MOLECULAR EPIDEMIOLOGY OF *STAPHYLOCOCCUS AUREUS* FROM HOSPITAL PATIENTS, HIV POSITIVE AND NEGATIVE ABATTOIR WORKERS AND ANIMALS IN BUSIA COUNTY, KENYA

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2021

Declaration

I declare that this doctor of philosophy thesis is my original work and it has not been presented to any other learning institution for any academic award.

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ABBREVIATIONS

Acetyl coenzyme A acetyltransferase gene		*yqiL*
Acquired immunodeficiency syndrome		AIDS
Alpha		A
Ventilator-associated pneumonia		VAP
Busia County Referral Hospital		BCRH
Beta		B
Carbamate kinase gene		*arc*
Cassette chromosome recombinase		*CCR*
Clonal complex		CC
Community acquired Methicillin-resistant *Staphylococcus aureus*		CA-MRSA
complicated skin and skin structure infections		cSSSI
Daptomycin		DAP
Daptomycin resistance		DAP-R
European Antimicrobial Résistance Surveillance System		EARSS
Fibronectin-binding proteins		FnBP
Gamma		Γ
Glycerol kinase		*glpF*
Guanylate kinase		*Gmk*
Healthcare-associated pneumonia		HCAP
Hospital acquired Methicillin-resistant *Staphylococcus aureus*		HA-MRSA
Human immunodeficiency virus		HIV
Immune evasion gene cluster		IEC
Livestock acquire Methicillin-resistant *Staphylococcus aureus*		LA-MRSA
Staphylococcus aureus		*S. aureus*
Staphylococcus sciuiri		*S. Sciuiri*
Methicillin-resistant *Staphylococcus aureus*		MRSA
Methicillin-sensitive *Staphylococcus aureus*		MSSA
Microbial surface components recognizing adhesive matrix molecules		MSCRAMMs
Minimum inhibitory concentration		MIC
Mobile genetic elements		MGEs
Multidrug-resistant		MDR
Multilocus sequence typing		MLST
Multipeptide resistance factor gene		mprF
N-acetylglucosamine		NAM
N-acetylmuramic acid		NAG
Negative		- VE
Panton-Valentine leukocidin		PVL
Penicillin-binding protein 2a		PBP2a
Penicillin-binding proteins		PBP
Penicillin-binding proteins 2		PBP2
penicillin-resistant staphylococci		PRS
Phosphate acetyltransferase		*Pta*

Positive		+ VE
Pulsed-field gel electrophoresis		PFGE
Sequence types		ST
Shikimate dehydrogenase		*aroE*
Staphylococcal cassette chromosome *mec*		SCC*mec*
Staphylococcal enterotoxins		SEs
Staphylococcal protein A typing		Spa
Staphylococcus aureus		*S. aureus*
Sulphamethoxazole and trimethoprim		SXT
Tetracycline		*Tet*
Toxic shock syndrome		TSS
Toxic shock syndrome toxin-1		TSST-1
Trimethoprim		TMP
Triosephosphate isomerase		*Tpi*
Whole genome sequencing		WGS
The International Commission on Microbiological Specifications for Foods		ICMSF

ABSTRACT

Hospitals, Human immunodeficiency virus (HIV)-infected outpatient clinics, cattle farms and abattoirs provide environments that promote acquisition and spread of *Staphylococcus aureus* (*S. aureus*), increasing risk of colonization and infection in the communities. It is documented that establishment of molecular epidemiology of *S. aureus*, with respect to infection control strategies, is important in preventing and controlling *S. aureus* transmission. The terms antimicrobial, antibiotic, and anti-infective encompass a wide variety of therapeutic agents for the treatment of bacterial, fungal, viral, and parasitic infections (Leekha *et al*., 2013; Roberts *et al*., 2012). For the purpose of this study, antibiotic will be referred to as agents against bacterial infection, while the term antimicrobial, will be used for the antibiotic sulfamethoxazole-trimethoprim used for the treatment and prophylaxis of a yeast-like fungus *Pneumocystis jiroveci* pneumonia.

Excessive use of antibiotic empirical therapy in hospital settings has been reported as a contributor to emergence of bacterial resistance to antibiotics (Paterson and Rice, 2003; Laxminarayan *et al*., 2013). Information on empirical antibiotic use, antibiotic susceptibility prevalence of multi-drug resistance (MDR), virulence molecular epidemiology of *S. aureus* isolates in Western Kenya is limited.

This cross sectional study was carried out to establish *S. aureus Staph. aureus* carriage in hospitalized patients, abattoir workers (HIV/AIDS positive and negative) and livestock in Busia County, Kenya.

The justification of this study was as a result of high empirically use of antimicrobials, in hospital patients and livestock rearing and high prevalence of HIV of primary meat handling personnel in Busia County, taking in consideration some of HIV-positive individuals are employed as abattoir workers in various Slaughterhouses spread across the rural areas of Busia County. It was hypothesized that there is a possibility that the HIV-positive abattoir workers form a key link of *S. aureus* transmission in the hospital setting, abattoir environment, general community meat consumers ,as well as among livestock, necessitating usage of a multidisciplinary approach such as One Health, with a view to mitigate the

problems caused by *S. aureus* infections in Busia County.

Main objective was to establish molecular epidemiology of *Staphylococcus aureus* from Inpatients; abattoir workers (HIV-positive and HIV negative), and animals in Busia County, Kenya. The specific objectives being identification and documentation of empirical antibiotic treatment administered to inpatients at Busia County Referral Hospital (BCRH). Isolation and determine prevalence of *S. aureus* in human inpatients, abattoir workers and livestock, and respective phenotypic susceptibility profiles, in Busia County, Kenya. Determine presence of the *pvl*, *tsst-1*, *mec-A* and *sasX* genes in the *S. aureus* isolates. Conduct *S. aureus* molecular typing and whole genome sequencing in order to establish *S. aureus* genotypes circulating among inpatients, abattoir workers (HIV-positive and HIV-negative) and livestock in Busia County, Kenya.

Methods

Study Sites, Busia county referral hospital with a bed capacity of 160, Busia County, and parts of Bungoma, Siaya and Kakamega Counties for Abattoir workers and livestock study population

Study Population included 738 abattoir workers, 261 Inpatients and 103 livestock (Cattle, goats, sheep and pigs).

Sample Collection, Handling, Bacterial Isolation and Identification, All participants were informed of the project objectives and protocol by medical and clinical officers who collected signed informed consent. Nasal samples were collected by rotating a sterile swab five times in both anterior nares, from consenting inpatients, abattoir workers and livestock. The swabs were inoculated in tryptone soya broth/6% salt and transported in cool boxes to the lab for culturing.

The swabs were streaked on mannitol salt agar (MSA) and incubated at 37 °C overnight. Suspect *S. aureus* colonies (those that fermented mannitol, producing yellow colonies) were stocked in tryptone soya broth with 10% glycerol and stored at −40 °C; they were later transported on dry ice to Kenya Medical Research Institute (KEMRI) laboratories in Nairobi. The presumptive *S. aureus* isolates were further cultured onto MSA and repeatedly sub-cultured to get pure culture. The *S. aureus* isolates were identified using Gram reaction (Gram-positive cocci in clumps), catalase, and coagulase (tube method using rabbit plasma) and DNase tests.

Phenotypic antimicrobial Susceptibility Testing was also performed using the VITEK 2 instrument (bioMerieux, Marcy-l'Étoile, France), for benzylpenicillin, cefoxitin, oxacillin, ciprofloxacin, erythromycin, chloramphenicol, daptomycin, fusidic acid, gentamicin, linezolid, mupirocin, nitrofurantoin, rifampicin, teicoplanin, tetracycline, tigecycline, trimethoprim, vancomycin, clindamycin,and inducible resistance to clindamycin. Multi-drug resistant *S. aureus* were defined as isolates that were resistant to three or more antimicrobial. When stocking the pure S. aureus growth; a long sweep of the colonies was done to allow preservation of genetic diversity of nasal carriage of the participant. During whole genome sequencing of the sample, if

sequences from multiple isolates were detected, these samples were cultured and single colonies selected for sequencing.

Molecular Genotype Testing included, DNA extraction from *S. aureus* isolates was performed using QIAGEN DNeasy ® Blood & Tissue Kit,(Staphylococcal cassette chromosome (SCC) mec typing was performed Isolates were screened for *pvl* and *tsst*-1 genes using PCR.

Genomic libraries were generated and sequenced on an Illumina HiSeq 2000, Illumina reads were analyzed based on *S. aureus* MLST database, analysis of virulence and antimicrobial resistance genes were conducted using the virulence finder database. Paired-end Illumina reads were mapped to the S. aureus reference genome ST22 strain HO 5096 0412 using Snippy v4.6.0.

Whole-genome alignments were created by keeping a version of the reference genome using Snippy's.consensus.subs.faoutput files. The resulting core-genome phylogeny was plotted with isolate metadata using ggtree v.3.0.4 and ggtreeExtra v.1.2.3 on R v4.1.0. HIV testing was performed on whole blood using the SD Bioline HIV 1/2 Fast 3.0 test strips

Statistical analysis was performed using the chi-squared test. A p-value <0.05 was considered an indication of significant difference.

Ethical approval was issued Kenya Medical Research Institute scientific steering Committee and Ethical Review Committee .

This study produced a number of key findings: Cefotaxime was found to be the most commonly prescribed empiric antibiotic therapy in patients admitted at Busia County Referral Hospital (BCRH), accounting for 60.0%, 37.7% and 78% in pediatric, medical and surgical wards, respectively. Metronidazole was the second most prescribed at 23.3%, 22.6% and 76.3 in the

pediatric, medical and surgical wards respectively. Among patients admitted at BCRH, the prevalence of methicillin-sensitive *S. aureus* (MSSA) was 10.7 %; it was higher than that of methicillin-resistant isolates (MRSA; 0.8%).The hospital isolates were susceptible to Chloramphenicol, Daptomycin , Fusidic Acid, Linezolid , Mupirocin, Nitrofurantoin, Rifampicin, and Teicoplanin. The MSSA isolates were highly resistant to Penicillin (96.6%), Trimethoprim (73.3%), Cefotaxime (40.0%), but resistant to Ciprofloxacin, Clindamycin, Vancomycin, Trimethoprim/Sulfamethoxazole, Erythromycin, Gentamicin, and Tetracycline, with some isolates, exhibiting multi-drug resistance (MDR). Eleven MLST ST strains were identified in BCRH; they included: ST1633, ST22, ST152, ST188, ST80, ST8, MRSA ST140-MRSA, ST580, ST25 (new ST), ST573 and ST5. Additional new STs (sequence types) identified in BCRH included ST241 –MRSA and ~ST508. These sequence types were clustered into 8 clonal complexes (CC): CC125, CC22, CC1, CC80, CC8, CC508, CC25 and CC5. For abattoir workers, nasopharyngeal prevalence of *S. aureus* was 16.0 %; HIV - positive workers had a higher *S. aureus* colonization rate (25.8%) than their HIV - negative counterparts (14.6%). Low percentages of antibiotic resistance were detected in the abattoir workers' isolates: The *S. aureus* isolates from this study were susceptible to Chloramphenicol, Daptomycin, Fusidic Acid, Linezolid, Mupirocin, Nitrofurantoin, Rifampicin, and Teicoplanin. In contrast to isolates from the hospital, the *S. aureus* isolated from abattoir workers were susceptible to Linezolid, Gentamicin, Ciprofloxacin and inducible Clindamycin resistance at 99.2 % , 98.4 % , 98.4 and 96.8 % , respectively. Increased antibiotic drug resistance was identified towards Penicillin (97.7%), Trimethoprim (65.1 %) and Tetracycline (25.6%). HIV-positive abattoir worker populations significantly carried more MDR- *S. aureus* isolates compared to HIV-negative ones ($p < 0.000418$); two MDR *S. aureus* isolated from HIV-positive abattoir workers had

combination patterns of Penicillin-Erythromycin- Trimethoprim- Inducible Clindamycin resistance - Trimethoprim/Sulfamethoxazole (PEN-E-TMP-ICR-SXT) and Penicillin-Ciprofloxacin-Erythromycin-Trimethroprim-Inducible Clindamycin resistance - Trimethoprim/Sulfamethoxazole (PEN-CIP-E-TMP-ICR-SXT); one, with a combination of Penicillin-Erythromycin-Trimethroprim—Gentamycin-Inducible Clindamycin resistance (PEN-E-TMP-Gent-ICR), was isolated from a HIV –negative abattoir worker. There was a strong association between members of ST 8 genotype with higher phenotypic resistance to Trimethoprim/Sulfamethoxazole (SXT) and HIV infection ((P > 0.00001). Forty three percent (43%) of *S. aureus* isolates, majority of which were PVL gene-positive, belonged to the ST 152 genotype, which accounted for 9.0% of the *S. aureus* isolates in study HIV-positive abattoir workers and 7.1% of *S. aureus* isolates in study HIV - negative abattoir workers. Other ST-type *S. aureus* that carried PVL genes were ST1633, ST30, ST22, ST80, ST2430 and ST1. Toxic shock syndrome toxin-1 gene (*TSST-1*) was associated with ST 72 (Crossley *et al*., 2009); these strains were exclusively found among HIV-negative abattoir workers. Phylogenetic analysis of ST8 and ST152 *S. aureus* strains showed a possible transmission of these strains among BCRH inpatients and that two distinct clones of ST152 were circulating in Western Kenya; ST152 *S. aureus* strains had *dfrG* (Trimethoprim resistance gene) only in Clade A, while all but one of the ST8 isolates had the *dfrG* gene. A novel ST661, assigned to a new clonal complex 661 was identified in one isolate, four new MSSA sequence type variants: 88~(CC8), 1290~ (CC221), 1292~ (CC9) and 8~ (CC80) were recovered from abattoir workers; a new MSSA ST 508~ variant and ST241-MRSA-III, hosting a virulence-associated gene *sasX*, with a single-locus variation from ST239-MRSA-III, were isolated from inpatients in BCRH. These new variant strains had slight mutations in allele of their known housekeeping gene. There was presence of

human genotypes ST152, ST8, ST1290, and ST30 among isolates from livestock nares, indicating the dispersal of human genotypes ST152, ST8, ST1290, and ST30 in livestock and identified ST1925-MSSA as a possible livestock associated *S. aureus*, implying that some MSSA clones have ability to cross the species barrier and jump from humans to several livestock species (Ostojić and Hukić, 2015). Findings of this study will directly improve clinical management and diagnosis of MDR MSSA and MRSA infections among HIV/ AIDS patients, hospital patients, abattoir workers and cattle in Kenya and prevent zoonotic transmission in Kenya and possibly in other low- and middle-income countries in similar situations.

1. INTRODUCTION

This study applied a one health approach, combining human inpatients, abattoir workers, those with and without human immunodeficiency virus infection (HIV)(del Amo *et al.,* 2013; Justiz Vaillant and Gulick, 2022) and livestock, in establishing presence of colonization of methicillin sensitive and methicillin resistant *Staphylococcus aureus* (MSSA and MRSA) strains, by nasal screening. These strains are potential infectious pathogens in the populations studied and may also pose a larger public health challenge to the community beyond the inhabitants of Busia County.

Staphylococcus aureus is causal agent of skin and soft tissue infections in humans and cattle (Fluit, 2012); it can also cause food poisoning and more serious *Staphylococcus aureus* systemic

Infections (Foster, 1996; Pollitt *et al.,* 2018; Reddy *et al.,* 2019). Most infections acquired in hospitals today are caused by microorganisms; since laboratory results may not be available for up to 72 hours, antibiotic choices for the initial treatment of infection will be driven by the clinical presentation (World Health Organization, 2002; Leekha *et al*., 2011). A standard approach is to use broad-spectrum antibacterial agents as single or antibiotic combinations as initial empiric therapy when the infecting agent (bacteria) is unknown, for both community- and hospital-acquired infections (Leekha *et al*., 2011). Some empirically used antibiotics or antibiotic combinations include cefotaxime plus metronidazole, amoxicillin/clavulanate, ciprofloxacin, ciprofloxacin plus metronidazole, trimethoprim/sulfamethoxazole, norfloxacin and ceftriaxone (Rowe-Jones *et al*., 1990; Mettler *et al*., 2007).

Hospital-acquired-MRSA (HA-MRSA) strains were the first to emerge in nosocomial setting, followed recently by community-associated-MRSA (CA-MRSA) strains (Barber, 1961; Choo, 2017), has greatly increased the significance of these bacteria in the hospital and community settings due to the increased mortality associated with the infection (Benner and Kayser, 1968; Choo, 2017), especially in immune-compromised individuals. MRSA has become more common; with some developing resistance to multiple classes of antibiotics, (Malhotra-Kumar *et al.*, 2008), converting these strains to being multidrug-resistant (MDR), multidrug-resistant strain are *S. aureus* isolates that are resistant to three or more classes of the tested antibiotics. (Malhotra-Kumar *et al.*, 2008). As a result, doctors and nurses in many areas of Sub-Saharan Africa have limited antibiotic treatment options since most of them are ineffective against MRSA infections (Ashley, 2011). It is, therefore, important to know how widespread MDR MRSA is in Kenya. This can only be done through a systematic epidemiological study of antibiotic susceptibility/resistance studies.

Some of these MRSA strains and particularly CA-MRSA are capable of producing a virulence cytotoxin known as Panton-Valentine Leukocidin (PVL); discovered in 1936 by Wright (Wright, 1939). This is a cytotoxin that has been associated with severe necrotizing infections involving the skin or mucosa and necrotic hemorrhagic pneumonia (Gillet *et al.*, 2002). Many infections originating from CA-MRSA have been linked to PVL (Shukla, 2005). Another concern is the presence of strains resistant to sulfamethoxazole-trimethoprim especially among individuals infected with HIV; the drug is used as a prophylaxis against *Pneumocystis jiroveci* pneumonia (PJP), in patients with low CD4 counts and other diseases caused by organisms such as *Salmonella*, *Toxoplasma*, *Haemophilus* and *Staphylococcus* (Dworkin *et al.*,2001). This drug is invaluable in resource - limited countries such as Kenya. Studies have shown that HIV-positive

patients infected with *S. aureus* strains resistant to sulfamethoxazole-trimethoprim had a higher chance of dying (Pate *et al*., 2009). Sub-Saharan Africa remains the region with the majority of HIV infections in the world, with nearly one in every 20 adults infected, accounting for 69% of people living with HIV worldwide (Kharsany and Karim, 2016). Kenya has approximately 1.6 million HIV positive individuals, who are more susceptible to acquiring nosocomial and community MRSA (Doyle *et al.,* 2012). If these MRSA strains are also MDR, sulfamethoxazole-trimethoprim resistant and carry the PVL toxin, then it is highly probable that infected HIV-positive patients will have an increased risk of morbidity and mortality, especially in the case when empiric first line antibiotics are used prior to changing to second line, based on antibiotic susceptibility information. It can also be assumed that this will be an even greater threat in developing countries where choices of antibiotic therapies are limited due to cost and availability, coupled with limited laboratory capacity to test for antibiotic resistance in many areas. Emergence of community associated MDR *S. aureus* strain, ST398 (Waters *et al.*, 2011), have been documented among livestock and abattoir workers in food animal production industries (Fluit *et al.*, 2012; van Loo *et al*., 2007). In the United States of America, MDR-MRSA strains have been reported to be resistant to ciprofloxacin, clindamycin, erythromycin and oxacillin (Waters *et al*., 2011). Another study found that up to 93% of *S. aureus* isolated from milk collected from communal and commercial farms in South Africa were resistant to methicillin, with many isolates showing MDR properties (Ateba *et al.*, 2010). To date and to our knowledge there are no published reports on MDR- sulfamethoxazole -trimethoprim resistant-PVL toxin producing *S. aureus* isolated from human inpatients and abattoir workers in Kenya and greater East African region.

The role of livestock associated (LA)-MRSA and its zoonotic transmission has also not been studied in Kenya. It is possible that livestock could potentially be reservoirs for human infection; the strains may be transmitted from cattle to the abattoir worker or farmer and cause MRSA infection or colonization. And when the abattoir worker or farmer is hospitalized, he/she may spread the MDR- strain in the hospital setting; the *vice versa* is also possible. This study has, for the first time, used an integrated, one-health approach, molecular epidemiology and phylogenetic analysis of *S. aureus* to determine associated molecular links between MSSA and MRSA genotypes in inpatients, abattoir workers, and livestock. These strains are potential infectious pathogens in the targeted populations and may also pose public health challenges to inhabitants of Busia County. The study has laid a foundation for future epidemiological studies in Kenya and particularly in Busia County. It has also shed light on the MRSA and MSSA population structures, circulating virulence factors and antibiotic susceptibility patterns for Busia County. It is hoped that the availed data will help in curtailing the spread and increasing virulence of MSSA and MRSA strains; that it will also assist in the prevention of *S. aureus* infections in cattle, abattoir workers, human patients, as well as community as a whole. Thus, the study will raise the level of awareness and importance of *S. aureus* infections in low income countries.

1.1 Study Objectives

1.1.1 Main Objective

To establish molecular epidemiology of *Staphylococcus aureus* from humans [hospital patients; abattoir workers (HIV-positive and HIV negative)], and livestock (Cattle, goats, sheep

and pigs) in Busia County, Kenya

1.1.2 Specific Objectives

a. To identify and document the empirical antibiotic treatment administered to patients admitted in various wards in Busia Referral County hospital (BCRH)

b. To determine the prevalence of *S. aureus* in human inpatients, abattoir workers (HIV-positive and HIV-negative) and livestock (Cattle, goats, sheep pigs) and respective phenotypic susceptibility profiles, in Busia County, Kenya.

c. . To determine presence of the *pvl, tsst-1, mec-A* and *sasX* genes in the *S. aureus* isolates

d. To conduct *S. aureus* molecular typing and whole genome sequencing in order to establish *S. aureus* genotypes circulating among inpatients, abattoir workers (HIV-positive and HIV-negative) and livestock in Busia County, Kenya.

1.2 Hypotheses

a. Use of empirical antibiotic treatment is common among inpatients in Busia referral County hospital

b. *Staphylococcus aureus* is prevalent in human inpatients, abattoir workers (HIV-positive and HIV-negative) and livestock in Busia County, with some strains exhibiting resistance to various antibiotics.

c. There are virulent genes amongst *S. aureus* isolates from hospital inpatients and abattoir workers and livestock population in Busia County.

d. There are genetic relationships between *S. aureus* isolated from hospital inpatients and abattoir workers and livestock population in Busia County

1.3 Justification for the study

Staphylococcus aureus antibiotic resistance poses a significant global challenge. To address this problem requires a concerted One Health research approach on *S. aureus* infections in hospitals, livestock handlers and livestock. There is worldwide recognition of *S. aureus* as a "superbug" (Foster, 2004), due to its ability of developing resistance to multiple classes of antibiotics in both hospital and community settings (Foster, 2004), some of *S. aureus* infection cause high rates of morbidity and mortality (van Hal *et al*., 2012). Infections by *S. aureus* present is a regional disease burden to both humans and livestock industry (Azevedo, 2017), in low income rural communities of the sub-Saharan region (WHO, 2013). The rational of this study was as a result of high HIV prevalence in Busia County (National AIDS Control Council, 2018), taking into consideration some of HIV-positive individuals are employed as abattoir workers in various slaughterhouses, and spread across the rural areas of Busia County. It was hypothesized that there is a possibility that the HIV-positive abattoir workers form a link of *S. aureus* transmission in the hospital setting, abattoir environment, general community meat consumers and livestock, necessitating usage of a multidisciplinary approach such as One Health, with a view to mitigating the problems caused by *S. aureus* infections in Busia County.

2. LITERATURE REVIEW

2.1 Biology of *Staphylococcus aureus*

In 1884, Rosenbach characterized and named two pigmented colony types of staphylococci; *S. aureus* (yellow golden colonies) and *S. epidermidis* (white). Taxonomically, the genus *Staphylococcus* is in the bacterial family *Staphylococcaceae* (Harris *et al.,* 2002) which belong to the order Bacillales, grouped under the class of Bacilli, under the phylum, Bacillota (Harirchi *et al.,* 2022 ;Parks *et al.,* 2020). Genus Staphylococci consist of more than 20 species, three its species are considered to be pathogenic; they include *S. saprophyticus, S. epidermidis* and *S. aureus* (Cohen, 1986). Staphylococci species are characterized on the basis of their coagulase production and resistance to novobiocin (Cohen, 1986). Some species of this genus are known to have developed resistance to methicillin causing serious infection in the nosocomial environment, *S.aureus* being the notable culprit (Haque *et al.,* 2018).

S.aureus organisms are facultative anaerobic, Gram- positive, spherical with microscopic cluster-arrangement similar to that of grapes (Cruickshank *et al*., 1975; Quinn *et al*., 2002). They appear as yellow colonies on rich medium, and hemolyse blood agar. They are oxidase negative and catalase- positive, and grow at 15 - 45˚C at high NaCl concentrations. Almost all strains of *S.aureus* produce the enzyme coagulase (Kloos *et al.,* 1975).

Gram stain has been used to identify Gram positive bacteria via microscopy. Confirmation test requires inoculation onto mannitol salt agar, a selective medium that promotes *S. aureus* growth selectively. When incubated overnight at 37˚ C, typical *S. aureus* colonies are golden yellow. Further, biochemical tests are conducted to confirm the identity of *S. aureus* isolates

(Cruickshank *et al*., 1975; Quinn *et al*., 2002). *S. aureus* standard biochemical characteristics include: Lactose fermentation 100% positive reaction, catalase test 100% positive reaction, gelatin liquefaction 100% positive reaction, urease test 100% positive reaction, 35 % positive reaction protease production, 100% positive reaction galactose hydrolysis, 50 % positive reaction starch hydrolysis and for coagulase test, majority are positive (Chakraborty *et al*., 2011).

2.2Virulence factors and pathogenesis

2.2.1 Background of staphylococcal virulence factors and pathogenesis

Staphylococcus aureus has a number of virulence factors that support its pathogenesis/pathology. Originally, researchers directed their efforts toward cell surface (capsule) virulence factors, such as capsule but they have now appreciated the significance of exoproteins, including cytolysins and superantigens. Staphylococcal exoproteins are involved in the commencement and spread of infections, through direct tissue damage of the skin and mucosal membranes (Wardenburg 2008; Brosnahan *et al*., 2009).

Antibiotics employed to treat *S. aureus* -related illnesses such as Beta (β) -lactams for MSSA and vancomycin for MRSA, kill *S. aureus* by lysis or inhibition of cell wall biosynthesis. Antibiotics have no capability to inhibit or neutralize *S. aureus* produced exoproteins or other toxic effects on host cells, and if insufficiently used, β-lactams may stimulate the production of virulence-related exoproteins, leading to worse clinical outcomes (Stevens *et al*., 2007).

Due to the critical roles that *S. aureus* exotoxins play in pathogenesis; strategies have been directed toward understanding the modes of action of the virulence factors (exotoxins), with renewed focus being placed on prevention of *S. aureus* related infections as a result of virulence factors associated in morbidity and mortality.

2.2.2 Cell surface factors

2.2.2.1 Capsule

Staphylococcus aureus cell wall-associated virulence factors consist of capsular polysaccharides, staphyloxanthin (carotenoid pigment) and a set of proteins designated as Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Roughly 90% of clinical *S. aureus* isolates produce capsular polysaccharides; serotypes CP5 and CP8 isolates account for approximately 75% of this CPs (O'Riordan *et al*., 2004). The key role of capsules is to prevent neutrophilic phagocytosis; capsules also boost bacterial colonization and help in survival of *S. aureus* on mucosal surfaces (O'Riordan *et al*., 2004). Staphyloxanthin, the golden pigment produced by *S. aureus* plays a vital role in resisting killing of *S. aureus* cells by neutrophils via a process known as reactive oxidant-based phagocytosis (Song *et al*., 2009). Inhibition of staphyloxanthin production exposes the *S. aureus* isolates to innate immune clearance, as observed in mouse model, where mice were challenged intraperitoneally by staphyloxanthin producing *S. aureus*; there was marked reduction in numbers of infective *S. aureus* cells in animals'kidney (Song *et al*., 2009).

2.2.2.2 Fibronectin-binding proteins

Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), include collagen adhesion, protein A, clumping factors (Clf), and fibronectin-binding proteins (FnBP). These proteins initiate infection through microbial binding to host proteins (i.e., fibronectin, fibrinogen and collagen) an inhibit dectection by the host immune system (Foster, 2005). Clumping factors and FnBP are able to trigger platelet activation leading to clotting, while Protein A attaches to the Fc segment of immunoglobulin to inhibit opsonization (Foster, 2005).

2.2.3 Secreted factors

Virulence factors disrupt the host immune system, causing it to release nutrients that are used by the bacteria to proliferate. These secreted virulence factors are made of four major classes: super antigens, pore-forming toxins, a variety of exoenzymes and assorted protein (Tam and Torres, 2019).

2.2.3.1 Lipases, Nuclease, Hyaluronidase and Staphylokinase

There are a number of extracellular enzymes secreted by *S. aureus*; they include: nuclease, glycerol ester hydrolase (lipase), hyaluronidase and staphylokinase (Tam and Torres, 2019). .

2.2.3.2 Cytolysins

The cytolysin, Gamma (γ)-toxin and the leukocidin family comprise two distinct secreted proteins, known as fast (F) and slow (S) eluting components respectively. The Amino acid sequences representing the S-components are "HlgA, HlgC, LukM, LukE, LukS-PV", while "HlgB, LukD, LukF-PV" represent F (Menestrina *et al*., 2003; Kaneko and Kamio, 2004). Fast (F) and slow (S) eluting components do not have biological activity on their own, but can assemble to create heptamers (with stoichiometry of 3:4 or 4:3) or hexamers (3:3). The γ-toxin (made of HlgA or HlgC, and HlgB) is able to lyse both eythrocytes and leukocytes, whereas PVL (made of LukS-PV and LukF-PV) is more toxic to leukocytes as compared to erythrocytes (Kaneko and Kamio, 2004). These toxins when injected into the rabbit eye and skin have the ability to induce various levels of inflammatory reactions (Siqueira *et al*., 1997; Gravet *et al*., 1998). Gamma -toxin is produced by over 90% of clinical isolates while PVL accounts for less than 5% of clinical isolates. Other leukocidins, such as LukE/D represent approximately 30% of clinical isolates (Foster, 2005); LukM/F-PV is rarely isolated in *S. aureus* strains infecting humans (Menestrina *et al*., 2003).

Panton–Valentine leukocidin is associated with CA-MRSA isolates (Lina *et al*., 1999), however it is not know if PVL is responsible for the virulence of these isolates. Experimental mice model conducted by Labandeira-Rey and his team using isogenic PVL-positive strains, points to the association between PVL toxin and necrotizing pneumonia, but they did not observe the same association between isogenic PVL-negative strains with necrotizing pneumonia (Labandeira-Rey *et al*., 2007), this notion was disputed by Voyich and his team disputed (Voyich *et al*., 2006). Voyich and his team found that PVL is not an important virulence factor according to their experiments (Voyich *et al*., 2006); they reported that, genetically related PVL-positive and PVL-negative strain, were similar as far as mortality and skin damage was concerned. With presentation of these two theories, it is difficult to say if PVL and other cytolysins clinically play a role in *S. aureus* pathology in humans. It is, therefore, important for this matter to be scientifically settled; since it leaves a huge gap in public health management of *S. aureus* related necrotizing pneumonia in humans.

2.2.3.3 Super antigens

Super antigens are a class of *S. aureus* exotoxins with ability to induce a number of human diseases; one of them known as Toxic shock syndrome {TSS} (Xu and McCormick, 2012). Over 20 different *S. aureus* super antigens have been characterized; they include SEs, enterotoxin-like proteins, and TSS toxin-1. Over 60% of clinical *S. aureus* isolates have at least a minimum of one super antigen (Becker *et al*., 2003; Schlievert *et al*., 2007). These super antigens activate T lymphocytes and antigen-presenting cells (APCs), including macrophages and dendritic cells. Consequently, super antigens can activate 5–30% of T cells compared to 0.001% activation of T cells by an ordinary antigen. These super antigens provoke massive production of cytokines and chemokines from T cells and APCs, leading to TSS (Bernal *et al*., 1999). Clinical characteristics

of TSS are: fever (>of 38.9°C), rash, peeling and loss of the skin of palms of hands and feet, hypotension, and multi-organ dysfunction. Toxic shock syndrome may be split into two groups: menstrual or non-menstrual, depending on the site of infection. Menstrual TSS ordinarily strikes within 2 days after the beginning of menstruation or within 2 days after the end of menstruation, and it is linked to the use of tampon in women colonized in their vagina by super antigen-producing *S. aureus, TSST-1,* which accounts for over 90% of menstrual TSS (McCormick *et al*., 2001). On the other hand, non-menstrual TSS arises following surgical procedures, including cases such as burns or post-influenza pneumonia. Toxic shock syndrome toxin-1 accounts for roughly half of non-menstrual TSS cases with the bulk of the remaining cases attributed to *SEC* and *SEB* (Brosnahan *et al*., 2009; McCormick *et al*., 2001). This super antigen (*TSST-1*) is secreted by between 5 to 25% of *S. aureus* strains (Zumla, 1992).

Toxic shock syndrome toxin-1 is considered as a super antigen and it is produced by between 5 to 25% of *S. aureus* strains (Zumla, 1992), *TSST*-1 toxin induces the immune system, to produce enormous of interleukin-1, interleukin-2 and cytokines tumor necrosis factor-alpha (Salyer, 2007; Takeuchi *et al*., 1998). Large amounts of these factors result in systemic toxicity and suppress the human acquired immune response, with a classical clinical manifestation of multiple organ dysfunction syndromes and finally a fatal shock (Celie Truant *et al*., 2020; Noli Truant *et al*., 2022).

The prevalence of the *TSST-1* gene (*tst*) in clincal isolates is apprximately 25% and over 80% of MRSA USA isolates carry this gene, whereas the prevalence of SEB and SEC in clinical isolates is approximately 10%; MRSA USA isolates are also known to produce either SEB or SEC (Foster, 2005).

Lack or inadequate quantity of neutralizing antibodies is a risk factor for TSS, hence children and young women are at risk; it is common to observe TSS, menstrual and non-menstrual in patients who are unable to form neutralizing antibodies (McCormick *et al*., 2001; Andrews *et al*., 2001).

Super antigens also operate as allergens, stimulating IgE production through T-cell dependent, B-cell activation, in mast cell activation and local inflammation (Yarwood *et al*., 2000). Furthermore, super antigens are able to induce steroid resistance by activating MAPK cascades leading to the expression of glucocorticoid receptor (Li *et al*., 2004; Li *et al*., 2006). Glucocorticoid receptor GRβ is linked to autoimmune diseases such as Kawasaki syndrome (Curtis *et al*., 1995; Leung *et al*., 1993), rheumatoid arthritis (Yarwood *et al*., 2000), psoriasis (Leung *et al*., 1995), atopic dermatitis (Leung *et al*., 1995; Bunikowski *et al*., 2000), nasal polyps (Bachert *et al*., 2001), and asthma (Semic-Jusufagic *et al*., 2007). The mechanisms that this super antigen uses to cause disease have not been identified (Lin and Peterson, 2010).

2.2.4 Proteases

Staphylococcus aureus produces several proteases such as cysteine and serine proteases, and aureolysin (metalloenzymes). Other extracellular enzymes secreted by *S. aureus* include staphopains that disrupt host tissues and/or inactivate host antibiotic defense system such as lipids, defensins, and antibodies and complement mediators; this assists in bacterial dissemination (Lin and Peterson, 2010). Other types of serine proteases, though not as prevalent

as other extracellular proteases, are called Exfoliative toxins (ETA and ETB). In contrast to the other proteases, ETs are the main toxins causing bullous impetigo and staphylococcal scalded skin syndrome (Amagai *et al*., 2000). *Staphylococcus aureus* also secrete additional protein-based virulence factors such as chemotaxis inhibitory protein, staphylococcal inhibitor of

complement, extracellular adherence protein and staphylococcal super antigen-like proteins (Foster, 2005; Rooijakkers et *al*., 2005; Harraghy *et al*., 2003).

Staphylococcus aureus strains do produce a number of different types of enterotoxins (Mansour *et al.*, 2017), these enterotoxins are usually resistant to heat, pepsin digestion and have super antigenicity. These enterotoxins do cause staphylococcal food poisoning, whose symptoms include: increased saliva, vomiting, abdominal cramping, and diarrhea (Loir *et al*., 2003; Pinchuk*et al*., 2010). Contamination of food with *Staph.aureus* can lead to food poisoning, which is a health risk, especially when foods are not stored at a recommended temperature (Kadariya *et al*., 2014).

2.3 Methicillin-resistant *Staphylococcus aureus* (MRSA)

A Scottish surgeon Sir Alexander Ogston was the first person to describe staphylococci in pus in a knee joint abscess in 1882 (Ogston, 1881). Two years later, Friedrich Julius Rosenbach, a German physician, differentiated staphylococci using the pigmentation appearance of their colonies, *S. aureus*, for golden ones, and *S. albus* (currently known as *Staphylococcus epidermidis*) for white ones (Ogston, 1881). Medical treatment for *S. aureus* infections was possible with the discovery of antibiotics, in the 1940s, which was effected immediately (Appelbaum, 2006), but penicillin resistance developed. Then, methicillin was used to treat penicillin-resistant *S. aureus* infections; shortly thereafter in 1961; the first strains of *S. aureus* showing resistance to methicillin were reported by British scientists (Barber, 1961). MRSA is resistant to the class of antibiotics known as beta-lactams, with some strains acquiring resistance to additional classes of antibiotics (Appelbaum, 2007). The first strain of *S. aureus* resistant to vancomycin was identified in the USA (Centers for Disease Control and Prevention, 2002), it is an intravenous antibiotic which is among a handful of antibiotics which are used as the final option for effective treatment of *S. aureus* infections (Chang *et al*., 2003).

Methicillin-resistant *Staphylococcus aureus* is now approaching pandemic scale, beginning with the spread of hospital-acquired *S. aureus* (HA-MRSA) clones in 1960s, followed by the rise of community-acquired *S. aureus* (CA-MRSA) clones in the 1990s and emergence of livestock-acquired *S. aureus* (LA-MRSA) clones from the 2000s; MRSA clones have been reported to be prevalent from different continents of the world (Stefani *et al.*, 2012), with the Africa regions reporting a prevalence of below 50%, though there are some reports showing a rising trends in some countries (Kesah *et al*., 2003), with the exception of South Africa (Falagas *et al*., 2013).

2.4 Phylogenetic tree

Phylogenetic tree is a diagrammatic drawing showing evolutionary relationships of a group of organisms; it is centered on similarities and differences in their physical or genetic traits. These groups of organisms are known as taxa (singular: taxon) and are considered to have a common ancestor (Brown, 2002).

There are two types of phylogenetic trees, rooted and unrooted, and the choice of constructing a phylogenetic tree depends on the research objectives (Kinene *et al*., 2016). In rooted phylogenetic tree, the root represents the recent common ancestor of the entire taxon in a tree, making it the oldest part of the tree and it gives the direction of evolution, showing the flow of genetic information starting from the root up to the external nodes (tip) of each successive generation; this tree is made of both nodes and branches (Baldauf, 2003). It is important to accurately root a phylogenetic tree given that it provides the direction of evolution and enhances the power to interpret genetic changes between sequences (Pearson *et al*., 2013). Nodes stand for the taxonomic unit; it is the location where two branches diverges, making the node recent common ancestor depending on their position on the phylogenetic tree, with branches showing the relationship between different taxonomic units (Baldauf, 2003). To generate a phylogenetic tree root, one must identify an out-group, for example a baboon gene can act as an out-group to root a phylogenetic tree of human, chimpanzee, gorilla and orangutan genes as illustrated in Figure 1 below (Brown, 2002). According to fossil records, baboons are shown to have evolved away from human, chimpanzee, gorilla and orangutan lineage earlier than common ancestor of this species as portrayed in figure 1 below (Brown, 2002).

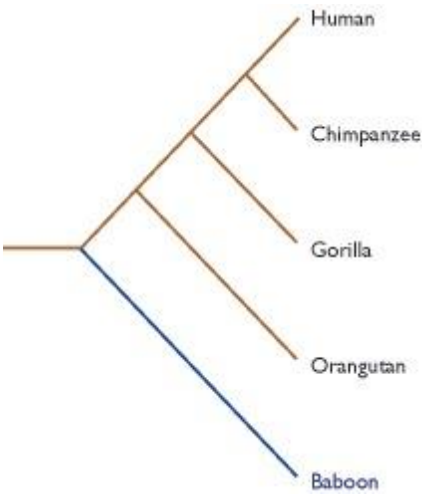

Figure 1: Diagram portraying evolvement of baboons

Unrooted phylogenetic tree is required when dealing with closely related groups (i.e. DNA sequence comparison) or when the main interest is based on relationships among the taxa; this type of phylogenetic tree does not provide information on the direction of evolutionary changes, that is: does not prove the sequences of evolutionary events leading to these genes. Unrooted phylogenetic trees are useful in showing clusters of related DNA sequences, establish the conservancy among a set of DNA sequences and finally show the diversity among a set of DNA sequences (Kinene *et al*., 2016). Constructing phylogenetic trees based on molecular data have many advantages, because it gives accurate representation of the species tree as compared to morphological comparisons that are likely to be ambiguous (Nadler, 1995).

Gene trees are not the same as the species tree and their internal nodes are not comparable, the internal node in a gene tree symbolizes the mutation of an ancestral gene into two genes with different DNA sequences, while internal node of a species tree is a speciation event separating a population of the ancestral species into two groups that cannot interbreed; thus, mutation and speciation are not likely to occur at the same time (Brown, 2002).

Molecular epidemiology research combines aspects of molecular biology typing, evolutionary biology, population biology and epidemiology (Galvani, 2003), with tracking of the spread of pathogens as its central goal. Consequently, a broad range of genotyping methods have been developed to assist in monitoring the spread of *S. aureus* strains; both localized outbreaks and international disseminations (Bayliss *et al*., 2017). It is important to understand when to apply these genotyping methods, because, some of these methods do not illustrate *S. aureus* strains evolutionary history and may present misleading accounts of their spread. Current research has shown that staphylococcal evolution occurs rapidly and the dynamics of *S. aureus* spread can be explained in details using genome-wide single-nucleotide polymorphisms {DNA sequence variation} (Nübel *et al*., 2011). Whole genome sequencing (WGS) aids in Single Nucleotide Polymorphisms (SNPs) analyses; it provides the best molecular typing method with the highest discriminatory power. These advances in sequencing technology have provided many advantages and opened new opportunities in the management of infectious diseases (Bayliss *et al*., 2017). DNA sequences obtained from WGS are fed into bioinformatics pipelines; the bioinformatics data can then be used in constructing phylogenetic trees (Pabinger *et al*., 2014).

2.5 Evolution, treatment and associated factors of MRSA clones

Bacterial clones are genetically identical cells originating from a common ancestor; eventually, differentiation may occur due to point mutations, recombination, gaining or deletion of mobile genetic elements (MGEs) (Bobay *et al*., 2015). This differentiation may lead to acquisition of pathogenic characteristics, for instance, antibiotic resistance genes. Consequently, genetic variation leads to genomic and phenotypic diversity (Croucher *et al*., 2014). Molecular typing methods are usually used to distinguish MRSA clones and help in mapping the spread and evolutionary path of MRSA clones (Deurenberg *et al*., 2007; Donnio *et al*., 2007).

2.5.1 Antibiotic resistant *Staphylococcus aureus* clones

Antibiotic resistant clones, were detected 4 years after the introduction of penicillin as an antibiotic therapeutic against bacterial infection (Ito *et al*., 2004; Davies and Davies, 2010).The detected MRSA clone shared similar genetic characteristic with MSSA clones causing epidemic in Europe (Crisóstomo *et al*., 2001).

Initially MRSA were considered as nosocomial pathogens, but later, they were isolated in community settings blurring molecular epidemiology of MRSA (Gordon and Lowy, 2008).

Clonal genetic structure of MRSA and MSSA clones can be differentiated by their choice of antibiotic treatment (Hassoun *et al*., 2017), epidemiological status and genetic makeup (Abd El-Hamid *et al*., 2022).

2.5.2 MRSA international clones

The invention of new advanced molecular epidemiology typing techniques, have helped to identify *S. aureus* clones that spread fast in local geographical areas, within countries , between

countries and globally (Ayliffe, 1997 ; Zurita *et al*., 2016; Strauß *et al*., 2017). The five internationally established lineages of HA- MRSA clones are; Pediatric NY/Japan, Iberian Hanover-, EMRSA-15 Barnum-, EMRSA-16, USA200 and USA600 Berlin (Gordon and Lowy, 2008).

In other circumstances, MRSA strains thought to have been confined in hospitals have been subsequently isolated in farming communities, resulting into the colonization of livestock, demonstrating the expansion of MRSA hosts in one heath scenario (Pantosti, 2012).

Human nosocomial MRSA, have been found to be similar to those colonizing or infecting companion animals (Petinaki and Spiliopoulou, 2015),this is quite different in food animals such as pigs, cattle, goat, sheep, etc that seem to have specific animal-adapted MRSA clones (Voss *et al*., 2005).

Livestock-associated MRSA ST398 have been reported in pigs, farm animals, such as horses, cattle, and poultry, owning its designation as LA-MRSA (Köck *et al*., 2005), although they rarely infect hospital inpatients, but infect persons working closely with these food animals (Pantosti, 2012).

It is important to note that there are some MRSA clones, that have specifically colonized livestock, such clones include ST1 and ST9 (Grundmann *et al*., 2010), in addition, a bovine MRSA isolate belonging ST130 and carrying a novel *mecA* gene have also been isolated in in England (García-Álvarez *et al*., 2011)

2.5.3 Hospital associated MRSA (HA-MRSA)

The main cause of multi-drug resistant (MDR) nosocomial infection is HA-MRSA (Köck *et al*., 2010). Nasal MRSA colonization is usually high among patients with diabetes mellitus,

intravenous drug users (IVDUs), AIDS, ICU, surgical and long-term care patients (Weinke *et al*., 1992; Mest *et al*., 1994; Pujol, 1996; Brumfitt and Hamilton-Miller, 1989). Due to high incidence of HA-MRSA in hospitals worldwide, the organisms have acquired resistance to essential antibiotics, rendering the antibiotics ineffective for prevention and treatment of HA-MRSA infections. MRSA is a MDR pathogen and its nasal carriage among hospitalized patients is higher than in community dwellers. It is estimated that MRSA carriage among community dwellers is below 1% (Graham *et al.*, 2006) compared to prevalence of MRSA nosocomial bacteremia, which may account for over 50% in some countries (The European Antimicrobial Resistance Surveillance System EARSS, 2007). Multi-drug resistant pathogens in hospitals have led to a huge economic problem for health authorities; besides, it increases morbidity and mortality in hospitals, as reported in some European countries (Stefani and Goglio, 2010), where the authorities spend approximately 380 million euros per year on the MRSA menace (Köck *et al.*, 2010). Health care providers need to establish strategies to control HA-MRSA infections by encouraging hand-washing, appropriate antibiotic use policies, introducing screening and decolonization programs (Henderson and Nimmo, 2018); these interventions can help, but they cannot eradicate incidences of MRSA infection, they can reduce in the hospital settings (Henderson and Nimmo, 2018). It has been established that *Staphylococcus aureus* can acquire a relatively stable mobile genetic element referred to as Staphylococcal cassette chromosome *mec* (*SCCmec*), integrating it into its chromosome; this element encodes for methicillin resistance (IWG-SCC, 2009). Staphylococcal cassette chromosome contains a variety of genes, which include *mecA* gene which is responsible for broad-spectrum beta-lactam resistance among all MRSA strains, conferring resistance to all lactam antibiotics, such as flu-oxacillin, cephalosporins and carbepenems (Utsui and Yokota, 1985). Some MRSA strains are associated

with hospital environment, giving them definition of Hospital associated –MRSA (Chen *et al*., 2012), HA-MRSA is known to carry SCC*mec* type I, SCC*mec* type II, or SCC*mec* type III (Asghar, 2014).

2.5.3.1 Molecular epidemiology of HA-MRSA

Antiquated MRSA clones were reported in European hospitals in the 1970s with some cases appearing in hospitals in the United States (Crisóstomo *et al*., 2001; Barrett *et al*., 1968; Bran *et al*., 1972). During this period, the rest of the world was generally spared, with MRSA failing to colonize the communities in these countries (Chambers and DeLeo, 2009). In the 1980s this antiquated MRSA clone vanished from European hospitals (Chambers and DeLeo, 2009).

Pedigrees of this antiquated MRSA clone such as the Iberian and Rome clones (Mato *et al*., 2004) and other prosperous MRSA lineages then emerged (Deurenberg and Stobberingh 2008; Enright *et al*., 2002).

There are five major HA-MRSA clones lineages circulating globally, CC5, CC8 (ST239), CC22, CC30 (ST36) and CC45 (Aires-de-Sousa, 2017) with each lineage possessing distinct epidemiological characteristics (Peng *et al*., 2018), genes encoding for surface proteins (McCarthy and Lindsay, 2010) and immune evasion genes (McCarthy and Lindsay, 2010).

2.5.3.2 Treatment of hospital-acquired MRSA infection in humans

Some MRSA infections, such as skin abscesses, may be lanced and drained without systemic antibiotic treatment (Boucher *et al*., 2010; Centers for Disease Control and Prevention, 2005). The location, severity, speed of progression of the infection, age and health of the patient are factors that may determine the type of treatment to be applied (Waness, 2010). While invasive staphylococcal infections require antibiotics (Boucher *et al*., 2010; Public Health Agency of Canada, 2001), antibiotic susceptibility testing must always form the basis of antibiotic treatment in human infections (Boucher *et al*., 2010). A limited number of antibiotics are capable of treating infections caused by HA-MRSA (Middleton *et al*., 2005), due to their resistance to commonly used antibiotics such as aminoglycosides, chloramphenicol, fluoroquinolones, macrolides, and tetracycline's (Carlson-Banning and Zechiedrich, 2013Lee *et al*., 2003; Centers for Disease Control and Prevention 2005; Middleton *et al*., 2005).

Serious and multiple drug resistant HA-MRSA infections are treated by Vancomycin, Linezolid, Tigecycline, Quinupristin/ Dalfopristin and Daptomycin; there are already reports of emerging *S. aureus* resistance to daptomycin, and linezolid Vancomycin (Amberpet *et al*.,2019), Linezolid (Tyson *et al*., 2018), and Daptomycin (Stefani *et al*., 2015), which means researchers must increase efforts for discovering new antibiotics (Centers for Disease Control and Prevention 2005; Boucher *et al*., 2010; Catry *et al*., 2010).

2.5.3.3 Nosocomial risk factors associated with *Staphylococcus aureus* infection

Risk factors associated with an increased risk of nosocomial acquisition of MRSA and MSSA are well known, for example patients in long-term health care facilities are at a higher risk of acquiring nosocomial associated MRSA infection (Thompson *et al*., 1982). Other risk factors

associated with nosocomial associated MRSA/MSSA infection, include: recent hospitalization, surgery, dialysis, weakenedimmune system (e.g., HIV/AIDS, cancer) and individuals who are in close contact with health care workers **(**Sydnor *et al*., 2011; Ojulong *et al*., 2008; Onorato *et al*., 1999).

2.5.4 Community-associated Methicillin-resistant *Staphylococcus aureus*

Community-associated MRSA can be differentiated from HA-MRSA, by a MRSA infection detected from an outpatient or inpatient within 48 hours of hospitalization (David and Daum, 2010; Loewen *et al*., 2017; Klevens *et al*., 2007).

The other CA-MRSA infection inclusion criteria are patients who are diagnosed with CA-MRSA infection, but did not have prior HA-MRSA health -associated infection; risk factors include surgery, hemodialysis, and hospitalization in the preceding 12 months, residing in a long-term care facility, having an indwelling catheter or a percutaneous device. The rest of MRSA infections are deemed to be HA-MRSA (Centers for Disease Control and Prevention, 2005; Morrison *et al*., 2006). This case definition was originally utilized to determine MRSA infections in healthy individuals in the community, lacking health-care related exposure (Fridkin *et al*., 2005; Naimi *et al*., 2003).

Methicillin-resistant *S .aureus* (MRSA) isolates were previously limited in hospitals, health care settings, and patients visiting these facilities. However in the mid-1990s, there had been a sudden increase in the number of MRSA infections registered in populations without risk factors associated with health care system (Adcock *et al*., 1998). The new clones associated with the community are now recognized as CA-MRSA. Community acquired MRSA strains can be differentiated from Hospital acquired strains based on their genetic characteristics,

epidemiological traits, or microbiological profiles, clinical symptoms and antibiotic susceptibility patterns (Loewen *et al*., 2017).

Community acquired MRSAstrains are usually disseminated quickly among healthy individuals in the community; these strains occasionally cross into nosocomial settings causing typical CA-MRSA infections in hospital settings (David and Daum, 2010). Community acquired MRSAcan be differentiated from HA-MRSA by molecular biology techniques. The main characteristic of HA-MRSA strains include a large staphylococcal chromosomal cassette *mec* (*SCCmec*) belonging to *SCCmec* type I, II, or III. Hospital acquired MRSAstrains are normally resistant to various classes of non-β-lactam antibiotics, their cassettes harbor the *mecA* gene which is universal among MRSA isolates but rarely PVL genes (David and Daum, 2010). On the other hand, CA-MRSA strains differ by harboring a smaller staphylococcal SCC*mec* cassette; they are characterized as *SCCmec* type IV or type V which is apparently more mobile as a result of harboring a smaller staphylococcal *SCCmec* cassette (Berglund and Söderquist, 2008). Community acquired MRSA are resistant to a smaller number of non-β-lactam classes of antibiotics and often harbors PVL genes (David and Daum, 2010). Genotypic characteristics of CA-MRSA strains direct these strains to infect and colonize a different population in contrast to HA-MRSA, presenting well-defined clinical symptoms. Community acquired MRSA infections have a tendency of infecting healthy younger individuals, where they cause mainly skin and soft-tissue infections (SSTIs), necrotizing pneumonia and severe sepsis (Buckingham *et al*., 2004; Munckhof *et al*., 2008; Naimi *et al*., 2003). On other hand, HA-MRSA strains tend to infect or colonize older patients in hospital setting, causing pneumonia, bacteremia, and invasive infections. Obscuring the epidemiological background, in some cases, HA-MRSA strains trigger MRSA infections in the community setting; such instances could arise from discharged patients

treating HA-MRSA acquired infections in hospital at home, complicating the epidemiology stemming from the transmission of these escaped HA-MRSA strains in the larger population (David and Daum, 2010). This has serious repercussions in the community as some MRSA isolates based in the hospital surviving beyond health care setting, subsequently causing MRSA infections in the community (Creech *et al*., 2005; Farley *et al*., 2008; Hidron *et al*., 2005).

The rise of CA-MRSA strains has serious implications, since a large pool of MRSA isolates does survive beyond health care setting (Creech *et al*., 2005; Farley *et al*., 2008; Hidron *et al*., 2005).

A good example of dealing with MRSA is by applying current conventional infection control methods as established in health care facilities in the United States. This strategy may not be achieved devoid of a parallel MRSA infection control program in the community settings.

Treatment of MRSA infections is very costly compared with their MSSA counterpart, especially MRSA bacteremia followed by pneumonia and surgery site infections (Yuasa *et al*., 2019), when compared with their MSSA counterpart (Charlebois *et al*., 2004; Cooper *et al*., 2004; Liu *et al*., 2008; Purcell *et al*., 2006).

There are fairly few antibiotics which can be used to treat MRSA infections, with some groups having significant limitations; the unfortunate thing is the slow pace in development of new antibiotics worldwide. Faced with increased resistance to different classes of antibiotics, it is theoretically possible that a fatal MDR *S .aureus* infection may emerge in the near future (Daum and Seal, 2001; Talbot *et al*., 2006; Wenzel, 2004; Dowzicky *et al*., 2000; Luh *et al*., 2000; Meka *et al*., 2004; Rose and Rybak, 2006; Marty *et al*., 2006).

2.5.4.1 Treatment of CA-MRSA infection in humans

Community acquired MRSA can be treated effectively with antibiotics other than methicillin; these antibiotics include macrolides and azalides. Some CA-MRSA strains have shown rising resistance to fluoroquinolones and tetracycline (Centers for Disease Control and Prevention, 2005; Tenover *et al*., 2009; Boucher *et al*., 2010). In the case of staphylococcal food poisoning, antibiotic treatment is not recommended, since the illness is caused by enterotoxins and not replication of *S. aureus* harbor the enterotoxins genes (Zeaki *et al*., 2019) as the illness is self-limiting (Public Health Agency of Canada, 2001; Jones *et al*., 2002).

2.5.4.2 Risk factors associated with *Staphylococcus aureus* infection in the community

Risk factors for CA-MRSA are: athletes and contact sports participation, skin to skin contact, contaminated surfaces, shared items, compromised skin, and poor hygiene (Green *et al*., 2012). Crowded conditions in child care units, military barracks, and prisons also at increased risk of CA-MRSA infection (Tong*et al*., 2011).

2.5.5 Livestock Associated- Methicillin-resistant *Staphylococcus aureus*

Livestock Associated- MRSA is a rising public health concern in veterinary medicine (Anjum *et al*., 2019), with MRSA infections being reported in horses, dogs, cats, pet birds, cattle and pigs (Crespo-Piazuelo and Lawlor, 2021). This type of MRSA, first reported from mastitis milk samples from Belgian dairy cattle in 1972 (Devriese *et al*., 1975), this shows that MRSA infection is not restricted in human beings alone (Devriese *et al*., 1975). This type category of MRSA infection is prevalent in livestock, abattoir workers and livestock handlers, having been reported in many European countries, Canada and Singapore (Wulf *et al*., 2008; Vandenbroucke-Grauls *et al*., 2006).

Community acquired MRSAclones, originally deemed to colonize and infect humans in the community are currently being isolated from livestock and becoming a worldwide problem (Smith *et al*., 2009; van Cleef *et al*., 2011). Some countries such Netherlands and Denmark had previously reported low prevalence of MRSA (van de Sande-Bruinsma *et al*., 2015; Danish Health Authority, 2016), but that is no longer the case now, there have been reported increase of LA-MRSA infections particularly strains belonging to CC 398 (Karlsen *et al*., 2021).

LA-MRSA CC398 strains have been reported to the most dominant causing invasive infections in humans (Fetsch *et al*., 2021), such as endocarditis, osteomyelitis, and ventilator-associated pneumonia (VAP) in humans in Europe and North America (Ekkelenkamp *et al*., 2006; Mammina *et al*., 2010), such as endocarditis, osteomyelitis, and VAP in humans in Europe and North America (Ekkelenkamp *et al*., 2006; Mammina *et al*., 2010).

In Asia, the predominant CC ST9 has been reported to cause invasive disease in humans (Graveland *et al*., 2011). Farmers and food handlers who are in direct contact with LA-MRSA-positive animals, are particularly at risk of becoming colonized with LA-MRSA (Davies *et al*., 2012).

Isolation of CA-MRSA infection in an inpatient in hospital setting and similarly the isolation of HA-MRSA strain in the community have led to distortion of the epidemiological characteristics of HA-MRSA (Kateete *et al*., 2019).

Similarly LA-MRSA may cause MRSA infections in both community (Cuny *et al*., 2015) and health care settings, despite being originally associated with livestock (Cuny *et al*., 2015), which has further complicated the epidemiological characteristics of LA-MRSA in both community and healthcare settings (Huijsdens *et al*., 2006; Lewis *et al*., 2008; Layer *et al*., 2012).

Advances in molecular epidemiological analyses have contributed to better understanding of molecular characterization and epidemiology of LA-MRSA (Lakhundi and Zhang, 2018). Coupled with advances in next generation sequencing technique, has provided a rapid cost effective way to analyze of whole bacterial genomes (Price *et al*., 2012), simplifying understanding of the evolution and spread of LA-MRSA (Harrison *et al*., 2013; Spoor *et al*., 2013).

2.5.5.1 Molecular epidemiology of LA-MRSA

Phenotypic characterization was earlier used to differentiate *S. aureus* isolates from humans and animals into distinct biotypes (Meyer, 1967).

Currently, 87% of *S. aureus* isolates associated with human colonization and infections have been characterized into 11 extensively disseminated Clonal complexes: CC1, CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45, CC51 and CC121 (Silva *et al*., 2022). Eight clonal complexes: CC15, CC22, CC30, CC45, CC30, CC45 (Rasmussen *et al*., 2014) and the rare CCs: CC80 and CC152 (Senok *et al*., 2020) are mainly related to human isolates. In the case of LA-MRSA, their genetic background, in combination with their antibiotic resistance, has shown host specificity among livestock (Cuny *et al*., 2010). This view has, however, changed owing to some studies based on comparative genome analysis revealing that CC130 and CC398 display low host specificity. Clonal complex 97, CC133, CC522 and clonal lineage ST151 are reported to specifically colonize and infect ruminants, with clonal lineage ST 385 being linked to poultry infection and colonization (Köck *et al*., 2014; Cuny *et al*., 2010; Pantosti 2012; Fitzgerald, 2012; Espinosa-Gongora *et al*., 2014).

It is believed that *S. aureus* isolates from ST5 human and poultry infections and those of CC398 isolates from livestock originated from ancestral human isolates (Price *et al*., 2012; Lowder *et*

al., 2009),with ST91 human-associated isolates being traced back to ruminant ancestry (Spoor *et al*., 2013). In fact, some companion animals have been found to be colonized by this sequence type; further, in-depth studies have established colonization factors responsible for its host specificity (Walther *et al*., 2012; Pantosti 2012).

Genetic alterations responsible for *S. aureus* animal host adaptations are associated with the accessory rather than the core genome, MGEs or genomic pathogenicity islands (Malachowaand DeLeo, 2010), with LA-MRSA CC398 strains retaining the ability to infect humans in an area of high human /livestock population density (Price *et al*., 2012; Lowder *et al*., 2009; Uhlemann *et al*., 2012; McCarthy *et al*., 2011; Utter *et al*., 2014; Witte *et al*., 2007).

Among the well characterized LA-MRSA strains, the most extensively disseminated is CC398, followed by CC9, which has spread globally and appears more prevalent among several livestock species in Asia. Other characterized strains include ST5 isolated recently in pigs in the USA and CC1 strain which appears to have low host specificity (Chuang and Huang, 2015; Molla *et al*., 2012; Elstrøm *et al*., 2019). MRSA CC 1 strain has been has been isolated in in tertiary hospitals in Italy, Spain and Romania, from patients presenting with MRSA infections at the emergency department located (Bouchiat *et al*., 2017).

Multidrug-resistant CC1s MRSA and MSSA strains associated with cattle in Italy, show close genetic relatedness (>90–100% PFGE similarity) to human isolates (Alba *et al*., 2015); this LA-MRSA CC1 zoonotic potential to cause disease in humans; this a is serious public heath issue and prevention measures must be taken to prevent spill-over at farm-level (Grundmann *et al*., 2014; Alba *et al*., 2015). Clonal complex 1 strains harboring PVL gene were first reported in the USA in the late 1990s, followed by several worldwide citations (Deurenberg and Stobberingh, 2009).

Clonal complex 130 that contains homologue *mec*C in place of *mecA* has attracted attention in the recent years (Monecke *et al*., 2016; Silva *et al*., 2020). MRSA CC130 have so far been isolated from domestic animals, an indication of its host specificity characteristic (Cuny *et al*., 2012), it has been isolated from domestic animals such, cattle, goats, sheep (Paterson *et al*., 2014), dogs, and cats (Walther *et al*., 2012; Loncaric *et al*., 2019) and wild animals such as roe deer, chamois, hares, brown rats, and ,seals (Paterson *et al*., 2012; Espinosa-Gongora *et al*., 2015).

Curiously, the strain was detected in wildlife and domestic ruminants living in the same surroundings, signifying possible reciprocal infections (Loncaric *et al*., 2014). Clonal complex 130 has ability to infect humans though in rare instances as documented in few peer reviewed journals (Cuny *et al*., 2011; Paterson *et al*., 2014). There have been reports of the ST 425 harboring homologue *mec*C, with a MSSA variant of ST 425 being linked to mastitis in dairy cattle. Sequence type 425 strain has been isolated from nasal swabs of wild boars; the same swab also contained ST133, being a co-colonizing strain (García-Álvarez *et al*., 2011; Meemken *et al*., 2013). Sequence type ST22 strains, associated with HA-MRSA, have been reported to cause nosocomial infections in veterinary clinics (Harrison *et al*., 2014; Walther *et al*., 2009), while ST8, *spa* type t064, initially isolated in a Canadian equine clinic, has been reported in other countries; it was suspected to have originated from a nosocomial-associated ST8 subpopulation (Weese *et al*., 2006)

2.5.5.2 LA-MRSA in Livestock

The first report of LA-MRSA CC398 colonization of pigs came from France; the citing was subsequently followed by detections of this strain in Netherlands, Denmark, Germany, and France, Italy (Armand-Lefevre *et al*., 2005; European Food Safety Authority {EFSA} 2009).

LA-MRSA CC398 is found as a colonizer in pigs in North America (Molla *et al*., 2012), Northern Africa (Chairat *et al*., 2015), Asia (Chuang and Huang 2015) and Australia (Groves *et al*., 2014). This clone is not only found in pigs, but also from veal calves (Groves *et al*., 2014), poultry and dairy cattle (Nemati *et al*., 2008; Vanderhaeghen *et al*., 2010).

The prevalence of LA-MRSA infection in livestock correlates with the size of the farms (Mascaro *et al*., 2019; Alt *et al*., 2011; Graveland *et al*., 2011), this correlation also apply to farming systems conventional *vs* alternative (Cuny *et al*., 2012; Van de Vijver *et al*., 2014) and the use of disinfectants and zinc in feeds (Slifierz *et al*., 2015). The spread between farms is related to animal trading (Broens, 2011). Raw meat products have been shown to be contaminated during processing. MRSA contamination was detected in pork (11%), beef (15%), veal (15%), lamb (6%) and turkey meat (35%) in the Netherlands, while in Germany only 2.8% of pork end products were contaminated (de Boer *et al*., 2009; Beneke *et al*., 2011). MRSA meat contamination has also been reported in Canada (Weese *et al*., 2010) and the United States of America, with significant rates of contaminated retail meat being reported in Netherlands and Spain (O'Brien *et al*., 2012; van Loo *et al*., 2007; Lozano *et al*., 2009). CC398 as a food intoxicant has not been reported to date and isolates attributed to this CC rarely harbor enterotoxin genes (Kadlec *et al*., 2009; Argudín *et al*., 2011), although there is a possibility that CC398 may re-acquire immune evasion gene cluster {IEC} (Pérez-Moreno *et al*., 2017).

The immune evasion gene cluster can contain *sea* or *sep* as discovered in the Netherlands by Van Wamel *et al.* (2006), Immune evasion gene cluster containing *sea* or *sep* were first reported in Netherlands (Wamel *et al*., 2006), Cuny *et al.* in 2015 found that about a fifth of LA-MRSA CC398 infections do occur in humans beings, and these strains also carried the IEC gene cluster {n = 99}(Cuny *et al* in 2015), with only one of these isolates containing *sea* (Cuny *et al*., 2015).

Immune evasion gene cluster -containing isolates have not yet been detected in pigs, however, IEC-containing isolates are frequently detected in hospitalized equines, horses and their caretakers are considered to reservoirs of CC398 carrying immune evasion gene cluster (Bierowiec *et al*., 2016), with a single strain harboring a *sea* gene (Cuny *et al*., 2015).

MRSA CC398 strain has been found in tank milk (Paterson *et al*., 2012); linked to possible udder colonization and possibility of subclinical mastitis in dairy cattle in Germany (Schnitt and Tenhagen, 2020).

Livestock associated MRSA CC398 strain has also been isolated from industrial rabbit farms and pet rabbits (Agnoletti *et al*., 2014; Loncaric *et al*., 2013).

2.5.5.3 LA-MRSA CC398 in Animals other than Livestock

In some Belgium pig farms, colonization by LA-MRSA CC398 in pigs, goats, cats, dogs, mice, rats and humans (Pletinckx *et al*., 2013), has surfaced as a nosocomial pathogen in equine clinics in the Netherlands, Belgium, and Switzerland (Cuny *et al*., 2008; Van Duijkeren *et al*., 2010; Van den Eede *et al*., 2009; Sieber *et al*., 2011).

2.5.5.4 Risk factors associated with LA-MRSA infection in Livestock

Exposure to MRSA-infected livestock is a risk factor for humans handling them or their contaminated meat products. Individuals living in areas with livestock population density have an increased of risk LA- MRSA ST398 infection (Feingold *al*., 2012).

2.6 Pathology caused by *S. aureus,* diagnoses and treatments

2.6.1Pathology caused by *Staphylococcus aureus* in humans, respective diagnoses and treatments

2.6.1.1 Skin abscess

2.6 .1.1.1Furuncles

A skin abscess occurs when pus accumulates in hair follicles, skin tissues, or underneath the skin. A furuncle, commonly referred to as a boil, presents as a painful infection developing in the vicinity of a hair follicle and contains pus, in contrast to carbuncle which is a group of boils formed underneath the skin. Once *S .aureus* infects hair follicles, the follicles swell and are transformed into boils and carbuncles (Stevens *et al*., 2014).

Furuncle begins as a red lump, which is rapidly filled by pus; this lump may swell to the point of bursting (Stevens *et al*., 2014; Ibler and Kromann, 2014). Furuncles and carbuncles usually infect thighs, armpits, buttocks, face, and neck, and mostly affect persons who have an impaired immune system (Carneiro-Sampaio *et al*., 2011); young adults are particularly susceptible to furuncles as compared to children or older adults; males being more frequently affected than females (Yamasaki *et al*., 2005). Overcrowded and unhygienic environment are the risk factors associated with furuncles (boils) and carbuncles (Dellit and Duchin, 2007).

Furuncles may heal without any treatment; they usually burst and heal without a scar after few days or at most within 3 weeks. Carbuncles are not as frequent as furuncles, but they are larger and approximately 4 inches in diameter with one or more openings that pour pus onto the skin (Hay and Morris-Jones, 2016).

2.6 .1.1.2 Carbuncles

The main cause of carbuncle is *S .aureus*, the infection leads to generalized body symptoms that include: a fever of 38° C or higher, showing signs of weakness and exhaustion (Hay and Morris-Jones, 2016). The infection may be disseminated in various regions of the body as well as to other individuals, especially the household members. Carbuncles are usually on the back, thighs and neck. This type of infection usually takes longer to develop; it is deeper and more serious than furuncle infection and with higher risk of scarring (Hay and Morris-Jones, 2016).

2.6.1.1.3 Treatment, complications, and prevention

There are several measures that are utilized to mitigate the symptoms of skin abscesses, they include allowing pore dilation and discharging of pus; medical treatment includes puncturing abscesses and draining the pus (Stulberg *et al*., 2002); an abscess can also be surgically removed. Antibiotics may only be used as recommended by a medical specialist.

Staphylococcus aureus may be disseminated to other areas of the body where it can cause secondary infection; cellulitis being one such example (it is a serious secondary infection). The best way of preventing boils and carbuncles includes maintaining high standard of hygiene; including washing the skin, cleansing wounds, cuts, and grazes promptly, by dressing any cut using a sterile bandage to prevent infection (Stulberg *et al*., 2002).

2.6.1.2 Septicemia (blood poisoning)

Sepsis also known as bacteremia, septicemia or blood poisoning, is a medical emergency that can result in a life-threatening condition. Septicemia results in damage to the body tissues, that are often far away from the initial site of infection (Singer *et al*., 2016). Prompt treatment and

mandatory one-to-one care enhances the patient's chances of survival. In the case of mild sepsis, many patients do recover, with a mortality rate of about 15%, while for patients with severe sepsis or septic shock; the mortality rate may rise to between 20 to 50% (Levy, 2004; Dellinger *et al*., 2004; Pastagia *et al*., 2012).

Some patients with *S .aureus infections* may develop sepsis as they wait for their clinical blood culture results or as they await admission to the hospital. Thus, hospitals and laboratories are advised to expedite diagnosis by using rapid DNA PCR testing for *S .aureus* (screening) identification; the rapid and accurate testing may prevent deaths and diminish the impact and destruction caused by the *S .aureus* sepsis (McAdow *et al*., 2011). In this case, it is recommended that medical personnel prescribe vancomycin and other intravenous (IV) antibiotics that are suitable in the treatment of *S .aureus* septicemia (Lalani *et al*., 2008; Wunderink *et al*., 2008). Most sepsis cases occur in patients in a nosocomial setting, especially those who are admitted in the intensive care unit (ICU). Risk factors associated with sepsis include: age, black race, immunodeficiency (i.e. HIV, cancer), hospitalization associated with use of invasive medical devices (i.e. breathing tubes, urinary catheter, and artificial joints), pneumonia, diabetes and serious injuries (Wisplinghoff *et al*., 2004; Mayr *et al*., 2014). There are many symptoms associated with sepsis such as high fever 39.4°C, high heartbeat of above 90 beats per minute, rapid breathing, edema (swelling), and altered mental state (Hay and Morris-Jones, 2016). Signs of severe sepsis are: organ dysfunction, difficulty in breathing, irregular heart function, sudden change in mental status, spotted skin and reduction in platelet count (Guclu *et al*., 2013). Severe sepsis may lead to septic shock as well as very low blood pressure. Another complication associated with sepsis is manifested as reduction of blood flow that may lead to blood clots in vital organs (heart, kidneys, brain) and in the extremities; this condition

may lead to various stages of tissue death and organ failure (Dellinger *et al*., 2004). Sepsis can be detected using blood and urine tests, and imaging scans such as; x-rays, ultrasound, CT, MRI or (Yanagihara *et al*., 2010; Tissari *et al*., 2010; Palestro *et al*., 2007). Sepsis may be treated using antibiotics via intravenous route, intravenous fluids, vasopressors or other immunemodulators and remedies to stablise the sugar levels (Wunderink *et al*., 2008).

2.6.1.3 *Staphylococcus aureus* and Pneumonia

2.6.1.3.1 Pneumonia

Staphylococcus aureus may cause pneumonia in any setting and many antibiotics are effective against infections caused by MSSA and MRSA, although only a few are approved to treat staphylococcal pneumonia.Vancomycin had been the preferred drug for the past 60 years despite its difficulty to attain the right serum levels and increased nephrotoxicity **(**Rubinstein and Keynan, 2014); some recent researches have indicated worse outcome even with higher minimum inhibitory concentrations (MICs) of vancomycin against some strains of MRSA, thus compelling scientists to search for other effective treatment options (Holmes and Howden, 2014).

There are other antibiotics available for the treatment of MRSA pneumonia including: linezolid, telavancin, ceftaroline, and ceftobiprole. Tedizolid, dalbavancin, and oritavancin are being assessed for the treatment of pneumonia and other infections, with the exception of skin and soft tissue infections (Peyrani and Ramirez, 2015). A key advantage of linezolid is that it can be given orally and does not need dosage adjustments in patients with renal failure or therapeutic drug monitoring.

2.6.1.3.1.1 Hospital-acquired pneumonia(HAP)

Staphylococcus aureus inhabit the airways asymptomatically, but at times, depending on the interaction of patient, environmental and bacterial factors (Defres *et al.,* 2009), the organisms may become pathogenic; bacterial virulence overcoming the host immune defense system, leading to severe pneumonia (Defres *et al.,* 2009). Healthy individuals who had no prior risk factors of colonization may also be susceptible to MRSA pneumonia (Defres *et al.,* 2009). Hospital-acquired pneumonia is customarily defined as that which developed 48 hours after admission, without intubation at the time of admission (Shebl and Gulick, 2023).

Vancomycin and Linezolid are recommended for the treatment of MRSA in the case of healthcare-associated pneumonia (HCAP), hospital-acquired pneumonia, and ventilator-associated pneumonia, initialed as VAP (American Thoracic Society, 2005). It is, however, important to take note of conflicting results in some recent articles comparing choices between Vancomycin and Linezolid therapy (Kalil *et al*., 2013; Caffrey *et al*., 2014; Jiang *et al*., 2013; Peyrani *et al*., 2014). It is also imperative, to continue conducting research in search of new antibiotics that can provide better therapeutic options for the treatment of pneumonia caused by MRSA (Tremblay *et al*., 2013; Scott, 2013; Arshad *et al*., 2014; Casapao *et al*., 2014; Awad *et al*., 2014; Barriere, 2014; Torres *et al*., 2014; Pasquale *et al*., 2015).

2.6.1.3.1.2 Clinical manifestation of Ventilator-associated pneumonia

Pneumonia that developed 48 hours after insertion of endotracheal intubation and/or mechanical ventilation, and was not observed before intubation, is classified as a Ventilator-associated pneumonia (Defres *et al.,* 2009; American Thoracic Society, 2005). There are two types of VAP among MRSA: early and late onset. Early-onset disease takes place within 4–5 days of

admission and it is caused by CA-MRSA, while late-onset disease is caused mainly by HA-MRSA (Defres *et al*., 2009).

2.6.1.3.1.3 Healthcare-associated pneumonia (HCAP)

Classification of HCAP was introduced recently to include: already-ill patients residing in nursing-home, patients in long-term facility care, patients attending hemodialysis clinic, or a recent receipt of intravenous antibiotic treatment (Kollef, 2009), chemotherapy or wound care within 30 days before the current infection (Kollef, 2009; Shorr *et al*., 2008).

2.6.1.3.1.4 Community-acquired pneumonia

Community-acquired pneumonia (CAP) is caused by CA-MRSA. These strains cause a very severe pneumonia, with a higher probability of mortality compared to HA-MRSA and they require empiric treatment as an integrated part of its treatment management (Minejima *et al*., 2014; Wunderink, 2013). Symptoms of this type of pneumonia occur as a result of CA-MRSA infection, commencing before or within 48 hours of admission to hospital (Defres *et al*., 2009), despite the patients having no prior healthcare interaction. Community-associated linked CAP typically appears in young, formerly healthy persons with swiftly progressive pneumonia or a person with severe respiratory disease as shown in Figure 2below (Masters*et al*., 2017).

Toxin produced by aggressive CA-MRSA strains course significant damage in normal lungs, this condition has a mortality rate of between 30 to 40 percent (Siddiqui and Koirala, 2022), despite prompt treatment by appropriate antibiotics (Rubenstein *et al*., 2008).

The role that CA-MRSA plays in Community acquired pneumonia, poorly understood (Masters *et al*., 2017), despite being suspected as the causative of CAP in UK and Europe (Nathwani *et al*., 2008); a similar strain having been initially reported in the United states of America. This,

therefore, implies that some of CA-MRSA strains found in Europe and America, had acquired capability to cause severe necrotizing pneumonia (Gillet *et al*., 2002; Bradley, 2005; Liu and Ji, 2020; Healy *et al*., 2004; Francis *et al*., 2005; Dufour *et al*., 2002; Kravitz *et al*., 2005).

Primary cause of community-acquired pneumonia (File *et al*.,2012), is due to direct inoculation of the bacterial pathogen into the lungs (Pahal *et al*.,2022), the other route is as a result of haematogenous dissemination to the lungs from other initial infective sources (Torres and Cillóniz, 2015),such as endocarditis or bone and joint infection (Defres *et al*., 2009).

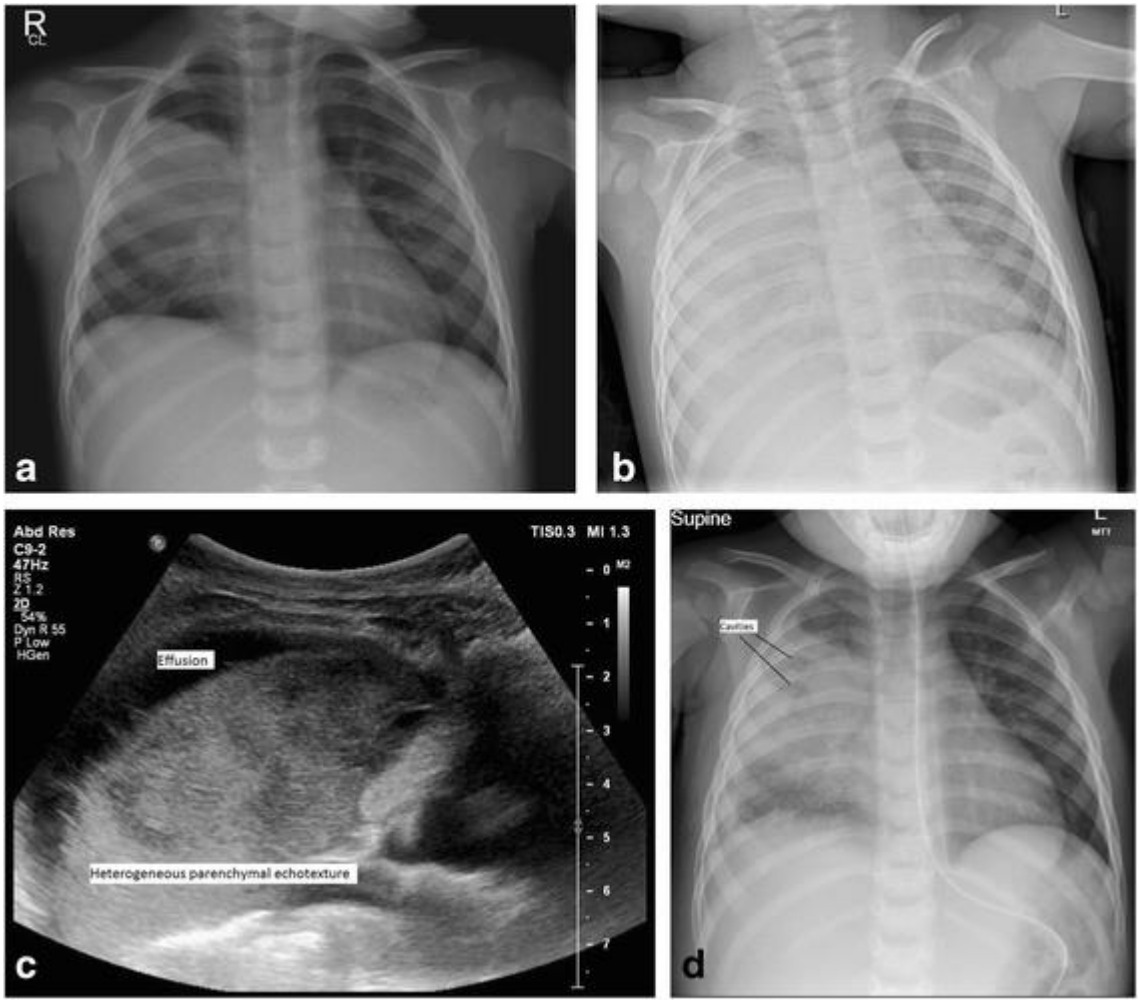

Figure 2 Necrotizing pneumonia on chest radiograph and ultrasonography (Defres *et al*., 2009)

a Initial plain chest radiograph showing right mid-zone airspace opacity, consistent with pneumonia (Masters*et al*., 2017).
b Radiograph of the chest (after 24h) demonstrating right-sided pleural effusion (Masters*et al*., 2017).
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c Ultrasonography image of the lung taken immediately afterwards demonstrating pleural effusion and heterogeneous parenchymal echo texture, consistent with necrotizing pneumonia (Masters*et al*., 2017).
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d Radiograph 4 days later of the chest following thoracoscopy and removal of chest drains showing right upper zone cavities within the region identified previously by ultrasonography
(Reproduced from Masters*et al*., 2017).

2.6.1.3.1.5 Diagnosis of Staphylococcal pneumonia

In the case of diagnosis of ventilator-associated pneumonia (VAP), broncho-alveolar lavage specimens are given preference to endotracheal cultures for the isolation of the causative agent, while diagnosis of healthcare-associated pneumonia (HAP) requires cheap, non-invasive and rapid sampling method, with minimal expertise in determining the microbiological diagnosis, for example non-bronchoscopy directed (blind) broncho-alveolar lavage (Masterton *et al*., 2008; Rubenstein *et al*., 2008).

In cases of secondary pneumonia, 90 % blood culture samples tend to be positive as compared with 20 % positivity rate from patients suffering from primary pneumonia (Bradley, 2005); blood cultures taken from patients suspected to have VAP tend to be positive at 24–36% as compared to patients suspected of HAP 5–15% (Kollef, 2006; Fagon *et al*., 1988).

Since blood cultures often give negative results in cases of VAP, it is important to collect sufficient respiratory tract specimens, which should be collected before commencing antibiotic treatment (Defres *et al*., 2009; Han and Lazarus 2016; Leibovici *et al*., 1998). The best type of specimen for the diagnosis of VAP, are endotracheal sampling or pleural fluid as compared to sputum (Ioanas *et al*., 2001), the reason being that pathogen usually colonize the respiratory secretions of healthy human beings (Defres *et al*., 2009).

2.6.1.3.1.6 Virulence factors associated with community-acquired pneumonia

Staphylococcus aureus has a variety of virulence factors which include the cell-surface-associated adherence (Foster and Höök 1998), and secreted (exotoxins) factors that contribute to pathogenesis such as attachment to surfaces/tissues(Tam and Torres, 2019), evade or attack the immune system and cause toxic effects to the animal or human host (Pietrocola *et al*., 2017).

One of the virulence factors, protein A, facilitates the attachment and colonization of host tissues, while others, leukocidin, kinases and hyaluronidase, facilitate the organism's ability to penetrate tissues. Haemolysins, leukotoxin and leucocidin are toxins that cause damage and lysis of eukaryotic cell membranes, while TSST-1 causes toxic shock syndrome (Dunyach-Remy *et al*., 2016; Nhan *et al*., 2011).

Exfoliation toxin operates as "molecular scissors" advancing bacterial skin invasion, while PVL induces lysis of leukocytes leading to permeability of the target cells followed by the lysis of phagocytes (monocytes-macrophages and neutrophils), keeping in mind that ther are two competing opinion on PVL association with necrotizing pneumonia as ealier mentioned; these virulence factors aid *S .aureus* to evade the immune system (Dumont *et al*., 2011; Ventura *et al*., 2010).

Toxicity studies, in different mouse models, have also implicated protein A and α-hemolysin in pathogenesis of pneumonia in their lungs (Defres *et al*., 2009).

The importance of PVL was further demonstrated by Labandeira-Rey *et al.* (2007), who found that it was PVL positive strains of MRSA and not the PVL negative ones that caused necrotizing pneumonia in experimental mice. The exact function of PVL in pulmonary infections is, however, not well understood, because of contrary results obtained by other scientists, for

example, Wardenburg *et al* (2007), using a different experimental pneumonia mouse model, observed that MRSA -PVL positive *agr* and *spa* mutants did not cause fatal lung infections; this experiment suggested that α -haemolysin and protein A were the only factors which might have been involved in parenchymal damage. In support of the latter observation, DeLeo and Otto (2008) managed to protect mice against lethal pneumonia through immunization against α-haemolysin. Thus, further research based on α- haemolysin may lead to development of a vaccine against this type of pneumonia in the future (DeLeo and Otto, 2008).

2.6.1.3.1.7 The antibiotics for treatment of Staphylococcal pneumonia

Antibiotic susceptibility testing of oxacillin and cefoxitin is used to determine whether the *S .aureus* is an MRSA or MSSA strain; in addition, various types of antibiotics are usually tested to find out whether they are clinically effective against *S .aureus* or not .

Initially methicillin resistant strains were confined to the hospital environment; these strains were resistant to all β lactams (Garoy *et al*., 2019), with some exhibiting multi drug resistant traits to a range of antibiotics (Aura *et al*., 2014; Hiramatsu *et al*., 2002; Bai *et al*., 2021).

As opposed to HA-MRSA, CA-MRSA strains are resistant to b-lactams and usually susceptible to other classes of antibiotics (Pantosti, and Venditti, 2009; Bukharie, 2010), they also differ in their epidemiology, genetic characteristics, and clinical presentation (Bukharie, 2010).

It has been observed, that after a period of time CA-MRSA strains may acquire resistance genes, and this has lead to difficulty in differentiating HA-MRSA strains from CA-MRSA strains using conventional *in vitro* antibiotic susceptibility testing (Otto, 2012).

Therefore it is important to conduct molecular-based rapid tests parallel to phenotypic /routine antibiotic susceptibility, such as PCR to detect *mec*A and SCC*mec* type IV genes, respectively (Kondo *et al*., 2007; McClure *et al*., 2006).

2.6.1.3.1.8 Radiological investigations

There are no specific radiological characteristics for staphylococcal pneumonia, in the initial stages of *S .aureus* CAP disease progression; there are minimal infiltrates, although there could be rapid progress within hours.

Chest radiograph can be used differentiate CA-MRSA pneumonia from HA-MRSA pneumonia, in CA-MRSA pneumonia show bilateral consolidation or bilateral infiltrates, associated with

rapid progression and clinical deterioration particularly in PVL-producing CA-MRSA (Morikawa *et al*., 2012), whereas, in the case of HA-MRSA pneumonia, CXR infiltrates are cavitate (Monaco *et al*., 2005).

Further confirmation is usually performed by computed tomography scan (McClure *et al*., 2006; Tronci *et al*., 2007). Other commonly observed findings include pleural effusions, pneumatoceles and pneumothoraxes.

Ventilator-associated pneumonia has no radiological characteristic that differentiates it from any other causative pathogens, with 26% of cases of VAP being missed on CXR (Rubenstein *et al*., 2008). When VAP patients show a more severe disease and poor response to antibiotic treatment, MRSA should be suspected as the most likely causative agent; also when radiological progression appears to be faster than other pathogens (Vidaur *et al*., 2008).

2.6.1.4 Osteomyelitis

Osteomyelitis is usually classified as acute or chronic depending on histopathology rather than, infection duration. Acute osteomyelitis is linked with bone inflammation, caused by bacterial pathogens, with classical symptoms appearing within two weeks of infection. Necrotic bone is usually seen in chronic osteomyelitis, onset of its symptoms appearing after six weeks following the commencement of the infection (Mylona *et al*., 2009). Bacterial pathogens causing osteomyelitis differ depending on the patient's age (Momodu and Savaliya, 2022); it has been observed that *Staph. aureus* is the main etiological agent causing acute and chronic bloodborne osteomyelitis in adults and children (Momodu and Savaliya, 2022); and the pathogen being considered the leading pathogen in bone and prosthetic joint infections (Momodu and Savaliya, 2022). Group A streptococci, *Streptococcus pneumoniae* and *Kingella kingae* are recognized as the other important etiological agents responsible for causing this type of infections; *Kingella*

kingae rarely causing infections in adults, but are pathogenic to immunocompromised patients; Group B streptococcal osteomyelitis is considered to primarily infect newborns (Kaplan, 2005). Methicillin-resistant *S .aureus* is frequently isolated in patients suffering from osteomyelitis and it accounts for a third of all staphylococcal isolates linked to bone infections (Aragón-Sánchez *et al*., 2009). While *Staphylococcus epidermidis*, *Serratia marcescens*, *Escherichia coli* and *Pseudomonas* spp are the main causative agents in contiguous infections, in majority of chronic cases; fungal and mycobacterial infections have been observed in some patients, mainly seen in immune compromised patients (Kohli and Hadley 2005; Ohl and Forster, 2015).

2.6.1.4.1 Clinical features of Osteomyelitis

Acute hematogenous osteomyelitis is an outcome of bacteremic inoculation of bone. Children are predisposed to acute hematogenous osteomyelitis in the metaphyseal regions of the long bones, which are vastly vascular and prone to minor trauma. This type of osteomyelitis accounts for slightly above 50% of acute hematogenous osteomyelitis seen in children less than five years of age (Gutierrez, 2005).

Clinical symptoms of osteomyelitis are nonspecific and difficult to diagnose and they present as chronic pain, malaise, poor wound healing, and persistent sinus tract or wound drainage, and fever (Gutierrez 2005; Hatzenbuehler and Pulling, 2011)

2.6.1.4.2 Diagnosis of Osteomyelitis

Clinical diagnosis of acute osteomyelitis in children is primarily based on the rapid commencement and localization of symptoms such as fever, lethargy, and irritability. On the other hand physical examination usually depends on identifying outcomes, such as erythema, soft tissue swelling or joint effusion, decreased joint range of motion, and bony tenderness.

Slightly over 50% of resultant cultures are positive, making it difficult to identify the etiological agent responsible for the bone infection (Chen *et al*., 2010). In adults, diagnosis of osteomyelitis is difficult, as high index of clinical suspicion is needed in combination with clinical symptoms, laboratory results and imaging techniques (American Society of Plastic Surgeons, 2011).

Bacterial cultures are crucial in diagnosis and treatment of osteomyelitis; these include a positive culture from bone biopsy and necrosis related histopathology (American Society of Plastic Surgeons 2011; Lipsky *et al*., 2006).Treatment of osteomyelitis relies on suitable antibiotic determined by culture and antibiotic susceptibility results and in most cases surgical removal of infected and necrotic tissue (Roblot *et al*., 2007; Karamanis *et al*., 2008). Empiric treatment of acute osteomyelitis, especially in children, must include antibiotics targeting *S .aureus* , of which β-lactam are considered as first-line treatment except in cases where MRSA is a suspect. As a matter of caution, MRSA must be considered in primary antibiotic treatment (Bachur and Pagon, 2007). Vancomycin is the first choice for intravenous treatment, while fluoroquinolones are used as alternate treatment in patients with diabetic foot infections or penicillin allergies due to staphylococcal infections.

2.6.2 Pathology caused by *Staphylococcus aureus* in animals, respective diagnoses and treatments

2.6.2.1 *Staphylococcus aureus* infections in animals

Bumble foot is the predominant staphylococcal infection found in poultry (McNamee and Smyth, 2000; Wobeser and Kost 1992). It presents as a chronic inflammation as a result of bacterial infection through an abrasion or ulceration, leading to swelling of the plantar pads. The clinical signs associated with Bumble foot include pain, which hampers walking, leading to limited access to water and food. Dissemination of the infection to the internal tissues

(mesoderm, tendons and bones) leads to osteomyelitis, synovitis, and at times death (McNamee and Smyth, 2000). Wet litter or nutrient deficiency may worsen the incidence of bumble foot (Burger *et al.*, 1984; Martrenchar *et al*., 2002). Bumble foot causes major economic losses in the poultry production chain, mainly as a result of poor growth, field rejection and carcass rejection (Wobeser and Kost, 1992). Thermographic imaging is currently used to diagnose bumble foot; this method can be used to screen avian populations for early signs of bumble foot, leading to improved recovery percentages and bird well-being (Wilcox *et al*., 2009).

The principal cause of economic losses in dairy production is mastitis, which leads to economic losses as a result of poor milk yields, costs of veterinary treatments, discarding of spoiled milk and probably culling. Also, contaminated milk may contain pathogens and/or antibiotic residues (Peton and Le Loir, 2014). The main pathogen causing mastitis in milk producing countries is *Staphylococcus aureus* (Monistero *et al*., 2018; Haran *et al*., 2012), including those countries with strict mastitis control programs (Rajala-Schultz *et al*., 2021). Potential sources of *Staphylococcus aureus* contamination leading to mastitis include infected udders, teat canals, teat lesions, teat skin, muzzles, and nostrils. *Staphylococcus aureus* are spread to uninfected quarters due to poor hygiene via teat cup liners, milker's hands, washcloths, and flies (Capurro *et al.*, 2010). Prophylactic and control methods used to control contagious mastitis are: proper disinfection of teat after milking, separation of infected animals from healthy herds, and prompt detection and diagnosis of causative pathogens (Cobirka *et al.*, 2020); contagious mastitis causative agents , include *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative staphylococci (CNS), *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Streptococcus agalactiae* (Ali *et al*., 2021); this should be followed by appropriate antibiotic treatment administered via the teat canal (intra-mammary route) or in combination with parenteral therapy

(Peton and Le Loir, 2014; Carrillo-Casas and Miranda-Morales, 2012; Sommerhäuser *et al*., 2003).

To control mastitis on surfaces or objects, the following antiseptic or disinfectants are recommended, sodium hypochlorite (Hansen, 1971), alcohols (Neave *et al*., 1969), quaternary ammonium compounds (Boddie and Nickerson, 2002), iodophors (Pearson, 1975), phenolics (Chew *et al*., 1985), glutaraldehyde and formaldehyde (Sharun, *et al*., 2021) or a combination of iodine and alcohol and heat (Public Health Agency of Canada, 2001; Sehulster *et al*., 2004).

2.6.2 2 Treatments in Animals

Meat producers usually feed growth-promoting antibiotics to livestock (Landers *et al*., 2012) and to increase their body weight before slaughter (Graham *et al*., 2007; Graham *et al*., 2007). Antibiotics have been used to prevent development of infectious diseases caused by bacteria like: *S. aureus*, *E.coli*, *Salmonella* and enterococci (Barragry, 1994; Arello, 1998). This habit has, however, lead to emergence of antibiotic resistance in the animal bacterial strains (Baquero *et al*., 1996). It is, therefore, advisable to always carry out antibiotic susceptibility testing before administering antibiotic therapy (Arsla *et al*., 2017).

MRSA strains isolated from different species of animal, displayed a variety of antibiotic susceptibility patterns (Jayaweera *et al*., 2017), with a majority of LA-MRSA CC398 being resistant to Tetracycline, and Trimethoprim (Catry *et al* 2010; Van den Broek *et al* 2009).

A good example was a LA-MRSA associated mastitis cases in Germany, that was resistant to ten different antibiotics (Lienen *et al*., 2021), with 41% being resistant to beta-lactams and tetracycline (Fessler *et al*., 2010), similar diversity of antibiotic resistance patterns was witnessed

among LA-MRSA CC398 strains isolated swine collected from different parts of Germany (Kadlec *et al*., 2009).

Some MRSA strains do appear susceptible to Clindamycin *in-vitro*, despite such strains harboring a gene that renders them resistant to treatment (Prabhu *et al*., 2011). This phenomenum is known as Inducible Clindamycin resistance and is common among erythromycin-resistant isolates (Prabhu *et al*., 2011), this types of MRSA strains have been isolated in dogs and cats in Canada (Faires *et al*., 2009). Chlorhexidine, povidone iodine or glycerol are effective against localized MRSA infections resistant to Clindamycin (Catry *et al*., 2010; Block *et al*., 2000), it is important to monitor this types of infection to prevent localized progression or systemic spread (Catry *et al*., 2010).

2.7. Infection of humans by LA-MRSA

2.7.1 Transmission of LA-MRSA to Humans

Though *S .aureus* is *a* benign colonizer, it can also be an opportunistic pathogen in immune - compromised individuals and patients with advanced cancer (Powell *et al*., 2016; Yamashita *et al*., 2013). Apart from its extraordinary ability of colonizing body surfaces and dodging the immune system, the organism can contaminate animal food products in the course of handling, preparation and processing.

It is able to grow at both low and high temperatures (7◦ to 48.5◦C; most favorable being 30◦ to 37◦C) and can survive in pH ranges of 4.2 to 9.3, with 7 to 7.5 pH is regarded to be the most favorable; it can also grow comfortably in 15% NaCl (Omololu, 2017). In addition, *S .aureus* can survive dry and stressful environments e.g. human nares and skin, inanimate surfaces, which include hospital equipment, bedrails, stethoscopes, medical charts, and ultrasound machine (Omololu, 2017). Contaminated inanimate surfaces, can contaminate hands of health care workers by direct contact, contaminated hand or hands of the health care or come into direct contact with the patient, raising likelihood of transmission of pathogen to patient or inanimate surfaces and equipment in the patient's zone [covering patient and his/her surrounding(s)], clothing and surfaces that will come into direct contact with the patient (Chaibenjawong and Foster, 2011; Russotto*et al*., 2015). Finally this previously nosocomial *S .aureus* infection may enter into the general community including those working in butcheries (Yakubu *et al*., 2013, Thom *et al*., 2013).

In abattoirs and butcheries, contamination depends on a number of factors, such as knowledge of food safety issues, personal hygiene of abattoir workers, hygienic handling of carcasses and

meat, equipment and maintaining hygienic conditions of the meat processing plants and butcheries (Yakubu *et al*., 2013).

Physical contact is the one of the mode for the transmission *S .aureus* strains between hosts (Knox *et al*., 2015); pig barns with highly contaminated dust containing MRSA isolates droplets from MRSA colonized pigs, presents an environment with increased risk of MRSA colonization of individuals working in these farms through inhalation of MRSA-contaminated dust (Verstappen *et al*., 2014).

Previous work have indicated a nasal colonization rate ranging between 77%–86% in individuals working in MRSA- positive stables (Cuny *et al*., 2009; Van den Broek *et al*., 2009); this colonization appears to be caused by exposure and intensive contact with carrier animals (Graveland *et al*., 2011); families living in same locality, but not exposed to the animals, had a low colonization rate of between 4%–5% (Cuny *et al*., 2009). It was also found that a significant percentage of the farmers have persistent MRSA colonization even as stable exposure is interrupted (van Cleef *et al*., 2011; Köck *et al*., 2012). Farmer's contact with pigs in Belgium, Denmark, and Netherlands has also been identified as a risk factor for acquisition of MRSA to respective farmer's household members (Cuny *et al*., 2015). In fact, an enhanced MRSA carriage rate was witnessed among family members from Belgium, as a result of close contact between the pig farmers and pigs (Garcia-Graells *et al*., 2013).

In the Far East, Taiwan, LA-MRSA ST9 nasal carriage in pigs was reported to be higher in large farms compared to smaller farms (Fang *et al*., 2014).

In Germany and Belgium, slaughterhouse workers have been found to be colonized with LA-MRSA; this is evidence that occupation in slaughterhouse is considered to be a major risk factor.

Whole-genome analysis of LA-MRSA isolates obtained from slaughterhouse workers and their families, showed high colonization rate of LA-MRSA in the workers and respective household members, meaning that there was possible transmission of the organisms between slaughterhouse workers and their families (Van Cleef *et al*., 2010; Mulders *et al*., 2010; Cuny *et al*., 2009; Hermes *et al*., 2012; Garcia-Graells *et al*., 2012; Verkade *et al*., 2014; Bosch *et al*., 2015).

2.7.2 LA-MRSA Infections in Humans

Livestock associated MRSA CC398 enjoys the same virulence status as *S .aureus* from humans, and might lead to hospital setting linked skin and soft tissue infections, which may at times require surgical interventions. Among patients working in close contact with livestock, approximately 13% of LA-MRSA is associated with severe skin and soft tissue infections (Layer *et al*., 2012).

Livestock associated MRSA can be introduced in hospital settings via patients infected by LA-MRSA. To control this type of infection in the nosocomial settings, the affected patients must be treated using appropriate antibiotics; it must also include the decolonization of their nares, since LA-MRSA nasal colonization may lead to nosocomial infections of surgical sites, ventilator associated pneumonia or septicemia (Cuny *et al*., 2015).

Human-to-human transmission of LA-MRSA CC398 strains in hospital settings has been reported. When these strains are typed by *spa*-typing or MLST, they present themselves as small clusters of infection (Wulf *et al*., 2008). Although LA-MRSA CC398 is rare in comparison to HA-MRSA, there is sufficient alternative antibiotic treatment of LA-MRSA CC398 infection; going by the current antibiotic resistance profiling of LA-MRSA (Wassenberg *et al*., 2011).

There are also reports showing LA-MRSA resistance to β-lactams, Macrolides, Lincosamides, Streptogramins, and Tetracylines and in part to Fluoroquinolones, as well as to Sulfamethoxazole -Trimethoprim, while they tend to be susceptible to Glycopeptides, Daptomycin, Tigecycline, Rifampicin, Fusidic acid, Fosfomycin and Linezolid (Cuny *et al*., 2015).

2.8Antibiotic resistance

Antibiotic discovery has been a key development in medicine. In the past 6 decades, their usage in animal husbandry and veterinary medicine has led to better growth, while making livestock rearing to be productive as well as safeguarding the well-being of both livestock and humans. Their usage has been employed vastly in livestock rearing, in prevention, control, and treatment of infections in livestock (Angulo *et al*., 2004; Silbergeld *et al*., 2008). Overuse or abuse of antibiotics is responsible for the emergence of antibiotic resistant bacteria; the foremost public health problem in many parts of the world (Davies and Davies, 2010).

Antibacterial resistance is caused mainly by selection pressure on susceptible bacteria as a result of use of antibiotics; by selecting the wild resistant strains. A number of social and administrative factors play a role in the emergence and spread of resistance; including over-prescription of antibiotic(s) by physicians, patient self-medication and noncompliance with antibiotic treatment regimens (Knobler *et al.*, 2003). Hospital settings (including various referral and teaching hospitals) have been identified as the main fertile grounds for the emergence and proliferation of resistant pathogenic and non-pathogenic bacteria due to high numbers of outpatients and inpatients on antibiotic therapy (Knobler *et al*., 2003).

Majority of antibiotic-resistant bacteria were initially found in hospitals; these bacteria have now found their way to the general community (Levy, 2002). A good example is the CA-MRSA, that have caused sepsis, impetigo, erysipelas, cellulitis, and folliculitis in children, athletes and military recruits (Napierkowski, 2013; Boyce *et al*., 2005; Campbell *et al*., 2004; Maltezou and Giamarellou, 2006; Robinson *et al*., 2005).

It has been acknowledged that the HIV pandemic is responsible for the spread of antibiotic resistance from the hospital to the community especially among *S .aureus*. This was well captured by a study conducted in a public hospital in Cape Town between January 2002 and June 2006 (Jaspan *et al*., 2008), where they found that, majority of *S .aureus* isolates were methicillin resistant, with some being acquired from the community. Extensive use of prophylaxis has worsened the situation, giving rise to sulphamethoxazole-resistant *S .aureus* among HIV-infected patients in Soweto (Madhi *et al*., 2000). In addition, 8%–60% of MRSA isolated from this hospital seemed to have originated from the community (Fridkin *et al*., 1996).

There are several different pathways, in which these resistant strains can invade a human host and cause serious infection, they include, ingestion of infected meat products. This is an occupational risk to abattoir worker while handling contaminated meat, such contact may be disseminated into the larger community. The contaminated meat find its way into a nosocomial setting , further disseminate these resistant strains in a number of ways, either through direct contact or via the food chain, affecting health of livestock and their handlers(Landers *et al*., 2012; EFSA, 2017; Mkize *et al*., 2017).

Factors leading to emergence of antibiotic resistant strains within the community are inappropriate use of antibiotics (Larson, 2007), ineffective infection control and hygiene practices and homeless immunocompromised individuals, institutional settings such as military camps, prisons, sports teams, and day care centers (Kazakova *et al*., 2005; Mathews *et al*., 2005; Nguyen *et al*., 2005).

Environmental or policy factors also contribute to the increase in antibiotic resistant bacteria, which include: poorly manufactured antibiotics, counterfeit antibiotics, use of expired antibiotics and use of antibiotics in agriculture, all of which contribute to presence of antibiotic

resistant bacteria in communities, particularly in the high agricultural and livestock rearing regions (Landers *et al.*, 2012). Increased usage of antibiotics in livestock rearing regions as growth promoters, prevention of bacterial infection(s) and disease treatment, have increased antibiotic intake and have been cited as drivers for the emergence of antibiotic resistance in livestock rearing settings and meat product processing institutions (Landers *et al*., 2012). These antibiotic-resistant strains are likely to be selected, leading to their dissemination into the environment via livestock waste. Antibiotics and antibiotic resistance genes appear to have independent roles to antibiosis in the environment; antibiotic concentration levels secreted by environmental bacteria are known to be extremely below the minimum inhibitory concentrations i.e. approximately 200 times below MIC values (Larsson and Flach, 2022). It is assumed that these sub-inhibitory concentrations still help in selection for antibiotic-resistant bacteria (Andersson and Hughes, 2012). This reservoir of environmental resistant bacteria can easily exchange resistance genes, through transposons or integron vectors, with potential pathogenic strains (Barlow, 2009), which may end up being transmitted directly or indirectly to humans via food consumption (Ventola, 2015); various antibiotic resistance genes are integrated into plasmids. Pathogenic resistant bacteria such as *S .aureus* may, therefore, lead to significant health problems, which cannot be easily treated. Horizontal gene transfer is mediated via three main mechanisms: transformation, transduction or conjugation (Barlow, 2009). Once pathogenic bacteria acquire the antibiotic resistance genes, they become difficult to treat resulting in higher morbidity and mortality rates (Knobler *et al*., 2003).

2.9 Human immunodeficiency virus/acquired immune deficiency syndrome and MRSA

2.9.1Overview of Human Immunodeficiency Virus/ Acquired Immune Deficiency (HIV/AIDS) Syndrome and MRSA

Dr. Robert C. Gallo and his co-workers identified the first human retroviruses, HTLV-1 and HTLV-2 in 1984 (Gallo *et al*., 1984). He demonstrated that retrovirus HTLV-1, also known as human immunodeficiency virus (HIV)-1, was the cause of Acquired Immune Deficiency Syndrome (AIDS) (Lasker, 2014). AIDS is a chronic, life-threatening condition caused by the HIV virus; this virus damages the immune system and interferes with the patient's ability to fight organisms that may cause disease (Alimonti *et al*., 2003). *Staphylococcus aureus* infections cause a significant morbidity in persons infected by the HIV virus (Jacobson *et al*., 1988; Witt *et al*., 1987; Onorato *et al*., 1999). *Staphylococcus aureus* colonize the nares of HIV-infected patients more frequently than it does the general population (Hidron *et al*., 2010), leading researchers to hypothesize that this higher colonization might translate into a higher incidence of infections (Sakr *et al*., 2018; Raviglione *et al*., 1990; Weinke *et al*., 1992). The higher colonization of MRSA in HIV-infected persons is as a result of immunosuppression (Hidron *et al*., 2010; Utay *et al*., 2016), recent exposure to antibiotics (Hsu *et al*., 2020), illicit drug use (Szumowski, *et al*., 2009), recent hospitalizations (Kyaw *et al*., 2012), prior MRSA colonization, infection, or chronic skin disease (Shet *et al*., 2009; Szumowski *et al*., 2009).

Trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis seems to protect against MRSA colonization (Cenizal *et al*., 2008), although clonal outbreaks of MRSA resistant to TMP-SMX have been described (Rodríguez-Noriega and Seas 2010). Human immunodeficiency virus experts have also noted an increase in the number of HIV patients with community-acquired

MRSA infections (Ahuja *et al*., 2009; Miller *et al*., 2003), some of them having been exposed to HA-MRSA in hospitals due to frequent admissions, which have direct relation to high rate of antibiotic therapy (Gorwitz *et al*., 2006).

2.9.2Risk factors associated with *Staphylococcus aureus* infection in humans with HIV/AIDS

Risk factors associated with MRSA colonization include: frequent hospitalization or antibiotic use, previous MRSA infections, low CD4 count, and other behavioral risk factors (Kyaw *et al*., 2012; Tumbarello *et al*., 2002; Villacian *et al*., 2004). HIV is known to be an independent risk factor for colonization with *S .aureus* and infections can result in substantial morbidity and mortality in HIV- positive patients (Kyaw *et al*., 2012; Dworkin*et al*., 2001; Hidron *et al*., 2005; Senthilkumar *et al*., 2001).

2.10Antibiotic target and mode of Resistance

2.10.1 Sulfamethoxazole and trimethoprim (SXT TMP)

Sulfonamides were discovered in 1932, followed by its approval for use as the first antibacterial agents in 1935 (Jeśman *et al*., 1995). Sulfonamides have been used to treat *S .aureus* infections in humans (Sköld, 2000), a combination of TMP and SXT has synergistic effect, resulting in the inhibition of bacterial biosynthesis of tetrahydrofolic acid (Vilchèze and Jacobs, 2012). This Synergistic antimicrobial effect have been used to treat *Staphylococcus aureus* infection especially CA-MRSA infections (Zinner and Mayer, 2015; Paul *et al*., 2015). Sulfamethoxazole/ trimethoprim can also be combined with cephalexin to treat community skin and soft tissue infections caused by *Staphylococcus aureus* and *Streptococcus* species (Scholar and Pratt, 2000; Schneider *et al*., 2003).

For a successful TMP / SXT synergistic bactericidal effective against the pathogen, the concentration ratio of TMP to SXT ranging from of 1:5 and 1:40 (Thiebault, 2020); the minimum inhibitory concentration value of TMP / SXT must correspond to those that kill the pathogens (Patel and Welling, 1980).

Trimethoprim-sulfamethoxazole (TMP-SXT) is a very effective antimicrobial agent for the treatment of *Pneumocystis jiroveci* infections (Butler-Laporte *et al*., 2020), which determine its use in the prophylactic management of patients at high risk of developing such infections including HIV/AIDS patients (Wormser *et al*., 1982)

2.10.1.1 Trimethoprim-sulfamethoxazole combination's mode of action

There are two ways that inform the Trimethoprim-sulfamethoxazole mode of action, the first one involves sulfamethoxazole that inhibits dihydropteroate synthetase (DHPS), DHPS role is to

catalyze the formation of dihydrofolate from para-aminobenzoic acid (pathway (Eliopoulos and Huovinen, 2001)), the second mode involves Trimethoprim, which inhibits dihydrofolate reductase (DHFR), DHFR catalyzes the formation of tetrahydrofolate from dihydrofolate in the subsequent step of the folate biosynthesis pathway (Eliopoulos and Huovinen, 2001). Due to the slight difference in the antibacterial mode of action of SXT and TMP, this agents when used in combination, increases the efficacy of the drug combination, due to synergistic effect, as opposed with when they are used separately(Eliopoulos and Huovinen, 2001).

2.10.1.2 Trimethoprim-sulfamethoxazole single agent resistance mechanisms

SXT and TMP resistance is mediated via five different principal resistance mechanisms: permeability barrier and/or efflux pumps that act against both TMP to SXT (Navarro-Martínez *et al*., 2005); presence of naturally insensitive DHFR enzymes which also exist in *Bacteroides* species, *Clostridium* species, *Neisseria* species, and *Moraxella catarrhalis* (Huovinen, 1987); regulation changes in the target enzymes; mutational or recombinational changes in the target enzymes; and acquired resistance by antibiotic -resistant target enzymes.

2.10.1.2.1 ***Trimethoprim resistance mechanisms***

There are two types of TMP resistance mechanisms in *S .aureus*; the first one is point mutations in the chromosomal *dfrA* gene (Vickers *et al*., 2009). Point mutations confer low- to moderate-level resistance and it is the predominant TMP resistance mechanism found in *S .aureus* clinical isolates (Dale *et al*., 1997); these resistant isolates are usually selected even in the presence of SXT (Dale *et al*., 1997). Genetic factors leading to TMP chromosomal resistance in *Staph. aureus*, is not well understood (Vickers *et al*., 2009). The horizontally acquisition of plasmid-borne resistance genes , by *S. aureus*, is fully understood, and it is mediated by genes encoding

resistance variants of DHFR types S1, S2 and S3 enzymes (Adrian and Klugman, 1997; Dale *et al*., 1997; Sekiguchi *et al*., 2005).

Mutation in promoter gene has been cited as the cause of chromosomal DHFR resistance, leading to permeability mediated barrier resistance towards both sulfonamides and TMP, as observed in *E. coli*. Among *S .aureus* and *Streptococcus pneumoniae,* resistance to TMP is due to a single amino acid substitution in the *dhfr* gene; this substitution is linked to the alteration of chromosomally encoded DHFR (Dale *et al*., 1997; Pikis *et al*., 1998). Among TMP-resistant *Hemophilus influenzae,* mutations have been found both at promoter and coding regions of the *dhfr* genes (de Groot *et al*., 1996). Trimethoprim permeability mediated barrier resistance may also be acquired as in the case of *Klebsiella pneumoniae* and *Serratia marcescens*, with high-level TMP transferable resistance being observed in staphylococci (Huovinen, 1987**).**

2.10.1.2.2 ***Sulfonamides resistance mechanism***

In sulfonamide-resistant *Streptococcus pneumoniae*, resistance occurs due to 2 amino acid duplications in the *fol*P gene (a *dhps* gene) leading to the alteration of the tertiary structure of the enzyme (Padayachee and Klugman, 1999). However, there is high possibility that alterations in the *dhps* gene is as a result of transformational recombination rather than by a series of mutations (Shrestha and Sharma, 2013); Swedberg *et al*., 1998), these mutations are also prevalent in nature among many clinically important bacteria such as *S .aureus* (Eliopoulos and Huovinen, 2001). Transferable resistance to sulfonamides in *E. coli* and *Shigella* species was detected in the late 1950s in Japan; this type of resistance is mediated by 2 drug-resistant DHPS enzymes encoded by *sulI* or *sulII* genes (Akiba *et al*., 1960; Sköld, 2000).

2.10.2 Penicillin and Methicillin

The introduction of penicillin for bacterial infection treatment in1942, marked the new era of antibiotic treatment. In early years after its discovery, Penicillin prove be effective against many bacterial infections (Rammelkamp and Maxon, 1942; Gaynes, 2017; Samanta and Bandyopadhyay 2020). However, in the same year, penicillin-resistant staphylococci (PRS) were observed in hospitals (Samanta and Bandyopadhyay 2020; Rammelkamp and Maxon, 1942).

Penicillin resistant strains (PRS) first emerged in hospitals and then spread into the community (Rammelkamp and Maxon, 1942); in fact, eighty percentage of hospital-acquired and community staphylococcal isolates were resistant to penicillin by the late 1960. This trend has recurred with each new wave of antibiotic resistance (Chambers, 2001).

In 1944, William M. M. Kirby first demonstrated that penicillin was inactivated by penicillin-resistant strains of *S .aureus* (Kirby, 1944); a year later, Bondi and Dietz discovered the specific role penicillinase plays in deactivating penicillin (Bondi and Dietz, 1945). Subsequently *S .aureus* penicillinase-producing strains spread fast in hospitals and later into the community (Plorde and Sherris 1974). Introduced in 1959, Methicillin was found to be very effective in treating patients with penicillinase mediated bacterial infections; however, the first cases of MRSA strains were detected in 1961.

2.10.2.1 Penicillin Mode of action

Like other bacteria, *S .aureus* has a cell wall as the outer layer (Francius *et al.*, 2008). The major component of the cell wall is peptidoglycan; this component is made-up of a net-like structure that is responsible for the rigidity and support of the cell wall. Penicillin and others in the beta-lactam antibiotic group are made-up of beta-lactam ring (Romaniuk *et al.*, 2015).

Penicillin's mode of action involves stoppage of the beta-lactam ring binding to DD-transpeptidase; this leads to inhibition of its cross-linking function and prevention of formation of a new cell wall. In the absence of a peptidoglycan cell wall, *S .aureus* cells are vulnerable to water and molecular pressures; this leads to death of the bacterium (Romaniuk *et al.*, 2015).

2.10.2.2 Penicillin and methicillin resistance mechanisms

Penicillinase mediated resistance is due to production of the penicillinase enzyme by gene located in a plasmid (Bennett, 2020), in other cases, methicillin resistance is due to alteration of the penicillin-binding protein 2a {PBP2a} (Fishovitz *et al*., 2014; Farzana and Hameed, 2006).

2.10.2.3 Methicillin resistance mechanisms

Methicillin resistance is due to the expression of methicillin-hydrolyzing β-lactamase 5 and through the alteration of Penicillin-binding proteins 2 (PBP), which significantly reduces PBP binding affinity for beta-lactam antibiotics, leading to high rates of unbinding antibiotic compound (Moya *et al*., 2009) *mecA* gene, that is hosted in *S .aureus* encoded penicillin binding protein (PBP2a) resistance functions differently from Penicillin resistance based on the production of β-lactamase Fishovitz *et al*., 2014); it thus inhibits non-stable β-lactams such as those of penicillin and ampicillin (Hardy *et al*., 2004). Mechanism of methicillin resistance in *S .aureus* is mainly due to the expression of foreignpenicillin-binding proteins (PBP) and PBP2a (not penicillin-binding proteins 2 {PBP2}); PBP2a is resistant to the action of methicillin that usually takes over the transpeptidation (cross-linking) reactions of the host PBPs. Production of PBP2a is regulated and is usually maintained at low levels, however the level of synthesis may be increased as result of mutations in the regulatory genes. Penicillin-binding protein 2a is resistant to the action of methicillin; it protects *S .aureus* against the actions of methicillin.

MRSA isolates are usually also resistant to the other classes of beta-lactams, leading to limited treatment options (Hardy *et al*., 2004).

Penicillin-binding protein 2a is encoded by the *mecA* gene, a gene hosted in mobile genetic element known as staphylococcal chromosome cassette *mec* (*SCC mec*); *mecA* gene expression enables *S .aureus* to grow and multiply in the presence of methicillin and other β-lactam antibiotics. *Staphylococcus aureus* strains which carry *mecA* gene are referred to as MRSA; some other *Staphylococcus* spp and *Streptococcus pneumoniae* strains are also resistant to penicillin-like antibiotics (Magilne *et al*., 2008).

Staphylococcal chromosome cassette (SCC *mec*) is hosted in mobile genetic elements, which are integrated into the *S .aureus* chromosome. The ease of transfer of this genetic element explains the rising prevalence of resistance to β-lactam antibiotics such as penicillin, its chemical derivatives and cephalosporin drug (Huletsky *et al*., 2004).

Staphylococcal cassette chromosome is the principal determinant for broad-spectrum beta-lactam resistance and is made of two key parts: cassette chromosome recombinase gene complex (*ccr*) comprising *ccr* genes {*ccrAB or ccrC*} (Lakhundi and Zhang, 2018), and its surrounding open reading frames (ORFs). The other is the *mec* gene complex, which is composed of the *mecA* gene, regulatory genes, and insertion sequences upstream or downstream of *mecA* (Hiramatsu, 2004; Ito *et al*., 2003).

2.10.3 Quinolones

Nalidixic acid is a naphthyridine and it was the first member of the quinolone class of antibiotic to be isolated by George Lesher and team in 1962; this was a byproduct of chloroquine synthesis (Lesher *et al*., 1962). This antibiotic was introduced for use in the 1960s for the treatment of

uncomplicated enteric bacterial urinary tract infections. In the 1970s, a number of first-generation quinolones, such as oxolinic acid were approved for use (Emmerson and Jones 2003; Mitscher 2005; Andriole, 2005).

Prior to the early 1980s, the quinolones were underutilized; it was after early 1980s that second generation of quinolones were developed (Emmerson and Jones 2003; Mitscher 2005; Andriole, 2005). The newer quinolones included: norfloxacin, ciprofloxacin, and ofloxacin; this generation of quinolones exhibit a significant improvement of activity against gyrase; they have a better penetration against *S .aureus* and improved pharmacokinetics / pharmacodynamics (Emmerson and Jones, 2003; Mitscher, 2005; Andriole, 2005).

2.10.3.1 Quinolone mode of action

Quinolones' mode of action involves converting their targets, gyrase and topoisomerase IV, to toxic enzymes that break up the bacterial chromosome leading to the inhibition of DNA synthesis (Hooper, 2001; Gould *et al*., 2007).

2.10.3.2 Quinolone mechanisms of resistance

Resistance to quinolone is caused by spontaneous point mutations in specific locations inside the bacterial chromosome, making it unaffected by the antibiotic. The chromosomal areas which are responsible to the respective resistance are referred-to as quinolone resistance-determining regions (QRDRs).

There are three mechanisms that mediate quinolone resistance. The first is the target-mediated resistance, which is due to specific mutations in gyrase and topoisomerase IV leading to diminished interactions between quinolones and these enzymes. The venerable mutated amino acids, in this case, are the serine and acidic residues that are anchored to the water–metal ion

bridge, causing disruption of the water–metal ion bridge, leading to quinolone resistance (Robicsek *et al.*, 2006; Strahilevitz *et al*., 2009; Wohlkonig *et al.*, 2010).

The second resistance mechanism is plasmid-mediated quinolone involves extra chromosomal genetic elements, such as *qnr* genes , acquired from aquatic bacteria chromosomal genes, belonging to genus *Aeromonas, Photobacterium, Shewanella,* and *Vibrio* , this genes are then being integrated into the plasmid and merged into *S. aureus* sul1-type integrons (Ye *et al*., 2020).

These extra chromosomal genetic elements encode a protein that leads to disruption of quinolone–enzyme interactions, which may cause alteration in the drug metabolism or upsurge of quinolone efflux. There are three classes of genes that are linked to this type of resistance. The first are *qnr* genes which encode proteins that contain approximately 200 amino acids, as part of the pentapeptide repeat protein family. About100 *Qnr* variants have been characterized; they are designated into five distinct subfamilies and have similarities to *McbG* and *MfpA,* which mimic the DNA (Robicsek *et al.*, 2006; Strahilevitz *et al*., 2009). The *Qnr* proteins cause quinolone resistance via two different mechanisms: the first mechanism causes decrease in attachment of gyrase and topoisomerase IV to DNA, protecting the bacterial cell, by lowering the number of available enzyme targets on the chromosome. These proteins also have ability of binding gyrase and topoisomerase IV, preventing quinolones from accessing the enzyme cleavage complexes (Tran and Jacoby, 2002; Xiong *et al*., 2011).

The second mechanism involves a plasmid-encoded protein known as *aac (6')-Ib-cr*. This protein is linked to quinolone resistance and is a variant of an aminoglycoside acetyltransferase. It has two specific mutation points: one at *W102R* and another at *D179Y*. The protein *aac (6')-Ib-cr*

acetylates the substituted nitrogen of the C7 piperazine ring in norfloxacin and ciprofloxacin, decreasing *W102R* and *D179Y* activities (Bush, 2020).

Plasmid-encoded quinolone resistance proteins constitute the third quinolone resistance (Aldred, *et al*., 2014), the mechanism involves efflux pumps, *OqxAB, QepA1* and *QepA2;* QepA1 and QepA2 are associated with efflux pumps (Aldred *et al*., 2014).

The third resistance mechanism is chromosome-mediated. It can be caused by the expression of porins or the over-expression of cellular efflux pumps, leading to decrease in cellular concentrations of quinolones (Mitscher 2005; Robicsek *et al*., 2006). In the case of Ciprofloxacin resistance in *S. aureus* initially begins by mutations in *gylA* gene,that confer low-level resistance to Ciprofloxacin, this is followed by mutationsin the *gyrA* gene, which is associated with a high level of ciprofloxacin resistance (Ferrero *et al*., 1995; Ng*et al*., 1996). In some other cases, a number of efflux pump systems cause Fluoroquinolones resistance in both MRSA and MSSA (Costa*et al*., 2011). In other cases topoisomerase IV is deemed to be the primary target, while, DNA gyrase have been identified as the secondary target (Jacoby, 2005).

Quinolone resistance emerged quickly among *S. aureus*, especially in those organisms which were also resistant to methicillin (MRSA); difference in rates of developing the quinolone resistance being different between MSSA and MRSA strains, although the cause of the difference has not been established. It is suspected that antibiotic selective pressure plays a major role in this difference, especially in hospital setting; the pressure leading to selection and spread of the more antibiotic-resistant MRSA strains (Lowy, 2003).

2.10.4 Vancomycin

Dr. E. C. Kornfield, an organic chemist at Eli Lilly in 1952, received a dirt sample and isolated *Streptomyces orientalis*, which produced a substance referred-to as "compound 05865"; it was effective against most Gram-negative organisms, including PRS. This compound was named vancomycin; it was approved for human therapy by US Food and Drug Administration in 1958 (Levine, 2006). However, due to its apparent toxicity, vancomycin was prescribed only to patients with serious β-lactam allergies or infections caused by organisms that were resistant to other agents (Levine 2006; Traber and Levine, 1981). Its increased usage came about in 1980s when it was found to have excellent effect in treating pseudomembranous enterocolitis in humans, which is caused by*Clostridium difficile*. This led to 100-fold increase in usage of the drug within the next 2 decades (Kirst *et al*, 1998). With its foreseeable action against MRSA, vancomycin has become the drug of choice (Mohr *et al.*, 2007), ushering a new era in the history of the antibiotic (Levine, 2006).

Vancomycin is the antibiotic of choice for selected clinical infections, which include: serious staphylococcal infections as a result of resistance to penicillin and cephalosporin, and streptococcal endocarditis in patients intolerant to penicillin G. Vancomycin is also used in combination with aminoglycoside for treatment of enterococcal and non-enterococcal infections caused by *Streptococcus bovis*, viridans streptococcal endocarditis, corynebacterial endocarditis and staphylococcal ileocolitis (Geraci, 1977).

2.10.4.1 Vancomycin mode of action

In synthesis of the bacterial cell wall, peptidoglycan layer, are made of peptides monomers of N-acetylmuramic acid (NAG) and N-acetylglucosamine (NAM), with the help of the enzyme

autolysin, gaps are created to form the insertion of additional building blocks (DeMeester *et al.*, 2018).

These monomers (NAG-NAM–peptide) are then joined to the growing end of the bacterial cell wall with transglycosidase. This process is repeated many times leading to the formation of a rigid, highly cross-linked cell wall structure (Watanakunakorn, 1984).

Vancomycin resistance is occur as a result of the antibiotic binding to monomers and prevents transpeptidase insertion of new monomers, interrupting the crosslinking of NAG-NAM–peptide in preceding peptidoglycan layer, weakening the cell wall fabric, leading to leaking out of bacterial cellular contents and finally death of the bacterium (McGuinness *et al.*, 2018; Lee *et al.*, 2018)

2.10.4.2 Vancomycin resistance mechanisms

Methicillin-resistant *Staphylococcus aureus* strains with reduced susceptibility to vancomycin were originally cited in Japan around 1997(Hiramatsu *et al*., 1997); methicillin-resistant *Staphylococcus aureus* strains with modest increase of MIC for vancomycin, ranging from 3 to 8 μg/ml are known a vancomycin-intermediate sensitive *S. aureus* {VISA}(Moses *et al*., 2020)*;* they do not harbor imported foreign genetic materials (Howden *et al*., 2010); instead, their increased vancomycin MIC values is linked to mutations which emerge in the invading pathogen in the course of vancomycin *in vivo* therapy (Howden *et al*., 2010). This type of resistance has been cited with increasing frequency all over the world (Gardete and Tomasz, 2014). Despite moderate increases in MIC value, outcome of vancomycin therapy against VISA isolates often ends in treatment failure (Liñares, 2001; Fridkin *et al*., 2003).

High-level vancomycin resistant *S. aureus* (MIC value greater than 100 μg/ml) was originally described in the United States of America in 2002, with the shocking revelation that it was no longer possible to treat vancomycin- resistant *S. aureus* (VRSA) using vancomycin (Gardete and Tomasz, 2014; Sievert *et al*., 2008).

High-level vancomycin resistant *S. aureus* is mediated by acquisition of plasmid-borne Tn1546 transposon, which was acquired from vancomycin-resistant *Enterococcus faecalis*. Transposon Tn1546 alters the cell wall structure and metabolism of *S. aureus*. The mode of action of vancomycin resistance is centered on the high affinity for the d-alanyl-d-alanine (D-ala-D-ala) residue, which is a part of the bacterial cell wall precursor Lipid II (Gardete and Tomasz, 2014).Transposon Tn1546 carries *vanA* operon which is responsible for type VanA high-level

glycopeptides resistance. Thus, transposon Tn1546 may either be carried on plasmids or integrated in the bacterial chromosome (Murray and Nannini, 2010).

The *vanA* and *vanB* operons have been extensively studied among vancomycin-resistance gene clusters (Chang *et al*., 2003) which have key genes, *vanHAX* and *vanHBBXB,* encoding for these essential proteins which mediate glycopeptide resistance. Another resistance mediating enzyme is dehydrogenase which is encoded by *VanH/VanHB* gene; this enzyme reduces pyruvate into D-Lactate, a ligase that is responsible in the synthesis of d-Ala–d-Lac, d-alanyl–d-lactate and a d,d-dipeptidase (VanX or VanXB) which hydrolyzes peptidyl-d-alanyl–d-alanine (d-Ala–d-Ala) terminus of peptidoglycan precursors located at the cell surface; this action effectively terminating cell wall synthesis (Arthur and Quintilian, 2001). This operon also encodes for a dipeptidase that hydrolyses D-alanine-D-alanine precursors *(VanX/VanXB*), significantly lowering affinity for the vancomycin (Arthur and Quintilian, 2001). There are six other recognized vancomycin-resistance genes (McKessar *et al*., 2000), including: *VanA, VanB, VanC,*

VanD, *VanE*, and *VanG* ; *VanA* (McKessar *et al*., 2000), resistance gene cluster is made up of seven genes, *vanS, vanR, vanH, vanA, vanX*, *vanY*, and *van Z* (Arthur and Courvalin 1993; Arthur *et al*., 1993; Handwerger and Skoble, 1995). The emergence of VISA and VRSA strains has necessitated development of alternative treatment against VISA and VRSA related infections. Alternative treatments to vancomycin are the new therapeutic options for invasive MRSA infections that include: Daptomycin, Linezolid, Tigecycline and Quinupristin/Dalfopristin (Micek, 2007).

2.10.5 Daptomycin

Daptomycin is a cyclic lipopeptide antibiotic produced by soil bacterium *Streptomyces roseosporus;* it is effective against majority of Gram-positive bacteria, especially against vancomycin-resistant enterococci and MRSA. Daptomycin was approved by US-FDA in 2003 and indicated for the treatment of complicated skin and skin structure infections (cSSSI) (Barry *et al*., 2001; Streit *et al*., 2004).

2.10.5.1 Daptomycin mode of action

Daptomycin mode of action involves disruption of membrane potential, with rapid bactericidal activity against *S. aureus*. This makes it an attractive antibiotic against serious Gram-positive infections (Steenbergen *et al*., 2005).

The detailed mode of action of DAP involves the insertion of the lipophilic DAP tail into the bacterial cell membrane, initiating a rapid membrane depolarization and a potassium ion efflux. This leads to the termination of DNA, RNA and protein synthesis, resulting in the pathogen's death (Wiedmann, 2011; Silverman *et al*., 2003; Silverman *et al*., 2003 B). These organisms

usually die in less than one hour when exposed to daptomycin; this is proof of its rapid bactericidal effect against Gram-positive pathogens (Cha and Rybak, 2004; Fuchs *et al*., 2002).

2.10.5.2 Daptomycin resistance mechanisms

Citations of *S. aureus* daptomycin resistance (DAP-R) are fairly recent and the mechanism of resistance appearing to be fairly diverse. The resistant strains frequently display a build-up of single nucleotide polymorphism in the multipeptide resistance factor gene (*mpr*F) and the *yycFG* components of the *yyc*FGHI operon. The function of *mpr*F gene in *S. aureus* is to transform phosphatidylglycerol with l-lysine to lysyl-phosphotidylglycerol, leading to reduced *S. aureus* affinity for cationic antimicrobial peptides (Ernst et *al*., 2009), bringing about spontaneous resistance of *S. aureus* to daptomycin (Friedman et *al*., 2006); whereas *yyc* operon responds to stressors caused by DAP. There are other molecules on DAP-R strains cell membrane that facilitate d DAP-R resistance via cell membrane depolarization (Silverma et *al*., 2003), permeability and reduced surface binding of DAP (Silverma et *al*., 2003). DAP-R strains resistance can also occur by the modification of the cell wall (Bertsche et *al*., 2013), by increased production of cell wall teichoic acids and d-alanylation (Bertsche et *al*., 2013); this is mediated by genes located in *dlt* operon (Mishra et *al*., 2014), leading to thickening the cell membrane (Bayer *et al*., 2013)

2.10.6 Oxazolidinones

Oxazolidinones (e.g. Linezolid) were identified as monoamine oxidase inhibitors in the late 1950s and parts of the 1970s, E.I. duPont de Nemours discovered their antibiotic properties, but were discontinued due to liver toxicity (Brickner, 1996; Livermore, 2000).

Linezolid was approved by The U.S. Food and Drug Administration (FDA) in 2000 due to its pharmacokinetic advantage and was recommended for treatment of severe forms of MRSA infections such as deeper soft tissues and pneumonia (Leong et *al*., 2018; Barbachyn and Ford, 2003).

2.10.6.1 Mode of action of Oxazolidinone/Linezolid

Linezolid inhibits bacterial protein synthesis through a new mechanism that stops the initiation of protein synthesis (Pletz *et al.,* 2010); the mechanism also prevents cross-resistance with other protein synthesis inhibitors (Burkhardt *et al.,* 2007).

2.10.6.2 Mechanisms of Linezolid resistance

The first clinical *S. aureus* resistance to Linezolid was reported in 2001 (Tsiodras *et al*., 2001), though a 2006 surveillance program in the USA pointed out that 99.55% of *S. aureus* were still susceptible to the Linezolid (Jones *et al*., 2007).

Linezolid resistance mechanisms in Gram positive bacteria is through point mutation in linezolid 23S rRNA binding site (G2576T) and ribosomal proteins L3 /L4 of the peptide translocation center of the ribosome (Gu *et al.,* 2013). Gram positive bacteria may also be resistant to Linezolid by acquiring plasmid-borne ribosomal methyltransferase gene, *cfr* (Gu *et al.,* 2013; Zhu *et al*., 2007; Meka *et al*., 2004).

2.10.7 Tetracyclines

Chlortetracycline was discovered by Benjamin Duggar in 1948 marking the beginning of tetracycline (Tet) family therapy (Duggar, 1948; Duggar, 1949). Chlortetracycline was approved for use in 1952, followed by approvals of its derivatives doxycycline and minocycline in 1967 and 1972, respectively (Del, 1972). Tetracyclines (minocycline, doxycycline, and tetracycline)

are used for non-intravenous treatment of infections caused by both MRSA and MSSA such as papulo-pustular rosacea (Goldgar *et al*., 2009), with doxycycline and minocycline exhibiting superb oral bioavailability, tissue penetration, and tolerability (Klein and Cunha, 1995).

2.10.7.1 Tetracycline mode of action

Tetracyclines inhibit bacterial protein synthesis by blocking the binding of aminoacyl-tRNA to bacterial ribosome (Brodersen *et al*., 2003) generally by attaching to 30S ribosomal subunit in the mRNA translation complex (Chopra *et al*., 1992; Schnappinger and Hillen, 1996).

2.10.7.2 Tetracycline resistance mechanisms

Staphylococcus aureus strains involve active efflux of Tetracycline by acquiring *tet* K and *tet* L genes, which are harbored on a plasmid. The second mode of mechanism of transfering Tetracycline resistance involves ribosomal protection; a mode of resistance that is facilitated by *tet* M or *tet* O genes, which are located on a transposon. The third mode of mechanism to tetracycline resistance is chromosomally encoded via *tet* M gene; this confers resistance to the entire Tetracycline group of antibiotics. Some *S. aureus* strains bearing *tet* K gene are resistant to Tetracycline (Milatovic *et al*., 2003) while, susceptible to minocycline (Warsa *et al*., 1996; Bismuth *et al*., 1990; Tracinski *et al*., 2000).

2.10.8 New generation of Tetracycline; Tigecycline

The US Food and Drug Administration fast-tracked the approval of Tigecycline in 2005 for treatment of complicated intra-abdominal, cSSSI and community-acquired pneumonia (US Food and Drug Administration, 2011; Waknine, 2009). In 2010, FDA issued a warning concerning

Tigecycline, linking it to increased risk of death compared with other antibiotics (US Food and Drug Administration, 2011).

2.10.8.1 Tigecycline Mode of action

Tigecycline is a new generation of Tetracycline belonging to glycylcyclines class; it is a derivative of minocycline, which has modified side chain. Tigecycline has an enhanced binding ability to the 30S ribosomal subunit; ability which leads to inhibition of protein synthesis. It is effective to a wide range of pathogens, including MRSA (Rose and Rybak, 2006).

2.10.8.2 Tigecycline resistance mechanisms

The structural modification of Tigecycline overcomes resistance mechanisms that negatively affect Tetracycline and other antibiotics in this class. Its resistance occurs due to the inhibition of protein synthesis; Tigecycline prevents the entry of aminoacyl-tRNA into the A site of the ribosomal RNA (Micek, 2007; Scheinfeld, 2005).

2.10.9 Macrolides and Lincosamides

Erythromycin belongs to the class of macrolide antibiotics; it was discovered by Dr. Abelardo Aguilar in 1949, Dr. Abelardo sent soil sample to J. M. McGuire for further analysis (Eli Lilly's research team).This team isolated the metabolic by product from *Saccharopolyspora erythraea,* that was eventually commercialized and launched in 1952 (Lewis, 2013; Hibionada, 2014). Erythromycin is used to treat less serious skin and soft tissue infections caused by *S. aureus* (Rayner and Munckhof, 2005) or patients with similar infections but are hypersensitive to penicillin (Rayner and Munckhof, 2005).

Clindamycin belongs to the class of antibiotics called Lincosamides; it was first used in 1967. Despite clindamycin and erythromycin being structurally different, they are functionally similar,

since they attach to the 50 S ribosomal subunit, blocking the nascent peptides from leaving the ribosome (Wiley and Sons, 2014; Tenson *et al*., 2003; Leclercq, 2002).

Clindamycin is used in the treatment of more serious infections such severe respiratory tract infections, serious skin and soft tissue infections, moderate to severe diabetic foot infections, septic arthritis and osteomyelitis. Clindamycin has been used as a substitute to penicillins for dental infections, endocarditis prophylaxis, necrotizing pneumonia and penicillin-allergic patients (Smieja, 1998; Wiley and Sons, 2014).

2.10.9.1 Mode of action of macrolides and lincosamides

Both Erythromycin and Clindamycin cause dissociation of peptidyl-tRNA from the ribosome (Tenson *et al*., 2003), with Clindamycin further extending it to the peptidyl transferase center (Tenson *et al*., 2003), leading to dissociation of peptidyl-tRNAs containing two, three or four amino acid residues(Tenson. *et al*., 2003), unlike Erythromycin, that does not reach the peptidyl transferase center but induces dissociation of peptidyl-tRNAs containing six, seven or eight amino acid residues (Tenson *et al*., 2003; Leclercq, 2002).

Among *S. aureus*, erythromycin resistance is due to ribosomal modification of 23S rRNA methylases, mediated mainly by erythromycin A (*erm*A), erythromycin B (*erm*B), or erythromycin C (*erm*B) genes. These three genes encode for enzymes that bestow inducible or constitutive resistance to Macrolide-Lincosamide-Streptogramin, by active efflux of these antibiotics using an ATP-dependent pump driven by *msr*A gene (Prabhu *et al*., 2011; Duval, 1985; Jánosi *et al*., 1990; Novick and Murphy, 1985; Ross *et al*., 1990). Erythromycin A gene is harbored on the transposon Tn554, which also encodes for spectinomycin resistance (Chikramane *et al*., 1991; Krolewsk *et al*., 1981), while erythromycin B gene is harbored on

transposon Tn551 and in the penicillinase plasmid, pI258 (Mitsuhashi *et al*., 1963). The other genes like erythromycin C gene are located on small plasmids with the approximate sizes between 2.4 to 5 kb (Horinouchi and Weisblum, 1982; Iordănescu, 1976).

Testing for susceptibility using conventional test method does not detect inducible clindamycin resistance, due to presence of erythromycin genes; a situation that can lead to treatment failure; this requires a separate susceptibility test to detect its resistance. *Staphylococcus aureus* erythromycin-resistant and clindamycin susceptible strains are identified using a double-disk susceptibility test to detect inducible clindamycin resistance, this test is known as D test (Fokas *et al*., 2005).

Clindamycin mode of action includes attachment to the 50S ribosomal subunit of bacterial pathogen leading to the breaking down of protein synthesis; it interferes with transpeptidation reaction, thus inhibiting initial chain elongation (Merck and Company, 2005; Kohanski *et al*., 2010). Clindamycin also acts by disrupting bacterial protein synthesis. This triggers changes in the cell wall surface by decreasing the ability of bacteria to adhere to host cells and intensify intracellular killing of organisms (Smieja 1998; Pankey and Sabath, 2004).

In veterinary medicine, Clindamycin is used to treat osteomyelitis and skin infections in dogs and cats (Rich *al*., 2005); it is also used to treat toxoplasmosis in immunocompromised cats (Davidson *al*., 1996; Kahn *et al*., 2005, A; Kahn *et al*., 2005, B).

Use of Clindamycin is not generally recommended on erythromycin-resistant MRSA and MSSA because of a single-step mutation via Macrolide-Lincosamide-Streptogramin mechanism leading to Clindamycin resistance. Thus, Clindamycin may be used only against erythromycin-susceptible strains (Gemmell *et al*., 2006). Furthermore, Clindamycin is an attractive alternative

against MSSA and MRSA due to its efficacy and advantageous bone and tissue penetration and its antitoxin effects (Patel *et al*., 2006).

2.11 Cultivation of *Staphylococus aureus*

The growth and survival of *S. aureus* is reliant on the following conditions that include, environmental factors, such as temperature, water activity, and pH, presence of oxygen and composition of the food. The organism's physical growth parameters vary among different *S. aureus* strains (Stewart, 2003). *Staphylococus aureus* can grow at a pH of between 4.0 and 10.0 and can cope in both acidic and/or alkaline environment (Anderson, *et al*., 2010), pH 6 to 7 is considered ideal in the cultivation of *S. aureus* (Valero *et al*., 2009), the organism can withstand low temperature; therefore, survive in food stored below -20°C (Stewart, 2003). *Staphylococus aureus* can survive in low water activity (Valero *et al*., 2009; Medveďová *et al*., 2019) and are resistant to high salt concentrations (Medveďová *et al*., 2019), and the organisms can withstand osmotic stress (Medveďová *et al*., 2019).

It is a facultative anaerobe, growing both in aerobic and anaerobic conditions; however, its growth is slower under anaerobic conditions (Stewart, 2003).

2.12 Antibiotic susceptibility testing methods

2.12.1 *In vitro* antibiotic susceptibility testing

Antibiotic susceptibility *in vitro* testing can predict the clinical response to treatment and guide the selection of antibiotics. The *in vitro* methods include disk diffusion (Bauer*et al*., 1966; Barry *et al*., 1970; Hubert *et al*., 1998), broth microdilution (Wayne, 2006) and E-test (Woods*et al*., 2000). National Committee for Clinical Laboratory Standards, now clinical and laboratory standards institute (CLSI) has developed standard laboratory methods to be followed in all microbiology laboratories; this include, bacterial growth media , incubation temperature ideal for bacterial growth and the right environment to grow bacteria, the amount of bacterial inoculum to use and quality control checks, such as growth and physical supporting characteristics, gel strength, purity of media and batch contamination checks (Basu *et al*., 2005); this ensures reproducibility of results internationally (Khan *et al*., 2019).

2.12.2 Phenotypic MRSA detection

Disc diffusion methods using oxacillin susceptibility are widely used for detection of MRSA isolates (NCCLS, 2003). However, there are conflicting recommendations on phenotypic MRSA susceptibility testing, due to significant heterogeneity among different strains used in the phenotypic testing of methicillin resistance (Tomasz *et al*., 1991). National Committee for Clinical Laboratory Standards (CLSI) has defined a standardized method for the detection of MRSA isolates using disc diffusion methods (CLSI, 2017). The development of cefoxitin disc diffusion tests may alter (CLSI) recommendations, as studies suggest that tests with cefoxitin are more reliable than those with oxacillin (Almohana *et al*., 2012; Cauwelier *et al*., 2004),

because in the case of cefoxitin there is no requirement for special medium or incubation temperature (Mougeot *et al*., 2001; Felten *et al*., 2002).

2.13 Molecular diagnosis of *Staphylococcus aureus* including MRSA

2.13.1 Molecular diagnosis

It is important to have a fast reliable molecular identification of *S. aureus,* since this is a major concern in clinical microbiological diagnostics of *S. aureus*-related infections (Hoegh *et al*., 2014). *Staphylococcus aureus*-specific gene detection is based on PCR and Multiplex PCR for *nuc* gene, which encodes thermonuclease using primers used for the identification of by PCR (Maes *et al*., 2002; Louie *et al*., 2002). In the Multiplex identification of *S. aureus*, *nuc* gene primers are combined with the *mecA* gene based primers to provide a fast screening or identification of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *Staph. aureus* {MRSA} (Elsayed *et al*., 2003; Costa *et al*., 2005; Thomas *et al*., 2007). The PCR method is sensitive enough for rapid detection in broth cultures from blood or swab specimens in 2 hours (Struelens, 2006), as compared to identification and susceptibility of *S. aureus* in blood cultures (BCs) which takes 24-48 hours (Cattoir *et al*., 2011). The Multiplex method has limited sensitivity and poor specificity in specimens that have a mixture of methicillin-resistant coagulase-negative staphylococci and MSSA (Struelens, 2006).

Real-time PCR combines conventional PCR and fluorescent probe detection of amplified product at the same time; this allows accurate and timely diagnosis of disease, which is important for therapeutic intervention, this is advantageous compared to the traditional PCR (Rodriguez-Lazaro and Hernandez, 2013). It has high sensitivity and specificity, low contamination risk and is associated with rapid results (Espy *et al*., 2006). This technology is an attractive alternative to culture or immunoassay-based testing methods for diagnosis of *S. aureus* and MRSA infection (Jonas *et al*., 2002).

2.13.2 Molecular typing techniques used for MRSA characterisation

Molecular typing methods are used to characterize MRSA clones; three of them are Pulsed-field gel electrophoresis (PFGE), Multilocus sequence typing (MLST) and Staphylococcal Cassette Chromosome *mec* (SCC*mec*) typing (Cookson *et al*., 2007). When these typing methods are combined with the epidemiological and clinical information, the combined data can assist in detection of MRSA clusters, identify outbreaks of MRSA and MSSA strains; information which may be used to provide a rationale for appropriate infection control (Elena *et al*., 2012).

2.13.2.1 Staphylococcal cassette chromosome *mec* (SCC*mec*) typing

Staphylococcal cassette chromosome *mec* is a mobile genetic element that carries the determinant for broad-spectrum beta-lactam resistance encoded by the *mec*A gene ((IWG-SCC, 2009).). The gene is hosted a *mec* operon {*mec* gene complex} (Hiramatsu *et al*., 2013) together with its regulatory genes *mec*I and *mecR*1 (Berger-Bachi and Rohrer, 2002), which are accompanied with unique site-specific recombinases, commonly known as cassette chromosome recombinases (*ccr)*. Staphylococcal cassette chromosome *mec* elements are classified depending on combination of *ccr* and *mec* gene complex genetic composition (Hiramatsu *et al*., 2001). A number of *SCCmec* types (*SCCmec* I-XI) have been described to date (IWG-SCC, 2009), with HA-MRSA strains carrying *SCCmec* type I, *SCCmec* type II, and *SCCmec* type III (Wylie *et al*., 2005) ,while those that are associated with a community setting carry *SCCmec* type IV or V (David and Daum, 2010). *SCCmec* typing relies on PCR or multiplex PCR (M-PCR) that was invented by Kondo and others (Kondo *et al*., 2007).

2.13.2.2 Multilocus sequence typing

Multilocus sequence typing is an ideal method used for both long-term global epidemiological and population genetic studies (Urwin *et al*., 2003). This method is based on sequence analysis of seven *S. aureus* housekeeping genes: carbamate kinase (*arcC*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), shikimate dehydrogenase (*aroE*), triosephosphate isomerase (*tpi)* and acetyl coenzyme A acetyltransferase (*yqiL)* (van Leeuwen *et al*., 2003). For each house-keeping gene, the DNA sequences are assigned distinct alleles. Alleles results at each of the seven loci define the allelic profile or sequence type (ST); closely related sequence types (STs) form clusters and these clusters are referred to as clonal complexes (CCs) (Enright *et al*., 2000). A series of profiles can then be recorded as the identification marker for a particular strain; this has permitted the creation of internet-based curate databases and made inter-laboratory data exchange possible (Enright *et al*., 2000). Multilocus sequence typing offers an exceptional inter-laboratory reproducibility and data portability; in addition, it provides valuable and basic knowledge of the population make-up of typed strains. When this method was combined with characterized MRSA *SCCmec* type results, they provided additional information on the evolutionary origins of major MRSA clones (Enright *et al.*, 2002; Robinson and Enright, 2003). A large number of ST has been identified using this method against a limited number of CCs currently stored in the internet-based curate databases. Clonal complexes 1, 5, 8, 15, 22, 30, 45, 59, 80, 97, 121 are the most predominant among populations of typed *S. aureus* strains in internet-based curate databases. These CCs exhibit a global distribution, as reported in local and national surveys worldwide.It is only a small number of STs that form a sizeable percentage among *S. aureus* population in both local and international data bases. These groups of STs are widely spread and include any respective imports from external sources into a local setting.

MLST typing insufficient discriminatory power, presents a problem in identification of the imported *S. aureus* strains (STs), from resident strains in a local region (Grundmann *et al*., 2002)

The global diversity of MRSA as determined by MLST is not much, as compared to the diversity in any representative sample from a local setting. This is because the current dataset available from the central MLST database (http://saureus.mlst.net/) is meant to assign STs and identify novel STs, leading to rare STs being over-represented in the database; in addition, very few studies on *S. aureus* conducted in populations are entirely typed using MLST (Nübel *et al*., 2011). Cost-effective Pulsed-field gel electrophoresis (PFGE) or *spa* molecular typing are usually preferred, instead of MLST typing due to the cost involved in DNA sequencing (Boers *et al*., 2012). In fact, Multilocus sequence typing is expensive, labour intensive and time consuming (Liou *et al*., 2020), the database is normally not a representative of a population sample (Urwin *et al*., 2003; Nübel *et al*., 2011). Multilocus sequence typing and SCC*mec* typing are currently recommended for multicenter surveillance of MRSA (Cookson *et al*., 2007), inter-hospital surveillance of MRSA, studies of international transmission of MRSA clones and evolution of MRSA strains (Cookson *et al*., 2007).

2.13.2.3 Pulsed-field gel electrophoresis (PFGE)

Pulsed field gel electrophoresis typing method is based on variation in digestion of restriction enzyme *Sma* I, resulting in bacterial DNA fragmentations. The resultant fragments are separated by a modification of the conventional agarose gel electrophoresis, enabling a distinct segregation of the larger DNA fragments (Mehndiratta *et al*., 2012) according to size (Ichiyama *et al*., 1991; Prévost *et al*., 1991). Comparison of PFGE patterns of different *S. aureus* strains provide a vital pointer as to whether or not this *S. aureus* strains are genetically related; matching PFGE patterns are clonal (i.e. the same strain) or most likely they have originated from the same ancestor.

Pulsed field gel electrophoresis was the first genotyping technique widely used for typing *S. aureus*. A lot of effort was put to standardize this technique internationally, so as to overcome shortcomings of inter-laboratory reproducibility. PFGE is still a popular technique for typing *S. aureus*, especially outside Europe; in fact in some regions, groupings analyzed by PFGE banding patterns collate with MLST at the cluster level (CC) (Cookson *et al.*, 2007; Grundmann *et al*., 2002; Strommenger *et al*., 2006). Some studies have shown PFGE as having a higher discriminatory power than MLST, since band patterns from PFGE, varied substantially within a MLST STs, with up to five band differences in an original MLST study (Enright *et al*., 2002); besides this, pattern variations within CCs are not always harmonious with the phylogenetic relationships among isolates (Harris *et al*., 2010; Nübel *et al*., 2008). Lack of homology between CCs phylogenetic relationships among isolates is as a result of band pattern changes following a variety of genetic events, such as gain or loss of mobile genetic elements (MGEs) and intra-chromosomal recombination (Tenover *et al*., 1995); therefore, specific MLST sequence types may not be assigned on the basis of PFGE band patterns (Grundmann *et al*., 2002; Nübel *et al*., 2010).

Pulsed-field gel electrophoresis has been employed mainly on local epidemiological studies and is very suitable for identification of MRSA strains during outbreaks of disease (Tenover *et al*., 1995). Weakness of this technique is that individual strains recognized by PFGE usually dominate local populations, making it difficult to differentiate between short-term outbreaks and endemic situations at regional and national level (Koch *et al*., 2017; Nübel *et al*., 2011; Ghebremedhin *et al*., 2007). Pulsed-field gel electrophoresis is also not a suitable typing method for long term epidemiological surveillance (Hallin *et al*.,2007), since this type of study requires long-term evolutionary history of the pandemic clones (Melles *et al*., 2007); because of its

detection of small genetic differences (Moore *et al*., 2015), compared to whole-genome sequencing detecting genetic differences between organisms (Price *et al*., 2013).

2.13.2.4 Staphylococcal protein A *(spa*) typing

Single locus sequence typing is used to establish the relationships between MRSA isolates based on the comparison of DNA sequence differences in a single target gene (Yamasaki*et al*., 2005). Staphylococcal protein *A* gene in MRSA strains contains 24 bp tandem repeats that can be compared in central database (http://spaserver.ridom.de). MRSA strains can be discriminated by determining the repeat sequence numbers within the X region of *spa* gene (Kareem*et al*., 2020). Although PFGE has superior discriminatory power than *spa* typing, *spa* typing and MLST depicted clusters are better than those of PFGE; these two typing methods correlate well at CCs level, making it possible to designate CC with sufficient confidence (Cookson *et al*., 2007; Strommenger *et al*., 2006). Staphylococcal protein A (Spa) typing has some advantages, for example , the method is simple, rapid and highly reproducible, there are online databases that exist for electronic submission and *spa* types designation (Mehndiratta and Bhalla, 2012). Spa typing is a perfect tool for national and international surveillance of *S. aureus* strains and is recommended for short term local epidemiological studies (Strommenger *et al*., 2008; Deurenberg *et al*., 2008;Ruppitsch *et al*., 2006; Harmsen*et al*., 2003; Hallin *et al*., 2007). This method is user-friendly, superior inter-laboratory reproducibility and portability of DNA sequences as compared to PFGE (Nübel *et al*., 2011), several reference laboratories in Europe have substituted PFGE with *spa* typing in MRSA infection surveillance (Cookson *et al*., 2007). The disadvantage with *spa* typing is, however, its susceptibility to high frequency of mutation in the *spa* locus; this usually results in evolutionary convergence. To illustrate its short coming: *spa* types mapped using high-resolution phylogenies as based on genome-wide SNPs, show a

number of *spa* sequences being assigned in two or more distinct phylogenetic sub-lineage STs; these unrelated strains are as a result of repeated evolution (convergence) of *spa* sequences, a phenomenon known as homoplasies (Harris *et al*., 2010; Nübel *et al*., 2008). Homoplasies weaken the effectiveness of *spa* typing in research targeting MRSA dissemination; thus *spa* sequence identity may mislead the investigator by suggesting a geographical spread of a single clone. A good example for this is identical *spa* sequencetypes t138 and ST239, isolated from Greece and Brazil, which were found to be unrelated through genome-based phylogenetic analysis (Harris *et al*., 2010). High mutation rate and the subsequent variability of the *spa* locus is a major limitation of *spa* typing, because *spa* sequences are not too conserved to efficiently monitor the spread of MRSA; the same limitation has been observed in MSSA (Grundmann *et al*., 2010; Strommenger *et al.,* 2008). Furthermore,*spa* typing offers very little discriminatory power for MRSA in numerous circumstances, such as, when an individual variant dominates a MRSA population, limiting its use in studying the spread of these pathogens from a local setting to an intercontinental transmission state (Khandavilli *et al*., 2009; Shore *et al*., 2010; Strommenger *et al*., 2008).

2.13.2.5 Whole genome analysis

Genome-wide single-nucleotide polymorphisms (SNPs) or whole genome analysis permits the comprehensive reconstruction of pathogen spread. Current studies based on long DNA sequences from internationally representative MRSA isolate collections indicates that MRSA genomes add one point mutation per every 6 - 8 weeks (Harris *et al*., 2010; Nübel *et al*., 2010; Smyth *et al*., 2010). This quantifiable accumulation of DNA variation may be used correctly to infer time-based dynamics of the targeted pathogen disseminate, over considerable epidemiologically timescales (Cottam *et al*., 2008; Jombart *et al*., 2011; Lemey *et al*., 2009). The

hereditary ties among MRSA isolates from a particular outbreak or epidemic can be recreated based on serially sampled DNA sequences, as long as the DNA sequences are sufficiently variable (Jombart *et al*., 2011). In addition, these hereditary relationships based on sampling dates, locations, time course, routes of transmission and dissemination of the isolates can then be confirmed and visualized by using heredity -based tracking system (Jombart *et al*., 2011; Lemey *et al*., 2009). A good example was recent revelation that MRSA ST225 clone which was introduced in Europe in the mid-1990s rapidly spread among hospitals throughout Central Europe in a complex manner as earlier displayed by *spa* typing (Nübel *et al*., 2010). Another interesting study of MRSA ST239 revealed that it is possible to track the exact route from person-to-person transmission, so long as the DNA sequences of the targeted strains are of sufficient length for analysis {i.e., whole genome sequencing; WGS}(Harris *et al.,* 2010).

Staphylococcus aureus genome sequences contain genetic information on the isolates' ancestral history from weeks up to years, together with their geographical narrative from the level of localized outbreaks to worldwide spread (Harris *et al.,* 2010). These recent discoveries offer very encouraging outlooks for future research on MRSA distribution and dissemination. Deoxyribonucleic acid (DNA) sequencing is currently considered to be a rapid and cost-effective technique, allowing DNA sequencing projects at levels that were considered untenable some few years ago (Harris *et al*., 2010; Parkhill, 2008). The cost of WGS continues to decrease and it appears that genome sequencing may soon become the new gold standard for genotyping and first-line typing technique for *S. aureus* and other bacterial pathogens (Bentley and Parkhill, 2015). Evidently, a whole-genome sequence offers the decisive discriminatory power for molecular epidemiological typing combined with its quantitative advances, sampling and the

comparative analyses of multiple long DNA sequences; this method has provided major theoretical progress in staphylococcal molecular epidemiology (Park *et al*., 2017).

Whole-genome sequences in combination with next-generation sequencing (NGS) are capable of transforming investigations of outbreak and infection control programs worldwide (Kwong *et al*., 2017). When these WGS and NGS are combined, they provide identification of clear-cut routes together with the timescale based pathogen transmission (Deurenberg *et al*., 2017). Recent headways in bench-top, next-generation sequencing {(NGS} (Dominguez *et al*., 2016) and bioinformatics have assisted in rapid whole-genome sequencing and nucleotide-level comparison of the evolutionary ties among pathogenic isolates (Dominguez *et al*., 2016), such *as S. aureus* in local outbreaks and linking them to international transmission (Dominguez *et al*., 2016).

2.14 Specimens for diagnosis: humans and livestock

Diagnosis of *S. aureus* infections begins with the collection of specimens, which also depends on the infected area of the human/animal body such as skin and soft tissue infections or throat, nostrils and wound infections with pus forming bacteria. The following specimens, feces, milk, feed material; joint, trachea, uterus, and meat are collected to isolate *S. aureus* (Lee *et al*., 2003) and diagnose suspected *S. aureus* MRSA/MSSA infection in beef cattle, dairy cattle, pigs, and chickens (Lee *et al*., 2003).

Specimens are obtained using sterile absorbent cotton swabs for pus and other discharges; in suspected urinary tract infection, urine samples in sterile containers are used; while in investigating blood infection, fresh stab blood samples are obtained then transferred to blood culture bottle.

2.15 Cleaning and disinfection of *Staphylococcus aureus*

2.15.1. Disinfecting surfaces or objects

Staphylococcus aureus is susceptible to many disinfectants such as: alcohols, sodium hypochlorite, quaternary ammonium compounds, iodophors, phenolics, glutaraldehyde, formaldehyde, and a combination of iodine and alcohol (Public Health Agency of Canada, 2001; Sehulster *et al*., 2004). *Staphylococcus aureus* is also susceptible to moist heat at 121°C for a minimum of 15 minutes or dry heat at 160-170°C for at least 1 hour (Public Health Agency of Canada, 2001). Chemical disinfectants destroy MRSA on surfaces or objects. Cleaning the surfaces before disinfection can further lower the risk of spreading infection; sodium hypochlorite is commonly used for this disinfection (Thorn *et al*., 2013).

2.15.2 Hand washing

Hand hygiene is important in the prevention of MRSA transmission via hands of healthcare workers, veterinary personnel and animal handlers (Boyce *et al*., 2002; Gorwitz *et al*., 2006). Hand hygiene intervention includes washing of hands with an antibiotic soap or using an alcohol-based hand rub and educating healthcare personnel and patients, stressing on the importance of hand hygiene in the prevention of infection and colonization by *S. aureus* (Denyer *et al*., 2004; Boyce *et al*., 2002).

2.15.3. *Staphylococcus aureus* decolonization in humans

Between 20–80% of the human beings are asymptomatically colonized with *S. aureus* in their anterior nares (Brown *et al.,* 2014); MRSA colonizing at much lower rates (Brown *et al.,* 2014). Nasal decolonization is applied to certain categories of patients colonized with MRSA; the most vulnerable groups include frequently hospitalized people, the aged, patients on dialysis, AIDS patients and diabetics (Coia *et al*., 2006). The antibiotic recommended for decolonizing MRSA in these vulnerable groups is mupirocin, which decolonizes *S. aureus* from the anterior nares for a period of few weeks, although relapses are common within several months (Coates *et al.,* 2009).

Doctors should undertake decolonization in two parts: First treatment for decolonization of MRSA using rubbing nasal ointment (mupirocin 2%), then bathing using a special soap that contains 4% chlorhexidine once daily for 5 days (McConeghy *et al*., 2009; Evans *et al*., 2010).

3. MATERIALS AND METHODS

3.1 Study area

This study was conducted in Busia County, which is located in Western Kenya. The county is within the Lake Victoria Basin, bordering Siaya County to the South, Uganda to the West and North, Bungoma County to the North East, and Kakamega County to the East (Ope *et al* 2013; Karanja *et al* 2017), with an estimated population of 823,504. Trade, agriculture, tourism, fishing and commercial businesses are main economic activities, mainly at the border towns of Busia and Malaba, between Kenya and Uganda (Wafula, 2018). This county has a total of 81 health facilities, with the former Busia District Hospital being upgraded to county referral status; it also hosts 6 sub-county hospitals, 12 health centers and 49 dispensaries (County Government of Busia, 2020). A census of all abattoirs (n=154) was conducted in the study area. Twelve abattoirs declined to participate. Participants were recruited from 142 abattoirs, 81 ruminants and 51 porcine slaughtering and processing? (Cook *et al* 2017).

3.2 Study populations

This study was done on three study populations (1) two sets of abattoir workers (HIV-positive and HIV-negative) in the selected 142 slaughterhouses;

A total of 738 abattoir workers were recruited into the study between February and November 2012 from a total of 1005 workers (73.3%) in the selected slaughterhouses. In abattoirs with 12 workers or less all consenting workers were recruited and in abattoirs with more than 12 workers a random selection of twelve workers were recruited

3.2.1 Human study populations

Inpatients at Busia Referral County Hospital and abattoir workers (HIV-positive +VE and HIV-negative) in Busia County, Kenya.

3.2.1.1 Inclusion criteria

Patients who eighteen years of age and above and willing to sign consent form were enrolled into the study. Assent form for children less than 18 years were signed by legal parent or guardian. Inpatients must have been admitted for 48 hours or longer.

3.2.1.2 Exclusion criteria

Any individual with recent nasal surgery or other medical conditions were excluded.

3.2.2 Animal study population

Livestock brought-in for slaughter at the abattoirs in Busia County, Kenya.

3.2.2.1 Inclusion criteria for animals

Livestock with no injuries or a recent surgery in their nasal cavity, animal livestock whose owners had consented for sample collection.

3.2.2.2 Exclusion criteria for animal

Livestock with injuries or a recent surgery in their nasal cavity; livestock whose owners had declined to consent for sample collection .

3.3 Study design

This research applied a one health approach to evaluate *S. aureus* molecular genotype and antibiotic resistance phenotype in humans and livestock in Busia County, Kenya. It was geared towards determining epidemiological and molecular factors linked with healthcare associated (inpatients) and community associated (abattoir workers) *S. aureus* infection and colonization. In addition, efforts were made to determine possible direct transmission link between human and animal infections by comparing livestock strains to abattoir workers and inpatients. Sub-populations of human inpatients and abattoir workers (HIV-positive and HIV-negative) were further evaluated to determine possible Sulfamethoxazole-Trimethoprim (SXT/TMP) resistance in the test individuals, since SXT/TMP was the most commonly administered antibiotic to HIV-positive patients in Busia. Bacterial isolation and characterization were carried-out; the isolated *S. aureus* was further analyzed for antibiotic resistance, toxin gene carriage, molecular typing of *SCCmec*, PVL gene detection and MLST typing. This study was based on a cross sectional random sampling design; the study period was between 21/05/2015 to 07/07/2015.

3.4 Clinical data collection

The number of hospital admissions at each unit at Busia Referral County Hospital (BCRH) was collected for the study period. Each consenting participantfilled th e questionnaire, giving data on gender, age, length of hospitalization, **w**ard admissions, empirical antibiotic treatment given and livestock ownership. Efforts were made to calculate the proportion of antibiotics used in empirical therapy to inpatients admitted to various wards at BCRH, from the hospital records.

3.5 Specimen collection, handling and processing,

3.5.1Nasal samples

For both humans (inpatients and abattoir workers) and animals, samples were collected from the two nostrils using one nasal swab per person/animal. The cotton swab was moistened with sterile Tryptic phosphate broth (Oxoid; Thermo Fisher Scientific Inc) and carefully inserted into the nostrils; the swab was then rolled five times along the mucosa inside the nostrils, one at a time. The swabs were then aseptically and separately put in transport bottles containing Amies transport (Eswab™) medium. The samples were placed inside cooler box, with ice, cold packs, to maintain the temperature below 6º C (Lowe *et al* 2020) and at the end of collection day transported to International Livestock Research Institute (ILRI) field stations in Busia County for processing and analysis. Livestock samples were collected by trained animal health personnel with expertise in nasal swab collection from the animals.

3.5.2 Blood samples

Blood samples were collected from abattoir workers for HIV test after consent was obtained and counseling conducted. Blood was collected by a clinical officer into 4ml EDTA vacutainers using a butterfly catheter (World Health Organization, 2010). Samples were transported in cool boxes to the laboratory in Busia. Whole blood samples were stored frozen at -40°C until being transported to the KEMRI laboratory in Nairobi for long term storage at -80°C.

3.5.3 Disposal of used material

Once the swab was used to inoculate media for cultivation and identification of *S. aureus*, it was disposed into appropriate autoclavable biohazard waste bags for autoclaving; single use gloves

were also disposed and autoclaved. Used goggles were first disinfected with 10% solution of household bleach (Sodium hypochlorite), rinsed and dried for reuse.

3.6 Culture and identification of *Staphylococcus aureus*

The samples were inoculated onto sterilized mannitol salt agar platE, by streaking with inoculating loop and incubated at 37°C for 24 to 48 hours (Garoy *et al*., 2019). *S. aureus* colonies appear as golden yellow colonies (after mannitol fermentation), were aseptically picked and characterized using standard microbiological methods, including biochemical characteristics (Cheesbrough, 2002). *Staphylococcus aureus* are Gram- positive cocci, arranged in grape-like clusters; they are also positive for catalase test, coagulase test and DNase test (Cheesbrough 2002).

Catalase Test

The catalase test is used to differentiate *streptococcus* spp which are catalase-negative and *Staphylococcus* spp swhich are catalase positive. The test was performed by applying a few drops 3% hydrogen peroxide on 0a suspected gram positive bacterial growth on an agar slant, if the culture produces bubbles, then the organism is presumed to be a *Staphylococcus* spp.

Deoxyribonuclease (DNase) test

A loopful of growth from a blood agar plate was inoculated on DNase agar and incubated at 37°C for 24 hours , After incubation, approximately 15 ml 1 N hydrochloric acid (HCl) was used to flood the plate. Excess hydrochloric acid was removed, organisms with ability to hydrolyze DNA, will be surrounded with a clear zone around the growth, indicating a positive result. *S. aureus* produces DNase and the test differentiates it from other *Staphylococci* spp.

Coagulase tube test

A plasma dilution of 1:10 with saline was made; 0.5 ml of diluted plasma was transferred into a pair of test tubes. The tubes were inoculated with bacterial cells, and incubated in a water bath at 35°C for between 1 to 4 hours, presence of coagulation was considered to a positive test, lack of it was consider negative. *S. aureus* strains are capable of coagulating plasma, this test was used to differentiate *S. aureus* strains from S. epidermidis and other coagulase-negative species

3.7 Phenotypic antibiotic susceptibility testing of the isolates

3.7.1 General Testingof *Staphylococcus aureus* isolates

The choice of antibiotics for testing was informed by the standard antibiotic treatment as recommended by WHO for various *Staphylococcus aureus* infections such as abscesses, cellulitis, pneumonia, endocarditis, bacteremia, meningitis and osteomyelitis (Denyer *et al*., 2004; World Health Organization, 2017). Specific antibiotics within various classes were tested on all isolates included: Gentamicin (10 µg), Rifampin/rifampicin-5 µg, Oxacillin 1µg , Ciprofloxacin 5 µg, Trimethoprim-sulphamethoxazole 1.25/23.75 µg,Fusidic acid 30 µg, Vancomycin 30 µg and Teicoplanin 30 µg, Clindamycin 2 µg, Daptomycin 256 - 0.015µg/ml, Erythromycin 15 µg, Linezolid 30 µg, Chloramphenicol 30 µg, Tetracycline30 µg and Doxycycline 30 µg. Trimethoprim-sulphamethoxazole was specifically picked for testing as it is widely prescribed as prophylaxis for *Pneumocystis jiroveci* and against opportunistic bacterial infection in people suffering from HIV/AIDS (Dworkin*et al*., 2001). Tetracycline, aminoglycoside gentamicin and penicillin were included because of their high level use and resistance in food-producing animals, livestock handlers and the environment in food animal production in Africa (Kimera*et al*., 2020).

Antibiotic susceptibility testing of the isolates was carried out using modified Kirby-Bauer technique (Boyle *et al*., 1973) and results were interpreted using the published Clinical and Laboratory Standards Institute (CLSI, 2017). The suspension of each isolate was standardized using the colony suspension to achieve 0.5 McFarland turbidity standards (Cheesbrough, 2002). The resulting suspension was swabbed on Mueller-Hinton agar plate to produce confluent growth.

For the purpose of this study, multidrug-resistant strain was defined as and those isolates that were resistant to three or more classes of the tested antibiotics.

3.7.2 Detection of MRSA

MRSA was identified using oxacillin susceptibility testing (1ug preliminary test for Methicillin resistance) and confirmed by antibiotic susceptibility testing for cefoxitin 30µg (for detection of MRSA). Isolates that were resistant to oxacillin and cefoxitin were classified as MRSA (Centers for Disease Control and Prevention, 2019).

3.7.3 Testing for inducible clindamycin resistance

Inducible clindamycin resistance was expressed using a simple double-disk diffusion test known as D test (Anstead *et al*., 2014). In this test, an erythromycin disk was placed 20 mm from a clindamycin disk (Anstead *et al*., 2014); at this distance erythromycin induces the isolates to express *erm* gene, leading to a formation of a D-shaped zone of bacterial growth around the clindamycin disk. A positive reaction was reported as being resistant to clindamycin. D test was used to detect that *S. aureus* isolates that were resistant to erythromycin and susceptible to clindamycin (Prabhu *et al*., 2011).

3.8 Testing for specific genes in the *Staphylococcus aureus* isolates

3.8.1Testing for presence of Methicillin resistance (*mecA*) gene

3.8.1.1 Nucleic acid extraction

This was done following the protocol provided by the manufacturer (Qiagen, 2015). *Staphylococcus aureus* were harvested in 1000-1500 µl Molecular Grade Water in a sterile 1.5 mL microcentrifuge tube. This was followed by centrifugation for 10 minutes at 14000 rpm, which led to the formation of a pellet containing cleaned *S. aureus* cells and a supernatant.

The supernatant was then discarded and the pellet re-suspended in180 µl prepared enzymatic

lysis buffer (20 mM Tris-HCl (pH 8.0); 2 mM sodium EDTA and 1.2% Triton X-100); the resulting suspension was vortexed and incubated for at least 30 minutes at 37°C, on a heating block.

The samples were removed from the heating block and vortexed to evenly mix them (Qiagen, 2015). This was then followed by adding 25 µl of proteinase K and 200 µl of Buffer AL [the composition of this traction Buffer include, 1 liter Final Concentrations SDS/NaCl Extraction Buffer – 1 liter ;100ml 1.0M Tris-HCl pH 7.5 0.1M Tris-HCl pH 7.5 200ml 1M Tris-HCl pH 7.5 = 0.2M (200mM);100ml 0.5M EDTA pH 8.0 0.05M EDTA pH 8.0 50ml 0.5 EDTA pH 8.0 please check these EDTA concentrations??= 0.025M (25mM);125ml 10% SDS 1.25% SDS 50ml 10% SDS = 0.5% and675ml ddH2O 50ml 5M NaCl = 0.25M (250mM)] (Qiagen 2015); this was further vortexed and incubated at 56°C for 30 minutes, on a heating block (Qiagen 2015).

The samples were removed from the heating block and vortexed to mix before the addition of 200 µl ethanol (96–100%), followed by thorough vortexing to form a homogenous mixture. The resulting solution, including any precipitate, was drawn off using a pipette into the appropriate labeled spin column. The spin columns were then centrifuged at 8,000-10,000 rpm for 1 minute; the resultant liquid portion was collected and discarded into collection tubes.

Nucleic acid deposited in the spin column were transferred into a new collection tube, nucleic acid deposited were mixed with 500 µl Buffer AW1; the tubes were then centrifuged for 1 minute at 8,000-10,000 rpm and the resultant liquid plus contents discarded into collection tubes. The spin columns containing nucleic acid deposit were placed in a new collection tube, 500 µl Buffer AW2 (which is a Tris-based ethanol solution that removes salts) was added, the mixturewas centrifuged for 3 minutes at 14,000 rpm, and the resultant liquid plus contents discarded into collection tubes. Finally the spin column with nucleic acid deposit was transferred into a sterile 1.5 ml microcentrifuge tube, then 100 µl Molecular Grade water was added so as to collect DNA sample elutes (Qiagen 2015), The spin columns containing nucleic acid deposit were left at room temperature for 1 minute, then centrifuged at 8000 rpm for a minute and elutes were collected in clean, sterile tubes and were stored at 4°C until used (Du *et al*., 2019)

3.8.1.2 Amplification of *mecA* gene

The *mecA* gene was detected by PCR, as described by Kondo *et al.* (2007), using respective primers. The procedure involved use of *mecA*: Forward primer 5' TGCTATCCACCCTCAAACAGG 3' and Reverse primer 5' AACGTTGTAACCACCCCAAGA 3'. A 25 ul of reaction mix containing PCR buffer (1X), Magnesium chloride [$Mgcl_2$] (3.5mM), deoxynucleotide triphosphates [dNTPs] (200uM), Taq DNA polymerase (0.5u) and 1ul of DNA extract in reaction tube were placed in a thermocycler machine. Cycling conditions were set at an

initial denaturation step (94°C for 2 minutes) and 30 cycles of denaturation (94°C for 2 minutes), annealing (57°C for 1 minute) and extension (72°C for 2 minutes) with a final elongation step (72°C for 2 minutes). The PCR products were visualized using 1.5 % of agarose gel, submerged in tank containing ethidium bromide and the bands were observed under Ultraviolet light (UV) Trans-illuminator. Size of the expected *mecA* gene band was 286bp.

3.8.1.3 Molecular Typing of *SCCmec*

The DNA extracted in section 3.8.1.1 was used as template based on a method described by Kondo *et al.* (2007).This method involved two separate runs of multiplex PCR, The first multiplex PCR was to detect the presence of the *mec*A gene as well as the genes identifying the *ccr* types (Appendix : Table 1). In this case the reaction mixtures contained 10 ng of chromosomal DNA, 0.1 µM of oligonucleotide primers as shown in Table 1, 200 µM of each dNTPs, a *Taq* buffer and 2.5 U of *Taq* polymerase, in molecular grade water forming a final volume of 50 µl. The concentration of $MgCl_2$ was 3.2 mM. The amplification conditions were set to conform to the initial denaturation step (94°C for 2 minutes) and 30 cycles of denaturation (94°C for 2 minutes) , annealing (57°C for 1 minute) and extension (72°C for 2 minutes), with a final elongation step (72°C for 2 minutes). The second M-PCR was used to identify the *mec* gene complex (Table 2). The mixtures were similar to those that were used for the first multiplex PCR except that the concentration of $MgCl_2$ was 2 mM and the annealing temperature was raised to 60°C for 1 minute (Appendix: Table 2). The *SCCmec* type controls (i.e. I to VI) were included in both multiplex PCR tests {Appendix: Table 1} (Kondo *et al*, 2007). Using the information gathered on *ccr* type and *mec,* class interpretation was done according to the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements so as to

assign MRSA strains to the respective SSCmec types as illustrated in Appendix: Table 1 and 2) (IWG-SCC, 2009).

3.8.1.4Testing for presence of PVL gene

To detect the gene encoding for PVL, genomic DNA was extracted from the isolates as described in section3.8.1.2. Oligonucleotide primers to amplify the PVL gene were designed according to the published sequences of the PVL genes (Lina *et al.,* 1999) and are as follows:

Luk-PV-F, 5′-ATCATTAGGTAAAATGTCTGGACATGATCCA-3′ (Lina *et al.,* 1999)

Luk-PV-R, 5′-GCATCAASTGTATTGGATAGCAAAAGC-3′ (Lina *et al.,* 1999)

(GenBank accession numbers X72700 and AB006796)

Amplification of the PVL gene was done in a thermocycler with initial denaturation was 94°C for 10 minutes (Lina *et al.,* 1999), followed by 30 cycles of denaturation for 94°C for 30 second (Lina *et al.,* 1999), annealing was at 55°C for 1 minute (Lina *et al.,* 1999) and extension was at 72°C for 2 minutes (Lina *et al.,* 1999), with a final elongation step at 72°C for 5 minutes (Lina *et al.,* 1999).The products were electrophoresed through 1% agarose gels (Lee *et al.*, 2012), the gel was stained with Ethidium bromide and visualized under Ultraviolet light (UV) Trans-illuminator (Lee *et al.,* 2012). The product size was 433bp (Lina *et al.,* 1999).

3.8.1.5 Multi Locus Sequence Typing

Seven housekeeping genes were used in the final MLST typing (carbamate kinase (*arc*C), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), shikimate dehydrogenase (*aro*E), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqi*L) following the procedure developed by Enright *et al* in 2000, followed by the fragments being amplified using the primers shown in Appendix Table 3. Polymerase Chain Reaction was used to amplify an internal fragment from the DNA extracted in section 3.8.1.2. of the housekeeping gene (about 500 bp). The PCR products were cleaned using ExoSAP-IT PCR clean-up protocol using procedure in **Appendix protocal 1** provided by the manufacture (Affymetrix). Enzymes used to amplify the indicated seven housekeeping genes using PCR were as given in Appendix Table 3.

Nineteen microliters of master mix were added to each well containing one lyophilized bead made of PCR buffer (1X), Magnesium chloride [Mgcl2] (3.5mM), deoxynucleotide triphosphates [dNTPs] (200uM), followed by the addition of 0.5 units of Taq DNA polymerase,2 ul, forward and reverse primers (Appendix Table 3) and final addition of 2 ul of DNA extract,making a total of 25ul reaction mix in reaction tube.The mixture was then placed in a thermocycler machine and arranged as indicated in Table 1.

Cycling conditions were a denaturation step at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing done at 55°C for 1 minute 30 seconds and final00000000 extension was performed at 72°C for 1 minute this was modified, as opposed to 5 minutes that was used by Enright *et al.* (2000), finally with an elongation step of 72°C for 2 minutes (Enright *et al.*, 2000). The PCR products were cleaned using ExoSAP-IT PCR clean-up protocol using procedure in **Appendix protocal 1** provided by the manufacture (Affymetrix). Enzymes used to amplify the indicated seven housekeeping genes using PCR were as given in Appendix Table 3

The MLST final sequences were aligned and analyzed for completeness and accuracy using ClustalW2, a multiple sequence alignment tool for the alignment of DNA, provided by EMBL-EBI, Wellcome Trust Genome Campus of UK. From the allelic profiles, the sequence type for each isolate was determined by matching the allelic profiles of known alleles deposited at the *S. aureus* MLST database from the MLST Database (Feng *et al.,* 2008; Francisco *et al.,* 2009). This was followed by identification of clonal complexes and their founder strains based on allelic profiles, achieved by eBURST analysis,which is based on principles originally described (Feil *et al.* 2004; Francisco *et al.,* 2009).

Table 1. Samples layout in the thermocycler machine

	1	2	3	4	5	6	7
A	I ARC	1 ARO	1 GLP	1 GMK	1 PTA	1 TPI	1 YQI
B	2 ARC	2 ARO	2 GLP	2 GMK	2 PTA	2 TPI	2 YQI
C	3 ARC	3 ARO	3 GLP	3 GMK	3 PTA	3 TPI	3 YQI
D	4 ARC	4 ARO	4 GLP	4 GMK	4 PTA	4 TPI	4 YQI
E	5 ARC	5 ARO	5 GLP	5 GMK	5 PTA	5 TPI	5 YQI
F	6 ARC	6 ARO	6 GLP	6 GMK	6 PTA	6 TPI	6 YQI
G	7 ARC	7 ARO	7 GLP	7 GMK	7 PTA	7 TPI	Dr YQI
H	Negative ARC	Negative ARO	Negative GLP	Negative GMK	Negative PTA	Negative TPI	Negative YQI

1 carbamate kinase (*arc*C), **2** shikimate dehydrogenase (*aro*E), **3** glycerol kinase (*glp*), **4** guanylate kinase (*gmk*), **5** phosphate acetyltransferase (*pta*), **6** triosephosphate isomerase (*tpi*), and **7** acetyl coenzyme A acetyltransferase (*yqi*L). A to H organisms being amplified using specific primers of housekeeping genes were in the final MLST typing

3.9 Whole Genome sequencing

Whole genome sequencing involved two steps the wet lab and dry lab.

3.9.1 Wet Lab step

DNA was extracted from the isolates as described in section 3.8.1.2. Fifty (50) ng of the extracted DNA was prepared for sequencing using the Nextera DNA Sample Prep Kit (Epicentre). This was followed by a library preparation step, where sequencing libraries from 500 ng of the DNA extracted from each *S. aureus* isolate (Pasquali *et al.,* 2019). Amplification was done using Kapa Hifi polymerase (Kapa Biosystems, Woburn, MA, USA). This was followed by Whole-genome sequencing, which was performed with an Illumina MiSeq (Illumina, San Diego, CA, USA) which generated 150 bp paired end reads. The reads were finally interpreted using MiSeq, Illumina's integrated next generation sequencing instrument (Ravi *et al.,* 2018).

3.9.2 Dry-lab step

This step included creation of a bioinformatics pipeline to be used for the sequence analysis. The sequence analysis was carried out to determine the make-up/sequence of the *Staphylococcus aureus* isolate whole DNA, which also revealed presence of antibiotic resistance genes, genes associated with virulence and pathogenesis and presence of novel and new sequence types, by mining allelic profiles. The sequence type for each isolate was determined by matching the allelic profiles of known alleles deposited at the *S. aureus* MLST database from the MLST Database (Francisco *et al.,* 2009).

Illumina's integrated next generation sequencing instrument (Ravi *et al.,* 2018).

3.9.3 Molecular genotyping

DNA extracted using protocol provided by the manufacture (QIAGEN). SCC *mec* typing were performed using previously described methods (Kondo *et al*., 2007). Isolates with the *mec*A gene were classified as MRSA (IWG-SCC, 2009; Kondo *et al*., 2007). Panton-Valentine Leukocidin (PVL) gene detection was done by PCR using previously described oligonucleotide primers (Lina *et al.,* 1999).

DNA extraction from *S. aureus* isolates was performed on a QIAcube, using the QIAamp 96 HT kit (QIAGEN). Genomic libraries were constructed sequenced on an Illumina HiSeq 2000 (Illumina Inc.) at the Wellcome Sanger Institute, UK. Illumina reads were analysed based on the *S. aureus* MLST database (http://saureus.mlst.net), analysis of virulence and antimicrobial resistance genes were conducted using virulence Finder database (https://cge.cbs.dtu.dk/services/data.php).

3.9.4 Genomic analyses

The *S. aureus* species core-genome had been previously derived (Coll *et al*., 2020) from a collection of 800 *S. aureus* from multiple host species (Richardson *et al.,* 2018). The portion of the reference genome (2.83 Mb) corresponding to the core genome (1.76 Mb) was kept from whole-genome alignments and used to generate maximum likelihood trees using IQ-TREE v1.6.10 with default settings. The resulting core-genome phylogeny was plotted with isolate metadata using ggtree v.3.0.4 (Yu, 2020) and ggtreeExtra v.1.2.3 on R v4.1.0 (Xu *et al.*, 2021).

3.10 Data Managementand statistical analysis

All subjects were assigned a subject identification number (SID). Data were double entered into the study databases and were only associated with a SID in password protected files. All paper research records were kept at the Center for Microbiology Research (CMR)-KEMRI in a password protected, locked filing cabinet located in a restricted-access room. Qualitative data such as the prevalence of MRSA and MSSA, MDR, SCC*mec* types, PVL TST, drug resistance, were expressed as frequencies and percentages while differences of prevalence of MRSA was determined using Chi square analysis and Fisher's exact test (two tailed), as appropriate. This analysis was done using SPSS Version 20 (Elshimy *et al.,* (2018) IBM Corp. Released 2021). Using the information gathered about the *ccr* type and *mec* class, the *SCCmec* type was assigned according to IWG-SCC. The MLST final sequences were aligned and analyzed for completeness and accuracy using ClustalW2, which is a multiple sequence alignment tool for the alignment of DNA provided by EMBL-EBI, Wellcome Trust Genome Campus of UK. The sequence type for each isolate was determined from the MLST Database. Alleles that may be new were sent to the MLST Database curator for further identification and assignment (Jolley *et al.* 2018). Identification of clonal complexes and founder strains were based on allelic profiles and accomplished by eBURST analysis, based on principles originally described by Feil *et al.* (2004). Minimum spanning trees were created using PubMLST and BioNumerics software.

3.11 Ethical consideration

This study was conducted according to the Declaration of Helsinki and International Conference on Harmonization Guidelines on Good Clinical Practice (ICH-GCP). The protocol and informed consent form was reviewed and approved by the Centre for Microbiology Research Centre, Scientific Committee, Kenya Medical Research Institute Scientific Steering Committee and Ethical Review Committee. Ethical approval for animal subjects was issued by Ministry of Agriculture, Livestock and Fisheries and the Faculty of Veterinary Medicine Ethical Committee. Participation in the study was voluntary and participants could withdraw at any time even after accepting to participate. Initials and coded numbers were used to identify the participants' study reports to maintain confidentiality. All study records were maintained in a secured location.

4. RESULTS

4.1 Inpatients admission and empirical antibiotic therapy at the Busia Referral County Hospital

4.1.1 Busia Referral County Hospital monthly admissions

The monthly total admissions results during study period are presented in Table 2. The results indicate that a total 3744 persons were admitted to various wards during the entire study duration of study, from 21/05/2015 to 07/07/2015, with the Maternity ward having the highest admission of 1069 patients. Three additional wards were included in the study: Pediatric, Medical and Surgical wards, totaling to 2674 inpatients, with average number of 891 patients each. A total of 261 patients, with respect to the three wards, participated in the study; this constituted 10 % of the 2674 inpatients. One hundred and seventeen (45.0%) were admitted in the surgical ward, 91 (35.0 %) in Medical ward and 53 (20.0%) in Pediatric ward. Majority of inpatients (35.0 %) were of the age of 50 years and above, followed by age range 24-50 years (30.0%), 13-24 years (14%), 6-12 years (13%) and 1-5 (8%). Overall, 54.0 % were male and 46.0 % were female; recruitment consisted of patients that had been admitted at the hospital for 72 hours to 30 days, or more. In this study, empirical antibiotic treatment will refer to antibiotic prescription without or before knowing the causative pathogen and its susceptibility to antibiotics. Thirty one (31) of the inpatients were not on empirical antibiotic treatment, while the other 230 inpatients were receiving empirical treatment.

Table 2 : Busia Referral County Hospital monthly total inpatients admissions during study period.

Total number of inpatient admission per month during the study duration from 21/05/2015 to 07/07/2015						
WARDS	May	June	July	Total number of inpatient admitted during the study period Unit	Total	Ward bed capacity
Ward one (Paediatric)	245	357	309	911	911	32 Beds
Ward two (Medical- Male)	93	163	127	383	865	27 Beds
Ward three (Medical - Female)	124	186	173	483		30 Beds
Ward four (Surgical - Female)	164	158	174	496	898	31 Beds
Ward five (Surgical - Male)	135	136	131	402		21 Beds
Maternity	347	350	372	1069	1069	32 Beds

4.1.2 Descriptions of the study population stratified by antibiotic empirical therapy

To investigate the proportion of inpatients receiving empirical antibiotics therapy,admitted to various wards, the use of antibiotic empirical therapy was calculated. Table 3 lists the inpatients' antibiotic empirical treatment as recorded in Medical, Pediatric and Surgical wards, for the patients admitted to BCRH within 96 hours to 30 days or more, between the study duration of 21/05/2015 to 07/07/2015.

Cefotaxime was the most prescribed antibiotic used for empirical therapy antibiotic in inpatients at BCRH; it was prescribed at rates of 60.0%, 37.7% and 78% of inpatients in Pediatric, Medical and Surgical wards, respectively, while Metronidazole was the second-most prescribed at 23.3%, 22.6% and 76.3 in Pediatric, Medical and Surgical wards, respectively. These two, Cefotaxime and Metronidazole, were also administered to 61.9 % of patients admitted in surgical ward. The third-mostly prescribed antibiotic was Erythromycin; it was used to treat patients admitted in the medical wards, while Benzyl penicillin and Gentamicin were preferred empirical antibiotics used to treat children admitted in the pediatric wards, at 30.2 % and 15.1%, respectively. Other antibiotics used at these facilities for empirical treatment among study populations were: Ampicillin and Cloxacillin, administered in 21 inpatients, followed by Clindamycin on 3 inpatients; two other inpatients were being put on Amoxicillin and one on Doxycycline.

Table 3: Description of the inpatients' antibiotic empirical treatment as recorded in various wards in Busia Referral County Hospital

Ward Type	No in patients on empirical treatment	CTX	CIP	E	Met	Gent	BeZ pen	CTX / Met	BeZpen/ Gent	E/CTX
Medical Ward	90	54 (60.0%)	5(5.0%)	7(7.7%)	21(23.3%)	0(0%)	2(2.2%)	13(14.4%)	0(0%)	5(5.5%)
Pediatrics	53	20(37.7%)	1(1.9%)	0	12(22.6%)	8(15.1%)	16(30.2%)	10(18.9)	8(15.1)	0(0%)
Surgical Ward	**118**	92(78.0%)	0(0%)	0(0%)	90	0(76.3%)	1(0.85%)	73(61.9)	0(0%)	0(0%)

Cefotaxime CTX, Ciprofloxacin CIP, Erythromycin E, Gentamicin Gent, Metronidazole Met, Benzyl penicillin BeZPen and Tetracycline TE

4.2 Prevalance and Antibiotic susceptability profiles of *S. aureus* isolates in Busia County

4.2.1 Prevalance of *S. aureus* among inpatients at Busia Referral County Hospital

Nasal swab samples were collected from 261 patients at BCRH; samples were obtained from inpatients who had been admitted for 48 h or longer. Of these 29 (11.0%) were colonized with *S. aureus*. One patient was colonized with two different *S. aureus* sequence types briging the total number of isolate to 30 isolates.

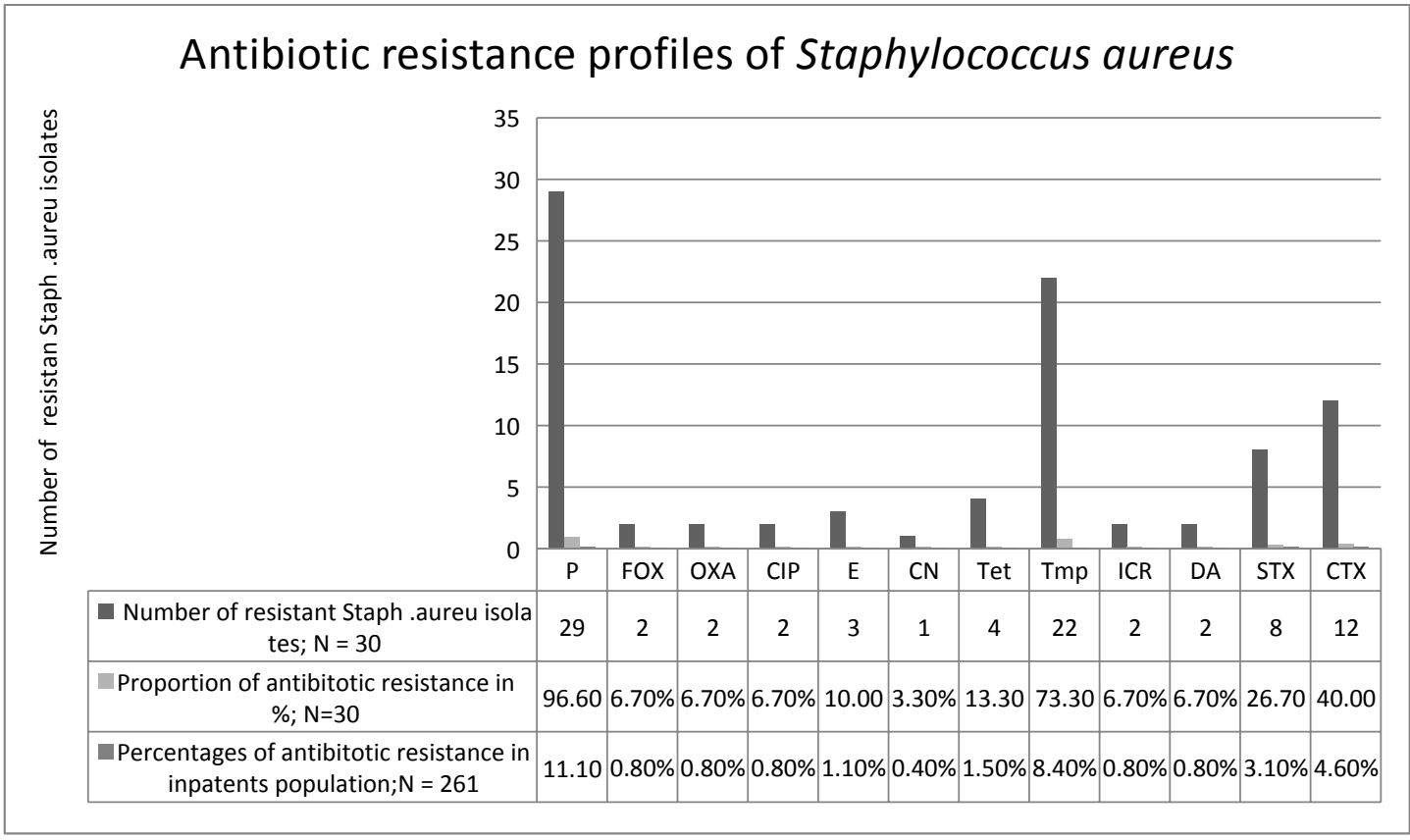
4.2.2 Antibiotic susceptabilty profiles of isolated from in patients at Busia Referral County Hospital

Staphylococcus aureus isolates from this study population were 100% susceptible to Chloramphenicol, Daptomycin, Fusidic Acid, Linezolid, Mupirocin, Nitrofurantoin, Rifampicin. MSSA isolates were mostly resistant to Penicillin (96.6%), TMP (73.3%), and Cefotaxime (40.0 %). The MRSA isolates were totally resistant to Clindamycin, Tetracycline, TMP/SXT, Erythromycin, Gentamicin and Ciprofloxacin. One MRSA and MSSA strains resistant to Ciprofloxacin also exhibited multi drug resistance to other antibiotics, constituting 6.7 % of all isolates; thus, 6.7% of the isolates exhibited multi drug resistance. Figure 3 and Table 4 give the antibiotic resistance patterns, with respect to the tested antibiotics. Two MSSA strains, isolated from two patients (2/261 = 0.8%; 2/30 = 6.7%) were found to be vancomycin resistant (results presented later), while none of the MRSA were vancomycin resistant.

Table 4 Antibiotic susceptability profiles of ***S. aureus*** isolates from inpatients at the Busia Referral County Hospital.

Antibitotics Tested	**P**	**FOX**	**OXA**	**CIP**	**E**	**CN**	**Tet**	**Tmp**	**ICR**	**DA**
Number of resistant *S.aureus* isolates (N = 30)	29	2	2	2	3	1	4	22	2	2
Percentage (%) of isolates resistant to antibitotic (N=30)	96.60% (29/30)	6.70% (2/30)	6.70% (2/30)	6.70% (2/30)	10.00% (3/30)	3.30% (1/30)	13.30% (4/30)	73.30% (22/30)	6.70% (2/30)	6.70% (2/30)
Percentages of antibitotic resistance inpatents population;N = 261	11.10% (29/261)	0.80% (2/261)	0.80% (2/261)	0.80% (2/261)	1.10% (3/261)	0.40% (1/261)	1.50% (4/261)	8.40% (22/261)	0.80% (2/261)	0.80% (2/261)

Penicillin (P), Cefoxitin (Fox),Oxacillin (OXA), Ciprofloxacin (CIP), Erythromycin (E), Gentamicin (Gent), Tetracycline (TET), Trimethoprim (Tmp), Inducible clindamycin resistance (ICR), Sulphamethoxazole and Trimethoprim (SXT), Clindamycin(DA) and Cefotaxime (CTX).



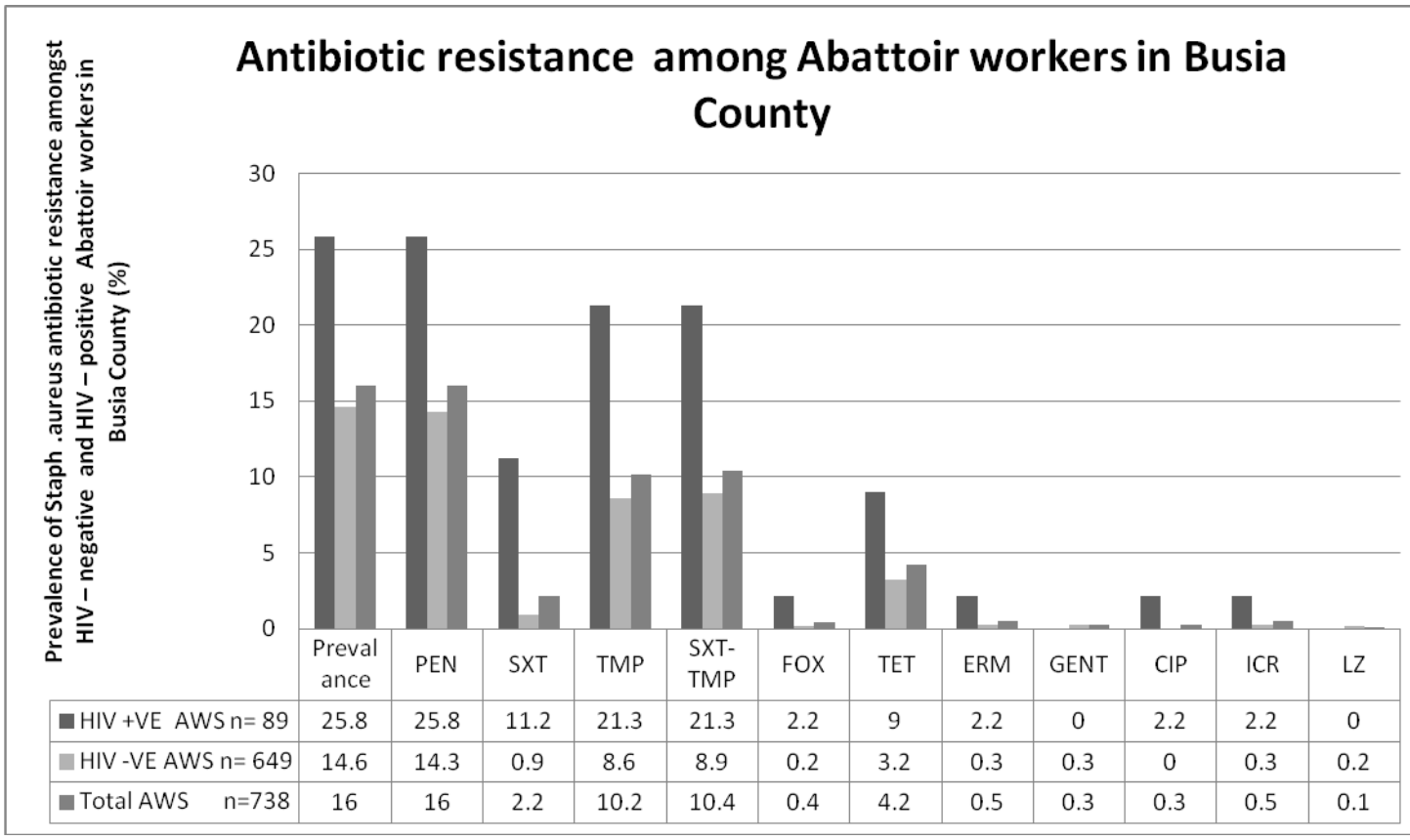
	P	FOX	OXA	CIP	E	CN	Tet	Tmp	ICR	DA	STX	CTX
Number of resistant Staph .aureu isolates; N = 30	29	2	2	2	3	1	4	22	2	2	8	12
Proportion of antibitotic resistance in %; N=30	96.60	6.70%	6.70%	6.70%	10.00	3.30%	13.30	73.30	6.70%	6.70%	26.70	40.00
Percentages of antibitotic resistance in inpatents population;N = 261	11.10	0.80%	0.80%	0.80%	1.10%	0.40%	1.50%	8.40%	0.80%	0.80%	3.10%	4.60%

Figure 3: Percentages of antibiotic resistant *S. aureus* isolates among inpatients admitted at Busia Referral County Hospital In a graphic form

Penicillin (P), Cefoxitin (Fox), Oxacillin (OXA), Ciprofloxacin (CIP), Erythromycin (E), Gentamicin (Gent), Tetracycline (TET), Trimethoprim (Tmp), Inducible clindamycin resistance (ICR), Sulphamethoxazole and Trimethoprim (SXT), Clindamycin(DA) and Cefotaxime (CTX).

4.2.3 Antibiotic resistance profiles of *S. aureus* isolated from HIV-positive and HIV-negative abattoir workers in Busia County

Out of the total number of tested *S. aureus* isolates, Linezolid resistance was at 0.8 %, Gentamicin resistance 1.7 %, Clindamycin resistance 3.4% and Ciprofloxacin resistance 1.7 %; these were the most effective antibiotics, to which over 90 % isolates were susceptible. The other resistances recorded were: 0.4 % to (MRSA);100 % to Penicillin-G; 65.3 %, to Trimethoprim, 26.3 % to Tetracycline, 13.5 % to SXT; as shown in Figure 4. Percent carriage of Penicillin-G, Cefoxitin, Trimethoprim and SXT resistant *S. aureus* isolates were significantly higher in HIV - positive abattoir workers compared to HIV – negative abattoir workers. All isolates were susceptible to Chloramphenicol, Daptomycin, Fusidic Acid, Nitrofurantoin, Mupirocin, Rifampicin, Teicoplanin and Vancomycin. HIV - positive abattoir workers were significantly colonized by *S. aureus* strains resistant to penicillin ($p < 0.006837$), Trimethoprim ($p < 0.000328$), Tetracycline ($p < 0.016338$) and higher phenotypic resistance to SXT ($p < 0.00001$).



	Prevalance	PEN	SXT	TMP	SXT-TMP	FOX	TET	ERM	GENT	CIP	ICR	LZ
HIV +VE AWS n= 89	25.8	25.8	11.2	21.3	21.3	2.2	9	2.2	0	2.2	2.2	0
HIV -VE AWS n= 649	14.6	14.3	0.9	8.6	8.9	0.2	3.2	0.3	0.3	0	0.3	0.2
Total AWS n=738	16	16	2.2	10.2	10.4	0.4	4.2	0.5	0.3	0.3	0.5	0.1

Figure 4 percentages of *S. aureus* antibiotic resistance amongst HIV-negative and HIV- positive Abattoir workers in Busia County

Penicillin(PEN), Trimethoprim/sulfamethoxazole(SXT), Trimethoprim(TMP), Cefoxitin (FOX), Tetracycline(TET), Erythromycin (E), Gentamycin (Gent), Ciprofloxacin (CIP), Inducible Clindamycin resistance (ICR), Linezolid(LZ) and Abattoir workers (AWS):

Foot note: Calculated as a percentage of resistant strains divided by the number of study subjects i.eHIV-negative and HIV- positive Abattoir workersin Busia County

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4.2.4 Resistance of *S. aureus* nasopharyngeal isolates to Linezolid. Teicoplanin, Vancomycin in Busia County

Two *S. aureus* strains belonging to sequence types ST25 and ST580 were recovered from patients admitted at Busia County Hospital, showing phenotypic resistance to Vancomycin; while a *S. aureus* ST707 strain, isolated from an abattoir worker from one of Busia Slaughterhouse showed resistance to Linezolid (Table 5). These results show that there is emergence of vancomycin, teicoplanin and linezolid resistance to MRSA, MSSA in Busia County in different settings such as inpatients, abattoir workers and livestock, though occurrences of these strains are still low.

Table 5 Linezolid, Teicoplanin and Vancomycin resistant *S. aureus* nasopharyngeal strains from Busia county.

Targeted Populations	Organism	ST	TRM RG	ERY RG	ERY PR	GENT RG	LIN PR	TEI PR	VAN PR
AW	*S. aureus*	707					R >=8 MIC-COP 2	S	S
BCRH	*S. aureus*	25	*dfrG*	*ermC*	R		S	S	R>=32 MIC-COP <=0.5
BCRH	*S. aureus*	580					S	S	R>=32 MIC-COP <=0.5
L	*S. aureus*	N/A					S	R 4 MIC-COP <=0.5	S

AW: Abattoir workers, BCRH: Busia Referral County Hospital, L: Livestock PR: Phenotypic resistance, RG: Resistance genes, ST Sequence type, N/A: Not applicable, *mecA*. TRM: Trimethoprim, E: Erythromycin, GENT: Gentamicin, LIN: Linezolid, TEI: Teicoplanin, VAN Vancomycin. MIC-COP: Minimum Inhibitory Concentration and Susceptible Cut-off Points.

4.2. Association of *Staphylococcus aureus* sulfamethoxazole /trimethoprim resistant isolates and with sequence types in HIV - positive and HIV – negative abattoir workers population

Overall, HIV-positive abattoir workers had significantly higher percentage (12.4%) of *S. aureus* isolates which were phenotypically resistant to SXT than HIV-negative abattoir workers (0.8%) ($p < 00001$), as presented in Figure 5. The same was the case for *S. aureus* isolate ST8 (10.1% for HIV-positive abattoir workers; 0.5% for HIV-negative ones) ($p < 00001$).Two ST 25 strains isolated from HIV-positive abattoir workers were resistant to SXT at 2.2% (2/89) while ST 25 strains isolated from HIV-negative workers were susceptible. Two sequence types ST80 and ST152 isolates isolated from HIV-negative abattoir workers were resistant to SXT, at a rate of 0.2% (1/649) respectively, while isolates of the same sequence types isolated from HIV-positive workers were susceptible to SXT; a single ST152 strain Isolated from an HIV-negative abattoir workers was found to be resistant to SXT, this strain also harbored a PVL gene.

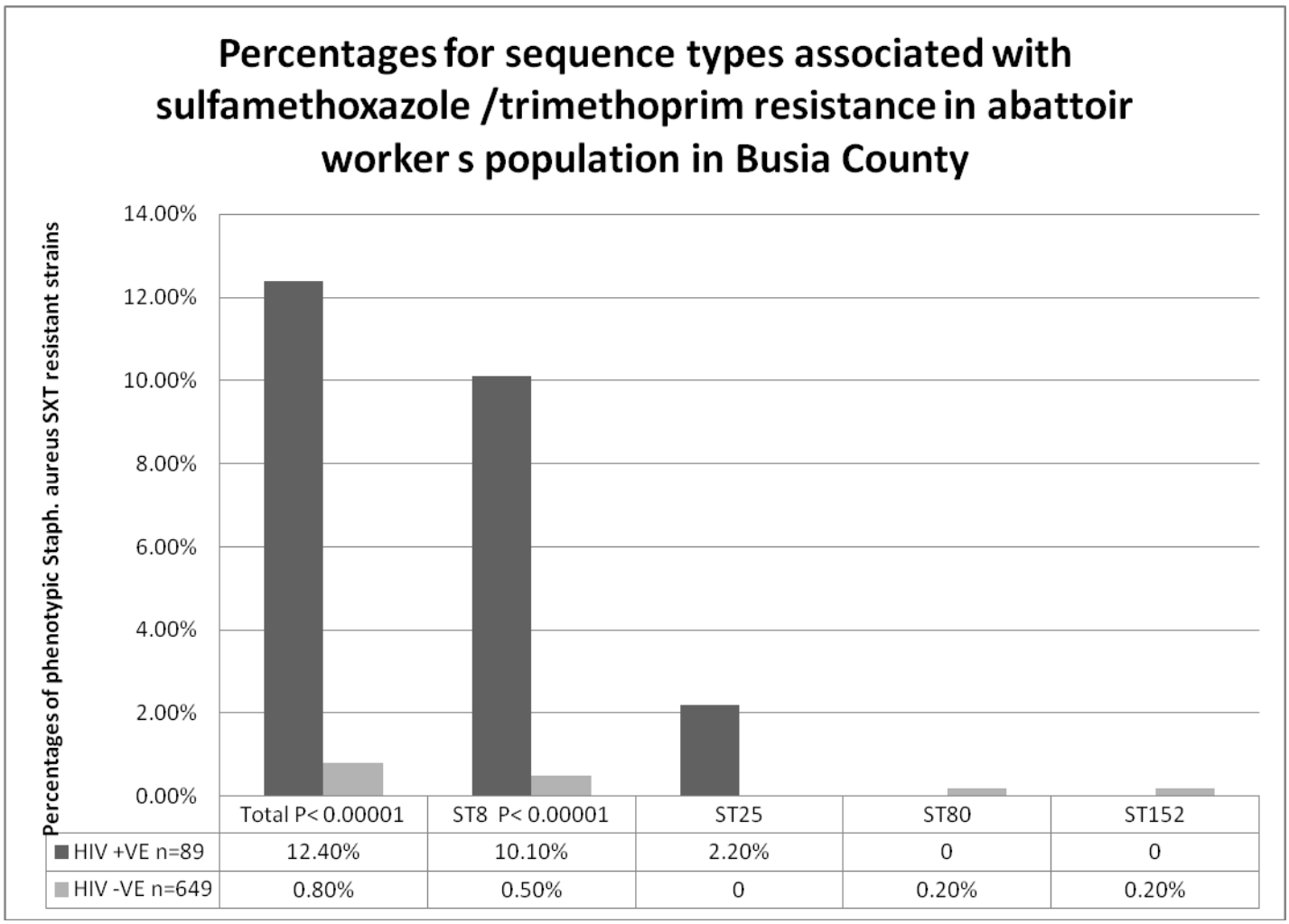

Figure 5: Association of *S. aureus* isolates in relation to SXT resistance and associated STs among HIV-positive and HIV- negative abattoir workers.

Footnote: ST = sequence type

4.3 Multi drug resistance patterns of *Staphylococcus aureus* isolates in Busia County

4.3.1 Multi drug resistance patterns of *Staphylococcus aureus* isolates obtained from nares of patients admitted at Busia Referral County Hospital

Pie chart (Figure 6), gives the graphical representation of the multi-drug-resistant antibiotic (MDR) patterns of MSSA and MRSA nasopharyngeal isolates recovered from the 30 inpatients out of 261 study patients admitted at BCRH. Thirty one percent (31%) of the *S. aureus* isolates were resistant to Penicillin- Cefotaxime - Sulphamethoxazole and Trimethoprim – Trimethoprim alone as indicated in the pie chart below (Figure 6). Highly resistant MDR *S. aureus* were isolated from patients admitted at the BCRH; there was one MRSA that was resistant to 11 antibiotics: Penicillin-Cefoxitin -Oxacillin (OXA)-Cefotaxime-Ciprofloxacin-Erythromycin –Gentamicin-Tetracycline (TET)-Trimethoprim -Inducible clindamycin resistance -Sulphamethoxazole and Trimethoprim (P-FOX-OXA -CTX-CIP-E-CN-Tet-Tmp-SXT-ICR) and one MSSA strain that was resistant to 9, but of combination: Penicillin-Cefotaxime-Ciprofloxacin-Erythromycin, Sulphamethoxazole and Trimethoprim –Trimethoprim alone- Vancomycin -Inducible clindamycin resistance –Clindamycin(P, CTX, CIP, E, Tmp, SXT, Van, ICR Da).One strain was intermediately resistant to Vancomycin, showing a potently emerging Vancomycin *S. aureus* resistance in BCRH.

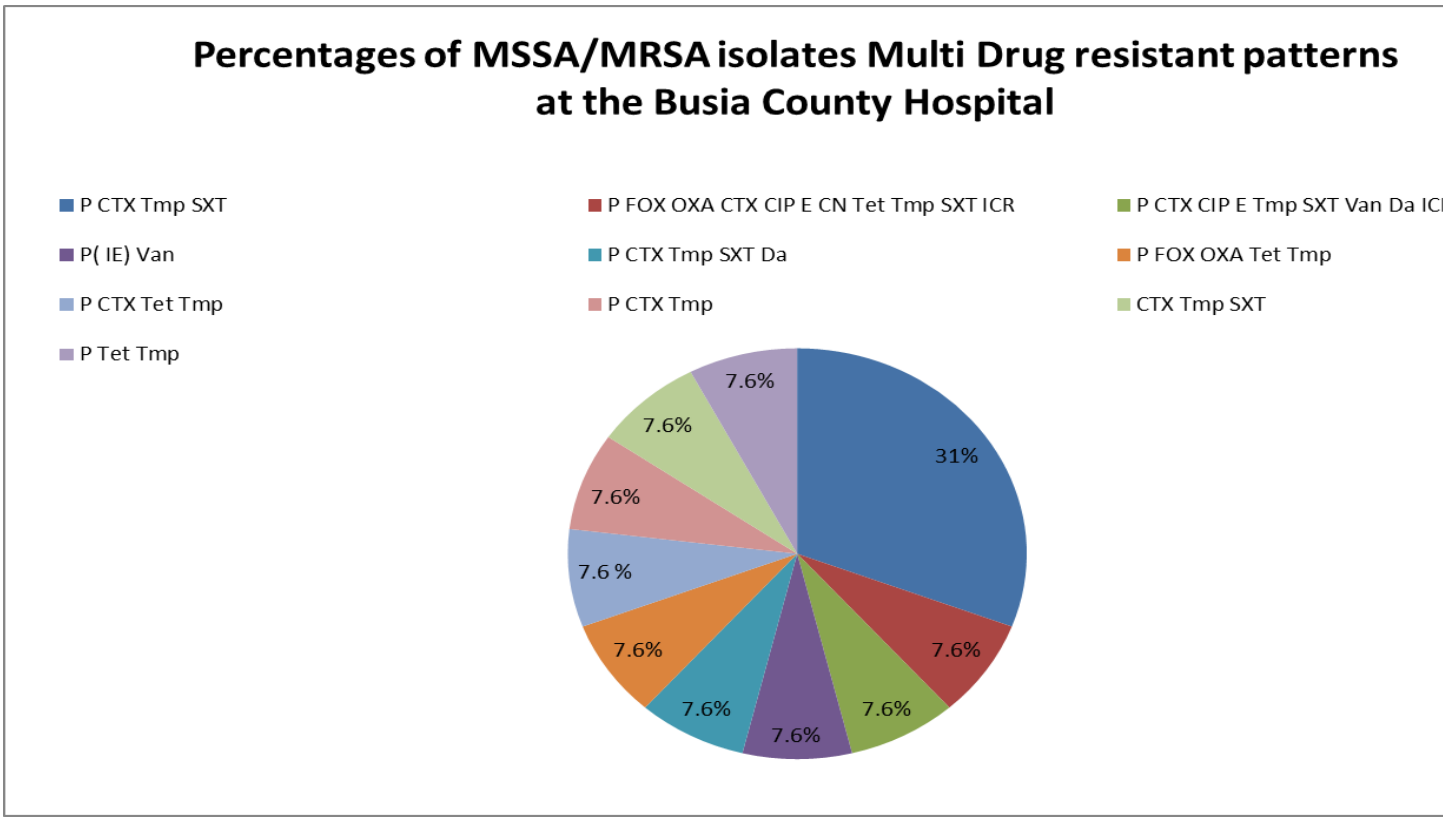

Figure 6: Percentage multi drug resistance patterns (antbiograms) of *Staphylococcus aureus* isolated from patients admitted at Busia Referral County Hospital

Penicillin (P), Cefoxitin (Fox), Oxacillin (OXA), Ciprofloxacin (CIP), Erythromycin (E), Gentamicin (Gent), Tetracycline (TET), Trimethoprim (Tmp), Inducible clindamycin resistance (ICR), Sulphamethoxazole and Trimethoprim (SXT), Clindamycin(DA) and Cefotaxime (CTX).

4.3.2: Multi drug resistance patterns of *Staphylococcus aureus* isolates recovered from HIV - positive and HIV – negative abattoir workers in Busia County

Prevalence of multi drug resistance *S. aureus* (MRSA and MSSA) was determined in HIV positive and negative abattoir worker study populations. Multi-drug resistant (MDR) *S. aureus* were defined as isolates that were resistant to three or more classes of antibiotics (Magiorakos *et al*., 2012). As recorded in Table 6, the commonest MDR resistant pattern was SXT/TmpPenTet, and it constituted 65% of all MDR strains isolated, accounting for a fifth of all *S. aureus* isolates in this study, at prevalence of 3.7% among abattoir worker study populations.HIV-positive abattoir workers were significantly colonized by MDR *S. aureus* exhibiting resistance to SXT/TmpPenTet pattern compared to HIV-negative abattoir worker population ($p < 0$ .004288). Likewise, HIV-positive abattoir worker population significantly carried more MDR- *S. aureus* isolates compared to HIV-negative ones ($p< 0.000418$); two resistant MDR *S. aureus* were isolated from HIV-positive abattoir workers; they had resistance combination patterns Penicillin-Erythromycin-Trimethoprim alone-Inducible clindamycin resistance -Sulphamethoxazole and Trimethoprim(PETmpICRSXT),and Penicillin- Ciprofloxacin - Erythromycin-Trimethoprim alone-Inducible clindamycin resistance -Sulphamethoxazole and Trimethoprim (PCIPETmpICRSXT). One MDR *S. aureus* resistant to Penicillin- Erythromycin- Gentamicin Trimethoprim alone-Inducible clindamycin resistance (PETmpGentICR) was isolated from an HIV –negative abattoir worker.

Table 6 The proportion and percentages of MDR Staph .aureus among all *S.aureus* isolates in HIV-negative and HIV- positive

No of MDR isolates	**Multidrug-resistance pattern**	**HIV- positive abattoir workers group** **Proportion of MDR *S. aureus* n=23(%)** **Percentages of MDR *S .aureus* n=89**	**HIV- negative abattoir workers group** **Proportion of MDR *S .aureus* n=95(%)** **Percentage of MDR *S .aureus* n=649**	**P values p < .05**
27	TmpPenTet (21) SXTTmpPenTet(4) MRSA-TmpPenFOXTet(2)	Proportion: 8/23 (34.9%) Percentage: 8/89(9.0 %)	Proportion: 19/95 (20.0%) Percentage: 19/649 (2.9%)	**p < 0 .004288**
2	SXTTmpPenErmICR(2)	Proportion: 1/23 (4.3%) Percentages: 1/89(1.1%)	Proportion: 1/95 (1.1%) Percentage: 1/649 (0.2%)	
1	TmpPenErmGentICR(1)	0	Proportion: 1/95 (1.1%) Percentage: 1/649 (0.2%)	
1	SXTTmpPenErmCIPICR, 1 isolates	Proportion: 1/23 (4.3%) Prevalence: 1/89(1.1%)	0	
31/738(4.2)	**Total MDR**	**Proportion: 10/23 (38.5%)** **Percentage: 10/89(11.2)**	**Proportion: 21/95(21%)** **Percentage: 21/649 (3.2%)**	***p*< 0.000418.**

Foot notes; Percentage of MDR *S .aureus* was calculated as a number of MDR *S .aureus* strains isolated divided by the number of study subject's i.e. HIV-negative and HIV- positive Abattoir workersin Busia County.
Proportion of MDR *S .aureus* was calculated as a number of MDR *S .aureus* strains isolated divided by total number of *S .aureus* isolates in each study population.Subjects.

4.4 Prevalence of Methicillin-resistant *Staphylococcus aureus* and Methicillin sensitive *Staphylococcus aureus* in Busia County

4.4.1 Prevalence of Methicillin-resistant *Staphylococcus aureus* and Methicillin sensitive *Staphylococcus aureus* amongpatients admitted at Busia Referral County Hospital

Thirty inpatients were colonized with *S. aureus* (11.5%); of these *S. aureus* isolates, 93.3 % (28/30) were MSSA while 6.7 % (2/30) were MRSA. These isolates were recovered from the following wards as follows: Surgical ward 12/30 (40%), Medical ward 10/30 (33%) and Pediatric ward 8/30 (27%).

4.4.2 Prevalence of Methicillin-resistant *Staphylococcus aureus* and Methicillin sensitive *Staphylococcus aureus* among HIV-positive and HIV-negative abattoir workers in Busia County

In this study, prevalence was calculated as the proportion of abattoir workers colonized with both or either of MRSA and MSSA. *Staphylococcus aureus* organisms were isolated from 16.0 % of 738 abattoir workers (118 of 738 samples). Of the 89 HIV-positive patients tested, 23 (25.8%) were positive for S. *aureus*; 2 (2.2 %) were MRSA and 21(23.6 %) were MSSA. Of the 649 HIV-negative patients tested, 1 MRSA strain (0.2 %) and 94 (14.5 %) MSSA isolates were recovered; a low prevalence of MRSA among of abattoir workers population was 0.4% (3/738) observed, compared to respective prevalence of MSSA which was15.6 % (Table 7). Prevalence of MSSA was, therefore, higher than MRSA amongst abattoir workers in Busia County, Kenya. There were 3 HIV positive abattoir workers who were co-infected by MSSA strains raising the total isolates to 26 and 5 HIV negative abattoir workers who were co-infected by MSSA raising the total isolates to100; these 8 strains were not included in prevalence calculation, to avoid double counting of an individual abattoir worker

Table 7 **Prevalence of MSSA and MRSA isolated from Abattoir workers in Busia County**

HIV - status	Number of abattoir workers	Prevalence of *S. aureus*	MRSA Prevalence	MSSA Prevalence
HIV positive (n=89)	89 (12.1 %)	23 (25.8%)	2 (2.2 %)	21(23.6 %)
HIV negative (n=649)	649(87.9 %)	95 (14.6%)	1 (0.2 %)	94 (14.5 %)
Total abattoir workers (n=738)	738 (100%)	118 (16.0%)	3 (0.4 %)	115 (15.6 %)

4.4.3 Analysis of Inpatients rearing various livestock in their households

To elicit possible animal contact for application of one health approach, inpatients were interviewed regarding contact with animals. One hundred and thirty four (134) inpatients out of the 261 (i.e. 51.3%) owned livestock; some owned more than one type of livestock. Total number of livestock collectively owned by inpatients was 163; majority rearing goats and cows, at 49 % and 41% respectively (Table 8 **);** However, when genotyped (results given later), none of the hospitalized patients were found to be colonized with LA-MRSA/LA-MSSA strains.

Table 8 Inpatients rearing various livestock

Keeping Cattle	Keeping Goats	Keeping Pigs	Keeping Sheep	Total number of livestock owned by of inpatients
N = 66(41.0%)	N = 80(49.0%)	N =12(7.0%)	N =5 3.0%	N=163

4.4.4 Presentation of *Staphylococcus aureus* isolates recovered from livestock's nasopharyngeal samples in Busia County

The findings show the presence of *S. aureus* isolates in different livestock, which were recovered more from pigs and cattle as compared to sheep and goats (Table 9). This was expected since the slaughterhouses handled more cattle and pigs than sheep and goats.

Table 9: Methicillin-resistant *Staphylococcus aureus* and Methicillin-sensitive *Staphylococcus aureus* MSSA isolated from animal nasopharyngeal samples in Busia County

	MRSAisolations: Number (%)of respective population	**MSSA isolations: Number (%)of respective population**	**Total *S. aureus* isolations: Number (%)of respective population**
Cattle (n=48)	2 (4.2%)	12 (25.0%)	14 (29.2%)
Sheep (n=6)	1 (16.7%)	3 (50.0%)	4 (66.7%)
Goats (n=9)	1 (11.1%)	2 (22.2%)	3 (33.3%)
Pigs (n=40)	**3 (7.5%)**	**14 (35.0%)**	**17 (42.5%)**
Total animal samples (n=103)	7 (6.8%)	31 (30.1%)	38 (36.9%)

MRSA = Methicillin-resistant *Staphylococcus aureus*
MSSA = Methicillin-sensitive *Staphylococcus aureus*
n = means number

4.5 Distribution of *pvl, tsst-1, mec-A* and *sasX* genes in isolates and related sequence types, from inpatients and abattoir workers in Busia.

4.5.1 Panton Valentine Leukocidin gene (*pvl*) and Toxic shock syndrome toxin-1 (TSST-1) genes identified among *S. aureus* isolates recovered from inpatients Busia Referral County Hospital

To determine MLST STs of MRSA and MSSA strains isolated from patients admitted at BCRH, MLST typing was conducted. Eleven MLST STs were identified from the 30 isolates, the sequence types were: ST1633, ST22, ST152, ST188, ST80, ST8, ST241 variant, ST140, ST580, ST508 variant, ST25, ST573 and ST5, as presented in Figure 7. Types ST241 variant and ST140 were MRSA; ST241 being recorded as a variant (alleles were slightly different from the original ST241). The rest were MSSAs; ST508 being recorded as a new sequence (alleles were slightly different from the original ST508). Sequence types are clustered into clonal complexes by their similarity to a central allelic profile (genotype), central genotypes are classified by heuristic means, BURST and split decomposition, together with information of sequenced housekeeping genes and allelic profiles from public health laboratories and epidemiologists (Liao *et al*. 2006; Feil *et al*. 2004). Sequence types ST580, ST508 variant, ST25, ST573 and ST5 were clustered into 8 clonal complexes: CC125, CC22, CC1, CC80, CC8, CC508, CC25 and CC5. This study also investigated the presence of Panton Valentine Leukocidin (PVL) gene, which is associated with both skin and soft tissue infection and probably necrotizing pneumonia among inpatients admitted in BCRH; and Toxic shock syndrome toxin-1 (TST gene) through PCR testing. The PVL gene was detected in 6 isolates, constituting 20 % of all the isolates(prevalence of 2.3 % of the study population of 261 patients); four ST152 strains (13% of isolates and 1.5 % of the study population of 261 patients), one ST1633 and one ST5.

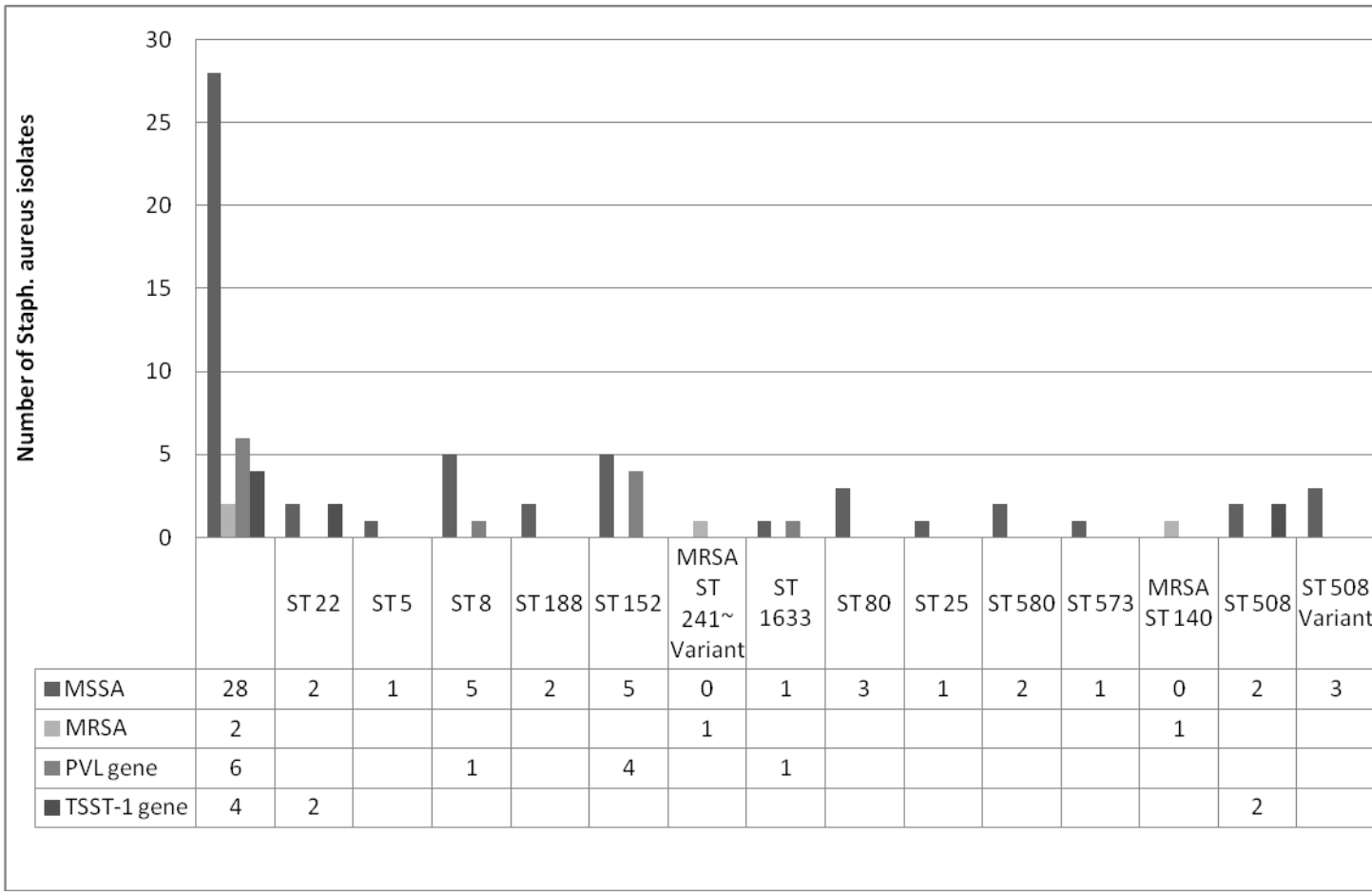

Figure 7 Breakdown of *S. aureus* sequence type isolates hosting PVL and TSST-1 genes, isolated from patients admitted at Busia Referral County Hospital

4.5.2 Panton Valentine Leukocidin gene (PVL) and Toxic shock syndrome toxin-1 (TSST-1) genes identified among *S. aureus* isolates recovered from Abattoir workers in Busia County

Multilocus sequence typing (MLST) was used to identify *S. aureus* sequence types (STs), in addition prevalence of PVL and toxic shock syndrome toxin-1 (TSST-1) genes carriage were determimed in the abattoir workers population. The data was then further analyzed to ascertain whether the two virulence factors were associated with particular sequence types or were associated with state of HIV-AIDS infection. Figures 8 and 9 show the prevalence of *S. aureus* sequence types harboring PVL and TSST-1 (*tst*) genes. Forty three percent (43 %) of the tested isolates carried were of Panton Valentine Leukocidin (PVL) gene, most of them belonging to ST152. All of the seven ST152 strains isolated from HIV-positive abattoir workers carried PVL genes; accounting for 7.9%; these strains did not carry the *tst* gene. The other sequence type that harbored PVL genes was a single ST30 strain; HIV-positive abattoir workers had a prevalence of 9.0% PVL gene carriage, a slightly higher prevalence compared to HIV - negative abattoir workers (7.1%); this difference was not statistically significant ($p > 0.518418$). Other strains harboring PVL genes were ST1633, ST30, ST22, ST80, ST2430 and ST1. Toxic shock syndrome toxin-1 gene was carried by all ST72 strains; other STs carrying this virulence toxin were ST707 and ST22. The prevalence of *S. aureus* toxic shock syndrome toxin-1 gene carriage in HIV-negative abattoir workers was 1.4%.

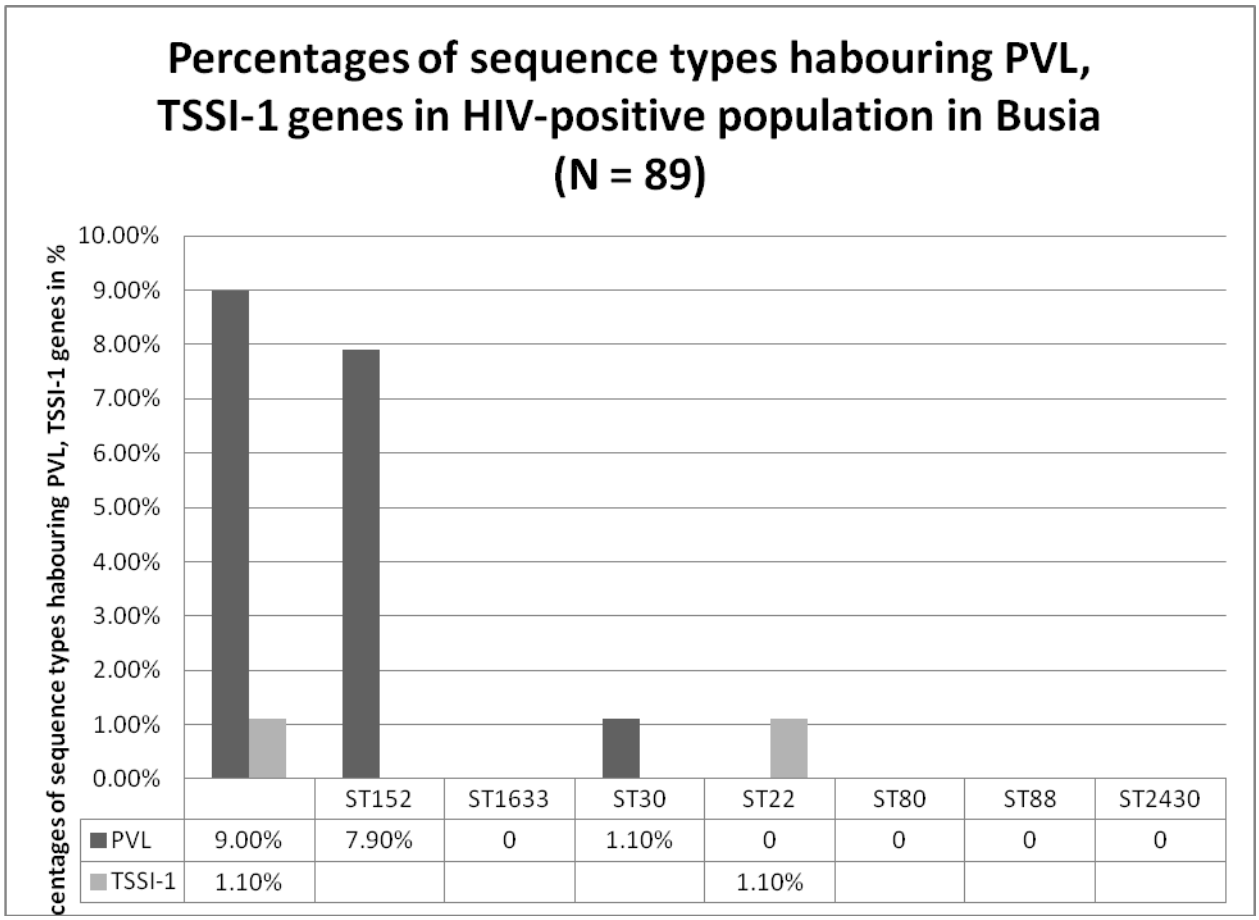

Figure 8 Percentages of *S. aureus* sequence types, Panton Valentine Leukocidin gene and Toxic shock syndrome toxin-1 (tst gene) in HIV-positive abattoir in Busia

Footnote: ST; Sequence type, PVL; Panton Valentine Leukocidin gene and TSSI-1; Toxic shock syndrome toxin-1

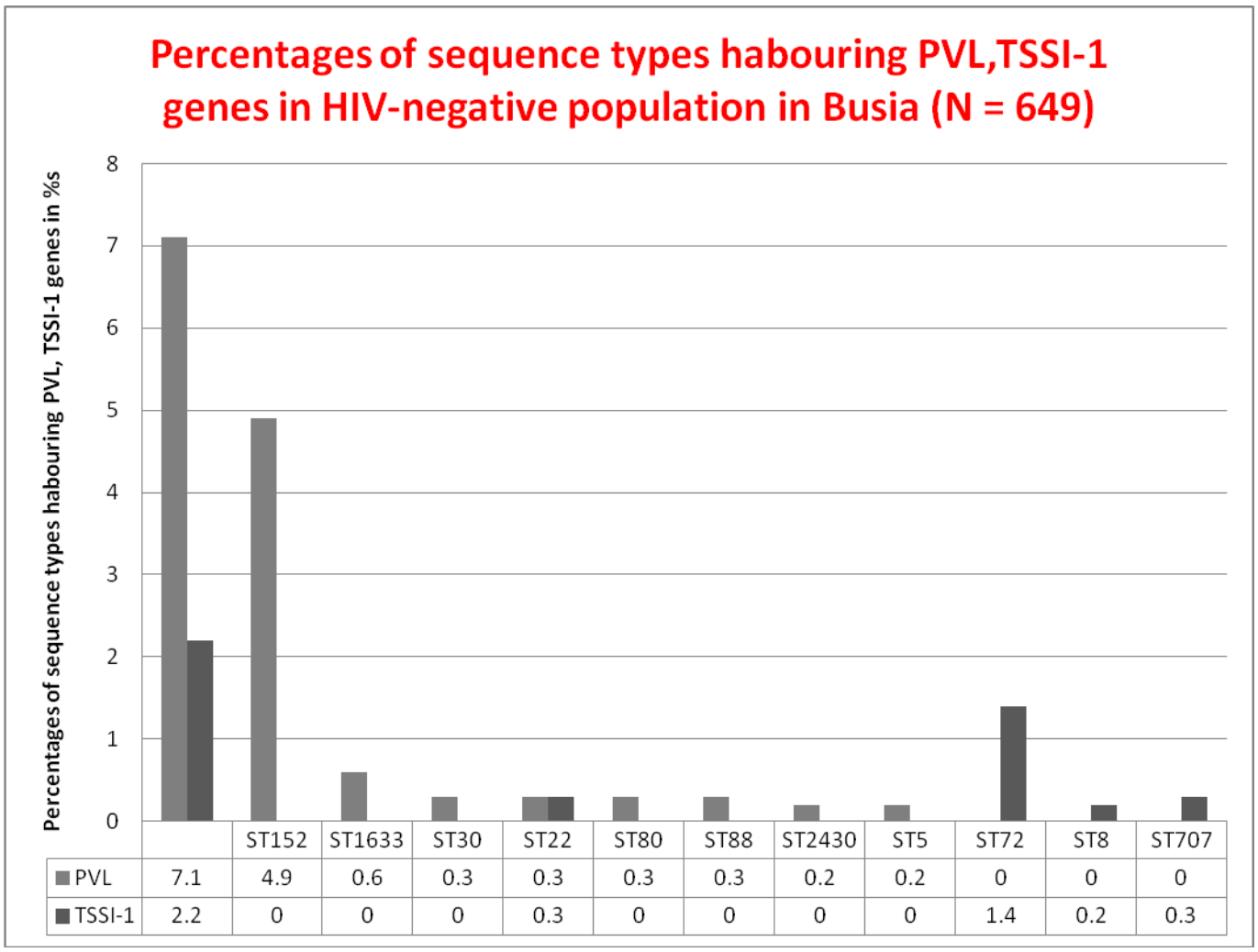

Figure 9 Percentages of sequence types, Panton Valentine Leukocidin gene and Toxic shock syndrome toxin-1 (tst gene) in *Staph. aureus* isolates recovered from HIV-negative population in Busia

Footnote: ST; Sequence type, PVL; Panton Valentine Leukocidin gene and TSSI-1;Toxic shock syndrome toxin-1

4.5.3 Differentt alleles found in ST508 strains and a new ~ST508 sequence type allele and associated virulence factors/genes

Staphylococcus aureus carriage of *tst* gene in isolates recovered from HIV-negative population abattoir workers in Busia was detected using PCR. Toxic shock syndrome toxin-1 (*tst* gene) was detected from 7 isolates, 2 strains belonging to ST508 and 3 strains of a new ~ST508 sequence type which is closely related to ST508 type. These strains differed only at a single allele of one the housekeeping genes (loci) coding for Acetyl coenzyme A

acetyltransferase {*yqil*} (Enright *et al*., 2000); they shared a similar antibiotic resistance pattern of being susceptible to all antibiotics with exception of Benzyl penicillin; they both also belonged to cluster CC508 (Table 10). The other sequence types, harboring TST gene, were in 2 strains of ST22 as indicated in Table 13.

Table 10: Different alleles found in ST508 strains and new variant ST508

						MLST alleles (~ indicates closest matching allele)*						
Strain name	**Sequence ID**	**Species**	**ST**	**CC**	**ST (**	**arcC**	**aroE**	**glpF)**	**gmk**	**pta**	**tpi**	**YqiL**
BH53	**20957_6#44**	***S. aureus***	**508~**	**CC508**	**New ST**	**10**	**40**	**8**	**6**	**10**	**3**	**211**
BH44	**20957_6#39**	***S. aureus***	**508**		**Known Assigned ST**	**10**	**40**	**8**	**6**	**10**	**3**	**2**

* Larse *et al*. (2012)

4.6 Genetic diversity of *Staphylococcus aureus* sequence types in Busia County

4.6.1 *Staphylococcus aureus* sequence types identified in Busia County

4.6.1.1 *Staphylococcus aureus* sequence types identified in patients admitted at Busia Referral County Hospital

To understanding the *S. aureus* clones circulating in BCRH; it was important to conduct molecular typing *SCCmec* typing, MLST and whole genome sequencing (Montelongo *et al*., 2022), translating this data into related sequence types and clonal complexes (Manara *et al*., 2018).

Their determination was done via eBURST program based upon related sequence types and the whole genome sequencing analysis, to identify the antibiotic resistance gene; phenotypic confirmation was done through antibiotic susceptibility technique. In this study ST 241, a single-locus variant of ST239, was isolated from a nasal swab of a child under five years of age. This variant normally carries *SCCmec* type III that exhibits multiple drug resistance to Ciprofloxacin, Clindamycin, Trimethoprim/Sulfamethoxazole, Erythromycin (*ermA* gene), Gentamicin (*aacA-aphD* gene), Tetracycline (*Tet M*, *Tet K* genes) and Streptomycin/spectinomycin (*aad 9* gene). In addition this strain harbored a mobile genetic element–encoded gene *sasX* which is involved in MRSA colonization and pathogenesis (Li *et al*., 2012). The other sequence type portrayed by the isolated MRSA strain belongs to ST 140 which normally harbors *SCCmec* type IV and is usually found in the community; it is also resistant to Tetracycline mediated by *tet M* gene (Table 11) and have been reported in Sweden. All the new STs isolated in this study had been submitted to the database so that they may be assigned as new types as indicated in Table 14.

Table 11 Comparison of MRSA new variant sequence type isolates from patients admitted at Busia Referral County Hospital with other known sequence types

							MLST alleles (~ indicates closest matching allele)						
Strain name	Sequence ID	Species	ST	CC	SCC mec types	New ST	*arcC*	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*
BH16	20957_6#26	*S. aureus*	241~	CC8		New ST	2	3	1~	1	4	4	30
97T-013	Sweden 1997	*S. aureus*	241		III Hospital		2	3	1	1	4	4	30
BH38	20957_6#37	*S. aureus*	140		IV Community		43	37	48	19	49	26	39
95T-049	Sweden 1995	*S. aureus*	140				43	37	48	19	49	26	39

4.6.1.2 *Staphylococcus aureus* sequence types identified in Abattoir workers in Busia County

A pie chart of 102 MSSA isolates and one MRSA strains and corresponding 24 distinct sequence types was constructed, it showed a high clonal diversity among MSSA isolates in the HIV-negative abattoir workers, as indicated in Figure 10. The most predominantsequence types among MSSA isolates were ST152 (48.0%), ST72 (12%) and ST8 (10%) in descending order.One *S. aureus* isolate, designated as MRSA ST88, because it was resistant to cefoxitin and harbored *mec*A gene positive, SCC*mec* typing assigned the strain to. *SCCmec* type IV (2B) group.

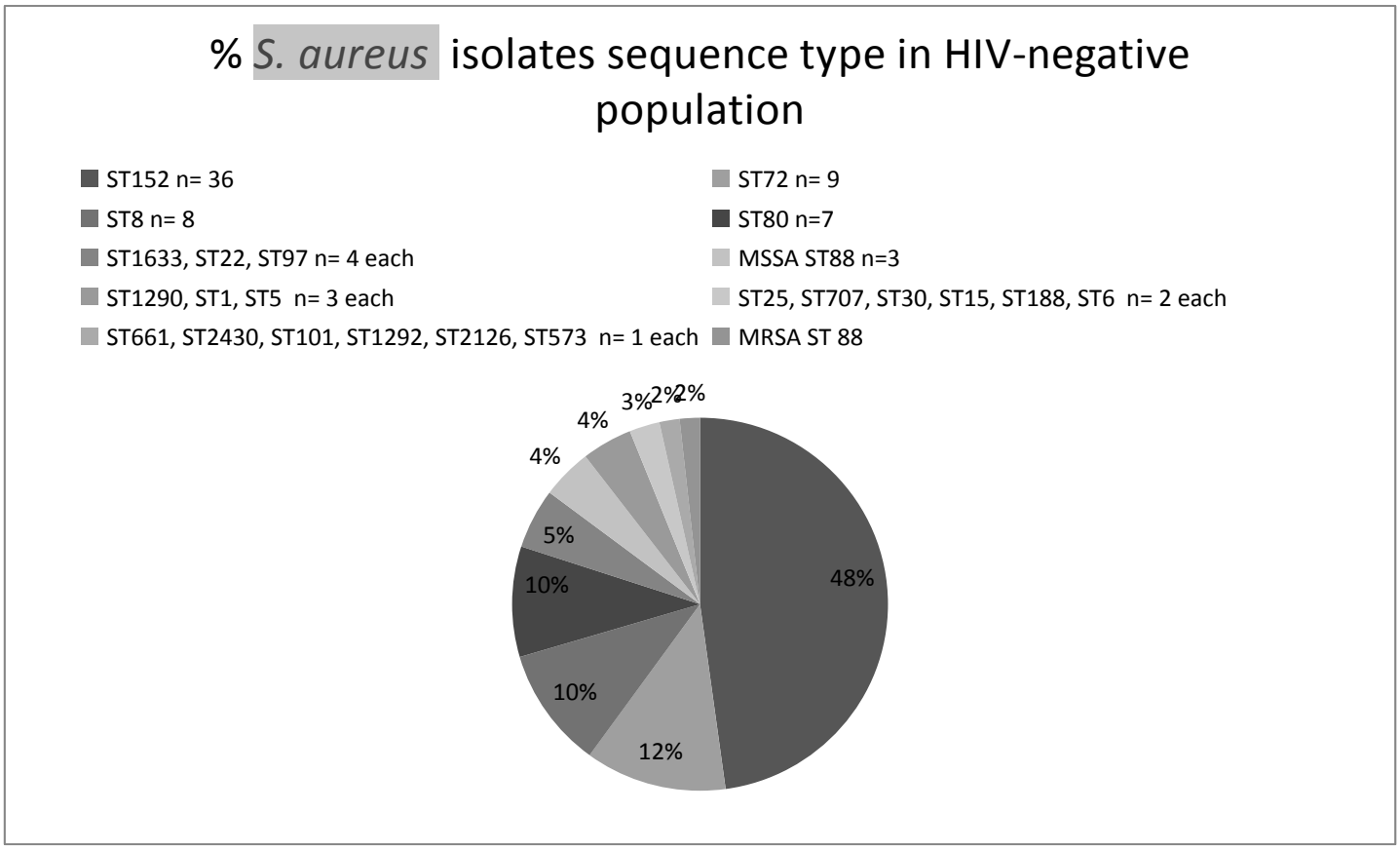

Figure 10 Pie charts for showing percentages of *S. aureus* sequence types in the HIV-negative abattoir workers population

4.6.1.3 *Staphylococcus aureus* sequence types shared between abattoir workers, inpatients and livestock in Busia County

A new ST ST1925~-MSSA variant was found in pigs, goats and cattle but no transmission to humans was evident, despite contact with humans (Table 12). In contrast, human-associated *S. aureus* sequence types ST152, ST8, ST1290 and ST30 were detected in livestock reared in Busia County, pointing toward a human-to-livestock transmission in Busia County (Table 15).

Table 12 Methicillin-sensitive *Staphylococcus aureus* sequence types shared between Abattoir workers, inpatients and livestock in Busia County

Study Population	ST152-MSSA	ST8-MSSA	ST1290-MSSA	ST30-MSSA	ST1925-MSSA ~
HIV-VE (AW) no = 649	ST152 no = 36	ST8 no = 8	ST1290 no = 3	ST30 no = 2	
HIV+ VE(AW) no = 89	ST152 no = 7	ST8 no = 11		ST30 no = 1	
BCRH no = 261	ST 152 no = 5	ST 8 no = 5			
Pig no = 40	ST152 no = 1			ST30 no = 3	ST1925~ no = 1
Goats no = 9				ST30 no = 1	ST1925~ no = 1
Cattle no = 48	ST152 no = 1		ST1290 no = 1	ST30 no = 1	ST1925~ no = 2
sheep no = 6		ST8 no =1			

AW: Abattoir workers, BCRH: Busia Referral County Hospital, MSSA; Methicillin-sensitive *Staphylococcus aureus* ST; Sequence type no, Number of isolates

4.6.1.4 Novel clonal complex and new sequence type variant *Staphylococcus aureus* strains from abattoir workers in Busia County

Allelic profiles were obtained and assigned into ST and eBURST to identify there clonal complex. One novel *S. aureus* strain sequence type, assigned novel clonal complex and was categorized as a separate CC, this novel CC was isolated from an HIV- negative abattoir worker. Other four new sequence type variants were identified, five strains from HIV- negative abattoir workerspopulation and one from an HIV- positive abattoir worker, as presented in Table 13.

Table 13: Novel Clonal Complexes (CCs) and new Sequence Type (ST) *S. aureus* strains from nasopharyngeal samples of abattoir workers from various slaughter houses in Busia County

Strain	ST	CC	New sequence type	*arcC*	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*	Antibiotic pattern
BA014 HIV -VE	661~	Likely Novel CC	New sequence type	201	111~	358~	66	221~	82	477~	**Pen**
	88	**CC8**	**Standard ST**	**22**	**1**	**14**	**23**	**12**	**4**	**31**	**Pen**
BA015 HIV-VE	88~	CC8	New ST variant	22	1	58~	23	12	4	31	**Pen**
	1290	**CC221**	**Standard ST**	**1**	**4**	**1**	**1**	**11**	**1**	**3**	
BA020 HIV-VE	1290~	CC221	New ST variant	1	4	1~	1	11	1	3	**Pen**
BA173 HIV-VE	1290~	CC221	New ST variant	1	4	1~	1	11	1	3	**Pen**
	1292	**CC9**	**Standard ST**	**3**	**38**	**1~**	**1**	**1**	**1**	**40**	**Pen**
BA067 HIV-VE	1292~	CC9	New ST variant	3	38	1~	1	1	1	338	**Pen**
	ST 8	**CC8**	**Standard ST**	**3**	**3**	**1**	**1**	**4**	**4**	**3**	
BA106 HIV -VE	**8~**	CC8	New ST variant	281~	3	1	1	4	4	3	**PenTmp**
BA107 HIV+VE	8~	CC8	New ST variant	3	3	1	1	145~	4	3	**PenTmpSXT**
BA170 HIV-VE	8~	CC8	New ST variant	3	3	1	1	145~	4	3	**PenTmp**

4.6.2 Comparison of *Staphylococcus aureus* Sequence types (STs) found in Busia, Kenya and those found in other African countries

4.6.2.1 Comparison of Methicillin sensitive *Staphylococcus aureus* Sequence types (STs) found in Busia, Kenya and those found in other African countries

A number of studies have reported MSSA and MRSA population structures in Africa, but MSSA and MRSA population structures in Busia County, Kenya remain unclear. Multilocus sequence typing was used to characterize genetic background of these strains, with an aim of shedding light on the population structure of MSSA and MRSA in relation to those found in other African countries as demonstrated in Table 14. The following three MSSA sequence types were found in other African countries but were absent in Busia County: ST45, ST852 and 1865 (Abdulgader *et al*., 2015; Egyir *et al*., 2014). Busia County shared the following STs with other regions of the African continent sequences types ST15, ST152, ST25, ST88, ST8, ST30 and sequencestypes, ST22 (Abdulgader *et al*., 2015). In contrast, there were 8 unique MSSA sequence types ST1, ST5, ST72, ST80, ST97, ST188, ST707 and ST1633 in Busia county.

Table 14 Genetic diversity of MSSA STs in Busia Kenya in relation to other sequence types found in other African countries

Region/countries	-MSSA STs absent in Busia, Kenya n = 3	MSSA STs shared with Busia, Kenya n = 7	MSSA STs unique for Busia, Kenya n=15
SOUTH AFRICA	**ST45,ST1865**	**ST22**	
NORTHERN AFRICA	**ST852**	**ST15,ST22,ST25,ST30,ST8**	**ST1,ST5,ST72,ST80,ST88,ST97,ST188,ST152,ST707,1633**
Algeria		**ST22 ,ST8**	
Egypt			
Morocco		**ST8 ,ST15**	
Tunisia		**ST15, ST22 ST30**	
AFRICA n= 3	**ST45 ,ST852,1865**	**ST15, ST152, ST25, ST8, ST30, ST22**	**ST1,ST5,ST72,ST80,ST97,ST188, ST707,1633**

4.6.2.2 Comparison of MRSA Sequence types (STs) shared by the study populations in both Busia, Kenya and other African countries

Table 15 shows genetic diversity of MRSA STs in Busia Kenya in relation to sequence types found in other Africa countries. In contrast to MSSA, the MRSA isolated in Busia, Kenya, shared less genetic relationship (STs) with those isolated from other African countries There were only two unique MRSA strains found in Busia county ST140,~ ST241- (new ST closely related to ST 241); the county shared only two MRSA strains ST with the rest of Africa, ST88 and ST 241; while there were more strains in other parts of Africa that were not found in Busia, including: 13 strains from Western Africa (ST8,ST88,ST5,ST72,ST250,ST94,ST37,ST39,ST152,ST1,ST247,ST772,ST2021), from Southern Africa n= 13(ST22,ST8,ST72,ST5,ST45,ST612,ST36,ST1173,ST1338,ST650,ST612, ST239 and from Central Africa n= 7 (ST8, ST5,ST72,ST45,ST1289,ST2629,ST789).

Table 15: Genetic diversity of MRSA STs in Busia Kenya in relation to other sequence types found in other Africa countries

Region/countries		Shared MRSA with Busia Kenya	Kenya Busia N=3
Eastern Africa no=4			**ST140**
Tanzania no=2	**ST88,ST8**	**ST88**	
Uganda no =1	**241**	**ST241**	
Madagascar no = 3	**ST30 ,ST8**	**ST88**	**ST140, ST241**
Western Africa no=13	**ST8,ST5,ST72,ST250,ST94,ST37,ST39,ST152,ST1,ST247,ST772,ST2021**	**ST241, ST88**	**ST140,**
Nigeria no =13	**ST152,ST1,ST247,ST772,ST8,ST5,ST2,ST239,ST250,ST94,ST37,ST39**	**ST88, ST241**	
Ghana no =6	**ST8,ST72, ,ST239,ST250,ST2021**	**ST88**	
Niger no =2	**ST239**	**ST88, ST241**	
Mali no =1		**ST88**	
Senegal no =3	**ST239, ST5**	**ST88, ST241,**	
CENTRAL AFRICA no = 7	**ST8, ST5,ST72,ST45,ST1289,ST2629,ST789**	**ST88**	**ST140, ST241**
Gabon no =3	**ST8,ST5,ST45**	**ST88**	
Cameroon no =3	**ST8,ST5,ST1289**	**ST88**	
Angola no =5	**ST8,ST72,ST5,ST2629,ST789**	**ST88**	
SOUTHERN AFICA n0 = 12	**ST22,ST8,ST72,ST5,ST45,ST612,ST36,ST1173,ST1338,ST650,ST612, ST239**		**ST140, ST241**
SOUTH AFRICA no =13	**ST22,ST8,ST72,ST5,ST45,ST612,ST36,ST1173,ST1338,ST650,ST612, ST239**		**ST140, ST241**

4.7 phylogenetic analysis

4.7.1 Phylogenetic analysis and transmission amonglivestock, inpatient and abattoir workers

There is evidence of intra-livestock transmission of ST 152 *S. aureus* between a cow BL 31 and a pig BL 32 as seen in Figure 11. Two isolates positive for tetracycline resistance genes are indicated with arrows. Phylogeny shows that two distinct clones of ST152 are circulating in Kenya (named for now - Clade A and Clade B). Small numbers of isolates very closely related – suggestive of transmission highlighted with blue boxes in Phylogenetic tree (Figure 11). Colored labels in Figure 11 represent *S. aureus* recovered from the three study populations, blue represent *S. aureus* isolated from a livestock, Red label represent *S. aureus* isolated from inpatients from BCRH and black colour represent represents *S. aureus* recovered from abattoir workers.

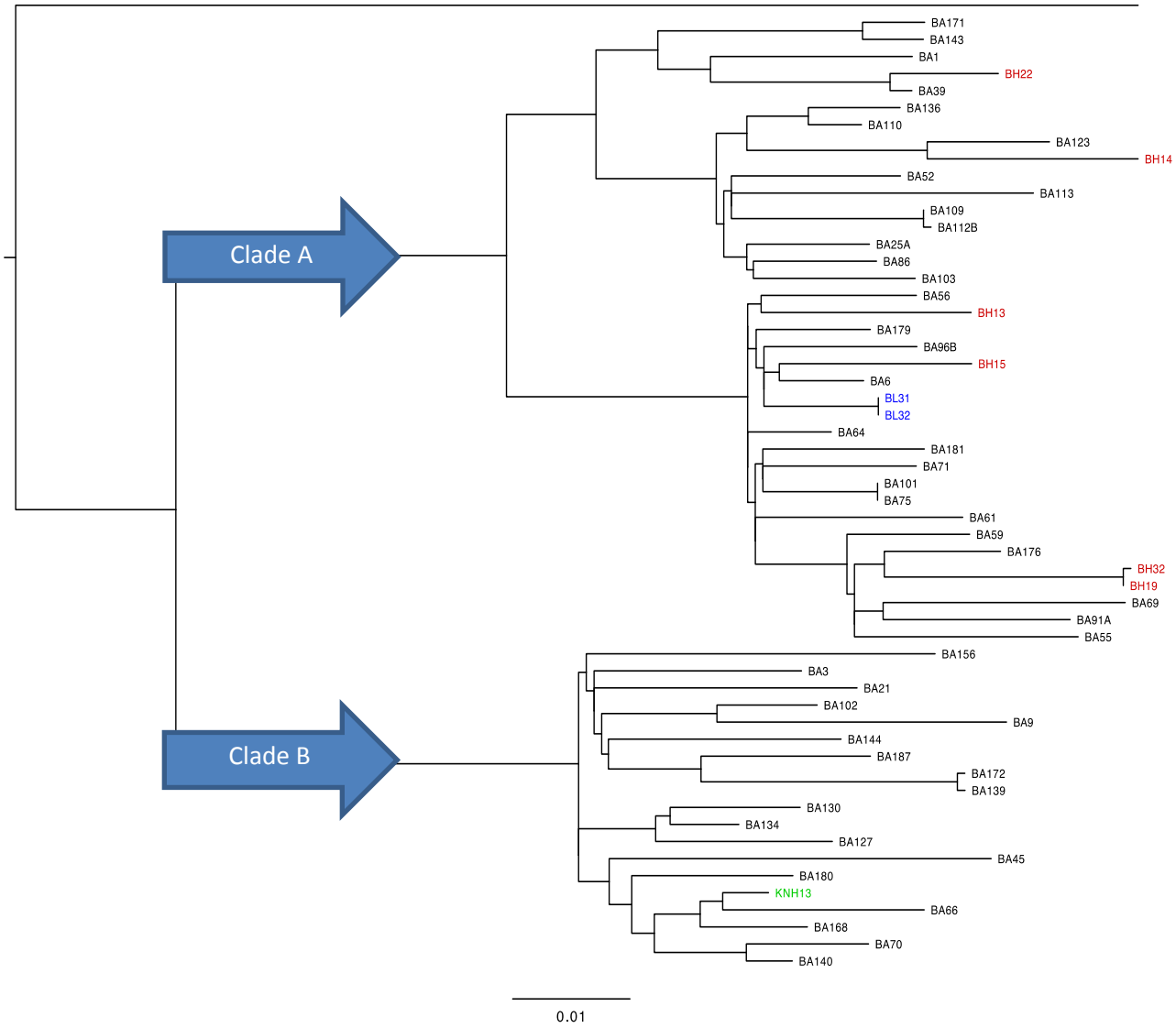

Figure 11: Differences in association of MSSA, SXT resistance and associated ST among HIV- positive abattoir workers and HIV- negative abattoir workers

4.7.2Ancestral phylogenetic analysis of PVL gene

Figure 12 indicates that PVL gene was probably acquired ancestrally before the two clades split; in addition a small number of isolates have completely lost the PVL gene in Busia County.

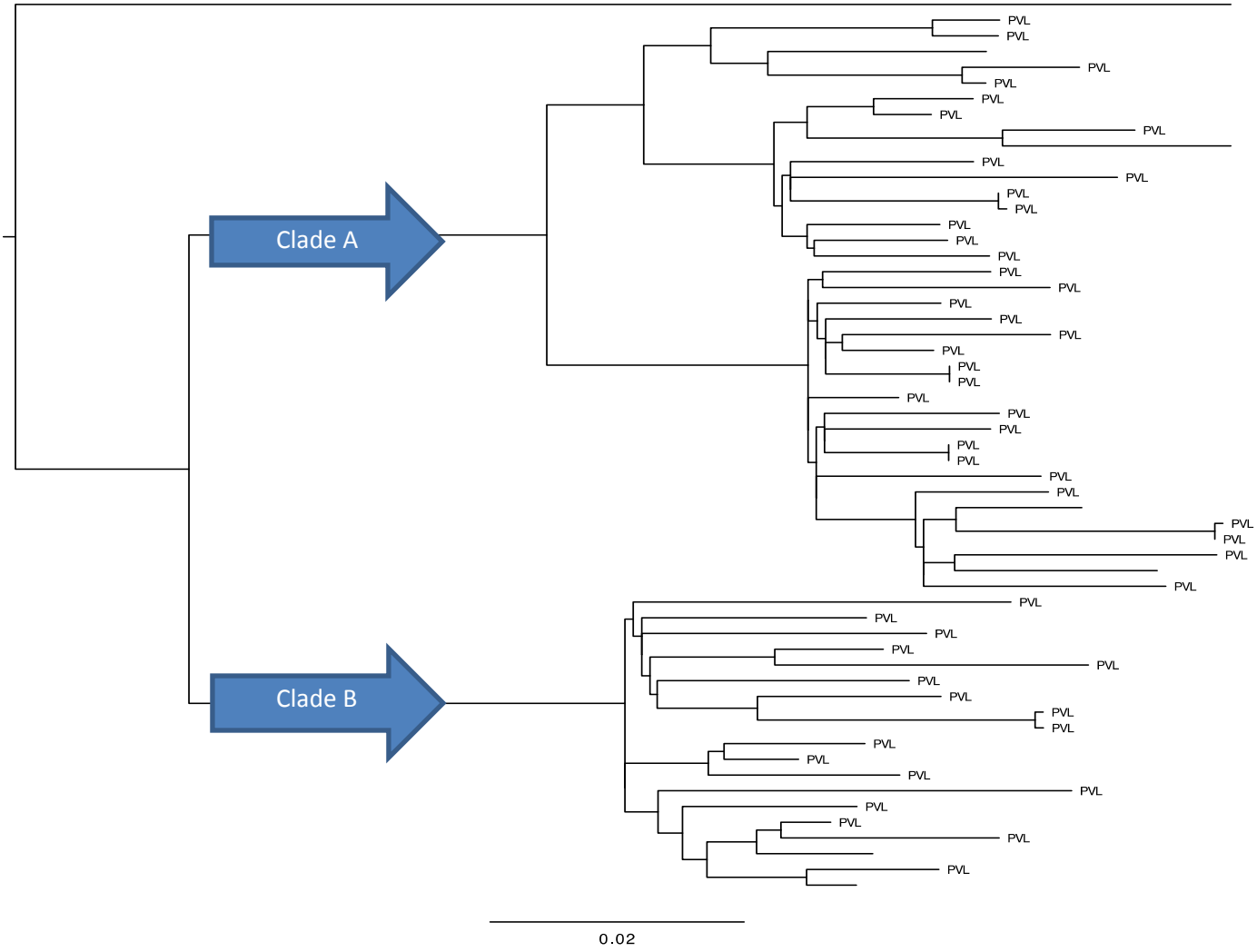

Figure 12 Phylogenetic tree showing presence of the PVL gene, probably acquired ancestrally before the two clades split.

4.7.3 Phylogenetic analysis of *Staphylococcus aureus* ST8 indicating higher possibilityof transmission in Busia County Hospital and among abattoir workers

Figure 13 points to the possibility of patient to patient transmission of *S. aureus* in BCRH (isolates 47, 48, 49) and between abattoir workers to abattoir worker transmission in abattoirs located in Busia County (isolates 29, 25B, 32, 99,112A), an HIV positive abattoir worker 25B, had a co-infection with a MDR ST 152 *S. aureus* that was resistant to PenTetTMP harboring a PVL Virulence factor gene. Figure 13 has colored labels, representing *S. aureus* recovered from the three study populations, blue represent *S. aureus* isolated from a livestock, Red label represent *S. aureus* isolated from inpatients from BCRH and black colour represent represents *S. aureus* recovered from abattoir workers.

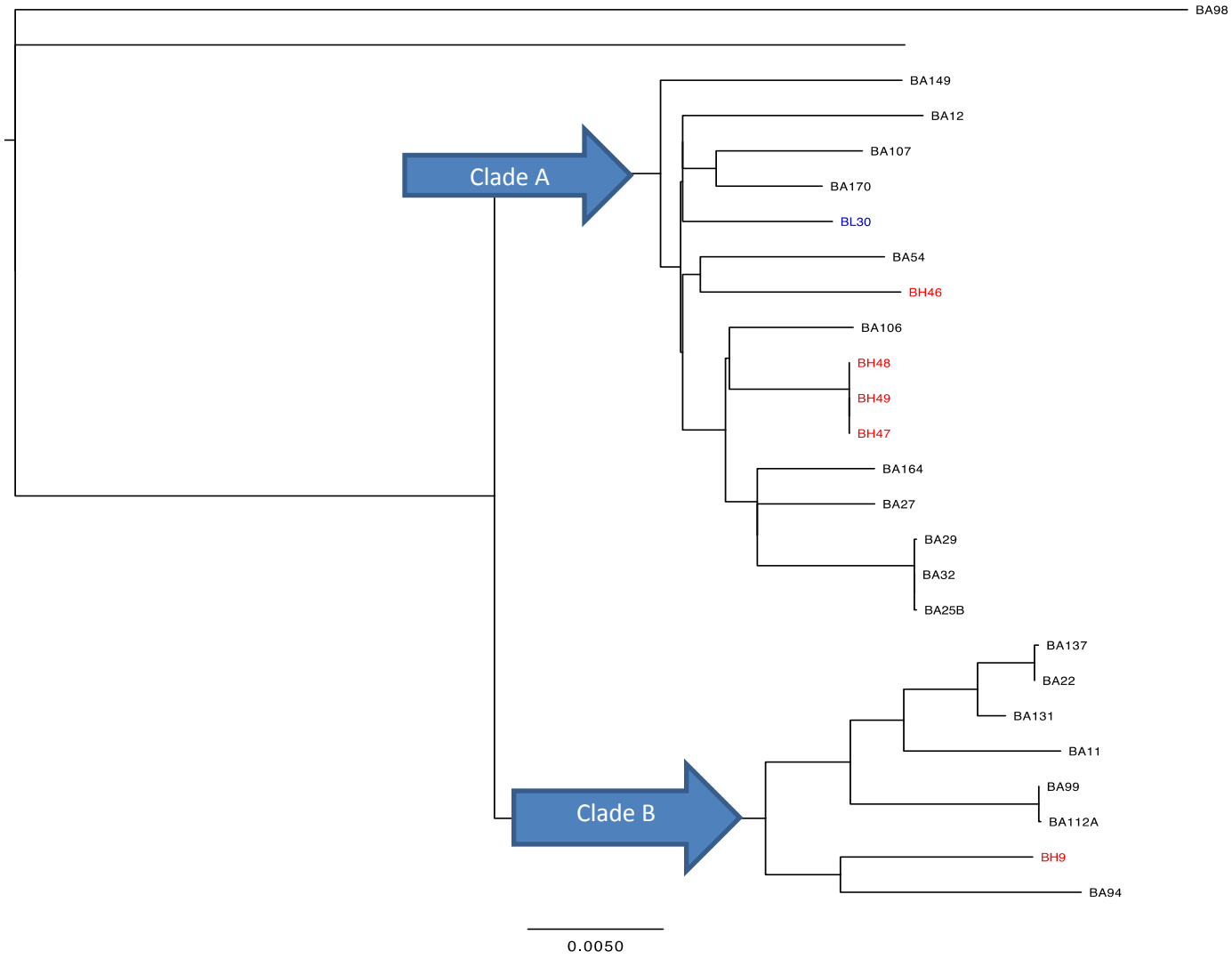

Figure 13 Phylogenetic analysis of *Staphylococcus aureus* ST8 indicating higher possibility of transmission in Busia County Hospital and among abattoir workers

4.7.4 Ancestral phylogenetic analysis of dfrG – trimethoprim resistance gene in *Staphylococcus aureus* isolated in Busia County

Phylogeny marked up with the presence of the *dfr*G – trimethoprim resistance gene shows that only Clade A has acquired the *dfr*G– this is shown as the larger of the two clades as indicated in Figure 14, suggesting the *dfr*G gene might have been helped with expansion of this clade A in Busia county. Coloured labels in Figure 14, represent *S. aureus* recovered from the three study population, blue represent *S. aureus* isolated from an livestock, Red label represent *S. aureus* isolated from inpatients from BCH and black colour represent represents *S. aureus* recovered from abattoir workers.

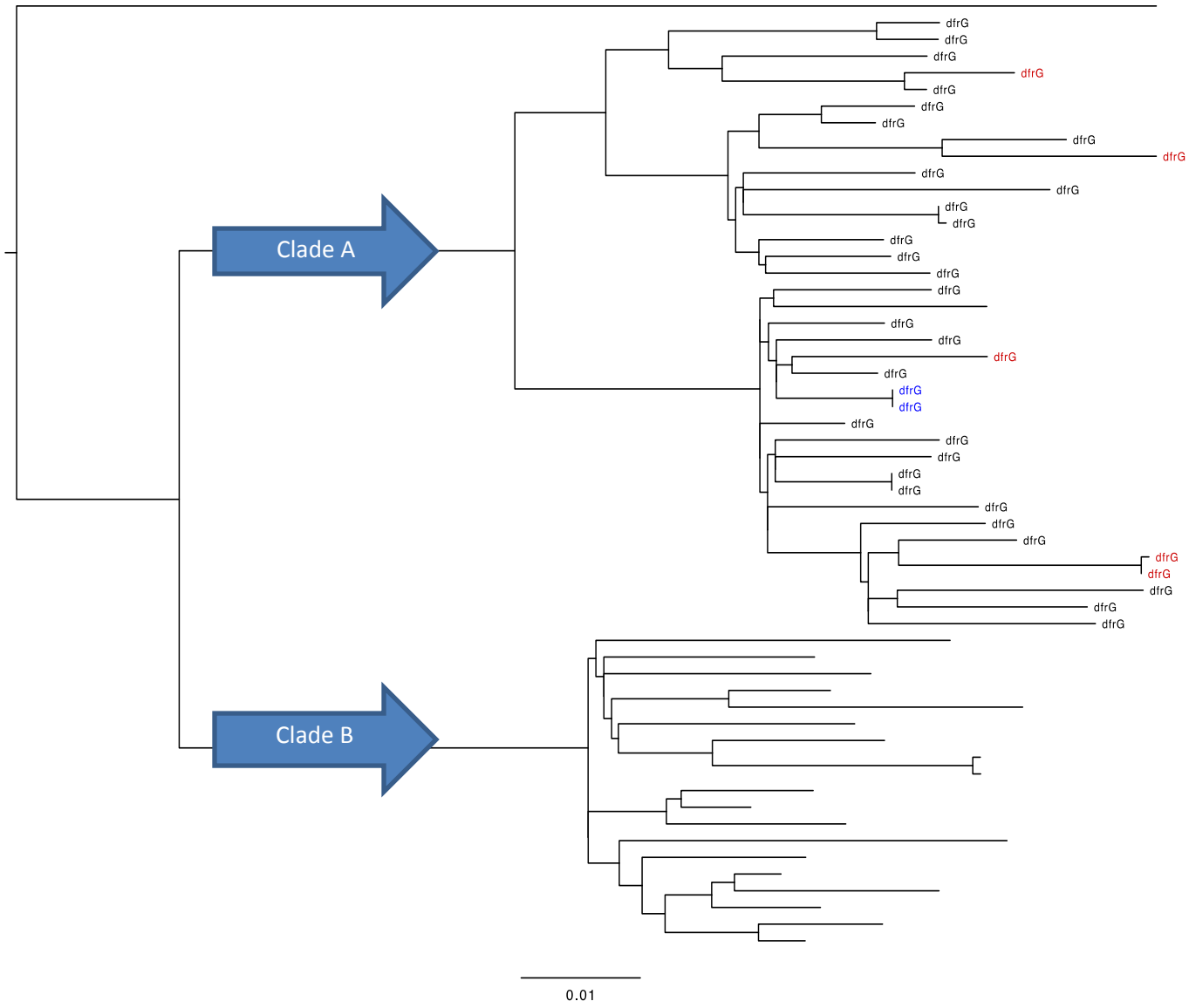

Figure 14 Phylogenetic analysis of *Staphylococcus aureus* ST8 indicating higher possibility of transmission in Busia County Hospital and among abattoir workers

4.7.5 Phylogeny of the *dfr*G – trimethoprim resistance gene in *Staphylococcus aureus* isolated in Busia County

All but one of *Staphylococcus aureus* ST8 isolates had the dfrG gene as shown in figure 15. Coloured labels in figure 15, represent *S. aureus* recovered from the three study populations, blue represent *S. aureus* isolated from a livestock, Red label represent *S. aureus* isolated from inpatients from BCRH and black colour represent represents *S. aureus* recovered from abattoir workers

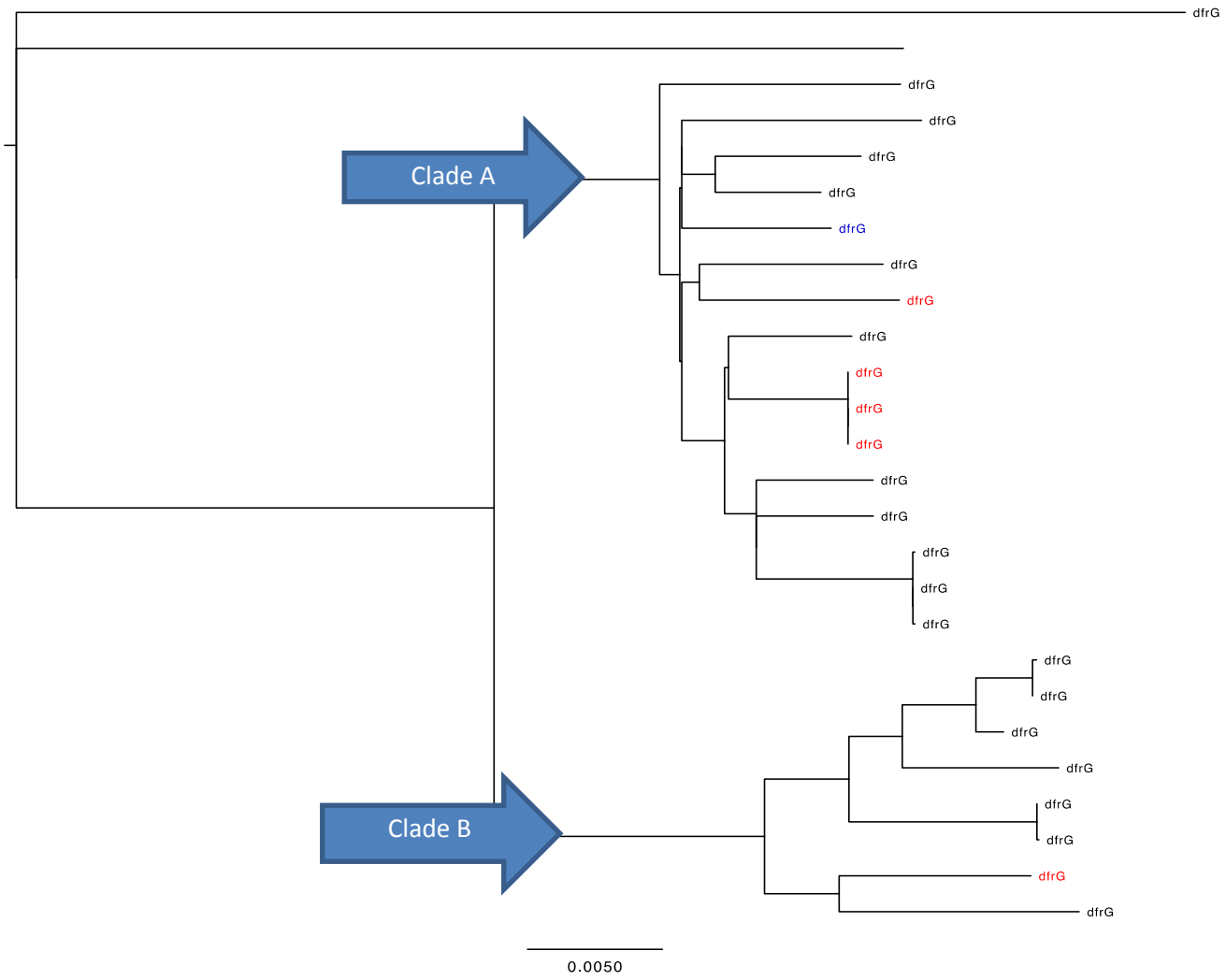

Figure 15 Phylogeny of *Staphylococcus aureus* ST8 isolates with trimethoprim resistance gene (dfrG gene) in Busia County

4.7.6 Phylogenetic analysis of *Staphylococcus aureus* ST CC1 isolates indicating higher possibility transmission in Busia County

Phylogenetic Figure 16 indicates a higher possibility of transmission of CC1 *S. aureus* isolates among abattoir workers in abattoirs located in Busia County (isolates29, 25B, 32, 99,112A). These strains showed a high degree of similarity, an indication of a possible person to person *S. aureus* among transmission abattoir workers

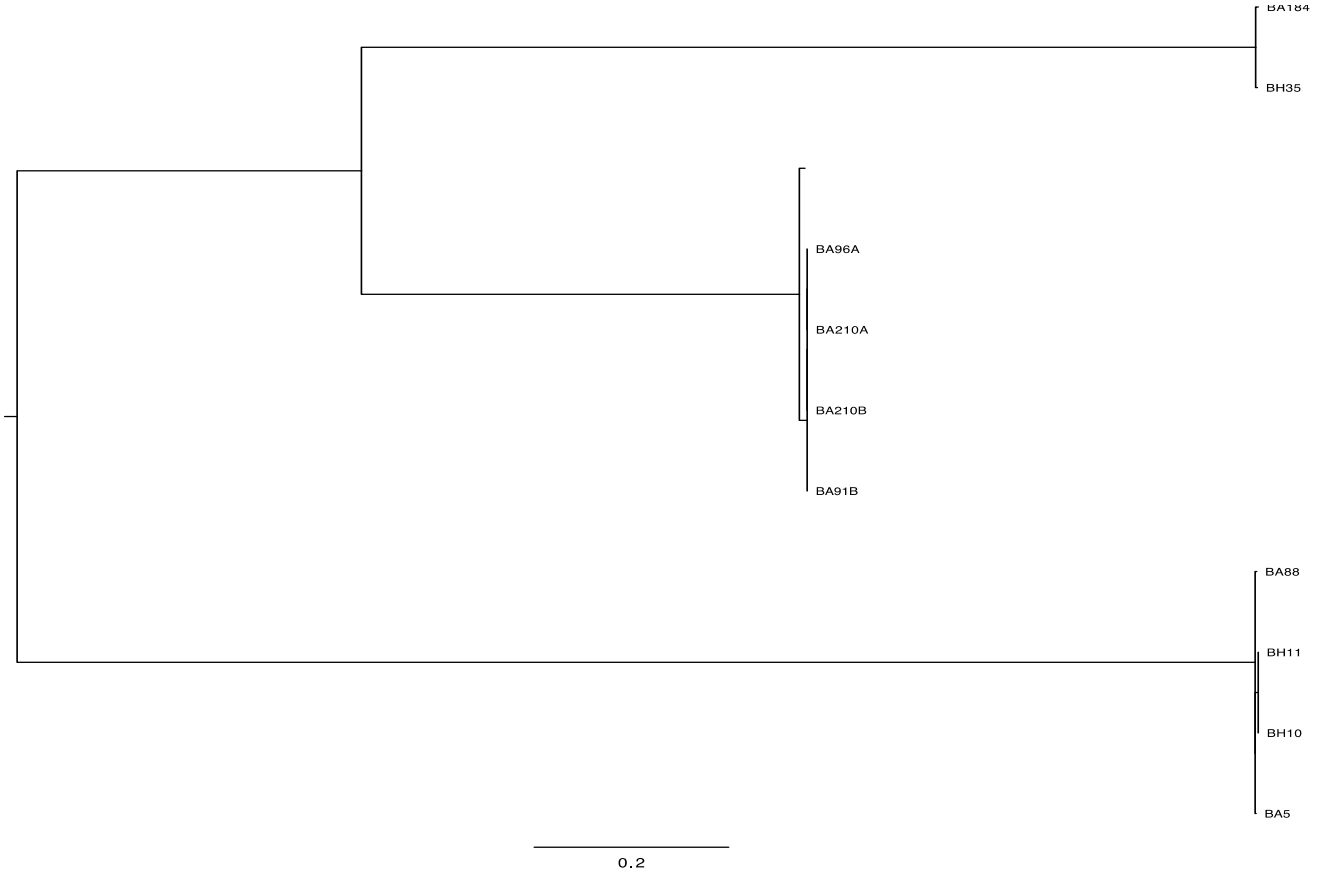

Figure 16 Phylogeny of *Staphylococcus aureus* ST CC1 isolates indicating higher possibility of transmission in Busia County

4.7.7 Phylogenetic analysis of the S. aureus isolates and antibiotic resistance from abattoir workers in Busia County

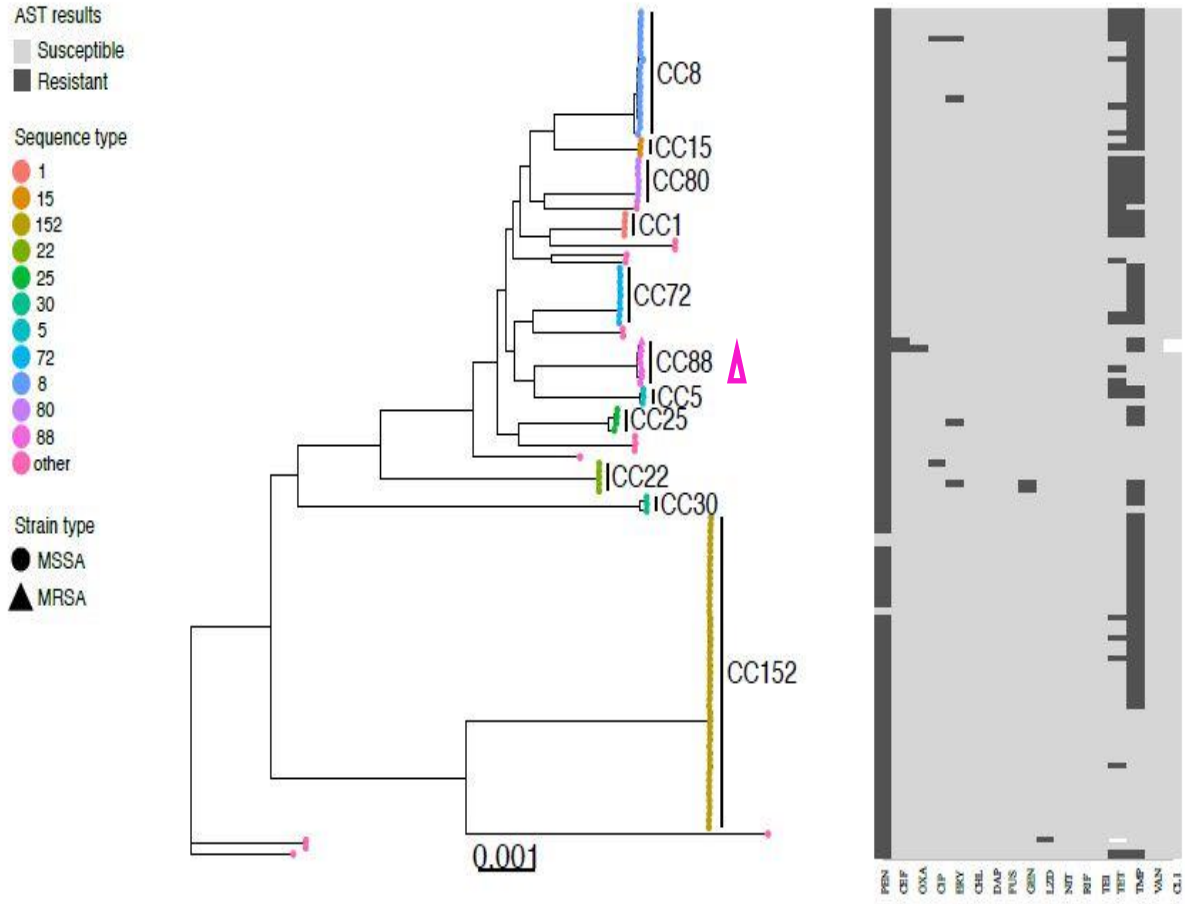

Figure 17 Phylogenetic analysis of *S. aureus* sequence types corresponding to clonal complexes and heatmap of their antibiotic and multi drug resistance pattern.

This diagram circle ➡ MSSA sequence types; strains; pink triangle ➡ MRSA strains; This diagram show the most predominant STs and the antibiotic susceptibility pattern in the following order ST 152, ST 8 and ST 72 . The three MRSA isolates were all ST 88

4.7.8 Phylogenetic analysis of the *S. aureus* isolates and antibiotic resistance from abattoir workers in Busia County

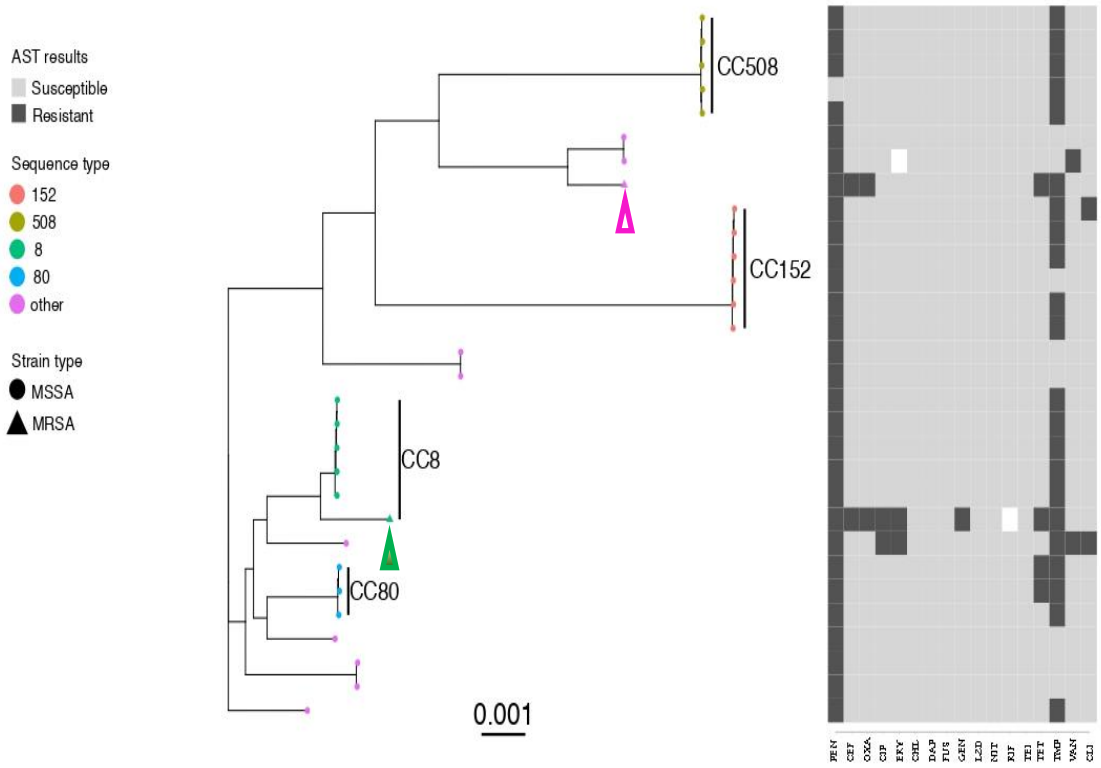

Figure 18 Phylogenetic analysis of *S. aureus* sequence types corresponding to clonal complexes and heatmap of their antibiotic and Multi Drug resistance.

This diagram shows the most predominant STs and the antibiotic susceptibility pattern yielded 9 clonal complexes and 13 STs. The predominant MSSA (Black circle) were ST 152, ST 8 and ST508. The MRSA sequence types were ST140 (first reporting in Africa, Pink triangle) and a new sequence type ST241 (Green triangle).

5. DISCUSSION

In BCRH, factors that may promote persistence and dissemination of MRSA, MSSA and MDR Gram negative bacteria exist. These factors include: (1) over usage of existing antibiotics such as Cefotaxime for empiric treatment; this antibiotic is one of the most commonly prescribed empirically in BCRH, presenting an enabling environment that may lead to the rise of adaptive resistance mechanisms by MRSA and MSSA in a nosocomial setting, (2) lack of proper guidelines on conventional microbiological diagnostics, in identification of causative bacterial pathogens, coupled with poorly equipped diagnostic microbiology laboratories, and (3) laboratory personnel who are not equipped with proper knowledge in isolation and proper identification of the pathogens (Kimang'a, 2012). These factors have contributed to doctors and other health personnel's continual use of broad-spectrum antibiotics empirical therapy, as seen in BCRH; a practice which is also common in hospitals in resource limited countries (Nicks*et al*., 2009). It is, therefore, important that, these hospitals conduct pathogen-specific antibiotic susceptibility testing, which will help them in choosing respective appropriate antibiotic(s), despite being in resource-constrained settings. Nasopharyngeal carriers of *S. aureus* are at a significant risk of developing staphylococcal infections, such as abscesses, cellulitis, folliculitis and serious necrotizing fasciitis etc (Ki and Rotstein, 2008), especially those suffering from HIV/AIDS disease such the abattoir workers of Busai Counry (Reid *et al*., 2017).Therfore, to regular assessment of nasopharyngeal *S. aureus* carriage may be used as an indicator, to gauge antibiotic resistance of *S. aureus* and MRSA in communities in either local, national or international settings (Yamamoto*et al*., 2010; Wertheim*et al*., 2005).

Inappropriate use of empirical antibiotic therapy increases selective pressure by killing susceptible bacterial strains, creating a thriving resistant bacterial strain population in the nosocomial environment (Ventola, 2015). *Staphylococcus aureus* has been implicated in different infections that are treated empirically. Physicians in many sub-Saharan regions in rural or semi urban healthcare settings including BCRH still rely on clinical

judgment; this is based on surveillance data obtained from other locations in the country or other countries in the region. These physicians have been reported to have poor perception of the importance of laboratory microbiological diagnostic testing; a perception that has been identified as a major barrier to laboratory use in this part of Africa (Knobler *et al*., 2003; Polage *et al*., 2006).

The current study, conducted in Busia County Referral Hospital (BCRH) in Western Kenya between May 2015 to July 2015, recorded prevalence of nasal carriage of *S. aureus* at 11%, among 261 recruited inpatients, giving prevalence of MSSA and MRSA at 10.7 % and 0.8 % respectively. The prevalence of nasal carriage of *S. aureus* found in Busia County Referral Hospital (BCRH) was lower or at par with those reported at two Kenyan hospitals, Aga Khan University Hospital (Dinda *et al*., 2013; Omuse *et al*., 2012; Omuse *et al*., 2014) and at Thika Level 5 Hospital.

The lower prevalence of *S. aureus* in BCRH could be attributed to a higher HIV/AIDS prevalence in Busia County as compared to other regions of Kenya (National AIDS Control Council, 2016); the extensive usage of Sulfamethoxazole and trimethoprim prophylaxis in these patients could be suppressing growth of MSSA and MRSA in the studied population. The lower prevalence could also be attributed to high usage of empirical antibiotic treatment especially cefotaxime in BCRH, which accounted for 60.0%, 37.7% and 78% usage in patients admitted at Pediatric, Medical and Surgical wards respectively. Cefotaxime was the most frequently prescribed antibiotic among patients admitted at BCRH; this antibiotic has been reported as the second most used empirical antibiotic treatment in some Asian countries (Atif *et al*., 2016). The justification for frequent cefotaxime use could be due to suspected multi-drug resistant Gram- negative pathogens in Kenya (Musila *et al*., 2021).

Johnson (2011) has commented that there is a considerable difference of MRSA and MSSA prevalence in different countries warranting research that compares prevalence of local set up such as Busia County against other strains regionally, continentally and globally. This approach can offer important information on the

dynamics of MRSA and MSSA strains, respective drug susceptibility patterns and genetic makeup for populations in a local setting, in comparison to other international circulating clones. The comparison is expected to give early signals of emergence of foreign MRSA and MSSA strains; it being of importance for regions situated along international borders such Busia County, Kenya. The strategic position of Busia County, being a border town/region, is of importance because the MRSA and MSSA data obtained from there, with respect to virulence and antmicrobial susceptibility of the circulating strains, can be extrapolated to the whole Eastern African region. This County serves a larger number of transient travellers, from neighboring countries, international tourists, livestock and their products.

To identify changes in the epidemiology of *S.aureus* in Eastern African region, this study compared prevalence of *S. aureus* between Busia county and neighboring countries. In Tanzania, a study involving 600 clinical specimens (pus, wound swabs and aspirates) obtained from inpatients admitted at surgical ward in Bugando Medical Centre (BMC) in Mwanza, reported a slightly higher prevalence for both MRSA at 4.3 % {26/600} and MSSA at 22.2% {134/600} (Mshana *et al*., 2009), than the prevalence of MRSA andMSSA obtained nasal sample from inpatients at BCRH. In Uganda, which borders Busia County on the Eastern side, a study on *S. aureus* nasal carriage among 500 adult patients in a regional referral hospital reported prevalence of MSSA at 29% and MRSA at 2.8% (Bebell *et a*l., 2017); this was slightly higher compared to *S. aureus* nasal carriage prevalence reported in patients admitted in BCRH. A retrospective study conducted in Eritrea, at the National Reference Laboratory, involving 278 clinical isolates, recorded 272 *S. aureus* isolates from pus samples and six isolates from ear discharge; the prevalence of MRSA among the pus specimens was 9% (Naik and Teclu, 2009). In Ethiopia, a meta-analysis study of 76 pooled *S. aureus*, involving a patient population of 4570, reported a higher MRSA prevalence of 47%.The data used in this meta-analysis was obtained from well-designed eligible studies, selected from online journal and research databases (Scribendi, 2022); with the isolates derived from various infection sites, such as: ear discharge, eye discharge, blood, wound infection, surgical site infection,

mixed samples, leprosy ulcer and urine samples. In an earlier study on surgical patients, in the same country, a prevalence of MRSA was reported to be slightly higher, at 55%, compared to the pooled prevalence. In both studies, there was no mention of MSSA prevalence (Abera *et al*., 2008; Deyno *et al*., 2017). Thus, the review on Eastern African cases showed that MRSA prevalence in hospital populations in East Africa are lower compared to prevalence in hospital populations in Eritrea and Ethiopia (Bebell *et al*., 2017). The difference in MRSA prevalence between Tanzanian/Ugandan and Eritrean/Ethiopian studies, could be attributed to the approach used to calculate prevalence; for example: in East African countries, prevalence was calculated as the number of MRSA isolates divided by the study population, multiplied by one hundred, while in studies carried out in Eritrea and Ethiopia, prevalence was reported as the percentage of MRSA *vis a vis* various clinical *S. aureus* isolates collectedover a period of time. The right definition of "Prevalence"is: "the statistical measure of the number of cases of a disease or carriage of a potential pathogen present in a particular population at a specified time" (Norman and Ryrie, 2018; Gorwitz *et al*., 2008). A study conducted between 1999–2002 in South Africa reported a prevalence of hospital associated MRSA of 23 %, which was higher than those reported in the East African countries (Perovic *et al*., 2006; Mshana *et al*., 2009; Bebell *et a*l., 2017; Dinda *et al*., 2013; Omuse *et al*., 2012; Omuse *et al*., 2014; Aiken *et al*., 2014). In Northern Africa, two hospital-based studies in Algeria reported a prevalence ranging from 35%-45%; other hospital-based studies conducted in Morocco between 2003–2005 and 2006–2008 reported a prevalence of 19 %, for MRSA; while a study conducted in nine hospitals in Egypt, representing 17 % of the country's population reported a 3 year increasing trend between 2003–2005. The observation of the latter study was based on *S. aureus* isolates recovered from blood cultures; the highest recorded prevalence of MRSA was 52% (Amazian *et al*., 2006; Borg*et al.,* 2007). Thus, prevalence studies of MRSA and MSSA in African countries point to an inconsistency in prevalence values, making it difficult to reach a definitive conclusion on a representative MRSA and MSSA prevalence in African continent.

Previous studies on *S. aureus* in the African continent are focused on MRSA infections in human clinical isolates; nevertheless, there are very few literature reviews focusing on MSSA infections.

This preference on MRSA research and reporting was clearly seen in Ethiopia and Eritrea; the reason could have been due to the notion that, MRSA infections cause longer hospitalization, resulting in higher cost of hospitalization, and tend to have worse clinical outcomes compared to MSSA infections; therefore MRSA infections are seen as a greater public health problem compared to MSSA infections (Perovic *et al.,* 2006).

Factors contributing to higher prevalence of MRSA in wealthier African nations include high usage of antibiotics, which are similar to developed nations in Europe and United States of America. However, in those countries that have introduced effective measures to control the transmission of MRSA, there is evidence of reduced incidence of MRSA infections (Humphreys, 2007). Implementaion of effective infection prevention and control measures in Europe has led to lower prevalence of MRSA in hospitals and health care centers (Humphreys *et al.,* 2009). These studies have also reported noticeable geographical variations in prevalence of *S. aureus*; proportions of MRSA among *S. aureus* clinical isolates, differing from country to country. In Europe, prevalence varies between 0.4% in Sweden to 48.4% in Belgium; with MRSA proportion among *S. aureus* isolates being greater than 25 % in at least one third of these countries (Johnson, 2011); 25 % of *S. aureus* bloodstream infections were caused by MRSA in several Central and Southern European countries (Messina *et al.,* 2013; European Centre for Disease Prevention and Control EARSS Annual Reports, 2010). Although efforts to bring down MRSA infection levels have been achieved in some European countries, it has not been successful in others (Messina *et al.*, 2013; European Centre for Disease Prevention and Control EARSS Annual Reports, 2010). However, there are, some few countries that have seen declining prevalence, for example: surveillance studies conducted in 2008 in United Kingdom, reported decrease of bloodstream MRSA infection ranging from 31% in 2007 to 19.3% in 2009; this was as a result of the UK government instructions to hospitals and health care facilities to bring down these rates by 50% (Health Protection Agency, 2009). A review of

MRSA prevalence in United States can be best analyzed by a United States national surveillance conducted in all states between 1st October to 16th November 2006; 8654 strains were characterized as MRSA, from bothpatients clinical and nasal carriage isolates, against a total of 187,058 patients admitted in various hospitals and health care facilities; this represented a national prevalence of 4.6 %. This national surveillance showed a higher prevalence of MRSA than previous studies (Jarvis *et al*., 2007).

Methicillin-resistant *S. aureus* remains an important public health concern despite the signs of decline in some countries in Europe. Public health authorities should take appropriate measures to control the spread of MRSA (Barton *et al*., 2006), these measures must help in stopping transmission of pathogen, by isolating individuals or patients who are already colonized or infected by MRSA (Coia *et al*., 2005), hand washing is another effective intervention that could help in stopping the spread of this super bug (Marimuthu *et al*., 2005), other measures include screening of patients for colonization, with an aim of decolonization of the colonized individual (Vos *et al*., 2005) and reduction of selective pressure through appropriate antibiotic prescribing (Duerden *et al*., 2015). Successful applications of these control measures vary; some Scandinavian countries and the Netherlands have lowered MRSA infection rates by applying the 'search-and-destroy' policy for MRSA (Björholt and Haglind, 2004; van Rijen *et al*., 2009), this has led to a lower prevalence rate of 2%, in Netherlands and Switzerland, as opposed to countries like Japan and Hong Kong that have not introduced this measure, with prevalence rates of > 70% (Fluit *et al*., 2001).

Looking into the pathogenicity of both MRSA and MSSA strains, there are different opinions among scientific communities, with some suggesting that MRSA are just as pathogenic as their MSSA counterparts, while others disagree and state that MRSA are just opportunists, and that their significance has been overstated (Rozgonyi *et al*., 2007). The reason why scientists do more research on MRSA than MSSA in both hospital and community settings is based on MRSA's resistance to standard antibiotics which are commonly used for empirical

treatment in both settings; the antibiotic selective pressure against MSSA, having favored the emergence of MRSA sub-populations (Rozgonyi *et al*., 2007).

Some studies have documented that nosocomial MRSA infections and the resulting bacteremia are more likely to cause death, as opposed to infection and bacteremia caused by MSSA; there is extensive literature on these studies, both in Africa and the world in general. Contrary to these findings, French and his team have demonstrated that there are no differences between MRSA and MSSA strains in animal model experiments, as far as virulence and pathogenicity are concerned (French *et al*., 1990). Their study, by design, adopted an unbiased approach and covered both MRSA and MSSA infections, basing its reasoning on the fact that both strains equally presented significant public health challenges (French*et al.,* 1990).

Two studies on MRSA and MSSA carriage at the Aga Khan University Hospital, Nairobi, Kenya, reported absence of MRSA strains from nares of 246 randomly selected healthcare workers (Omuse *et al*., 2012). The second study reported a prevalence of MRSA and MSSA at 10% and 30% respectively, from clinical samples obtained from surgical site infections (Dinda *et al* 2013); Omuse *et al.* (2014), worked on 731 *S. aureus* isolates from clinical specimens and found a much lower prevalence of MRSA at 3% compared to those reported in most African countries (Omuse *et al*., 2014). Another study conducted on nasopharyngeal *S. aureus* carriage in a county government hospital located about 50 km from Nairobi, Kenya, reported a prevalence of 10.1 % MSSA and 7.0% MRSA,which pointed at a low prevalence of MRSA in Kenya compared to other studies in Africa (Aiken *et al*., 2014).

In nosocomial setting, like BCRH, a quarter of healthcare-associated infections are caused by *S. aureus*, leading to increased hospitalization, which exposes inpatients to life threatening infections as opposed to patients with no *S. aureus* related infections (Lowy 1998; Wisplinghoff *et al*. 2004; Noskin *et al*., 2005). These organisms have been historically associated with nosocomial infections and, in recent past, they have also been associated with increased antibiotic resistance. However, it is important to note that antibiotic resistant *S. aureus*

strains have also been isolated from non-hospitalized individuals in some countries, most notably in the United States, and even smaller localities such as Busia county Kenya, as attested in this study

Empirical antibiotic treatment has been linked to increased prevalence of infections, especially MDR Gram negative bacterial pathogens. These pathogens pose a considerable threat to global public health, especially in hospitals like BCRH, where they could be the driving force behind increased antibiotic resistance towards commonly prescribed empirical antibiotic therapy, thus limiting treatment options against the historically slow rate of new antibiotic discoveries (Paramythiotou and Routsi, 2016). Furthermore, infectious MDR Gram negative and some MDR Gram positive strains such as *S. aureus* e.g. Hospital acquired MRSA have been linked to increased morbidity, mortality and prolonged hospitalizations, leading to a considerable burden on public health, hospital settings and other healthcare provisions (Llor and Bjerrum, 2014; Laxminarayan*et al*., 2013). Other common MDR Gram negative pathogenic bacteria of great concern in nosocomial environment belong to the family *Enterobacteriaceae* (especially *Klebsiella pneumoniae* and *Escherichia coli*), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Mehrad *et al.*, 2015). This was also witnessed in BCRH, where inpatients were treated empirically with broad-spectrum antibiotics such as Cefotaxime because susceptibility data was unavailable.

Despite BCRH having low prevalence of MRSA, factors that have led to high MRSA prevalence in wealthier African countries and some European countries, as a result of escalated antibiotic usage, have a potential of increasing the prevalence of MRSA in BCRH in the near future. Complicating matters further, some antibiotics targeting Gram-negative bacterial infections are similar to those used to treat Gram positive bacterial infections in western Kenya (Nyangacha *et al*., 2017).

Prevalence of high-level multi-drug antibiotic resistance among Gram negative bacterial pathogens, in the nosocomial settings in East Africa, Kenya in particular, are similar to those of other lower and middle-income countries (Ampaire*et al*., 2015; Ntirenganya *et al*., 2015; Maina *et al*., 2016). Broad-spectrum antibiotics such

as Cefotaxime, Sulfamethoxazole /Trimethoprim, Tetracycline-class, Gentamicin and ciprofloxacin (Fair and Tor, 2014), that are empirically targeted to treat bacterial Gram negative infections in both nosocomial and community settings (Tamma *et al*., 2012; Stevens *et al*., 2014), may exert unnatural selective pressure favoring both MDR MRSA and MSSA strains in these settings, accelerating their proliferation, followed by their dissemination, to the detriment of inpatients and the community (Fair and Tor, 2014).

In BCRH, percentages of antibiotic resistance and antibiotic susceptibility patterns between MSSA and MRSA differed significantly. With exception of Penicillin Benzyl penicillin, Trimethoprim and Cefotaxime, majority of MSSA isolates were susceptible to Chloramphenicol, Daptomycin, Fusidic Acid, Linezolid, Mupirocin, Nitrofurantoin, Rifampicin and Teicoplanin, while MRSA isolates were also resistant to non-β-lactam antibiotics such as ciprofloxacin, clindamycin, trimethoprim/sulfamethoxazole, erythromycin, gentamicin and Tetracycline.

Benzyl penicillin (penicillin G) is considered as an essential medicine, as published in both the Kenya Essential Medicines List 2016 and The WHO Model List of Essential Medicines (EML) (World Health Organization Model List of Essential Medicines, 2019). This antibiotic is currently used in many public hospitals in Kenya. It is prescribed for treatment of pneumonia, streptococcal throat infection, syphilis, necrotizing enterocolitis, diphtheria, gas gangrene, leptospirosis, cellulitis, and tetanus, caused by both Gram positive and some Gram - negative bacteria (Aksoy and Unal, 2008; World Health Organization Model List of Essential Medicines, 2019; World Health Organization:MF-2009). In BCRH, 16 % of children who were admitted at Pediatrics wards were empirically treated using it. A high of 99 % of MSSA and MRSA isolates resistant to penicillin have been reported by some researchers, including those from Kenya (Aiken *et al*, 2014; Omuse *et al*., 2014); this is in comparison toTrimethoprim.

In Western Kenya, MSSA PRS isolates were found to be highly prevalent. High level of *S. aureus* resistance to Penicillin-G among inpatients at BCRH was observed, with 96.6% of *S. aureus* isolates being resistant to

Penicillin-G; this very high Penicillin-G resistance is in line with other studies conducted in other hospital settings around the world , where proportions of Penicillin-G-resistant *S. aureus* isolates ranged from 72.2 %-100% (Dibah *et al*., 2014; Naik and Teclu, 2009; Santosaningsih *et al*., 2016; Shrestha 2013; Australia:CDI 2011; Rebiahi *et al*., 2011; Mshana *et al*.,2009).

Detailed literature between 2009 to 2016 indicate that, the percentages of MSSA PRS isolates recovered from clinical specimens such as pus, wound swabs and bacteremia, stood at around 72.2 %, 75 %, 83.5 %, 85.4 %, 86 %, 86.5 %, 87.3 %, 100% and100% in Iran, Eritrea, Indonesian, Nepal, Nigeria, Australia, Algeria, Tanzania and Korea respectively (Dibah *et al*., 2014; Naik and Teclu, 2009; Santosaningsih *et al*., 2016; Shrestha, 2013; Australia:CDI 2011; Rebiahi *et al*., 2011; Mshana *et al*., 2009). The high levels of penicillin resistance among Clinical *S. aureus* isolates, is worrying, as it renders this essential and cost effective antibiotic ineffective, in treating many life threatening *S. aureus* infections. It is, therefore, important for Health authorities to advice for more judicious use of Benzyl penicillin, so as to preserve its therapeutic advantage globally.

The current study has given the representative prevalence of Penicillin-G resistant *S. aureus* among abattoir workers in Busia, in a community setting. Although a small sample of the abattoir worker population was studied, the findings have shed light on the burden of Penicillin-G resistant *S. aureus* in this population, providing important data that will be useful for the public health authorities to come up with control measures in communities residing in Busia county and Kenya in general. The carriage rate of Penicillin-G resistant *S. aureus* isolates in HIV- positive abattoir workers was significantly higher than in HIV-negative abattoir workers($p< 0.005136$) in the county, and even higher than in patients admitted at BCRH ($p < 0.00074$). The prevalence of Penicillin-G resistant *S. aureus* inHIV- positive abattoir workers was recorded at 26.8 %, compared to 14.3 % in HIV-negative abattoir workers, while, prevalence of Penicillin-G resistant *S. aureus* in patients admitted at BCRH was 11.1 %, approximately 97% of *S. aureus* recovered from the abattoir workers population were resistant to penicillin-G resistant.

Generally, more than 70 % of *S. aureus* strains are sensitive to Gentamicin, with varying prevalence of Gentamicin sensitivity in MSSA strains reported in other regions. Variation of Gentamicin resistance in MSSA strains have been reported in Eritrea with approximately 30% of MSSA clinical isolates showing gentamicin resistance (Naik andTeclu, 2009). Other reports on MSSA Gentamicin resistance include: prevalences of 21 %,13.2 % and 11 .6 % in South Africa, Italy and Nepal respectively, and prevalences of less than 6.0 % in Indonesia , Nigeria , Australia and Iran (Smidt *et al*., 2015; Santosaningsih *et al*., 2016; Shrestha 2013; Shittu *et al*., 2011; Australia:CDI, 2011; Dibah *et al*., 2014).

All MSSA isolates from BCRH were susceptible to Gentamicin, indicating that, this antibiotic still maintains its activity against MSSA-associated endocarditis; high Gentamicin susceptibility among MSSA isolates is however not universal. Nationwide Survey in Tertiary-Care Hospitals in Korea previously reported a very high MSSA gentamicin resistance of 68.0 % out of 177 clinical isolates, necessitating the introduction of alternative treatment for the MSSA infection (Jeong *et al*., 2007). Countries such as Indonesian, Nigeria, Australia, Iran and Kenya (BCRH), with very low or non-existence of Gentamicin MSSA-resistant strains, are, therefore, encouraged to continue their prudent use of Gentamicin, to maintain its therapeutic advantages; this will help maintain its fallback role against MSSA-associated endocarditis in this regions (Dibah *et al*., 2014; Australia: CDI, 2011; Santosaningsih*et al*., 2016; Baddour *et al*., 2015; Hageman *et al*., 2006). Gentamicin is classified as an aminoglycoside and has been used to treat MRSA infections as far back as 1965; however, after a decade of use, there were reports of emergence of Gentamicin resistant MRSA strains in certain regions, leading to new waves of hospital outbreaks in late 1970s (Lacey, 1975; Speller *et al*, 1976). High level gentamicin-resistant MRSA strains have been reported in hospitals in Tehran (Iran); they were detected using Phene-Plate (PhP) typing (Rahimi, 2016).

Presence of Gentamicin resistant genes *aacA-aphD* in MRSA, can also lead to increased prevalence of Gentamicin-resistant MRSA strains among inpatients population, by depressing MSSA plasmid mediated

aminoglycoside resistant strains, which are susceptible to β-lactam antibiotics in the same environment (Martel *et al*., 1977'; Martel *et al*., 1983). This explains why prevalence of Gentamicin-resistant MRSA strains is much higher than plasmid mediated MSSA aminoglycoside resistant strains globally (Schmitz *et al*., 1999). In Europe, the proportion of MRSA aminoglycoside -resistant strains are 15-18 times higher (Schmitz *et al*., 1999), than those found in African countries, such as, Madagascar (1.9 %), Western Kenya (7.4 %) and Eritrea {12.0 %} (Breurec *et al.*, 2011; Naik and Teclu, 2009). Reasons for low prevalence of MRSA aminoglycoside resistance reported in these countries may be attributed to lower usage of empirical gentamicin therapy (Randrianirina *et al*., 2007). Cost-effectiveness of gentamicin- vancomycin compared daptomycin is similar (Bhavnani *et al*., 2009), making an expensive antibiotic in hospitals alike BCRH.

Proportions of MRSA aminoglycoside resistance in *S. aureus* clinical and nasopharyngeal isolates, vary considerably in both middle and high-income countries with exception of Nepal, which is regarded as a low income nation; from moderate proportions in Korea (28.0%) and Algeria of (30.3%) to highest proportions recorded in Indonesia (100%), followed by Italy (97%), Nepal (90-98%), South Africa (90%), Kuwait (81%), Iran (50-78.8%) and Australia (43.3%). This high prevalence could be due to easy access to antibiotics; for example in most parts of Asia, antibiotics are easily available from chemists; this, coupled with frequent self-medication, are the major drivers for increased high prevalence of MRSA aminoglycoside-resistant strains in this region and some parts of the middle eastern region (Alhomoud *et al*., 2017; Nickerson *et al*., 2017). Studies in Europe have also reported high prevalence rates of MRSA aminoglycoside-resistant isolates, with higher resistance being seen in Central and Southern Europe compared to Northern Europe countries; this has raised public health concerns in the European community (Schmitz *et al*., 1999). It is possible to reduce incidences of MRSA aminoglycoside resistance, as witnessed in Canada and North America (4.6 %), where collaborative efforts by the federal government, provinces and territories in the human and animal health division, paid off; these institutions, designed a policy, that was adopted by all to tackle antibiotic resistance by regulating and

promoting rational use of antibiotics; they also introduced infection prevention and control programmes and surveillance (Public Health Agency of Canada, 2017).

Therefore, there is need for sustained surveillance and adherence to well-designed antibiotic usage and infection control policies, which may prevent proliferation of MRSA aminoglycoside-resistant strains in low prevalence regions such as Western Kenya (Busia County), Madagascar, Eritrea (Breurec *et a*l., 2011; Naik and Teclu, 2009); lack of these interventions is the root cause of high prevalence of MRSA aminoglycoside resistance in European countries (Schmitz *et al*., 1999). Due to its low price, gentamicin can still be used in combination with other suitable antibiotics with exception of Benzyl penicillin for treatment on MRSA infection in BCRH, since, the rate of Benzyl penicillin resistance remains high among both MRSA and MSSA in the region. Furthermore, it is standard practice, to prescribe initial low-dose Gentamicin in combination with Penicillin or Cefazolin or vancomycin (Hidron *et al*., 2010) to treat patients presenting with a possible MSSA endocarditis, with physicians world over recommending addition of initial low-dose gentamicin for early clearance of MRSA in MRSA bacteremia (Baddour *et al*., 2015; Hageman *et al*., 2006).

Erythromycin and clindamycin are alternative antibiotics to gentamicin for the treatment of MRSA infections; more-so in cases of MRSA/MSSA aminoglycoside resistance. However, clindamycin tends to have cross-resistance with erythromycin; thus, such strains pose a difficulty in treating patients who have earlier been exposed to either of them, posing a challenge in treatment of the MRSA/MSSA infections (Japoni *et al*., 2010; Askarian *et al*., 2009). Erythromycin resistance genes are present in many species of bacteria and many of their determinants have already been identified (Arthur *et al*., 1987; Eady *et al*., 1993; Weisblum, 1995).

The D test was used to detect both erythromycin and inducible clindamycin among isolates from BCRH, the proportion of erythromycin resistant MRSA /MSSA strains among nasopharyngeal *S. aureus* isolates was 50.0 % /7.4% respectively, while inducible clindamycin resistance was 50%/3.7% respectively; these inducible

clindamycin resistant strains were resistant to erythromycin, while sensitive to clindamycin D-test. In the case of staphylococcal clindamycin constitutive resistance, 7.4 % of MRSA strains were resistant to both erythromycin and clindamycin, all MSSA were susceptible to clindamycin. These proportions were much lower than those reported at Bugando Medical Centre, Mwanza, Tanzania, at 61.5 % and 22% among MRSA and MSSA clinical isolates respectively (Mshana *et al.*, 2009). This difference could be attributed to the types of specimen: in the Tanzanian study; samples were from pus, wound swabs, aspirates, while those from BCRH were from nasopharynx. In contrast, proportions of MRSA /MSSA erythromycin resistance strains from the Tanzanian study were lower than those found at BCRH, MRSA /MSSA erythromycin resistance 29 % and 3.9% respectively. These observations emphasize the importance of including D test susceptibility testing on all *S. aureus* isolates showing MRSA /MSSA resistance to erythromycin and susceptibility to clindamycin, to prevent erythromycin based treatment failure (Mshana *et al*., 2009).

In addition, several other African countries, including Nigeria, Madagascar, Niger, Senegal, Cameroon, Ethiopia and Eritrea (Falagas *et al*., 2013; Breurec *et al*., 2011; Eshetie *et al*., 2016; Naik andTeclu, 2009), reported high proportions of erythromycin resistant MRSA and/or MSSA isolates among clinical samples at rates of MRSA 54.5%; MSSA 3.1 %, MRSA isolates 54 %, MRSA isolates 23-100 % and MSSA isolates 76.4 % respectively (Falagas *et al*., 2013; Breurec *et al*., 2011; Eshetie *et al*., 2016; Naik andTeclu, 2009). Countries which have reported higher prevalence of erythromycin- resistant MRSA and MSSA isolates than inducible clindamycin resistant MRSA and MSSA strains, but did not indicate whether they carried out D testing, included:

In another Asian country, Philippines, the rates of MRSA erythromycin-resistant strains were similar to those expressing clindamycin resistance at 56.0% and 56.4 % respectively (Juayang *et al*., 2014). In an Australian study, 70.9 % of MRSA isolates were resistant to erythromycin, compared to 35% that were resistant to clindamycin (Hidron *et al*., 2010; Nimmo *et al*., 2011). A study on MRSA antibacterial resistance in Kuwait

hospitals, found that all the isolates were susceptible to clindamycin (Hidron *et al*., 2010; Udo *et al*., 2006). In Saudi Arabia, which is located in the same region, a study on nasal carriage among patients admitted at Shaqra General Hospital, reported high erythromycin (85.4 %) and clindamycin (87.5%) resistance amongst MRSA isolates (Alhussaini, 2016). It is, therefore, recommended that laboratories in regions always include D testing; this will provide a more accurate picture on the burden of both *S. aureus* erythromycin and clindamycin resistance in respective population. Since clindamycin resistance is either constitutive or inducible, it is advisable that whenever clindamycin is anticipated to be used for the treatment of *S. aureus* infections, D-test be performed for differentiation (Seifi *et al*., 2012).The accuracy provided by D-test, is very important in the management of treatment of MRSA and MSSA infections; it prevents erythromycin-based treatment failure(Adhikari *et al*., 2017).

Among the Fluoroquinolones, Ciprofloxacin is the most extensively used antibiotic, due to its superior potency (Chakrakodi *et al*., 2016), but its usage in treating *S. aureus* infections has been compromised because of the rapid emergence of both MRSA and MSSA resistant strains (Lowy, 2003; Jacoby, 2005).

Very few MSSA and MRSA isolates exhibited a high resistance to ciprofloxacin in BCRH, Western Kenya. This in in contrast with most other studies which have reported significantly higher rates of ciprofloxacin resistance in MRSA compared to MSSA isolates, among clinical *S. aureus* isolates (Lindsay, 2013); they include studies in Tanzania, Eritrea, Nigeria and South Africa, which reported resistance rates of 54.0%: 0.0 %, 8.0%: 4.7 %, 72.7%:21.1 % and 91.0%:8.0 %, respectively (Mshana *et al*., 2009; Naik and Teclu, 2009, Falagas *et al*., 2013; Smidt *et al*., 2015). They, however, recorded a higher rate of MSSA ciprofloxacin- resistant isolates than MRSA ciprofloxacin-resistant ones; the case may have been due to MSSA clonal outbreaks (Dibah *et al*., 2014).

This study identified a novel variant strain MRSA ~ST241, which was isolated from an inpatient under five years of age; this variant is related to MRSA ST239/ST241-III [3A] that have previously been reported in

several African countries (Abdulgader *et al*., 2015). This variant MRSA ST241 was resistant to all beta-lactam antibiotics and also multiple classs of antibiotics, such as, ciprofloxacin, clindamycin, sulfamethoxazole /trimethoprim, erythromycin, gentamicin and tetracycline. In addition it was carrying virulence factor *sasX* (Xia and Wolz, 2014; Li *et al*., 2012); which assists the variantin colonization and pathogenesis, (Xia and Wolz, 2014; Li *et al*., 2012); it also supports immune system evasion (Foster *et al*., 2014; De Backer *et al*., 2012). The frequent use of Cefotaxime in empirical inpatient therapy in BCRH, against bacterial infections presents an ideal environment that may encourage rapid proliferation of novel MDR variant MRSA ~ST241; such a situation may expose inpatients at BCRH, to serious skin and surgical wound infections, if caused by the multidrug-resistant(MDR) strain, leading to prolonged hospital stay or even death (Kot *et al*.,2020).The rates of Cefotaxime empirical therapy found in all wards of BCRH are high, compared to rates of empirical gentamicin therapy at 15 %.

To mitigate this shortcoming, it is important to mobilize resources for the establishment of purposefully-built, well-conceived, cost-effective and efficient microbiology diagnostic laboratories to collect data, which will assist in accurate pathogen identification and establishment of their antibiotic susceptibility patterns, which will guide appropriate antibiotic prescription. The obtained data will assist in surveillance for these pathogens, resulting in improved antibiotic stewardship, which will assist in respective infection prevention and control.

This study has carried out a holistic study of *S. aureus* (MRSA/MSSA) antibiotic resistance, including both phenotypic patterns and presence of virulence genes, in human inpatients, abattoir workers and livestock populations in Busia County, Kenya.

Apart from HIV positive individuals being prone to opportunistic bacterial infections, occupational contact with livestock is also a known risk factor for human exposure to livestock- associated *S. aureus* infection (Leibler*et al*., 2016).; this organism has ability to infect cattle, hence abattoir workers (those who slaughter, act as butchery attendants and/or process livestock meat) are potentially exposed to infectious animal materials which

may contain livestock-associated *S. aureus.* There is scanty documentation on carriage of *S. aureus* among abattoir workers in beef production sector globally (Leibler*et al*., 2016). This study sought to demonstrate presence of livestock-associated strains of *S. aureus*among abattoir workers and livestock (brought for slaughter) in various slaughterhouses in Busia County. The study was based on phenotypic and molecular marker analysis. The results indicated a *S. aureus* nasal carriage of 16.0 % among the study population; this was slightly lower than the 20-30% prevalence documented in other studies (Preoțescu and Streinu-Cercel, 2013).

The study population in this community setting was made up of two groups: HIV-positive abattoir workers and HIV-negative abattoir workers. The prevalence of *S. aureus* nasal pharyngeal carriage of HIV-positive abattoir workers was slightly higher, at a rate of 25.8%; compared to 14.6% among their HIV-negative counterparts. Similar difference in nasopharyngeal carriage has been reported in Central and West African regions with similar study population structures; in Gabon, the prevalence of pharyngeal *S. aureus*in HIV-positive worker colonization was reported to be 20.6% compared to 18.0% in the HIV-negative population in a rural setting (Kraef *et al*., 2015), while in Lagos, Nigeria in a large urban setting, the nasal *S. aureus* carriage in HIV positive study subjects was reported to be 33% compared to 21% in HIV- negative individuals (Olalekan *et al*., 2012). These findings suggest that HIV individuals are predisposed to *S. aureus* nasopharyngeal colonization in the sub Saharan African region. Humans are asymptomatically colonized with nasal *Staph. aureus* in the range of between 20-30% (Preoţescu and Streinu-Cercel, 2013); colonization can cause opportunistic infection in immunocompromised individuals; these infections can be life-threatening if not treated promptly (Sakr*et al*., 2018);

With a higher prevalence of HIV- positive population than the national average (6.70% vs 5.90%), HIV-positive abattoir workers in Busia county are more likely to develop opportunistic bacterial infections due to their weakened immune systems, often ending up in hospital for treatment of these infections. HIV-positive abattoir workers, clinic attendance for check-ups, medication pick-up or re-filling of antiretroviral drugs and

sulphamethoxazole and trimethoprim prophylaxis, in clinics that are customarily hosted in hospitals, may introduce livestock-associated *S. aureus* into this nosocomial setting (Hidron *et al*., 2005; Cuny *et al*., 2015); they may, thus, transmit this livestock-associated pathogen to other outpatients, inpatients and health care personnel attending to them. They may also pass these strains to their fellow HIV- negative counterparts, meat consumers and possibly pass contaminated meat products to hospital settings and the general population.

The best way to address MRSA and MSSA infection in Busia County is by conducting regular surveillance of MRSA and MSSA nasal carriage; this will generate data for appropriate public health intervention. This type of surveillance should be introduced across Kenya, especially in rural abattoirs such as those found in Busia, which do not meet conditions set by the Meat Control Act of Kenya (2012).

Efforts to manage the dissemination of MRSA and MSSA must also be applied regularly, instead of a one -off reaction during outbreaks, as has been witnessed elsewhere (Abou-Shady *et al*., 2005). Lack of implementation of these controls may lead to increase of MRSA and MSSA infections, arising from occupation-related injuries among abattoir workers; laceration injuries are likely pathways for *S. aureus* to enter the body through breaks in the skin; that may in turn contaminate the meat, upon packing and serving, exposing their consumers to the risk of *S. aureus* infection. These consumers may in turn act as vehicles that spread these pathogens to the community and hospital populations (Cook *et al*., 2017).

Data from this study represents prevalence of MSSA and MRSA nasal carriage among abattoir workers community in Busia County in Western Kenya, and not for the general population. Information from this study may be used in future research as a baseline for *S. aureus* nasal carriage among abattoir workers in other regions of Kenya, as well as other parts of the world.

Resistant bacteria know no boundaries and spread via many routes from hospital environment into community, including inpatients - discharged, outpatients visiting the hospital for treatment or collecting prophylactic antibiotic in case of HIV-positive individuals. The issue of resistance as a result of empirical antibiotic

treatment is not limited to hospital settings but also to the community settings which include livestock handlers and animal rearing settings, these settings have seen increased incidences of this type of treatment (Malik and Bhattacharyya, 2019 ; Manyi-Loh *et al.*, 2018). The Eastern and Central African region where Busia County, Kenya is located, is designated as a high HIV prevalent areas (Kwena *et al.*, 2019), HIV positive individuals frequent hospitals more often than HIV negative individuals for the treatment of opportunistic bacterial infections as inpatients and outpatients.

Generally, higher Penicillin-G resistant *S. aureus* among abattoir workers may be attributed to, uncontrolled usage of Penicillin-G in livestock rearing is common in many part of sub Saharan Africa (Adesokan *et al*., 2013; Kimera *et al*., 2020): it could thus be responsible for the high prevalence of Penicillin-G resistant *S. aureus* among the abattoir workers. As mentioned earlier, these Penicillin-G resistant *S. aureus* strains may be passed to the general population via animal meat products, thus rendering infections by these strains untreatable by the antibiotic, despite its lower cost and low toxicity profiles (Fair and Tor, 2014; Cheng *et al*., 2016). Only few researches have assessed the prophylactic use of Penicillin-G in abattoir workers in low-resource settings (Kharsany and Karim, 2016), globally and in sub-Saharan region in particular; thus, this study has contributed data by shedding light on prevalence of antibiotic resistance in livestock handlers in Africa, a region considered as the global epicenter of HIVAIDS infection.

Data obtained from this study indicate a significant association between *Staph. aureus*SXT-resistant strains and HIV-positive abattoir workers($P > 0.00001$); these high prevalence rates could be due to selection pressure created by widespread empirical SXT use among HIV - positive abattoir workers (Dworkin*et al*., 2001); Tthis study did not investigate the extent of SXT prophylactic use in the general community).These findings are similar to another study carried out on HIV-positive individuals in Lagos, Nigeria, where there was a significant association ($P < 0.0001$) between HIV positivity and *S. aureus* SXT-resistant strains (Olalekan *et al*., 2012). The only difference between these two studies was that Lagos is the largest city in Sub-Saharan Africa with a

high human population, as opposed to Busia County. Another study conducted on HIV - positive patients in out-patient clinics in Lambaréné Gabon in Sub-Saharan Africa, found that SXT resistant *S. aureus* carriage was significantly associated with HIV-positive patients, compared with healthy controls. (Kraef *et al*., 2015). Similarity between these two studies and the one conducted in Busia County lends credence to the true effect of SXT prophylaxis in the increased spread of *S. aureus* SXT resistance among HIV -positive individuals in Sub-Saharan Africa. The same effect has been reported in the Indian sub-continent; HIV patients attending an outpatient skin clinic in Mangalore, India, had asignificantly higher nasal carriage of SXT-resistant *S. aureus* when compared to the general community (Chacko *et al*., 2009).

Livestock keepers usually administer antibiotics to animals to serve as growth promoters (assist them to survive stressful, crowded, and unsanitary environment). The abuse of these antibiotics, due to poor public health control policies, has been one of the lead promoters of emergence of antibiotic resistant bacterial strains in Africa (Kimang'a, 2012). It has also been established that thee inappropriate misuse of antibiotics in both humans and livestock , especially at sub therapeutic levels, led to emergence of antibiotic resistant strains such as MSSA and MRSA (Andersson and Hughes, 2012), and the resultant the generated resistant strains may end up causing infection in human population. The outcome of misuse of antibiotics is the rise and proliferation of resistant strains as a result of antibiotic selection pressure; it also aids in spread of these resistant strains into the environment;

Economic analysis, indicates that livestock keepers prefer using cheap antibiotics, such Tetracycline, Penicillins and Tylosin; it belongs to sulfonamide group; this common usage has in turn given rise to strains that are resistant to these commonly used antibiotics, which has lead to use of more expensive antibiotics, negatively affecting income of the livestock keepers(Yevutsey *et al*., 2017).

The study in Busia looked into presence of tetracycline resistant *S. aureus* among abattoir workers *vis a vis* inpatients population at BCRH. Tetracycline-resistant *Staph.aureus* strains were isolated at a significantly

higher rate in abattoir workers than in patients admitted at BCRH ($p< 0.043922$), with HIV-positive abattoir workers showing significantly higher rates of tetracycline resistant *S. aureus* nasal colonization compared to HIV- negative abattoir workers ($p< 0.016338$) and inpatients at BCRH ($p< 0.000219$). This difference could be attributed to the low usage of tetracycline as empirical antibiotic treatment among patients admitted in BCRH and higher prophylactic use of SXT in HIV-positive abattoir workers, giving rise to selective pressures favoring Tetracycline resistant *S. aureus* strains. This finding is in line with previous studies in other regions of the globe, linking over-usage of tetracycline in food-producing animals with emergence of livestock-associated tetracycline-resistant *S. aureus* strains. The linkage is a major concern to public health authorities, thus, results of the current studyare expected to encourage African governments to initiate surveillance studies on antibiotic resistance patterns of *S. aureus* strains affecting livestock and their handlers (Gundogan *et al*., 2005; Nemati*et al*., 2008). So far, there are few studies in Africa that have specifically addressed nasal carriage of *S. aureus* among abattoir workers

It is, however, noted that a number of respective surveillance studies have been carried-out in countries other than Africa (Johnson, 2011; Leibler *et* al**,** 2016); published results showing a steady rise in the incidence of antibiotic resistance in *S. aureus* strains isolated from livestock and their handlers.The incidence rise is attributed to misuse of antibiotics in both veterinary and human medicine (Aarestrup, 1999; Teuber, 2001). Findings of the current study among abattoir workers in Busia County mirrior with other studies conducted are elsewhere in the world, with one study conducted among beef packing workers in a Midwestern United States Slaughterhouse (Leibler *et al***,** 2016), which identified Tetracycline resistance in *S. aureus* as an indicator of livestock-association*;* raising a possibility of zoonotic transmission between abattoir workers and food-producing animals in Busia County.

Factors contributing to higher Tetracycline-resistant *S. aureus* carriage in abattoir workers , include; increased use of Tetracycline over the years, for the control of liver abscesses in ruminants , prevention of foot rot in

cattle, improved animal growth and feed efficiency and prevention of other bacterial infections in animals and chickens in the eastern African region (Kimang'a, 2012; National Research Council (US) Committee, 1980). Veterinarians have been blamed for the excessive use of antibiotics in livestock farming, which has given rise to antibiotic resistance problem in many parts of the world (Fortané, 2019), limiting the effectiveness of Tetracycline in treating human and animal infections (Chopra and Roberts, 2001). It is, however, encouraging that even with little resources provided by donors and governments for research in various African countries, there have been concerted efforts directed towards the introduction of control measures to reduce rise of antibiotic resistant bacterial strains (Ndihokubwayo *et al*., 2015). It is therefore important to encourage studies on zoonotic transmission of *S. aureus* in sub-Saharan region, for better formulation of control and management measures, towards curbing the spread of antibiotic resistance in humans and animals.

Occurrence of MDR *S. aureus* in Sub Saharan Africa poses a serious public health concern (Garoy *et al*., 2019); these strains were previously associated with nosocomial infections, but the epidemiology has changed, with the rate of MDR MRSA /MSSA isolation from infections, becoming quite prevalent in the community. These epidemiological changes raise the possibility of MDR MRSA /MSSA transmission to consumers via a contaminated animal meat products, particularly, unhygienic meat handling by a colonized abattoir workers, further complicating an already serious problem that were previously restricted to nosocomial situations (Lozano *et al*., 2016; Smith, 2015). Zeroing down to phenotypic and molecular markers of multi drug resistant *S. aureus* and MRSA among abattoir workers, antibiotic susceptibility testing established that HIV-positive abattoir workers were significantly colonized with MDR- *S. aureus* isolates compared to their HIV-negative counterparts ($p < 0.000418$), with MSSA-Penicillin,Tetracycline and Trimethoprimbeing the predominant MDR *S. aureus* resistance pattern. Similarly, the proportion of MDR MSSA strains isolated in various populations were significantly higher in HIV - positive abattoir workers compared to patients admitted in BCRH (*p*-value is .000256) and HIV – negative abattoir workers (*p*-value is .000498). HIV- positive abattoir workers were

significant carriers of MSSA-Penicillin,Tetracycline and Trimethoprim, MSSA - Penicillin, Sulphamethoxazole - Trimethoprim and Tetracycline and MRSA-Cefoxitin, Penicillin, Sulphamethoxazole-Trimethoprim, Erythromycin and Inducible clindamycin resistance, compared to HIV- negative abattoir workers ($p < 0.004288$); this could be due to selective action of SXT prophylaxis in HIV- positive abattoir workers. There are other reports of HIV infection being associated with multi-drug resistant bacteria (Marbou and Kuete, 2017). What is more worrying was the presence of a small number of highly resistant MDR *S. aureus* strains exhibiting resistance to MSSA-Penicillin, Sulphamethoxazole -Trimethoprim, Erythromycin - Inducible clindamycin resistance and MSSA-Penicillin, Sulphamethoxazole -Trimethoprim, Erythromycin, Ciprofloxacin and Inducible clindamycin resistance in HIV- positive abattoir workers in Busia. Check for possible repetition. There was an MDR MSSA strain isolates from an HIV – negative abattoir worker, that was resistant to Penicillin, Trimethoprim, Erythromycin, Gentamicin and Inducible clindamycin, elucidating that the MDR problem occurs across the population. This study has demonstrated a possibility of significant circulation of STX- Tet -resistant livestock-associated MRSA and MDRSA isolates in the HIV -positive abattoir workers ($p < 0 .004288$); meaning that there is a potential livestock-to-human transmission of *S. aureus* in Busia county; a food safety concern. In addition to this, there were two community acquired MRSA ST 88 strains belonging to SCC *mec* IV which were also resistant to TMP and clindamycin among Busia abattoir workers studied in a community setting. MRSA ST 88 strains were resistant to all -beta-lactam antibiotics, compounding the problem assocaied with MDR.

Multi-drug resistant *S. aureus* strains colonizing HIV - positive abattoir workers could enter into the meat supply chain through the meat and get transmitted to their fellow workmates and community meat consumers (Sáenz *et al*., 2001); in case the respective abattoir workers get hospitalized, the strains can be transmitted to hospital staff and other inpatients, threatening effective treatment of resultant infections (Smith *et al*., 2002). HIV - Positive persons, who could also in turn, introduce MDR *S. aureus* to the hospital environment, during

their regular checkups at HIV clinic, that are is traditionally located within hospital setting (Wringe*et al*., 2017). This has brought to light, the potential public health concern on food-animal production and antibiotic use (Kimang'a, 2012) and have demonstrated the potential danger of occupational exposure of MDR isolates of *S. aureus* to livestock handlers especially abattoir workers employed in these rural slaughterhouses.

Thus, MDR *S. aureus*-carrying abattoir workers pose a public health problem in hospitals, community and food industry in Busia county, Kenya and its neighboring communities,s taking into account Busia being a border town. Urgent measures should, therefore, be taken to prevent proliferation, spread and dissemination of livestock-associated MRSA and MDR *S. aureus* strains in these settings, as it has capability of limiting treatment options for MRSA/MSSA infections in livestock, community and hospital settings, in the already resource-constrained setting in rural Kenya (Ye *et al*., 2016a).The hospital management is encouraged, to develop strategies to prevent and control the spread of antibiotic resistance (Uchil *et al*., 2014), by forming a multidisciplinary team to implement local policies on use of antibiotics, infection control interventions, prompt detection and reporting of the antibiotic resistant strains, better surveillance and control of transmission of resistant bacteria, as practiced successfully in other hospitals (Struelens, 1998).

This study provides an insight on possible transmission risk of multi drug resistant MSSA and MRSA strains to the larger community in Busia, among whom are meat consumers; through interaction with infected abattoir workers or respectively contaminated meat products, raising a considerable public health problem in this area.

There have been varied efforts to control antibiotic usage with varying levels of successes in Europe. Efforts to control MRSA infection need to be sustained even in countries reporting declining prevalence. On the other hand, developing countries, especially in Africa, Asia and South America, need to adopt similar policy to fight the spread of MRSA and MSSA within their borders (Johnson, 2011).

Vancomycin, Daptomycin and Rifampicin are recognized alternative antibiotics to ciprofloxacin, for the treatment of *S. aureus* infections caused by ciprofloxacin-resistant MRSA and MSSA strains (Brumfitt and

Hamilton-Miller, 1989); a point that needs be brought-out and acted upon by public health and other health related authorities in the light of increased circulation of MRSA/MSSA quinolone-resistant strains, which pose serious limitations to Ciprofloxacin clinical effectiveness. These corrective measures need to be taken in order to control and stop the spread of MRSA/MSSA quinolone-resistant strains in Busia County, Western Kenya and the bordering countries. It is, however, encouraging to note that, results gotten in the current study indicate that ciprofloxacin is still a viable alternative to vancomycin, since; 93.3% of the MRSA/MSSA isolates in Busia were susceptible to it; these include isolates from HIV - positive abbatoir workers which showed resistance at 2.2 %, while all isolates from HIV- negative abattoir workers were susceptible. This may be due to very low usage of the antibiotic for empirical treatment among inpatients in BCRH. However, caution needs to be taken as a few MDR MRSA and MSSA strains were detected from 2 patients (0.8 %); meaning that the few resistant ones can easily multiply, in case of increased usage of the antibiotic for empirical antibiotic therapy.

However, many classes of antibiotics have been used to treat both humans and livestock, some antibiotics are approved for use in animals, others in human, and still others in animals and humans; these are also called shared class antibiotics (Phillips *et al.,* 2004). The classes used include: β-lactams (penicillins and cephalosporins); sulphonamides with and without trimethoprim; tetracyclines; macrolides, lincosamides and streptogramins; and quinolones including fluoroquinolones (Bager and Emborg, 2001); penicillin derivative , such as ampicillin, a broad spectrum derivative of penicillin and cloxacillin; Tylosin, belonging to sulfonamide group, used for the treatment of metritis and acute mastitis in cattle, sheep and goats, enteritis, pneumonia, erysipelas, and infectious arthritis in swine (Giguère *et al.,* 2013). It is also used to treat chronic respiratory disease in chickens.

Tetracycline and sulfamethoxazole and trimethoprim are two antibiotics which are mostly used as prophylactic remedies in livestock rearing, as growth promoter; it is also used in treating opportunistic bacterial infection in HIV/AIDS patients (Kimera *et al*., 2020). An estimated 1.6 million people live with HIV/AIDS in Kenya; the

disease is the fourth highest in term of global epidemic (UNAIDS, 2017 Data Book), yet very little is known about MRSA/MSSA genotypes or phenotypes in HIV-infected individuals, more so, those working in slaughterhouses located in many parts of the world.

Sulphamethoxazole and trimethoprim (SXT), is used as a primary or secondary prophylactic antimicrobial in HIV/AIDS patients, for the prevention of *Pneumocystis jiroveci* pneumonia and toxoplasmosis (Ribera *et al.*, 1999; White *et al.*,1999), as a standard care in the management of HIV-infected individuals. In early 1990s, developed nations, introduced standard care in the management of HIV-infected individuals in their countries, this was later adopted by many sub-Saharan countries (Dworkin *et al*., 2001), including the Kenyan government HIV/AIDS control program (Hamel *et al*., 2008; World Health Organization Technical Report Series: 2016). Apart from its prophylaxis use, SXT has been used as a broad-spectrum antibiotic against a wide range of aerobic Gram positive and negative bacteria, fungi and protozoa (Aksoy and Unal, 2008). It has been recognized for prolonging life span of HIV - positive individuals, in resource-limited settings such as Kenya (World Health Organization, 2014); its affordability makes it the antibiotic of the choice in countries with high prevalence of HIV (World Health Organization, 2014);

SXT can be used to treat a number of opportunistic bacterial infections in HIV-positive persons, (Sibanda *et al*., 2011). These findings present an opportunity for the public health authorities, to develop strategies that can reduce spread of SXT resistant *S. aureus* strains, taking into consideration that areas such as Busia County are characterized as high burden HIV/AIDS zones (National AIDS Control Council, 2016).

The importance of MSSA/MRSA as pathogens in Busia County cannot be underestimated, because pathogen can possess a number of virulence and other factors such as Panton Valentine Leukocidin (PVL) and Toxic shock syndrome toxin-1, giving it the superbug nature. Theseis organisms are causes of staphylococcal food-borne diseases in humans and they are found in hospitals, communities, environments, food products and food handlers, like the abattoir workers, in Busia County (Kadariya *et a*l., 2014; Smith *et al*., 2015).

Staphylococcus aureus harboring PVL gene, such as those that colonize abattoir workers in Busia County, are capable of causing infections like: skin, subcutaneous tissue, fascia, and muscle infections and cellulitis (Stulberg *et al*., 2002). Simple cellulitis, may rapidly progress to complicated skin and soft-tissue infections, such as necrotizing fasciitis, which may invade deeper tissues. If necrotizing fasciitis is not identified early and treated, it may lead to amputation of limb or loss of life (Espandar *et al*., 2011). HIV immune -suppression is one of the conditions, which have been identified as predisposing factors that can lead to the development of skin infections (Crum-Cianflone *et al*., 2012).

Looking at the virulence factors associated with *S. aureus* isolates among abattoir workers in Busia County, the prevalence of PVL genes carriage in HIV positive abattoir workers was slightly higher than that of HIV negative abattoir workers. There were no differences in prevalence of *S. aureus* PVL-positive strains carriage between HIV positive and HIV negative abattoir workers in Busia County ($p > 0.518418$). This compares well with also the case in Gabon, where the prevalence of *S. aureus* PVL-positive strains in HIV-infected population was higher than the study population not infected with the virus (Kraef *et al*., 2015). However, in Nigeria, the prevalence of *S. aureus* PVL-positive strains carriage were evenly distributed in both HIV-positive and HIV negative individuals (Olalekan *et al*., 2012). Regional differences in prevalence of *S. aureus* PVL-positive strains in populations residing in sub-Saharan region have been documented; lower prevalence was reported in Busia County, North, East and Southern Africa when compared with those found in West Africa (Breurec *et al*., 2011; Darboe *et al*., 2019). This study found that *S. aureus* PVL-positive carriage is endemic in abattoirs workers, as witnessed elsewhere in the Sub-Saharan Africa, exposing abattoirs workers, to opportunistic deep skin and soft tissue infections, particularly furunculosis, cutaneous abscesses, and severe necrotizing pneumonia (Breurec *et al*., 2011; Kraef *et al*., 2015; Vandenesch *et al*., 2003).

The study in Busia County, Kenya looked into the relationship between *S. aureus* SXT resistance and presence of PVL gene in HIV positive abattoir workers; it mirrored another study in Gabon, where the study population

consisted HIV - positive patients attending out-patient clinics. Both countries are considered high-HIV-burden areas, though Kenya has higher prevalence of HIV- positive population (5.9%) compared to Gabon with a prevalence of 3.6 % (The Central Intelligence Agency, 2016; National AIDS Control Council, 2016). The Gabonese researchers found that there was association between PVL- positivity and SXT resistance among *S. aureus* organisms isolated from HIV- positive population; they stated that, this association arose due the regular SXT usage for prophylaxis in the HIV infected population. The study went further and identified PVL -positive *S. aureus* clones associated with SXT resistance (Kraef *et al*., 2015). The current, Busia study, identified high association between *S. aureus* SXT resistant strains, HIV carriage and PVL carriage in the abattoir worker population. It was found that the majority of the SXT resistant strains, isolated from HIV-positive abattoir workers, belonged to ST8 MSSA strain/group which did not harbor PVL gene; the only one which was carrying PVL gene belonged to the group MSSA ST152 Similar strains (MSSA ST152 PVL-SXT), plus MSSA ST15 PVL-SXT, had previously been reported in Gabon in HIV- infected population (Kraef *et al*., 2015).

. Methicillin-susceptible *Staphylococcus aureus* ST15 and ST152 clones are the largest sequence types associated with PVL gene carriage in Africa (Donkor*et al*., 2015; Egyir *et al*., 2014); this was also the case in Busia County, where, similar *S. aureus* clones were found circulating among abattoir workers. Phylogenetic tree analysis of *S.aureus* ST152 clones in Busia showed presence of PVL gene in one clade in phylogenetic tree, this gene was probably acquired ancestrally before the split into two clades, with a few ST152 *S. aureus* clones losing PVL gene later.

In the course of their work, abattoir workers may be colonized by ST152 PVL- positive *S. aureus* clones via contact, from fellow workers or animals and their carcasses, presenting a possibility of *S. aureus* infection (Klous *et al*., 2016). Once an abattoir worker is colonized by PVL- positive *S. aureus* strain, there exists a possibility of such abattoir worker transmitting the PVL- positive *S. aureus* strain to his/her HIV - positive colleagues or HIV - positive customers, exposing these vulnerable groups to high risks of contracting skin,

subcutaneous tissue, fascia, muscle infections and cellulitis. Some of these infections can easily be transformed into complicated skin and soft-tissue infections, such as necrotizing fasciitis (Sáenz *et al*., 2001); in case the colonized abattoir workers get hospitalized, the strainmay be further transmitted to hospital staff and other inpatients, introducing this virulent strain in the hospital settings (Smith *et al*., 2002). Although, *S. aureus* harboring PVL gene are found in less than 5% of all clinical isolates, the strains have been previously linked to serious pneumonia known as necrotizing pneumonia, though debatable (Lina *et al*., 1999), which is often fatal primarily in young and healthy patients, with a 75% mortality rate, despite intensive medical treatment (Gillet *et al.,* 2002). There are two schools of thought related to PVL-associated *S. aureus* as the causative agent of necrotizing pneumonia in humans (Jappe *et al*., 2008; Gillet *et al*., 2002; Voyich *et al*., 2006; Labandeira-Rey *et al*., 2007; Shallcross *et al*., 2013).The sub-Saharan region is recognized as the world's epicenter ofHIV/AIDS , in addition, a virulence factor which is associated with skin and soft-tissue infections (Deurenberg *et al*., 2007) and has a debatable role in causing necrotizing pneumonia is prevalent in the region (Jappe *et al*., 2008; Gillet *et al*., 2002; Voyich *et al*., 2006; Labandeira-Rey *et al.*, 2007; Shallcross *et al*., 2013). However, peer reviewed publications continue to quote either of these contradictory scientific opinions, making it difficult to conclusively state whether or not PVL gene and other cytolysins play a clinical role in causing severe necrotizing pneumonia in humans. It is, therefore, important for the matter to be settled since it leaves a huge gap in public health management of *S. aureus* related necrotizing pneumonia in humans, especially among immunocompromised HIV - positive patients. Non-closure of this issue may lead to wrongful diagnosis, where HIV - positive patients presenting with serious necrotizing pneumonia caused by PVL-positive *S. aureus*, may be wrongly diagnosed as suffering from *Pneumocystis jiroveci* pneumonia or pulmonary tuberculosis and visceral leishmaniasis (Toledo and de Castro, 2001), such which easily be treated using sulfamethoxazole-trimethoprim in HIV-endemic areas of sub Saharan Africa (Huang and Crothers, 2009).

This is a very serious disease such as necrotizing pneumonia , especially in resource-constrained areas with weak health systems, like Busia County, taking into account that, a higher proportion of *S. aureus* isolates in Sub-Saharan Africa (57% in west Africa) are producers of Panton-Valentine leucocidin (PVL) compared to Europe and United States < 5 % (Chao *et al*., 2014; David and Daum, 2010; Holmes *et al*., 2005; Breurec *et al*., 2011). Diagnosis and establishment of the exact extent of a disease in patients, is critical for successful management of *S. aureus* infections, such as soft-tissue infection, bloodstream infections, pneumonia, or bone and joint infections.

Staphylococcus aureus organisms carrying a TSST-1 gene mainly reside in the vagina of infected women, but can also be found in other areas of the body such as the nasopharyngeal (Edwin *et al*., 1988). Toxic shock syndrome is a result of one of the enterotoxins, which belong to a group of pyrogenic exotoxins produced by *S. aureus* (Marrack and Kappler, 1990). It is produced and secreted by *S. aureus* carrying a TSST-gene (*TSST-1*), at the site of an infection, for example a cut skin (Spaulding *et al*., 2013).The toxin then enters into the bloodstream attacking the vascular system leading to inflammation, fever, symptoms of shock and tissue destruction (Talal *et al*., 1991).

The prevalence of *S. aureus* harboring TSST-1 gene among abattoir workers in Busia County was 1.4%. The gene was found in 13 % of *S. aureus* isolated from HIV- negative abattoir workers, though not statistically significant ($p \leq 0.6096$); these included all the nine MSSA ST72 strains isolated; other *S. aureus* sequence types harboring TSST-1 gene were MSSA ST707 and MSSA ST22.

Several studies have reported presence of *S. aureus* carrying a TSST-1 gene in food chains and among food handlers in Libya and Trinidad (El-Ghodban*et al*., 2006; Adesiyun *et al*., 1997), human milk in Libya, bovine milk in Hungary, mastitic milk samples in Iran and milk in Trinidad (El-Ghodban*et al*., 2006; Peles *et al*., 2007; Momtaz *et al*., 2010; Adesiyun *et al*., 1997),in food samples in Libya, Taiwan and Nigeria (El-Ghodban*et al*., 2006; Tsen *et al*., 1998; Adesiyun*et al*., 1992) and animals in Nigeria (Adesiyun *et al*., 1992).

Toxic shock syndrome toxin -1-carrying *S. aureus* may be transmitted to patients and health workers from meat products previously handled by abattoir worker; such zoonotic transmissions have been reported in Europe and United States (Smith, 2015; Price *et al*., 2017; Fasanmi *et al*., 2017a). *Staphylococcus aureus* infections do occur in inpatients or in the community, especially among the most vulnerable HIV- infected population, they may develop fever, rash, hypotension, multiple-organ-system dysfunction, and desquamation (Tong *et al*., 2015); some of these conditions are life threatening if not treated successfully (Reingold *et al*., 1982; Jamart *et al*., 2005). People in African countries do not receive much consumer protection compared to those in the European union countries; the developed nations have adopted an integrated approach to food safety, by monitoring meat products from the farm to the dining table, through risk assessments and management practices, (Sofos, 2008; Nørrung and Buncic, 2007).

Due to high morbidity and mortality associated with TSST-1 in individuals with immunosuppressed condition (Kulhankova *et al*., 2018), future introduction of recombinant vaccine, especially in the vulnerable group may be a life saving measure (Sharma *et al*., 2018). *Staphylococcus aureus* screening and decolonization should also be conducted in high risk groups such as abattoir workers, who are an important link in the food chain; these measures can prevent incidences of this life-threatening syndrome in sub Saharan region in future (Sharma *et a*l., 2018). The current study is the first in Eastern African region to report on presence of TSST-1 gene-carrying *S. aureus* in the nasopharynx of abattoir workers. It has provided useful insight on lineage of TSST-1 gene-carrying *S. aureus* circulating in Busia County and raised a possibility of transmission of this strain to vulnerable HIV- infected population, and contaminating meat; resulting in patients admitted at BCRH. Despite the inability of laboratories to detect TSST-1 carrying *S. aureus* in many hospitals in sub-Saharan region, this study provides baseline data for future research in the African continent, particularly in the area of meat hygiene in sub-Saharan region.

Abattoir workers in rural small-scale slaughterhouse settings in sub-Saharan Africa are unregulated, characterized by lack knowledge on foodborne diseases and their associated risks, poor personal hygiene, and they still use uncertified meat transportation methods, and these challenges have been observed in Busia County (Mann *et al*., 2013; Todd 2016; Cook*et al*., 2017).

The poor state of these slaughterhouses allows *S. aureus* contamination to persist on the floor, in inanimate surfaces, equipment and the abattoir workers' clothings. These unhygienic practices facilitate cross-contamination, from contaminated meat to the abattoir worker during carcass/meat processing (Fasanmi *et al*., 2018); passing-on foodborne pathogens to the meat products, after which the pathogens may find their way to food chains in the community and hospital settings (Abebe *et al*., 2018).

This study did not investigate the presence of *S. aureus* on the floor, inanimate surfaces, in the equipment used, the abattoir workers' clothing or level of environmental contamination in slaughter houses, which need to be investigated in the future.

Using phylogenetic analysis, this study provided evidence of cross-transmission of *S. aureus* between abattoir workers, in patients and livestock reared in Busia County. This evidence of possible direct transmission of *S. aureus* between inpatients, raises the possibility of further transmission to healthcare personnel's hands or gloves. This transmission could have a domino effect, putting other hospital personnel and their family members, outpatients; more so, the immune-compromised patients such HIV-infected and cancer patients attending clinics in the hospitals, at risk of nosocomial *S. aureus* infection. The immune - suppressed status puts the HIV-infected individuals at risk of getting opportunistic and healthcare-associated infections in hospitals, especially hospitals lacking infection control programs, as seen in a number of those in sub Saharan countries (Mehta *et al.*, 2014; Hall, 1998).

Taking into consideration the high prevalence of HIV in Busia County, which stands at 7.7 %, as compared to a national prevalence of 4.9% (National AIDS Control Council, 2018), a sizeable population of HIV infected

persons form a link or a route for possible transmission of nosocomial pathogenic *S. aureus* strains from hospital to the general community and vice *versa*; abattoir workers being a subset of the general population in a community setting. If infected, they can transmit this pathogen to their colleagues, customers and contaminate meat products.

As evident in this region, a number of HIV-positive abattoir workers were found to be harboring SXT resistant *S. aureus*; some of which were MDR, carrying virulence factors. These strains can find their way and contaminate meat products; they can also easily be transmitted to humans who eat this contaminated food.

This type of transmission has previously been observed in a longitudinal cohort study inCounty Hospital, Brighton, England, where the researchers used whole-genome sequence to link nasal *S. aureus* carriage transmission between health-care workers and patients (Price *et al*., 2017).This study identified health-care workers as the main source of *S. aureus* transmission to patients, highlighting importance of phylogenetic analysis application to ascertain the genetic relatedness of isolates. Phylogenetic analysis assists in identifying the source of infection to strain level. Target treatment may also reduce transmission of the identified *S. aureus* strain (Price *et al*., 2017). Effecting application of infection control methods by the BCRH authorities is a necessity.

Studies of genetic diversity of *S. aureus* in humans, animals and foods in Africa are important in determining the sources of *S. aureus* and MRSA in an ecological niche, the pathogen genetic makeup, and developing effective control strategies (Abdulgader *et al*., 2015; Okorie-Kanu *et al*., 2015). High ethnic, culture, religion and lifestyle diversity are drivers of the diversity seen in MSSA and MRSA strains in various communities residing in sub Saharan region (Abdulgader *et al*., 2015). While most studies on molecular characterization of MSSA and MRSA, particularly in African continent, have been geared towards human clinical isolates, little being known about the genetic diversity of MSSA and MRSA in abattoir workers and livestock populations (Falagas*et al*., 2013; Schaumburg *et al*., 2014; Abdulgader *et al*., 2015); this study endeavoured to establish

molecular characterization of the latter population in Busia County. It has revealed high clonal diversity among abattoir workers, inpatients and livestock populations.The results were in concurrence with those reported by Deurenberg *et al.* (2008), that there is more diversity in MSSA populations than in MRSA populations, globally.

Methicillin-sensitive *Staphylococcus aureus* clonal complex 152 (ST152 and ST1633) was the predominant lineage among abattoir worker population in Busia county, majority of them harboring PVL gene (< 90%).

This clonal complex has been reported to be endemic in Africa and the Caribbean as opposed to Europe (Sowash and Uhlemann, 2014; Ruimy *et al*., 2008). Seven sequence types ST15, ST6, ST88, ST72, ST1, ST97 and ST707, similar to those that were previously reported in Gabon and Senegal (Schaumburg *et al*., 2011) were isolated in the current study. Other sequence types isolated in both nosocomial (BCRH) and community settings in Busia included; MSSA ST1633, ST80, ST188, ST25, ST80, ST188, ST22*, ST25, ST188, ST80 and ST25.There were 8 sequence types (ST1, ST5, ST72, ST80, ST97, ST188, ST707 and ST1633) were circulating among inpatients and abattoirs workers populations in Busia County, Kenya, strains which have not been reported in other African countries; notably the sequence ST72 was harbouring TSST-1 gene. The study in Busia also recorded presence of a new variant, ~ST508, which had a mutation at Acetyl coenzyme A acetyltransferase (*yqil*) gene (loci). Those that are also present in other African countries were: MSSA ST15, ST152, ST25, ST88, ST8, ST30, ST22 and ST508. Sequence types that were found in other African countries, while absent in Busia (Abdulgader *et al*., 2015; Egyir *et al*., 2014) were: ST121, ST7, ST45, ST852 and ST 1865. There are variation in dominancy of MSSA clonal complexes in different continents, with CC121 being identified as being the most dominant CC in Asian (Ruimy *et al*., 2009), while CC30 was reported to be dominant in Europe (Feil *et al*., 2003); MSSA CC5 was the dominated clonal complexes in hospital settings in other parts of Africa (Abdulgader*et al*., 2015), whereas, CC152, CC8 and CC508 were the most dominant clonal complexes in BCRH; sequence types recorded for MSSA CC152 were ST1633 and ST152; those

recporded for CC8 and CC508 were ST8 and ST508, respectively. For community-acquired *S. aureus* isolates in Busia County, only two MRSA were recorded: ST88 and ST140. This is the first recorded case of the strain CA-MRSA ST140 in Africa; it was isolated from an inpatient at BCRH. Other reported cases are from outside Africa, for example: it was isolated from an outpatient residing in Örebro County Sweden and was classified as one singleton ST140 (Berglund*et al*., 2009). Community-acquired methicillin resistant *S. aureus* ST88 clone has established itself as the dominant MRSA clone in Africa, though its emergence and evolution in the continent is poorly understood and it is popularly referred-to as "African" CA-MRSA clone (Kpeli *et al*., 2017).The strain ST88 was isolated from some HIV- negative abattoir workers in Busia; the isolates contained a SCC*mec*-IV cassette, were resistant to all β-lactam antibiotics, Trimethoprim and showed intermediate resistance to Clindamycin. The isolates had different multi drug resistance characteristic compared to a recent emergent Ghanaian CA-MRSA clone, which has variable degree of resistance to erythromycin, clindamycin, trimethoprim, amikacin and streptomycin (Kpeli*et al*., 2017).

Community-acquired methicillin resistant *S. aureus* strains that have been previously identified in Africa include, CA-MRSA ST88, ST8, ST30, ST5, ST72, ST22, ST80, ST1010, ST1, ST728, ST250, ST94, ST37, ST39, ST152, ST247, ST772, ST2021, ST45, ST1289, ST2629, ST789, ST239, ST612, ST36, ST1173, ST1338, ST650, ST635, ST636, ST637, ST97, ST1819, ST2563, ST1440, ST45 and CA-MRSA ST153.This clonal heterogeneity is similar to molecular population structures ofCA-MRSA strains found in Asia and European countries (Chen and Huang, 2014).

The current study is the first to report on a new variant of MRSA, ST241 strain, carrying the *sas*X gene in Africa; it has a unique allele profile of the seven housekeeping genes (loci), with a distinct allele numbers assigned to one of the seven housekeeping genes (loci) encoding for glycerol kinase (*glpF*). This unique allele profile is slightly different from known MRSA ST241 profile.

Methicillin-resistant *Staphylococcus aureus* sequence types ST239 and ST241-III hospital-associated strains are widely disseminated in many African countries and they have been characterized from nine African countries (Abdulgader *et al.,* 2015) and has been reported to cause outbreaks in hospital settings (Holden *et al*., 2010); they have showed increased resistance to antibiotics (Okon *et al.,* 2009), and capacity for invasive disease (Wang *et al.,* 2009; Aires-de-Sousa *et al*., 2008; Feil *et al.*, 2008).

This study also found human genotypes ST152, ST8, ST1290, and ST30 in livestock reared in Busia County. This implies that some MSSA clones may have ability to cross the species barrier, resulting in cross-infections between humans to several livestock species. The link for the cross-infection may have been the abattoir workers; transmitting the organisms to the livestock, due to the close proximity between abattoir workers and livestock/meat products during slaughtering or processing (Swai*et al*., 2012).

Interestingly ST1925-MSSA strains were isolated exclusively in livestock population. They were previously detected in monkeys from Gabon (Schaumburg *et al*., 2015) and their findings pointed to a need for further research, for example: as to why this sequence type lacks the ability to colonize humans, despite the close proximity between monkrys and livestock/meat products, and, by extension, with abattoir workers.

Two sequence types, MSSA ST573 isolated from anabattoir worker and MRSA ST140 isolated from an inpatient from Busia County, have not been previously reported in the African continent, but they have been isolated in other parts of the world. Methicillin-sensitive *Staphylococcus aureus* ST573 has been previously reported in a community setting in Australia and Bangladesh, both in Asia (Monecke *et al*., 2013; Afroz *et al*., 2008); MRSA ST140 has been isolated at a neonatal ward in Sweden, Europe ; inferring a possibility of international transmission of these strains into Busia County or *vice versa*. This study also identified a

novel Clonal complex CC 661, represented by sequence type ST661 of unknown origin and new STvariants ST 88, ST1290, ST1292and ST8, which have not been reported earlier.These were found to also harbour factors

influencing host pathogen interactions; however, none of them contained any virulence genes such PVL and TSST-1 genes.

The role of whole genome sequences of the isolates in this study cannot be understated, his technique and its analysis revealed high clonal diversity among MSSA isolates in Busia County with MSSA clonal complex 152 (ST152 and ST1633) being the predominant lineage, majority of them harboring *pvl* gene. This clonal complex has been reported to be endemic in Africa and the Caribbean, as opposed to Europe (Sowash, and Uhlemann, 2014; Pantosti ,2012).Phylogenetic analysis using this method confirmed the possible human to human transmission both in the nosocomial and community setting inBusia County. If this technique is adapted in this region, it will simplify the accurate identification of virulence genes, assisting with an effective epidemiological, virulence and antibiotic resistance detection and surveillance system for prevention of disease outbreaks.

This is the first study to have provided data based on One Health genomics assessment of MSSA/MRSA isolates circulating in Western Kenya. This evidence will help Kenyan Government and policy makers to come-up withre spective intervention measures for this region, as well as other resource limited settings.

Future research investigation should also focus on role played by livestock in the transmission and dissemination of livestock-associated *S. aureus;* between different food animals, abattoir workers and healthcare workers and their genetic diversity. The collected data will further inform on *S. aureus* dissemination dynamics in Busia.

6. CONCLUSIONS

A. Status of empirical inpatient antibiotic therapy at the Busia Referral County hospital.

I. Cefotaxime, metronidazole, and erythromycin were the most frequently prescribed antibiotics for empirical antibiotic treatment among patients admitted at BCRH.

II. The study identified the following knowledge gaps at BCRH:

i. There was lack of local, national, or regional data, on the use of empirical antibiotic therapy used in BCRH.

ii. There were no standard empirical treatment guidelines for treatment of *Staphylococcus aureus* infection at BCRH, no evidence of surveillance and no assessment of the impacts of changes in antibiotic use on treatment of *Staphylococcus aureus* infections.

iii. There were also no prospective programs regarding appropriate empirical antibiotic therapy, including choice of the appropriate empirical antibiotic regimens, dosage, duration of therapy, and how to address and identify the emergence of *Staphylococcus aureus* antibiotic-resistant strains.

B. Prevalence of *Staphylococcus aureus* and antibiotic resistance in inpatients, abattoir workers (HIV+VE and HIV-VE) and livestock in Busia County.

i. The study recorded a higher rate of MSSA than MRSA carriers among patients admitted at BCRH.

ii. The study revealed that the percentage of *S. aureus* nasal carriage was higher in HIV-positive abattoir workers than that of their HIV-negative counterparts, although this difference was not statistically significant.

iii. This study provided anecdotal evidence on the presence of *S. aureus* nasal carriage in the livestock population in Busia County.

I. Findings of of *S. aureus* antibiotic resistant strains obantained from inpatients, abattoir workers (HIV+VE and HIV-VE) and livestock in Busia County.

i. MSSA strains isolated in Busia county were highly resistant to Penicillin, Trimethoprim and Cefotaxime.

ii. MRSA isolates obtained from inpatients at BCRH, exhibited high Multi drug resistance to commonly used antibiotics used within the hospital and were susceptible to ciprofloxacin, clindamycin, trimethoprim/sulfamethoxazole, erythromycin, gentamicin and Tetracycline.

iii. There was also an evidence of the possible emergence of vancomycin intermediate *S. aureus* in the hospital, with the potential for limiting antibiotic treatment options for MRSA/MSSA infections.

iv. Highly MDR MSSA strains colonizing the HIV negative abattoir worker population were detected. These strains showed multiple resistance patterns of PenicillinG-Ciprofloxacin-Erythromycin- Tetracycline - TMP and SXT-inducible clindamycin and Penicillin-G-Erythromycin- TMP- clindamycin inducible clindamycin.

v. Penicillin -Sulphamethoxazole and trimethoprim was the most common resistance pattern among MDR *S. aureus* strains associated with HIV- positive abattoir workers.

vi. On the basis of antibiograms obtained, Chloramphenicol, Daptomycin, Fusidic Acid, Linezolid, Mupirocin, Nitrofurantoin, Rifampicin and Teicoplanin may be used as alternative antibiotics for treatment of MSSA and MRSA infections in BCRH.

C. Association between *S. aureus* genotype, antibiotic resistance and virulence genes among inpatients and abattoir workers population in Busia County.

I. An association between *S. aureus* genotype and virulence genes, PVL, TSST-1 and *sas*X were observed among both inpatients and abattoir workers .

II. Carriage of MSSA ST72 was associated with TSST-1 gene among abattoir workers in Busia County; this may be the first study that reports this association in Africa region.

III. An association between possessions of virulence factors, *S. aureus* genotype and antibiotic resistance profile was found as follows:

   i. A HIV-positive abattoir worker was colonized by a panton valentine leukocidin -associated MSSA belonging to sequence type ST 152 and exhibiting resistance to Sulfamethoxazole / Trimethoprim.

   ii. An under five year old inpatient was colonized by a MRSA variant strain ST241 harboring a virulence factor *sas*X gene and exhibiting multi drug resistance to beta-lactam antibiotics, ciprofloxacin, clindamycin, sulfamethoxazole /trimethoprim, erythromycin, gentamicin and tetracycline.

   iii. There was an association between MSSA SXT resistance carriage and HIV-positive status among abattoir workers, majority of the SXT resistant strains belonged to MSSA ST 8.

D. *Staphylococcus aureus* genotypes circulating in Busia.

I. *Staphylococcus aureus* genotypes circulating in Busia County, Kenya:

   i. *Staphylococcus aureus* genotypes found to be circulating in Busia County, Kenya: MSSA(s)ST152,ST1633,ST8,ST80,ST1,ST188,ST573,ST72,ST30,ST22,ST25,ST508,ST580

,ST88,ST1290,ST5,ST1925;MRSAST88,ST707,ST15,ST6,ST661,ST2430,ST101,ST1292,ST2126,ST97 and MRSA (s) ST241, ST140 and ST 88.

ii. Methicillin-sensitive *Staphylococcus aureus* clonal complex 152 (ST152 and ST1633) was the predominant lineages among abattoir workers population in Busia county.

iii. The most dominant Clonal complexes in BCRH, were CC152, CC8 and CC508; MSSA CC152.

iv. The following sequence types were found in both nosocomial (BCRH) and community settings in Busia: MSSA ST1633, ST80, ST188, ST25, ST80, ST188, ST22, ST25, ST188, ST80 and ST25 ,

v. The study identified sequence types that have been reported in other countries in Africa, to include MSSA ST15, ST152, ST25, ST88, ST8, ST30, ST22 and ST508

II. First report of new (novel) *S. aureus* variants and respective virulence in Busia County

i. This is the first study to report nasal carriage of tetracycline resistant *S. aureus* among abattoir workers in Eastern Africa, a marker for the livestock-associated *S. aureus* colonization.

ii. The following novel MSSA Clonal complex and new *S. aureus* variant sequence types were identified: Clonal complex CC 661 represented by ST661 of unknown origin and new STs variants~, ST 88~, ST1290~, ST1292~and ST8

iii. A new MRSA variant of ST241 was identified, MRSA ST ~241 carrying a virulence factor *sas*X, this being the first isolation of MRSA ST ~241 carrying *sas*X in Africa, which belongs to SCC*mec* type III. It also exhibited high MDR to all beta-lactam antibiotics and ciprofloxacin; clindamycin; Sulphamethoxazole and trimethoprim; erythromycin; gentamicin and tetracycline.

iv. First to report of CA-MRSA ST 140 SCC*mec* type IV (Berglund*et al*., 2009) and MSSA ST573 (Monecke *et al*., 2013; Afroz *et al*., 2008) on the African continent.

III. Transmission of *S. aureus* between human and livestock in Busia County.

i. No direct evidence of *S. aureus* transmission, between livestock and inpatients in in Busia County was observed, although phylogenetic analysis indicated a possibility of zoonotic transmission in the County,

ii. There was detection of human genotypes ST152, ST8, ST1290, and ST30 in livestock reared in Busia County, implying that some MSSA clones may have ability to cross the species barrier, probable link being the abattoir workers.

iii. ST1925-MSSA strains were isolated exclusively in livestock population, despite the close proximity between abattoir workers and livestock/meat products.

7. RECOMMENDATIONS

A. Empirical antibiotic treatment of Staphylococcal infections BCRH.

I. Empirical antibiotic treatment guidelines and documentation of tsaphylococcal infection infections:

i. Busia County Referral Hospital should introduce empirical antibiotic treatment guidelines, which should be should be developed by the hospital; they should be consistently updated, with the aim of maintaining good medical practice in the treatment of staphylococcal infection and other infectious diseases.

ii. Introduction of both hard and electronic medical records empirical antibiotic treatment, for the documentation on pathogen identification and genotypes availability of this information will greatly improve quality reporting, evaluation of treatment methods and aid in future research activity in the region.

i. Empirical prophylaxis invokes a trade-off between possible benefit to the HIVAIDS individuals and the risk of emergence and spread of bacterial antibiotic resistance. There is need for the development of guidelines on sulphamethoxazole and trimethoprim prophylaxis in resource-limited settings e.g. Busia county, which should include, ways to prevent emergence and transmission of sulphamethoxazole and trimethoprim resistant bacteria in both hospitals and the community.

ii. Public health policy makers and their technical colleagues should develop national guidelines on the use of sulphamethoxazole and trimethoprim prophylaxis, an antibiotic that is cheap and effective against opportunistic bacterial infection in HIV/AIDS individuals and should be

spared for the treatment of confirmed bacterial urinary tract infections, MRSA skin infections, travelers' diarrhea, respiratory tract infections, and cholera, among others.

iii. Sulphamethoxazole and trimethoprim antibiotic should be administered only on severe immunosuppressed HIV/AIDS patients to prevent pneumocystis pneumonia and toxoplasmosis cases.

B. Antibiotic use in humans and Livestock.

i. Governments should introduce guidelines for appropriate antibiotic use, prioritizing research on antibiotic resistance and recommend policies and legislation to regulate drug approval , including laws on the manufacture, distribution, and prescription of antibiotics including veterinary sector, and for clinical use and surveillance.

ii. Antibiotic stewardship programs should be promoted to encourage prudent use of antibiotics, in order to preserve their effectiveness for serious and life-threatening infections.

iii. National and local governments with the help of scientific and health professionals should review existing scientific evidence and develop recommendations to limit the use tetracycline, including use in food animals.

iv. There is also need to monitor genetic makeup of MDR MSSA and MRSA strains in county, private and national hospitals in Kenya. The National, county health authorities should emphasize the importance of preserving important antibiotic, so as minimize antibiotic usage, this will assist in the prevention and control antibiotic resistant strains.

C. Prevention of the spread of virulent *S. aureus* strains.

I. To stop the spread of antibiotic *S. aureus* in Busia county, the public health authority must use multifaceted public health, which should include.

i. Screening and decolonization of *S. aureus*

ii. General hygiene and cleaning measures

iii. Antibiotic stewardship programs

II. Prevention of zoonoses

i. These authorities should put in place policies for monitoring and surveillance of animal diseases and zoonoses, by establishing laboratories that assist in conducting surveillance of zoonotic pathogens such as *S. aureus*, which are are of particular concern for at-risk groups such as the immunocompromised individuals including those that are employed in slaughterhouse.

ii. Public health authorities in Busia County should promote public health systems, that prioritize the detection of zoonotic pathogens, which will help in the prevention and management of infectious zoonotic diseases in humans and animals and curb the spread of antibiotic resistant *S. aureus*

iii. The authorities should provide a framework for future policy on food safety improvement that would cascade or transcend to better public health, this can be achieved through regular public health and sustained meat hygiene education by the veterinary public health practitioners, ensuring meat product food safety.

III. Establishment of functional microbiological laboratories for detection of virul *S. aureus* strains, surveillance, control, and management, the hospital management should make sure;

i. That all diagnostic procedures in Bacteriology Laboratories are centered on quality assurances, to ensure the reproducibility and reliability of the produced results.

ii. That quality checks should be maintained and documented at all stages in accordance with local and international laboratory standards.

iii. That, laboratory results are reported within turn-around-time (TAT) for timely initiation of rational antibiotic therapy.

IV. Capacity-building program targeting microbiology laboratory staff to ensure these staff are capable of;

i. Isolation of the bacterial pathogens, followed by accurate identification of bacterial genera and/or species by biochemical identification tests.

ii. Subjecting the identified bacteria, to an *in vitro* antibiotic susceptibility testing, so as to determine their susceptibility patterns, for the purpose of guiding rational antibiotic therapy.

iii. Preserves bacterial cultures in appropriate medium for future study.

iv. Isolating the bacterial pathogen, followed by its identification up to Genera and/or species level using biochemical identification tests.

D. Introduce a One Health collaborative initiative involving professionals working in human, veterinary and environmental health sciences programs; this collaboration should include:

I. Epidemiologic typing can be applied to identify

i. The transmission of an epidemic clone during an outbreak

ii. Long-term surveillance on the circulating *S. aureus* clone should be conducted at regular 3 yearly intervals

iii. Molecular typing methods should be introduced in the existing research facility to classify closely-related isolates and divergent, epidemiologically-unrelated isolates

II. Collaboration between these laboratory and the national and international research institutions

i. To know genomic diversity in this settings, requires close collaboration between this laboratory and national research institution such as Kenya Medical Research institute, donor organizations, and public private partnership involved in genotyping.

ii. This collaboration will provide data on.

a. Emergence of antibiotic *S. aureus* resistant strains,

b. Virulence factors associated with this strains,

c. Mapping the spread of this strains

d. Understanding their pathogenicity

iii. Molecular typing including *S. aureus* Sequences emanating from this work, will provide key information that will help in development of rapid diagnostic tools for better and more rapid characterization of *S. aureus* genotype at the local level, and thus complement phenotypic methods

III. One Health approach in Busia County will help.

i. The strengthening of public health systems at the human-animal-environment interface, which Will further help in protecting human and animal health at international, regional, national and county levels.

ii. Conduct continuous mentoring of the diseases, epidemiology, virulence factors and antibiotic resistance, which is the key for treatment of infections caused by *S. aureus* at international, regional, national and county levels, these will lead to substantial reduction morbidity and mortality associetd with *S. aureus*, as well as prevent major economic loses as a result by outbreaks.

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8. APPENDIX

Primer for PCR	Nucleotide sequence(5'→ 3')	Constructed on	Start position(s) in reference	Stop position(s) in reference	Reference *SCCmec or SCC* sequence(s)	Gene(s)or gene allel detected (primer pair)
mA1	TGCTATCCACCCTC AAACAGG	*mec*A	45813	45833	TypeII.1	*mec*A(mA1-mA2)
mA2	AACGTTGTAACCAC CCCAAGA (Kondo *et al*., 2007).	*mec*A	46098	46078	TypeII.1	
$^{\alpha}$ 1	AACCTATATCATCA ATCAGTACGT (Kondo *et al*., 2007).	*ccr*A1	24845	24868	TypeI.1	*ccr*A1-ccrB($^{\alpha}$1-$^{\beta}$c)
$^{\alpha}$2	TAAAGGCATCAATG CACAAACACT	*ccr*A2	26325	26348	TypeII.1	*ccr*A2-ccrB($^{\alpha}$2-$^{\beta}$c)
$^{\alpha}$3	AGCTCAAAAGCAA GCAATAGAAT	*cc*rA3	5486	5508	TypeIII.1	*ccr*A3-ccrB ($^{\alpha}$3-$^{\beta}$c)
$^{\beta}$c	ATTGCCTTGATAAT AGCCITCT (Kondo *et al*., 2007).	*ccr*B1,ccrB2,	25539, 27261	25518, 27240	TypeI.1	
		ccrB3	7276	7255	II.1,III.1	
$^{\alpha}$4.2	GTATCAATGCACCA GAACTT	*ccr*A4	8745	8764	Type VI	*CcrA4-ccrB4* ($^{\alpha}$4.2. $^{\beta}$4
$^{\beta}$4.2	TTGCGACTCTCTTG GCGTTT	*ccr*B4	10031	10012	Type VI	
$^{\gamma}$R	CCTTTATAGACTGG ATTATTCAAAATAT	*ccr*C	60319, 16838	60346, 16811	SCC mercury, type V	*ccr*C ($^{\gamma}$R-$^{\gamma}$F)
$^{\gamma}$F	CGTCTATTACAAGA TGTTAAGGATAAT	*ccr*C	60836, 16321	60810,16347	SCC mercury, type V	

Table 1 Multiplex-PCR 1 (for amplification of *ccr* gene complex type with *mec*A)

Multiplex–PCR 2						
Primer for PCR	Nucleotide sequence(5'3')	Constructed on	Start position(s) in reference	Stop position(s) in reference	Reference SCCmec or SCC sequence(s)	Gene(s)or allele(s) (primer pair)
mI6	CATAACTTCCCATTCTGCAGATG	*mecI*	42866	42888	Type II.1	*mec*A-*mecI(*r
IS7	ATGCTTAATGATAGCATCCGAATG	IS1272	28624	28647	Type I.1	*mec*A-IS1272 upstream *mec*A(mA7-I
IS2(iS-2)	TGAGGTTATTCAGATATTTCGATGT	IS431	8772	8748	Type V	*mec*A-IS431 of *mec*A(mA 2})
mA7	ATATACCAAACCCGACAACTACA	*mec*A	44830, 31450, 7969	44808,31428, 7991	TypeI.1, II.1,V	

Table 2 Multiplex–PCR 2 (for amplification of of *mec* gene complex

Table 3 Primers used to amplify the seven housekeeping genes using Polymerase Chain Reaction

Gene	Primer	Sequence (5′ → 3′)
Carbamate kinase (*arc*C)	*arc*C-Forward	TTGATTCACCAGCGCGTATTGTC
	*arc*C- Reverse	AGGTATCTGCTTCAATCAGCG
Shikimate dehydrogenase (*aro*E)	*aro*E- Forward	ATCGGAAATCCTATTTCACATTC
	*aro*E- Reverse	GGTGTTGTATTAATAACGATATC
Glycerol kinase (*glp*F)	*glp*F- Forward	CTAGGAACTGCAATCTTAATCC
	*glp*F- Reverse	TGGTAAAATCGCATGTCCAATTC
Guanylate kinase (*gmk*)	*gmk*- Forward	ATCGTTTTATCGGGACCATC
	gmk- Reverse	TCATTAACTACAACGTAATCGTA
Phosphate acetyltransferase (*pta*)	*pta*- Forward	GTTAAAATCGTATTACCTGAAGG
	pta- Reverse	GACCCTTTTGTTGAAAAGCTTAA
Triosephosphate isomerase (*tpi*)	*tpi*- Forward	TCGTTCATTCTGAACGTCGTGAA
	tpi- Reverse	TTTGCACCTTCTAACAATTGTAC
Acetyl coenzyme A acetyltransferase (*yqi*L)	*yqi*L- Forward	CAGCATACAGGACACCTATTGGC
	*yqi*L- Reverse	CGTTGAGGAATCGATACTGGAAC

1 PCR Product Cleanup protocal

ExoSAP-IT™ PCR Product Cleanup Brief Protocol

Product description ExoSAP-IT™ reagent treats PCR products ranging in size from less than 100 bp to over 20 kb with absolutely no sample loss by removing unused primers and nucleotides. Add ExoSAP-IT™ reagent directly to the reaction products following PCR. ExoSAP-IT™ PCR Product Cleanup is active in commonly used PCR buff□rsǰ so no buff□r exchange is required. After treatment, ExoSAP-IT™ reagent is inactivated by heating to 80°C for 15 minutes. The treated PCR products are now ready for subsequent analysis in applications that require DNA to be free of excess primers and nucleotides. PCR cleanup protocol Note: Store ExoSAP-IT™ reagent at –20°C in a non-frost-free freezer. 1. Remove ExoSAP-IT™ reagent from –20°C freezer and keep on ice throughout this procedure. 2. Mix 5 µL of a post-PCR reaction product with 2 µL of ExoSAPIT™ reagent for a combined 7 µL reaction volume. When treating PCR product volumes greater than 5 µL, simply increase the amount of ExoSAP-IT™ reagent proportionally. 3. Incubate at 37°C for 15 minutes to degrade remaining primers and nucleotides. 4. Incubate at 80°C for 15 minutes to inactivate ExoSAP-IT™ reagent. 5. The PCR product is now ready for use in DNA sequencing,

Manufacturer's address: Affymetrix Inc. | 3450 Central Expressway | Santa Clara, CA 95051 | USA