6%) HIV+ and 3(6%) HIV-, Pseudomonas; 2(3%) HIV+ and 2(4%) HIV- E. coli; 2(3%) HIV+ and 0(0%) HIV- (Table 2). There were no significance differences in isolation rate between HIV+ and HIV- patients.

S. aureus had multiple antimicrobial resistances in both groups (Table 3). This seem to agree with the study done on the prevalence of MRSA in eight hospitals selected from various African countries as cited by Kesah et al, where >60% of S. aureus were multi-resistant (14). Although the difference in MRSA between HIV + and HIV – patients was not significant, there was significant differences between S. aureus isolated from co-infected wounds (70-90%) and separately infected ones (44-50%) with [P-values = 0.045, odds = 5.5 (1.29-22); S. aureus versus S. aureus and E. coli], [P-values = 0.032, odds = 9.0 (1.0-80); S. aureus versus S. aureus and Proteus], [P-values = 0.046, odds = 8.0 (0.88-72.1); S. aureus versus S. aureus and Pseudomonas]. The odds ratios indicate that, S. aureus from co-infected wounds, are five to nine folds likely to become MRSA compared to S. aureus isolated individually regardless of patient’s HIV status (figure 1). There is no clear explanation to this observation in our study; however, gene mutation which is a common factor in S. aureus (22) could play a possible role. This point will need further elucidation in the future studies. These high MRSA in co-infection conditions is worrying and this indicates that, the isolates from such conditions could be resistant to all penicillin including penicillinase stable antibiotic and cephalosporins that are not widely used and expensive to the patients.

Generally, many studies done on MRSA have shown a gradual increase with time (11, 12, 19). In Kenya, MRSA was 21-30% (11) by the year 1996-1997. Astudy at Kenyatta National referral Hospital- Kenya showed MRSA prevalence of 33% (Kariuki et al; unpublished data) in 2001 and accordingly, our study has shown 56-57% and 70-82% from singly infected wounds and co-infected wounds respectively. MRSA prevalence in USA rose from 15-20% in 1997 to 28% in 1998 to 32% between 2001-2002 and 37% by 2007. Many of these studies however have been hospital base (4,5,11,12). Community based studies have shown an increase in frequency of MRSAisolated from clinical specimens than in hospital cases. Malonzia et al reported 59.2% MRSA prevalence from outpatients attending Kenyatta National referral Hospital. However, our results show almost a double increase by S. aureus isolated from co-infected wounds and almost same to those isolated from singly infected wounds. This has been attributed to indiscriminate usage of antibiotics leading to selection of resistance strains (19). MRSA are a frequent occurrence from specimens from pus and wounds (3,8) and this shows a characteristic that seem to agree with our findings.

Resistance of S. aureus from both singly infected and co-infected wounds to Chloramphenicol was higher in HIV positive (57-78%) than in HIV negative (19-40 %) (Table 3). Chi-square test established that there is significant in distribution of resistance between HIV+ and HIV- (P=0.027) Odds = 5.7(1.3-24.1) (figure 2). The odds ratio reveals that HIV+ patients are five folds likely to develop resistance to chloramphenicol compared to HIV − patients. The prescription of chloramphenicol drug due to frequent typhoid related symptoms in HIV infected persons in absence of laboratory confirmation may have contributed to high resistance of S. aureus from HIV/ AIDS positive individuals. Resistance of S. aureus from co-infected wounds to Ciprofloxacin, erythromycin, gentamicin, cefitoxime and cefuroxime was higher in HIV positive than in negative. Chi-square test established that there is significant difference in the distribution of resistance of S. aureus to antibiotics in patients who were HIV positive and negative, P- values of <0.05 (Table 1).

Resistance of S. aureus isolates to gentamycin, ciprofloxacin and cefuroxime was lower (<25%) in both groups compared to other antibiotics (penicillin, Erythromycin, cefitoxime) (Table 3). This is opposed
A slight increase of resistance (up to a maximum of 56%) of *S. aureus* to three antibiotics gentamicin, ciprofloxacin, cefuroxime is shown in co-infections (Table 3). The administration of gentamycin through intravenous and intramuscular injection (25), high cost of ciprofloxacin to many patients might have limited its indiscriminate use among both groups. The resistances of *S. aureus* to cephalosporins have also been reported in other studies (9,12,20). In our study, the resistance of *S. aureus* to Cefuroxime is <15% (Table 3) in singly infected wounds and between 20-40% in co-infected wounds (Table 3). The resistance of *S. aureus* to Cefitoxime is <40% (Table 3) in singly infected wounds and between 54-60% in co-infected wounds (Table 3). This shows that, with widespread use of these drugs, resistance may develop perversely, regardless of them being expensive and unavailable to many patients.

Resistances of *S. aureus* from co-infected wounds were higher than those of *S. aureus* from singly infected wounds (Table 3). This shows that, co-infections in wounds have an impact on the susceptibility of *S. aureus* to antibiotics hence pose threat to its treatment. *S. aureus* in presence of other pathogenic organisms could have acquired genes which might have mutated and assisted them in resisting commonly prescribed antibiotics. This is noted in the emergence of B-lactamase mediated resistance to antibiotics. Ampicillin (the first penicillin) was developed primarily to treat *E. coli*, within a few years, strains of *E. coli* became resistant by producing plasmid mediated B-lactamase designated TEM. After a short time *S. aureus* expressed a similar B-lactamase (22). Enterobacteriacea (*E. coli*, *Proteus mirabilis* and *Klebsiella*) have been found to exhibit a broad spectrum beta lactam resistance pattern (26). Epidemiological study has shown *E. coli* passing on the genes responsible for antibiotic resistance to *S. aureus* (27). In presence of other pathogenic organisms, especially in HIV/AIDS, *S. aureus* might have acquired already resistant genes. A good example has been also described by bacteriophages in *Staphylococci* for decades. This has been speculated that the high prevalence of B-lactamase production in *Staphylococci* has resulted from bacteriophage-mediated transfer of nonconjugative plasmids (28).

Administration of antibiotics to patients prior to laboratory testing might have had an impact on the growth of *S. aureus*. Eight HIV + and five HIV-patients whose wounds yielded *Proteus, Pseudomonas* and *E. coli* only had been treated with cloxacillin just a month prior to this study. The invasion of *S. aureus* into eukaryotic cells where MRSA is phagocytised by human macrophages, thus complete restoration of susceptibility to cloxacillin is shown and the strain becomes indistinguishable from MSSA due to the acid pH prevailing in Phagolysosomes (29,30) might have contributed to its growth. The three HIV-patients whose yielded nothing had been treated with at least three different antibiotics (cloxacillin, ampicillin and chloramphenicol) just three months prior to this study. These patients were between 50-70 years old. thus this implies that the older the person, the longer it takes for a wound to heal because the pH of the skin increases and lipid content decline inhibiting the permeability of nonlipophilic compounds, thus reducing the efficacy of some medications.

More studies need to be done to elucidate the pathogenic mechanisms leading to resistance of *S. aureus* in co-infected wounds. Both clinical and laboratory diagnosis should be employed in the management of *S. aureus* in order to minimize resistance.

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