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2 **ANTIBIOTIC SENSITIVITY OF *STAPHYLOCOCCUS AUREUS* ISOLATED**
3 **FROM PATIENTS ATTENDING RUIRU DISTRICT HOSPITAL, KENYA**
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12 **ABSTRACT**

Aim: To determine the sensitivity of *Staphylococcus aureus* to commonly used antibiotics in patients with skin, soft tissue and upper respiratory tract infections.

Study Design: Cross sectional

Place and Duration of Study: Samples were obtained from Ruiru District Hospital, Kenya between August and November 2012. The antibiotic sensitivity tests were done at the Laboratories of Jomo Kenyatta University of Agriculture and Technology at Juja, Kenya.

Methodology: The study included 100 in and outpatients with infections on clinical diagnosis, cellulitis, wound infection, ulcers, septic bruises, abscess (including furuncle / boil/ superficial skin abscess). A questionnaire was used to collect patient demographic data from patient records and cultures from the hospital laboratory collected and transported to the Jomo Kenyatta University Laboratories for identification of staphylococcal colonies by culture and biochemical tests. Disc diffusion test was used to determine *in vitro* antibiotic sensitivities of the *S. aureus* isolates as per the Kirby-Bauer diffusion technique

Results: The results indicate that the isolates were very resistant (10% sensitivity) to methicillin and gentamycin, moderately resistant (less than 40% sensitivity) to meropenem, erythromycin, oxacilin, ampicilin, penicillin, trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid but sensitive to minocycline (83.3%). The isolates were also sensitive to cefuroxime, ciprofloxacin, chlorphenicol and lincomycin (75, 58.3, 50 and 41.6% sensitivity respectively). In this regard, antibiotics such as gentamycin, chloramphenicol, trimethoprim/sulfamethoxazole and ampicillin, on the WHO and Kenyan essential drugs list are unlikely to offer help to the patients in Ruiru District hospital. Minocycline would thus be the antibiotic of choice against *Staphylococcus* infections followed by cefuroxime (75%) and ciprofloxacin (58.3%) as the next alternative drugs of choice.

Keywords: antibiotic, bacteria, sensitivity, resistance, *S. aureus*,

1. INTRODUCTION

The use penicillin as antibiotic was first described by Sir Alexander Fleming (1) and since then, antibiotics have been important therapeutic agents for mankind. In the past years there has been marked increase in the number of antibiotics prescribed as well as evolution of new antibiotics. Simultaneously there has been irrational and excessive use of antibiotics for chemotherapy in both humans and livestock has led to development of antibiotic resistance and even multiple drug resistance bacteria.

In most case, the initial antimicrobial prescribed for treatment of an infection is chosen on the basis of clinical impression after the physician is convinced that an infection exists and has made a tentative etiological diagnosis on clinical grounds. Rarely in developing countries such as Kenya are specimens are obtained for laboratory isolation of the causative agent and sensitivity testing to inform the antibiotic of choice. However, the identification microorganisms that are known to be susceptible to certain antibiotics in an area permit the selection of optimally effective drugs. The commonly performed tests are disks diffusion susceptibility tests (2).

Staphylococcus, a member of the Firmicutes, was first identified in 1880 in Aberdeen, United Kingdom, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint (3). One species, *Staphylococcus aureus* is frequently found as part of normal bacteria flora and is estimated that between 20-40% of the human population are long-term carriers in reservoirs in the anterior nares and thus produce nasal secretions and can infect others (4, 5, 6).

Although *S. aureus* is not always pathogenic, disease-associated strains cause a range of illnesses, from minor skin infections, boils (furuncles), cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. *S. aureus* is one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections especially in people with weakened defense systems or in patients undergoing major surgery. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection (7).

Bacteria either have intrinsic mechanisms for antibiotic resistance which they develop through mutation and selection processes or acquires resistance through genetic transfer from other bacteria in the vicinity. Joshua and Esther Lederberg showed penicillin and streptomycin resistant bacteria existed before penicillin

58 treatment and are believed that this emerged as a defense mechanism (8). The
59 prevalence of antibiotic resistant bacteria has not only increased with time but also
60 multi resistance **in regard** to antibiotics of similar or different mechanism of action.

61
62 Widespread use of antibiotics both inside and outside of medicine has however
63 played a significant role in the emergence of resistant bacteria, development of multi
64 drug resistant strains and the spread of resistance between bacterial species (9). This
65 has been facilitated by misuse, underuse and overuse of antibiotics by **physicians** as
66 well as patients. Of particular importance is “over the counter” dispensing of
67 antibiotics **believed to be rampant and thrives** due to poor regulatory implementation
68 as well as patients taking less than the recommended dosage or failing to complete
69 their doses. It has been reported that a large number of people do not finish a course
70 of a once daily antibiotic, 10% to 44 % saying that this is because they felt better (9).
71 These low or incomplete dosages result in decreased concentration of antibiotics in
72 the blood stream and tissues allowing the more resistant bacteria present to survive
73 and be selected leading to more resistant strains and a possible relapse with a more
74 severe infection that is more difficult to treat.

75
76 Despite the importance of antibiotic resistance, no comprehensive data base is
77 available regarding the status of clinical isolates in certain areas due to the dynamic
78 nature of emergence and distribution of resistance.

79
80 There are various mechanisms of antibiotic resistance for instance; penicillin
81 resistance mediated by penicillinase (a form of β -lactamase) production renders β -
82 lactam antibiotics, such as methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin,
83 and flucloxacillin ineffective; or mediated via the mec operon, lowering affinity for
84 binding β -lactams (penicillins, cephalosporins, and carbapenems). Glycopeptide
85 resistance on the other hand is mediated by acquisition of the vanA gene while
86 resistance to aminoglycoside antibiotics (kanamycin, gentamycin) is by
87 aminoglycoside modifying enzymes, ribosomal mutations, and active efflux of the
88 drug out of the bacteria (10, 11).

89
90 There are *S. aureus* strains resistant to many commonly used antibiotics. The β -
91 lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin, and flucloxacillin)
92 were developed to treat penicillin-resistant *S. aureus*, and are still used as first-line
93 treatment. Methicillin, the first antibiotic in this class introduced in 1959, was
94 ineffective only two years later, as the first case of **methicillin resistant *S. aureus***
95 **(MRSA)** was reported in England (12). Despite this, MRSA generally remained an
96 uncommon finding, even in hospital settings, until the 1990s, when there was an
97 explosion in MRSA prevalence in hospitals, where it is now endemic. In the UK,
98 only 2% of all *S. aureus* isolates are sensitive to penicillin, with a similar picture in
99 the rest of the world (13).

MRSA infections in both the hospital and community settings are commonly treated with non- β -lactam antibiotics, such as clindamycin and trimethoprim-sulfisoxazole. Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram-positive antibiotics, such as linezolid. First-line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics (vancomycin and teicoplanin). In Kenya, this is restricted for use in level 4 hospitals (14).

Vancomycin-resistant *S. aureus* (VRSA) also emerged the first case of vancomycin-intermediate *S. aureus* (VISA) reported in Japan in 1996 (15) and the first case of *S. aureus* truly resistant to glycopeptide antibiotics reported in 2002 (16). Three cases of VRSA infections had been reported in the United States as in 2005 (17). MRSA is the most frequently identified antimicrobial drug resistant pathogen in hospitals in the United States, with such hospital acquired *S. aureus* infections increasing from 2% in 1974 to 22% in 1995, and 50% in 1997 (18).

Antibiotic resistant pathogens including *S. aureus* have been observed in Africa. For instance, in a hospital study in Mogadishu, Somalia, multidrug *S. aureus* strains were observed (19) while in Nigeria, 40% of ear infections and bronchitis were shown to be resistant (20). Although *S. aureus* is responsible for illnesses ranging from bacteremia to infections of the central nervous system and respiratory tract, the prevalence of resistant isolates has not been documented for the majority of African countries (21).

In developing countries, the need for antibiotics is driven by high incidence of infectious diseases. There are also complex socioeconomic and behavioral factors such as irrational use of antibiotics by health professionals, unskilled practitioners and lay persons, poor drug quality, unhygienic conditions, poor drug regulation and inadequate surveillance (9). This bacterial exposure culminates in development, selection and spread of resistant organisms contributing to escalating problem of antibiotic resistance worldwide.

Of all surgical infection cases in Kenya, 30-40% were shown to be due to *Staphylococcus* approximately 50% being abscesses which culminated in mortalities in 30% of those untreated (4, 22). Patients with purulent wounds may infect others by invisible droplets containing bacteria Staphylococci through flies, equipment and fingers and clothing of the nursing and medical staff (22). Resistant strains of Staphylococci are common in Kenyan hospitals and may cause very serious infections particularly in hospitalized patients. It is believed that the excessive use and misuse of antibiotics has contributed towards antimicrobial resistance leading treatment failure and relapses with more severe infections that are more difficult to treat. This is particularly among chronically ill, surgical and hospitalized patients.

Determination of antibiotic sensitivity profiles of *S. aureus* in respect to commonly used antibiotics may vary in different hospitals from time to time. An institution

specific study on efficacy of antibiotics in treatment of infections caused by *S. aureus* is thus necessary to inform treatment plans that will achieve successful treatment outcomes. Furthermore, nationwide information from such studies can then be used to inform national policy such as the essential drug list. Overall, the emergence and spread of resistance to antibiotics used to treat infections caused by *S. aureus* will be minimized by rational use of antibiotics by health professionals.

To mitigate this resistance, it is important for laboratory identification of *Staphylococcus aureus* and antibiotic sensitivity of isolates from the site of infection or blood culture through commonly used methods which include coagulase (23), agglutination (24) and hybridization tests (25). This will provide comprehensive information on antibiotic sensitivities of *S. aureus* which is lacking in Kenya (26). The aim of the present study was to determine, among the most commonly dispensed antibiotics, which were the most efficacious in the treatment of *Staphylococcus aureus* infections in patients attending Ruiru District Hospital, Kenya.

2. MATERIAL AND METHODS

This was a cross sectional study done at Ruiru District hospital located in Kiambu County of Kenya using specimens from the hospital laboratory and demographic data from patient records. The population studied was both in and outpatient adults (18 years and above) attending the Ruiru District Hospital in the period between August and November 2012. This is a public hospital serving patients with middle to lower social economic status from a rural and peri urban area. Eligible patients for enrolment were those with skin, soft tissue and upper respiratory tract infections on clinical diagnosis, cellulitis, wound infection, ulcers, septic bruises, abscess (including furuncle/boil/superficial skin abscess). A sample size of 100 was determined using the following formula according to Fisher (27)

After obtaining ethical consent from the hospital administration, specimens were taken with sterile Bacron swabs from the hospital laboratory, put on Armies transport media (Oxoid, Unpath, Hemisphere England), transported to the Jomo Kenyatta University Laboratories, cultured on Blood Agar media or Mannitol Salt Agar, and then incubated at 37°C for 18-24 hours. The plates are then examined for typical staphylococcal colonies and gram staining, culture, biochemical tests (catalase, coagulase). The demographic data of the patients from patient records was captured in a questionnaire without including patient details that would jeopardize their privacy and confidentiality.

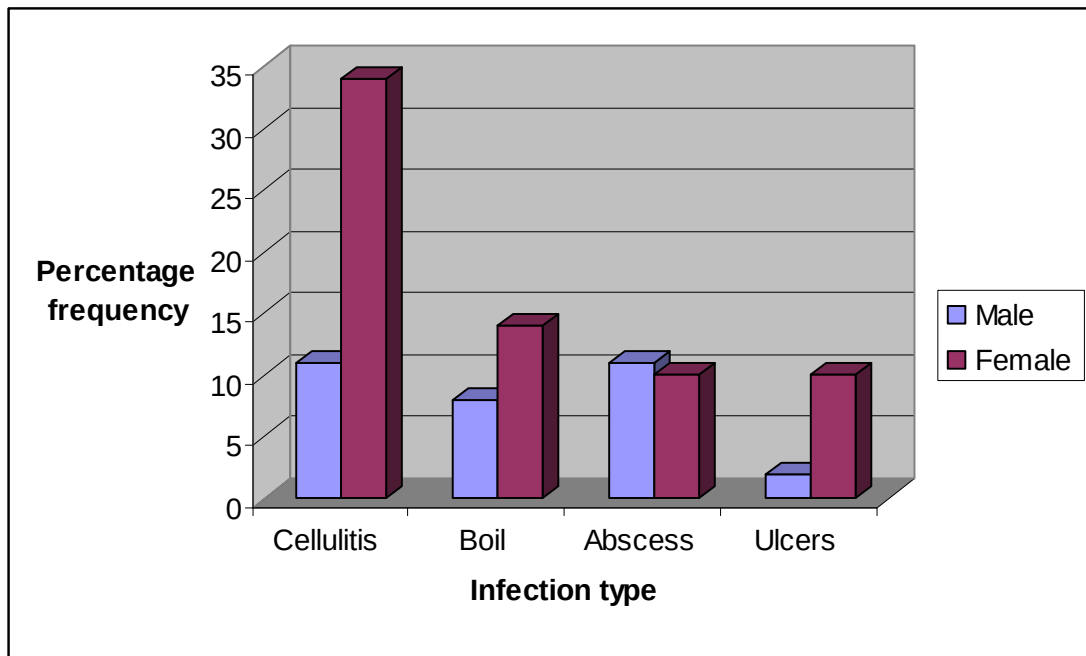
After identifying pathogenic *Staphylococcus aureus*, the disc diffusion test was used to determine *in vitro* sensitivities of the isolates to commonly used and easily available antibiotics (meropenem, erythromycin, oxacilin, ampicilin, penicillin, trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, cefuroxime, ciprofloxacin, chlorphenicol and lincomycin) as per the Kirby-Bauer diffusion technique (28) in accordance with CLSI (formerly, NCCLS) criteria, 2006 (29).

3. RESULTS AND DISCUSSION

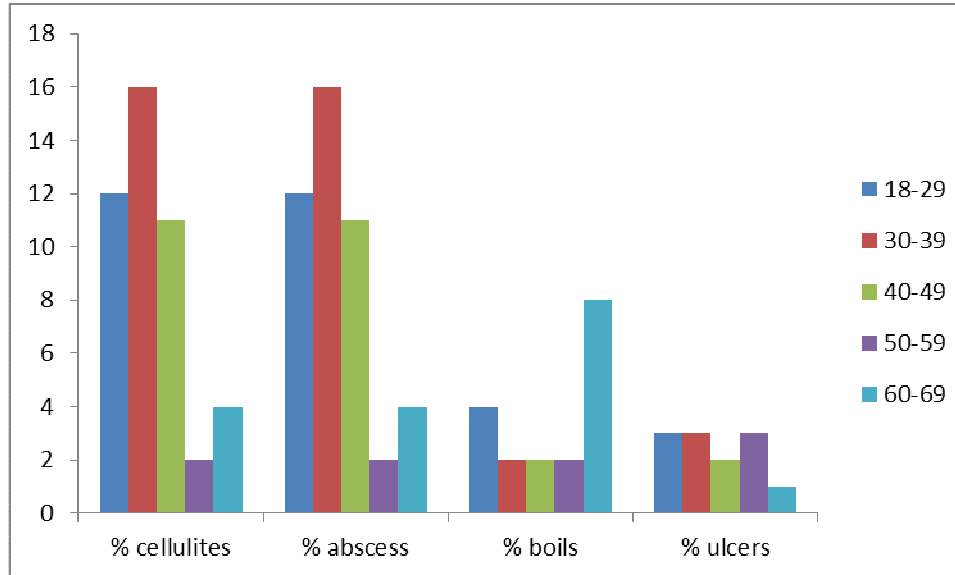
Samples were obtained from a total of 100 patients of mean age 18-69 years; 32 of which were male and 68 females. Among 100 patients sampled, boils, abscesses, cellulitis and ulcers were the major conditions (fig 1). The frequency distribution of ages of the patients ranged from 50-59 (12%) to 30-39 (29%). Although abscesses and cellulitis were most frequently found in patients aged 30-39 and boils in the 60-69 age group, these differences were not statistically significant (fig 2). Cellulitis and boils were more prevalent in females than males ($p < 0.001$ and $p < 0.05$). It may be that cellulitis and boils have a pre-direction for specific population based upon demographic factors such as sex.

The morphological and biochemical characterization showed that all 100 samples isolated were identified as pathogenic *Staphylococcus aureus* for they yellow medium sized colonies of gram positive cocci with catalase and coagulase activity.

Fig 1 Sex and infection of the patients from whom *Staphylococcus aureus* was isolated



218 **Fig 2 Source of *Staphylococcus aureus* according to age of patients**



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221 As shown in figure 3, the *S. aureus* isolated was most sensitive to minocycline
 222 (83.3%) while the least sensitive was the aminoglycoside Gentamycin and the β -
 223 lactam methicilin (8.3%). Other antibiotics had varying sensitivities; 75% sensitivity
 224 to the β -lactam cefuroxime; 58.3% sensitivity to the quinolone ciprofloxacin; 50% to
 225 the bacteriostatic chloramphenicol; 41.6% sensitivity to the lincosamide lincomycin;
 226 33.3% to the β -lactam meropenem; 25% the macrolide erythromycin as well as the
 227 β -lactams oxacilin, ampicillin, penicillin; trimethoprim/sulfamethoxazole and
 228 amoxicillin/Clavulanic acid 16.6%. There was no relationship between resistance to
 229 the antibiotics tested and sex or age of the patient nor the source of the isolate be it
 230 cellulitis, abscess, boil or ulcer.

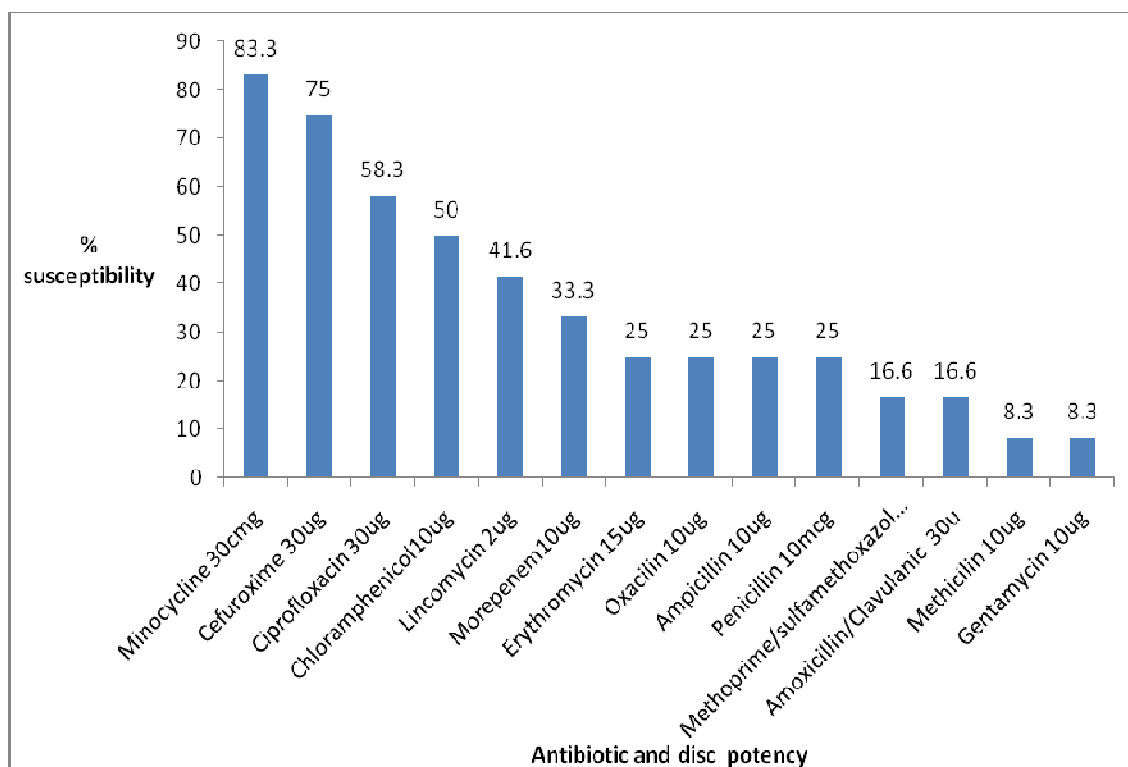
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233 All isolates were identified as *Staphylococcus aureus*, the most commonly isolated
 234 human bacterial pathogen found in skin and soft-tissue infections as well as sepsis
 235 (30). In 2009, Omuse (31) reported that 34% blood stream infections between 2003
 236 and 2008 at the Aga Khan University hospital in Nairobi, Kenya were due to *S.*
 237 *aureus*. Maina et al (32) reported that of all skin and soft tissue infections from
 238 patients in Nairobi hospitals, 65% of the boil isolates were *S. aureus* and so were
 239 23.3% of abscess cultures, 52.9% of cellulitis cultures and 44% of ulcer cultures.

240

241 **Fig 3. Sensitivity profiles of *Staphylococcus aureus* isolates to selected**
 242 **antibiotics**



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246 Unlike the UK where only 2% of all *S. aureus* isolates were reported in 2001 to be
247 sensitive to penicillin (13), in this study the prevalence of sensitive isolates was 25%.
248 In respect to methicillin, the 91.7% methicillin resistance (8.3% susceptibility)
249 observed in *Staphylococcus aureus* in this study is much higher than the 24.2%
250 found in hospital associated *S. aureus* in Texas in 2001 (33). Earlier studies in
251 American hospitals had tracked the increase of methicillin resistant *S. aureus* from
252 2.4% in 1975; 29% in 1991; to 50% in 1997 (34, 18). In addition to resistance to
253 methicillin, the resistance patterns of *S. aureus* observed in the present study to the
254 aminoglycoside gentamycin (8.3% susceptibility), the β -lactam/clavulanic acid
255 combination amoxicillin/clavulanic acid (16.6 % susceptibility) and other β -lactams
256 morepenem (33.3% susceptibility) oxacillin, ampicillin, penicillin (25% susceptibility)
257 can be compared with studies in varying countries. Boyee (1998) earlier reported that
258 methicillin resistant bacteria are usually resistant to **macrolides such as**
259 **erythromycin, clindamycin**, and frequently to gentamycin and ciprofloxacin.
260 Unlike this observation (35) however, the isolates in this study were moderately
261 resistant to ciprofloxacin (58.3%) as well as lincomycin a lincosamine similar to
262 clindamycin (41.6%).

263

264 In 2009, 49%, 44%, 39% and 50% of isolates *S. aureus* from chronic wounds in a
265 study in India were found to be resistant to ciprofloxacin, methicillin, oxacillin and
266 vancomycin respectively (36). Later in 2011, in another study (37), resistance of
267 MRSA to penicillin (100%), amoxicillin/clavulanic acid (70.83%), erythromycin

(66%), tetracycline (62.5%), cefotaxime (66%), cephalixin(58%) was reported. Unlike ~~the~~^{this} study ~~in-reported from~~ India, all MRSA strains in the present study were sensitive to vancomycin.

There are limited studies on antibiotic resistance in Africa in general and Kenya in particular. Vlieghe et al. (38) systematically reviewed published literature on bacterial resistance in Central African countries; Cameroon, Chad, Gabon, São Tomé e Príncipe, Congo-Brazzaville, Democratic Republic of the Congo, Central African Republi, Angola and Equatorial Guinea. What was observed was methicillin-resistant *S. aureus*, high-level penicillin-resistant *Streptococcus pneumoniae* and extended-spectrum beta-lactamases among Gram-negative pathogens. In another analysis, Kimang'a (39), observed there were resistant bacteria to ampicillin, amoxycillin, chloramphenicol, streptomycin, spectinomycin, rimethoprim/sulfamethoxazole, kanamycin, tetracycline and gentamycin in different parts of Africa.

An investigation of eight African hospitals noted comparatively higher levels of MDR MRSA in Kenya, Nigeria and Cameroon, with more than 60 % of isolates exhibiting resistance to at least three antibiotics (21). In Kenya, there was MRSA (28%) of all *S. aureus* tested in Nairobi city hospitals in 1997 with MRSA susceptibility to gentamicin and tetracycline extremely low (15 and 8% respectively). In addition, 2006, MRSA was found in 33 percent of *S. aureus* isolates in nosocomial infections at Kenyatta National Hospital in Kenya (40). A study on the public health threat of bacterial infections and antibiotic resistance at Kenyatta National Hospital during the same year found the prevalence of MRSA to be 40% of all *S. aureus* infections (31). Later, a study in 2009 reported that *S. aureus* isolated from healthcare facilities in western Kenya showed 25% resistance to commonly used antibiotics (41). A situational analysis of 2011 documented that at the Kenyatta National hospital *S. aureus* resistance to methicillin was 40%, 85% to gentamycin and 94 % to tetracycline (26). Even more recently, a study of skin and soft tissue isolates from patients attending Nairobi hospitals reported in 2013 that 84.1% were MRSA (32).

In Kenya, the bacterial infections that contribute most to human disease are often those in which resistance is most evident to antibiotics most frequently used (26, 42). Increased use of antibiotics thus appears to correlate with increased antibiotic resistance. In Kenya, penicillins are the most prescribed antibiotic class at 31 % (43, 44). Tetracyclines were the second most frequently consumed antibiotic class of which doxycycline was the most popular. Consumption of trimethoprim-sulfisoxazole increased from 1997 to 1999 possibly due to its prophylactic use among an increasing HIV positive population. Aminoglycoside consumption also rose steadily after 1999, of which gentamicin accounted for 78 percent of the mean annual amount. While macrolide use remained stable after 1999, Cephalosporin use from 1999 increased in 2000 but later dropped in 2001.

Multi-drug resistant (MDR) *S. aureus* towards routinely used antibiotics as observed in this study is similar to Kimang'a, (39) who reported that 71% of *S. aureus* isolates from Kenya demonstrated multiple drug resistance. This phenomenon may be attributed to widespread availability for gentamycin, trimethoprim/sulfamethoxazole, and erythromycin (25%) observed in this study to be least effective are recommended for use as a first level antibiotic thus together with methicillin and oxacillin are available in the numerous health centres categorized as level 2 (45, 14). In addition, the most effective cefuroxime (75%) and chloramphenicol (50%) are only found as second level drugs in level 4 district hospitals. However, ciprofloxacin with bacterial sensitivity of 58.3% was also widely available as a first level drug in health centres and amoxicillin/clavulanic acid which is available as a first level drug only in district hospitals was not effective (16.6% sensitivity).

Other factors that could be associated with antibiotic resistance of the *S. aureus* isolated could be improper use and patients not taking complete antibiotic course of treatment as prescribed. These habits are prevalent where there is dispensing of antibiotics over the counter (OTC) without the requisite prescriptive precautions. Such dispensing is particularly common among low and middle income such as found in the population likely to attend Ruiru District Hospital.

It has been noted that such indiscriminate use of antibiotics by over-the-counter access, not only gives rise to resistant strains of bacteria, but also provide opportunity for conjugative transfer of resistant plasmids to susceptible bacteria as has been observed in β -lactams and aminoglycosides (46, 47, 48). It may be possible that there may be sub-standard and counterfeit antibiotics in the market that may also work towards development of resistance as in the case reported in antimalarial drugs circulating in the markets of developing countries (49). Furthermore, there are suggestions that antibiotic resistance observed in pathogens isolated from humans may emanate from the livestock where the most popular antibiotic use reported was tetracycline (55%) followed by sulfonamides (21%) aminoglycosides (7%), β -lactam (6%), quinolones (0.6%) macrolides (0.2 percent) and tiamulin (43). The association between sex of the patient with cellulitis and boils is difficult to explain except as it may relate to low socio-economic status and thus hygiene standards of the people in this area. Such associations of antibiotic resistance with socio-economic status have been observed in the previous studies (4, 50).

4. CONCLUSION

This study has confirmed that ~~like as~~ in others parts of Kenya, there ~~are~~ is *S. aureus* ~~isolates~~ isolated at Ruiru District hospital that are resistant to commonly used antibiotics. In this regard, gentamycin, chloramphenicol, trimethoprim/sulfamethoxazole and ampicillin some of which are on the WHO and Kenyan essential drugs list are unlikely to offer help to the patients in Ruiru District

hospital. Overuse, misuse and underuse through lack of access, inadequate dosing, poor adherence, and substandard antimicrobials may have contributed to its emergence and spread. Furthermore, comprehensive local data on usage and antibiotic resistance needed to give a true reflection of the situation in Kenya and to form policy such as the essential drug list is lacking (26)

There is thus need for further research including establishment of prevalence and resistance patterns of *Staphylococcus aureus* in other geographic locations of Kenya. Since the isolates were also resistant to trimethoprim/sulfamethoxazole and lincomycin, the antibiotic of choice would be minocycline and cefuroxime (75%) and ciprofloxacin (58.3%) as the next alternative drugs of choice. These should be used carefully to avoid development of resistant *Staphylococcus aureus* strains. it is thus recommended and the essential drug list be subsequently reviewed.

Noting that currently sensitive microbes could also develop resistance to effective antibiotics, there is need for strictly enforcing quality and regulatory strategies, regularly updating prescription guidelines and emphasizing continued medical and public education to alleviate irrational use of antibiotics by health care professional, unskilled practitioners and patients. Where resources and expertise is available, the ultimate would be determination of bacterial antibiotic sensitivity prior to selection of treatment after isolation from a patient.

CONSENT (WHERE EVER APPLICABLE)

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Ethical approval for the study was obtained from the Medical Superintendent at Ruiru District Hospital.

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