DETECTION OF TWO *STAPHYLOCOCCUS* SPP. FROM DOOR HANDLES OF SOME ROOMS IN MALE HOSTELS IN CALEB UNIVERSITY

BY

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19/5927

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SUPERVISED BY

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JUNE, 2022

DECLARATION

I, CHIDERA OGUAYO, do hereby declare that this project is entirely my work and composition. The work embodied in this project has not been submitted in candidature for any degree and is not concurrently being submitted for any other degree. All references made to works of other persons have been duly acknowledged.

……………………… ………………………

SIGNATURE DATE

CERTIFICATION

This is to certify that this research project titled **"DETECTION OF TWO *STAPHYLOCOCCUS* SPP. FROM DOOR HANDLES OF SOME ROOMS IN MALE HOSTELS IN CALEB UNIVERSITY"** was carried out by **CHIDERA OGUAYO (19/5927)** under the supervision of the department of Biological Sciences and Biotechnology, College of Pure and Applied Sciences, Caleb University, Imota, Lagos State under my supervision.

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DEDICATION

This project is dedicated to my parents, Engr. (Mr.) Romanus Oguayo and Mrs. Dorris Oguayo.

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ABSTRACT

Staphylococcus spp. are found on all surfaces especially in public areas like hostels and on frequently touched areas like toilet and classroom door handles. The aim of this study was to determine the presence of *Staphylococcus* spp. in Door Handles of hostels in Caleb University. Twenty samples were obtained from male hostels of Caleb University, Lagos state using sterile swab-sticks. The swab samples were directly plated on Nutrient agar and Mannitol Salt agar and incubated at 37 °C for 18–24 h. Antibiotic susceptibility test was performed using the Clinical Laboratory Standard Institute's guidelines. A total of ten *Staphylococcus* spp. were isolated from all the hostel door handles, of which, 8 (80%) were *Staphylococcus aureus* and 2 (20%) were *Staphylococcus epidermidis* isolates. The percentage of isolates resistant to Ofloxacin, Cefotaxime, Ceftriaxone, Cefixime, Levofloxacin, Ciprofloxacin, Azithromycin, cefuroxime, Augmentin, Erythromycin, Imipenem and Gentamicin were 40%, 100%, 30%, 30%, 50%, 100%, 100%, 80%, 70% and 70% respectively. This data reveals that there is the presence of multiple drug resistant *Staphylococcus* spp. on the door handles of school hostels. Surveillance programs and researches should be done regularly to monitor the antibiotic sensitivity patterns of *Staphylococcus* spp. on surfaces as this will help curb the spread of multiple resistant strains.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Staphylococcus spp. are Gram-positive cocci that are catalase positive, coagulase positive, and non-motile (Schaumburg *et al.,* 2016). As part of normal microflora, the bacterium can be found on environmental surfaces, in the nasal nares of domesticated animals such as dogs, cats, and horses, and on human body surfaces (Akinrotoye *et al.,* 2019). *Staphylococcus* spp., on the other hand, can cause a variety of infections in animals, including mastitis in dairy cows, septicaemia and arthritis in poultry, and infections of the genital tracts in animals. The organism's large polysaccharide capsule protects it from being recognized by the cow's immune system (Yu *et al*., 2021).

In humans, they are found on the mucus membranes of around a third of the population, such as nasal passages and human skin, and they are well adaptable to antibiotic pressure, so they were able to colonize healthy individuals, which can be a source of infections and spread among people (Al-Abdli and Baiu, 2016). People who have their immunity suppressed due to the use of suppressive drugs or other diseases that cause immune diseases are more likely to become infected with *Staphylococcus* spp. *Staphylococcus* spp. can use a variety of bacterial immuno-evasive strategies to infect humans, including the production of virulence factors such as the carotenoid pigment Staphyloxanthine, which gives *Staphylococcus* spp. colonies their golden color on culture media. This factor aids the bacteria in avoiding reactive oxygen spp. (ROS) that the host uses to kill pathogens (Bhatta *et al.,* 2018).

Three types of infections can be caused by *Staphylococcus* spp. which include Pimples, impetigo, boils, cellulitis, scalded skin syndrome (SSS), and abscesses; toxicosis, such as food poisoning

and toxic shock syndrome (TSS); and systemic infections, such as endocarditis, brain abscesses, and meningitis, osteomyelitis, bacteremia, and sepsis (Roberts and No, 2014). Antibiotic resistance genes have been found in *Staphylococcus* spp., including the mecA gene, which causes methicillin resistance (Hammuel *et al.,* 2014). Penicillin resistance was first discovered in *Staphylococcus* spp. in 1947, four years after the drug was introduced to the market. A number of antibiotics, including beta lactam antibiotics like penicillin, amoxylin, and oxacillin, are resistant to *Staphylococcus* spp.

Treatment of *Staphylococcus* spp. infections has been difficult due to the bacteria's ability to resist commonly used antibiotics in hospitals; methicillin was the drug of choice, but due to significant kidney toxicity, it has been replaced by oxacillin (Colin *et al.,* 2018). Half of all *Staphylococcus* infections around the world are resistant to penicillin, methicillin, tetracycline, and erythromycin, leaving vancomycin as the only treatment option. Vancomycin-resistant strains have been identified in recent studies and are known as vancomycin intermediate resistance *Staphylococcus* spp. and vancomycin resistant *Staphylococcus* spp. (Safdari *et al.,* 2020).

Hand washing, use of disposable gloves in school environments to reduce skin contact are all options for preventing *Staphylococcus* spp. infection. Surfaces are disinfected with disinfectants such as ethanol, quaternary compounds, and sodium hypochlorite (Popovich *et al.,* 2021).

1.2 STATEMENT OF PROBLEM

The presence of *Staphylococcus* spp. in school hostels poses a significant risk of infection to the community, as well as the possibility of *Staphylococcus* spp. entering health care facilities. Immunocompromised students, who are also at risk of infection if they come into contact with

pathogen-carrying students in schools, are more likely to bring *Staphylococcus* spp. to the hostel with them. All other students who might spread the bacteria to other parts of the community congregate in the hostel.

There have been few investigations into the occurrence of Multiple Antibiotics Resistant *Staphylococcus* spp. in doorknobs. Multiple Antibiotics Resistant *Staphylococcus* spp. spread poses a serious threat to humans and causes a significant financial strain on healthcare resources. Only a few studies have been done to see how often Multiple Antibiotics Resistant *Staphylococcus* spp. isolates are in different door handles. Furthermore, the vast majority of studies focus primarily on specific surfaces and materials. As a result, this study was conducted at Caleb University hostels to determine the level of Multiple Antibiotics Resistant *Staphylococcus* spp. in practically all types of door handles.

1.3 AIM AND OBJECTIVES

The aim of this study was to determine the presence of *Staphylococcus* spp. on door handles of hostels in Caleb University

The specific objectives were to:

I. To identify the presence of *Staphylococcus* spp on the door handles of male hostels in Caleb University

ii. Determine the occurrence of *Staphylococcus* spp. on the door handles of male hostels in Caleb University

iii. Determine the antibiotics sensitivity profile of the isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Staphylococcus* spp.

Staphylococcus is a genus of Gram-positive bacteria of the family of *Micrococcaceae*. They are anaerobia facultative and do not form spores. Under the microscope, they appear spherical (cocci) and non-motile, and formed in grapelike clusters. *Staphylococcus* genus was associated to human infections for the first time in 1880, by the Scottish surgeon Sir Alexander Ogston, when he found grape-like groups of cocci in a knee purulent abscess (Moller *et al*., 2019). The name *Staphylococcus* , derived from the Greek staphyle (bunch of grapes) and kokkos (grain) was given based on their morphology, differentiating them from *Streptococcus* (clustered in chain). In 1884, the doctor Friedrich Julius Rosenbach differentiated two spp. thanks to their colonies colour: S. *aureus* (from Latin aurum, gold) and S, *albus* (from Latin, albus, white) now named S. *epidermidis* due to its presence in human skin (Carolus *et al.,* 2019).

Staphylococcus genus is composed of 51 spp. and 28 subspp. (http://www bacterio.net) divided in two groups: Coagulase-positive *Staphylococci* (CoPS, including the most pathogenic spp. S. *aureus*) and Coagulase-negative *Staphylococci* (CoNS). They are classified in function of their capacity to produce the coagulase enzyme responsible for blood coagulation, transforming fibrinogen into fibrine. The staphylococcal species have been classified according to their coagulase production capacity (Becker *et al*, 2014; Altunbulakli *et al*., 2018) (Table2.1)

Table 2.1: Species of the genus *Staphylococcus* classified according to their capacity to produce the coagulase enzyme.

Coagulase-positive *Staphylococcus*	**Coagulase-negative *Staphylococcus***	
S. aureus	*S. agnetis*	*S. felis*
S. *argenteus*	*S. argensis*	*S. jettensis*
S. scheitzeri	*S. arlettae*	*S. condiment*
S. pseudintermedius	*S. auricularis*	*S. rostri*
S. lutrae	*S. capitis*	*S. gallinarum*
S. intermedius	*S. caprae*	*S. muscae*
S. delphini	*S. carnosus*	*S. simiae*
	S. chromogenes	*S. xylosus*
	S. devriesei	*S. warneri*
	S. edaphicus	*S. vitulinus*
	S. epidermidis	*S. pettenkoferi*
	S. lentus	*S. nepalensis*
	S. equorum	*S. simulans*
	S. massilensis	*S. haemolyticus*
	S. scleiferi	*S. fleuretti*

Source: Altunbulakli *et al.* (2018)

Bacteria of the genus *Staphylococcus* are mesophilic and halotolerant. Specific growth media such as Mannitol Salt Agar (MSA) and Oxacillin Resistance Screening Agar Base (ORSAB, for methicillin-resistant isolates) are used often for their isolation and identification. MSA medium contains a high concentration of sodium chloride (7.5%) (for halotolerant isolates selection), phenol red as pH indicator, and mannitol. When the mannitol is fermented, the acid pH is indicated by the change of the red colour of phenol to yellow (CoPS). The ORSAB medium is also composed of a high concentration of salt (6.5%). In this case, the fermentation of the mannitol is observed by the blue colour of the isolates CoPS, due to the blue of aniline in an acid medium. The supplementation of oxacillin in the medium inhibits the growth of methicillin-sensitive isolates. Otherwise, the identification of staphylococcal spp. can be performed by API Staph gallery, MicroScan, VITEK identification cards or by mass spectrometry MALDI-TOF, among others (Madsen *et al.*, 2018, Jia *et al.*, 2021).

2.2 *Staphylococcus* spp. as Opportunistic Pathogens

Staphylococcal spp. are part of the natural microbiota of skin and mucous membranes of humans and most of mammals and birds. However, some of the spp. are described as pathogens involved in opportunistic infections of both 'humans and animals, being S. *aureus* the most important one (Otto, 2013; Rowe *et al.*, 2020).

2.2.1 S. *aureus*

Staphylococcus aureus is a commensal for humans and animals. Thirty to fifty percent of healthy adults are colonized, with 10 to 20 percent persistently colonized (Grundmann *et al.*, 2010). However, it is well-known for causing hospital-and community-acquired skin and lung infections. It is an important cause of endocarditis, osteomyelitis, *septicaemia* and toxic shock syndrome. S. *aureus* infection is a major cause of death in hospital-associated infections,

particularly when patients have underlying conditions such as immune deficiencies or primary infections caused by other pathogens (Otto, 2013). Furthermore, it can cause foodborne diseases and food poisoning, *Staphylococcal* food poisoning is due to the ingestion of enough amounts of *staphylococcal* enterotoxin (GEs) present in contaminated food (Argudin *et al*., 2010). Otherwise in animals, S. *aureus* is frequently involved in mastitis in livestock, exudative dermatitis in pigs and pets and arthritis in poultry (Baba *et al*., 2012).

2.2.2 Other *Staphylococcus* species.

Among CoPS, S. *pseudintermedius* is another spp. of importance which colonizes pets (Perreten *et al*., 2010; Paul *et al*., 2012; Gémez-Sanz *et al*., 2013; Stull *et al*., 2014). S. *pseudintermedius* causes skin and urinary infections and post-operative lesions in pets and is also responsible for some human infections (Bérjesson *et al*., 2015; Grinthal *et al*., 2015; Somayaji *et al*., 2016; Lozano *et al*., 2017). It has emerged recently, because it was misidentified as S. *intermedius* during more than 30 years until 2005 (Barjesson *et al*., 2015).

CoNS are considered less pathogenic than CoPS, but they represent one of the major nosocomial pathogens mainly in neonatal intensive care units (NICU), and can colonize implanted foreign bodies (Becker *et al*., 2014; Patel and Saiman, 2015; Osman *et al*., 2016). The species involved are mainly S. *epidermidis* and S. *haemolyticus*. Moreover, some spp. are related to genitourinary tract infections and endocarditis in humans (S. *saprophyticus* and S. *lugdunensis*) and bovine mastitis in animals (S. *chromogenes*, S. *epidermidis* or S. *simulans*) (Pyérali and Taponen, 2009; Taponen and Pydrala, 2009; Becker *et al.*, 2014).

2.3 Virulence Factors and Adaptation Capacity

Pathogenicity is defined as the ability of the microorganism to produce virulence factors which cause diseases or damage (colonization and cellular invasion, host cell tissues destruction,

avoidance of host immune defense proliferation etc.) (Gordon and Lowy, 2008). The virulence profile of *Staphylococcus* spp. is related to their cell wall components (mucoid capsule, adhesin, protein A, teichoic acid), enzymes (coagulase, hyaluronidase, catalase, and nuclease) and different extracellular toxins (Gordon and Lowy, 2008; Kong *et al*., 2016).

2.3.1 Colonization Capacity and Extracellular Enzymes

Staphylococci possess surface proteins, called "microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) that mediate adherence to host tissues through recognition of molecules such as collagen, fibronectin, fibrinogen and elastin. MSCRAMMs seem to be involved in initiation of endovascular infections, bone and joint infections, and prosthetic-device infections (Wertheim *et al*., 2005; Gordon and Lowy, 2008). Moreover, *staphylococci* produce various enzymes such as protease, lipase, and hyaluronidase that destroy tissues and enable the spread of infections to adjacent tissues. These enzymes can also take part in the formation of biofilms or in the inactivation of immune response proteins (Gordon and Lowy, 2008; Kong *et al*., 2016).

2.3.2 Toxins

Toxins are proteins secreted by the microorganisms into the extracellular matrix during the post-exponential and early stationary phases. Among the most common toxins secreted by staphylococci, are cytotoxins (hemolysins and leukocidins), exfoliative toxins (Ets) and pyrogenic superantigen toxins (SEs and toxic-shock syndrome toxin-1 (TSST-1)) (Gordon and Lowy, 2008; Kong *et al*., 2016).

2.3.2.1 Hemolysins

This family of peptides was discovered in the late 19% Century in a culture of *StaphIylococcus* that showed hemolytic activity. Hemolysins are toxins that lyse red blood cells. They are

classified in five types: alpha hemolysin, encoded by the gen hla, beta-hemolysin, delta hemolysin, gamma hemolysin and gamma variant hemolysin. Alpha hemolysin is the most studied and is associated to dermonecrotic and neurotoxic activity. In sepsis, its synergistic action on myeloid cells and platelets has been shown to kill a wide range of host animals. Upon binding of the toxin with its receptor, pore formation on cell membranes will cause necrotic cell death (Dinges *et al*., 2000; Kong *et al*., 2016). Beta hemolysin is non pore-forming, hydrolyses sphingomyelin and lyses monocytes. Even though its target cells are known, the toxin's mode of action is still unclear (Dinges *et al*., 2000; Kong *et al*., 2016). Delta hemolysin is produced by several *staphylococcal* strains such as S. *intermedius* and S. *epidermidis*. It is responsible for lysis of erythrocyte and a wide range of cells and organelles (Verdon *et al*., 2009). Gamma hemolysin is hemolytic to rabbit erythrocytes and its membrane damaging activity is also apparent in leukocytes. This group of hemolysins are bi-component, capable of lysing blood red cells (Dinges *et al*., 2000).

2.3.2.2 Leukotoxins

Discovered in 1932, the Panton-Valentine Leukocidin (PVL) is the toxin most virulent produced by S. *aureus* although it is only secreted by 2-4% of the strains (Prevost *et al*., 1995). This toxin is part of a bi-component Luk-Family. PVL consists of two protein subunits F and S, synthetized independently but acting synergistically on human cell membranes, leading to pore formation, alteration of the permeability and finally to the cell's destruction. Its presence is generally associated with skin and soft tissues infections (SSTIs), necrotizing *pneumonia*, *septicaemia* or *endocarditis* (Prevost *et al*., 1995; Lina *et al*., 1999; Balachandra *et al*., 2015).

S. *aureus* produces other leukotoxins of minor clinical relevance. The leukocidin M (kM and lukF) are associated with the destruction of polymorph nuclear leucocytes in ruminants, generally leading to bovine and ovine mastitis (Kaneko and Kamio, 2004).

LukED (lukE and IukD) causes dermonecrose but lacks an hemolytic activity (Gravet *et al*., 1998). Finally, LukPQ (lukP and lukQ) is a new phage-encoded leukocidin associated with horses and donkeys which preferentially destroys neutrophils in equine (Koop *et al.*, 2017).

S. *pseudintermedius* also produces a bi-component leukocidin composed of F and S subunits, similarly to S. *aureus* PVL. It is called Luk-I and destroys polymorph nuclear cells (Prevost *et al*., 1995; Gravet *et al.*, 1998). It is secreted by 90% of veterinarian isolates.

2.3.2.3 Exfoliative toxins and pyrogenic toxins superantigen

Staphylococcal exfoliative toxins are serine protease classified in five types: EtA, EXB, EtD, EtC and EtD2: EtA, EtB and EtD are responsible for *staphylococcal* scaled skin syndrome (SSSS) mainly in neonates and infants, but also in adults with renal dysfunction or immune-depression. The SSSS causes skin blistering and loss of superficial skin layers, dehydration and secondary infections (Dinges *et al*., 2000; Kong *et al*., 2016). EtC and EtD2 are generally associated with animals.

The toxic-shock syndrome was first named in 1978 in pediatrics and is caused by the TSST. It is characterized by headache, disorientation, hypotension, fever, skin eruption, diarrhoea etc., and may lead to coma. SEs are secreted by S. *aureus* strains in food and are one of the most common causes of food-borne diseases. There are more than 18 SEs identified which are heat-stable and low pH-tolerant, so that they are not degraded by cooking processes (Jarraud *et al*., 2001; Argudin *et al.,* 2010).

2.3.3 Immune evasion mechanism

The human innate immune system is responsible for the discrimination between self and foreign molecules, whether pathogen or not. It is mainly composed of phagocytes and the complement system. S. *aureus* possesses a human innate immune system evasion mechanism, which enables its adaptation and survival during the first stages of infection and/or colonization. S. *aureus* innate immune evasion cluster (IEC) is composed of up to five genes (sen, chp, sak, sea, sep), which combinations result in seven types of IEC (Van Wamel *et al*., 2006).

These genes are carried by the bacteriophage of the family 3, also known as beta hemolysin-converter. They are integrated in the 3'terminal of hlb, being able to truncate lb expression. Staphylococcal complement inhibitor (SCIN) (encoded by scn gene) is the most important component of IEC. SCIN prevents the ability of human neutrophils to phagocytose S. *aureus*. CHIPS (encoded by chp gen) is a bacterial chemokine receptor modulator that inhibits neutrophil chemotaxis. Both SCIN and CHIPS are important virulence factors that protect S. *aureus* from innate immune defence systems (Van Wamel *et al*., 2006). The staphylokinase (encoded by sak gene) has anti-opsonic activities and directly destroys defensins. SEA and SEP (encoded by sea and sep, respectively) are enterotoxins that can have a synergic effect with the other IEC mechanisms (Rahimpour *et al*., 1999). It should be noted that 90% of S. *aureus* of human origin carry the phage ϕ3 containing the gene scn, so that scn is considered a marker of human origin (Van Wamel *et al*., 2006).

Moreover, it has been recently described an equine variant of SCIN, eqSCIN (encoded by scn-eq), which is a potent blocker of equine complement system activation and subsequent phagocytosis of bacteria by phagocytes (De Jong *et al*., 2018). Whereas SCIN-A from human S. *aureus* isolates exclusively inhibits human complement, eqSCIN represents the first animal-

adapted SCIN variant that functions in a broader range of hosts (horses, humans, and pigs); it is carried by the phage ϕSaeq] (De Jong *et al*., 2018).

2.3.4 Host-related risk factors

The pathogenicity of a microorganism also depends on risk factors related to the host. Persons colonized with *Staphylococcus* are at increased risk for subsequent infections. In fact, hosts with higher risks of *staphylococcal* infections are those weaken by a chronic disease or an immune-depression. Important host-related risk factors are as follows i) the loss of primary barriers integrity such as the skin and mucous, considered the main defence mechanism against *staphylococcal* infections; i.) Clinical factors which involve an immune deficiency system ii.) The presence of foreign material such intravenous catheters, which are rapidly coated with serum constituents (Gibeinogen or fibronectin) and enable *staphylococci* to adhere through MSCRAMMs, facilitating colonization, etc. (Lowy, 1998; Gordon and Lowy, 2008).

2.4 ANTIBIOTIC RESISTANCE

Antibiotics aim to destroy the bacteria by interfering with essential cellular processes, leading to cellular death (bactericide) or inhibition of the microorganism growth (bacteriostatic). However, bacteria have demonstrated the ability to quickly respond to the antibiotics with the development of diverse resistance mechanisms. *Staphylococcal* resistance mechanisms include enzymatic inactivation of the antibiotic, alteration of the target with decreased affinity for the antibiotic, trapping of the antibiotic and efflux pumps. The mechanisms involve different cellular structures or metabolic pathways.

They can be innate (intrinsic resistance of the spp.) or acquired (spontaneous mutation of horizontal genetic transference) (Pantosti *et al.,* 2007). It should be highlighted that the acquired

resistance is the most important since the resistance encoding genes may be transferred between commensal, pathogenic and environmental bacteria.

2.4.1 Resistance to Beta lactams

Beta lactam antibiotics are the most used class of antibacterial agents in the infectious disease including penicilin, cephalosporins, monobactams and carbapenems. Penicillin is the first natural Beta Lactam to be used clinically, and for staphylococcal infections treatment. But few years after its discovery, it appeared resistance mechanisms mediated by the gene hlaZ (encoding a Beta lactamase enzyme) which inactivates the penicillin Beta Lactam ring by hydrolytic excision. It was then necessary to find other Beta lactam antibiotics, such as methicillin and oxacillin, which are semi-synthetic.

These antibiotics interrupt bacterial cell-wall formation as a result of covalent binding to essential penicillin-binding proteins (PBPs, transpeptidase), enzymes which are involved in the terminal steps of peptidoglycan cross-linking in both Gram-negative and Gram-positive bacteria (Bush and Bradford, 2016). Every bacterial spp. has its own distinctive set of PBPs that can range from three to eight enzymes per specie. In the case of *Staptylococcus* spp., they possess four intrinsic PBP's (PBPI-PBP4).

The PBP2 is the main target of Beta Lactam antibiotics, since it is the only PBP with transglucosylase activity in addition to transpeptidase. In fact the other PBPs lack the glycosyltransferase which is necessary for the peptidoglycan synthesis. *Staphylococcal* resistance to methicilin is due to an altered PBP2, named PBP2a which has a low affinity for Beta Lactam antibiotics, unlike the other PBPs (Sauvage *et al.,* 2008).

PBP2a is the product of the gene mecA and its regulatory genes mecl and mecR1. The mecA complex is included in a 30-60 kb element, denominated *staphylococcal* chromosomal cassette

mec (SCCmec). SCCmec, a mobile genetic element of horizontal transference, is most common in CoNS and can be considered an antibiotic resistance island as it can integrate additional mobile elements or resistance genes (Pantosti *et al*., 2007). Two mecA gene homologues with 80% and 91% nucleotide identities were found in *Staphylococcus sciuri* and *Staphylococcus vituinus* spp., respectively. The mecA gene homologue of S. *sciuri* is considered to have a ubiquitous presence among its spp. group (S. *sciuri S. fleureti, S. lentus, S. vitulinus*) and to be the evolutionary precursor of the mecA gene (Tsubakishita *et al.*, 2010; Becker *et al.*, 2014).

Recent studies have demonstrated that resistance to methicillin was mediated in *staphylococci* not only by mecA but also by homologous genes, mecC and mecB. The mecC, initially termed mecA $_{LGA251}$, Was discovered in 2011 in England in a S. *aureus* (LGA251) genome located in a novel staphylococcal cassette chromosome mec element, designated type-XI SCCmec (Garcia-Alvarez *et al*., 2011), It is thought to originate from CoNS (S. *sciuri* and S. *stepanovici*) (Paterson *et al.,* 2014). The mecC was 69% identical to its homologue mecA at the DNA level, and the encoded protein PBP2c was 63% identical to PBP2a. It was observed that the PBP2c exhibits a higher binding affinity for oxacillin. Moreover, the mecC-positive strains fail to be detected by current mecA laboratories detection methods, so that they were considered methicillin-susceptible (Ballhausen *et al.,* 2014; Skov *et al.,* 2014). Although the origins of mecC MRSA are not yet clear, there is good evidence that contact with animals poses a zoonotic risk and that mecC MRSA can be transmitted between spp. (Patterson *et al.,* 2014). On the other hand, the mecB gene was first described in a *Macrococcus caseolyticus* in 2009 (Becker *et al.,* 2014). However, it was recently reported a plasmid-encoded, and thereby transferable, methicillin resistance encoded by mec in an isolate of the genus *Staphylococcus* (Becker *et al.,* 2018).

2.4.2 Resistance to Non-beta lactams

For *staphylococcal* infections, other alternatives to beta lactams do exist and include diverse families of antibiotics such as aminoglycosides, macrolide-lincosamide-streptogramines (MLS), tetracyclines glycopeptides, mupirocins, fucidins, diaminopyrimidines, phenicols, oxazolidinones and fluoroquinolones. Unfortunately, strains with decreased susceptibility (or high-level resistance) to antibiotics of last resort (used for SARM severe infections), such as vancomycin have been described (Gardete and Tomasz, 2014). Furthermore, other antibiotics including those of last generation are getting less and less efficient. It is the case of linezolid, daptomycin, clindamycin, etc. (Chatterjee and Otto, 2013). Some bacteria, MRSA included, show a multidrug resistance (MDR) phenotype meaning that they are resistant to antibiotics of at least three distinct families; which makes staphylococcal treatment more difficult.

2.5 Environmental Decontamination

The essential concept and practice of sanitizing patient-care materials and equipment were created over 40 years ago (Rutala and Weber, 2011). This technique employs three classes for patient-care items and equipment. The first are critical objects that represent a significant risk of illness if contaminated. Because they are used in sterile bodily regions, these things must be sterilized before use. When semi-critical items come into contact with mucous membranes or non-intact skin, they must be thoroughly cleaned. Non-critical goods, on the other hand, are items that may come into contact with healthy skin and only need to be disinfected at a low level.

Non-critical items, such as environmental surfaces, must be disinfected on a daily basis at a low level. The Centers for Disease Control and Prevention (CDC) has issued disinfection and sterilizing standards for medical facilities (Egege *et al*., 2020). Despite the fact that Multiple Antibiotics Resistant *Staphylococcus* spp. has developed resistance to a variety of drugs, this

paper emphasizes that it can be readily removed with the use of detergents and disinfectants. According to the authors, cleaning solutions must be used at the proper concentrations, applied to all surfaces, and left in contact with the surfaces for an acceptable amount of time in order to kill bacteria. A minimum of 10 minutes of contact time is required by most cleaners (Egege *et al.*, 2020). However, these procedures are not always followed, which could result in active Multiple Antibiotics Resistant *Staphylococcus* spp. lingering on surfaces.

2.6 Multiple Antibiotics Resistant *Staphylococcus* spp. on Environmental Surfaces

Environmental surfaces can get contaminated with Multiple Antibiotics Resistant *Staphylococcus* spp. when germs are discharged into the environment by sick or colonized people. Sneezing, coughing, talking, eating, or obtaining basic medical attention can all cause this. Infected or colonized patients have previously been demonstrated to shed their specific strain of Multiple Antibiotics Resistant *Staphylococcus* spp. into the environment, resulting in high rates of contamination on surfaces and items near the patient (Igbinosa *et al.*, 2016; Aliyu *et al.*, 2020; Ajoke *et al.*, 2021). According to Igbinosa *et al.* (2016) Multiple Antibiotics Resistant *Staphylococcus* spp. was found in more than half of the floor samples, bed linen samples, and patient gown samples. They also discovered that Multiple Antibiotics Resistant *Staphylococcus* spp. contamination in the environment was found in 85 percent of patients with Multiple Antibiotics Resistant *Staphylococcus* spp-infected wounds or urine, compared to only 36 percent of patients with Multiple Antibiotics Resistant *Staphylococcus* spp. infection in the sputum, blood, or conjunctivae. Contamination rose as the number of culture-positive body locations increased. Infected patient rooms also had 32 percent contaminated surfaces, compared to only 20% in colonized patient rooms.

These germs will remain on environmental surfaces until they die or are wiped away. Multiple Antibiotics Resistant *Staphylococcus* spp. has been discovered to live for up to 318 days on environmental surfaces if they are not cleaned or removed (Igbinosa *et al*., 2016). A variety of factors influence the persistence of Multiple Antibiotics Resistant *Staphylococcus* spp. on environmental surfaces. According to recent studies, lower temperatures, lower humidity, and the presence of organic material, such as bovine serum albumin (used to mimic organic material), all increased the length of survival (Coughenour *et al*., 2011). In addition, the type of material utilized to make surfaces has an effect on longevity, with plastic and vinyl outlasting wood (Coughenour *et al*., 2011). Copper inclusion in surfaces has the potential to reduce life spans (Nworie *et al*., 2017). When comparing Multiple Antibiotics Resistant *Staphylococcus* spp. strains to non-resistant strains, survival times are comparable (Macori *et al*., 2017), while Multiple Antibiotics Resistant *Staphylococcus* spp. strains that are more prone to cause an epidemic have been shown to survive longer than ordinary Multiple Antibiotics Resistant *Staphylococcus* spp. strains (Ike *et al*., 2016). The importance of dirty ambient surfaces in the transmission of infections is still debated. Multiple Antibiotics Resistant *Staphylococcus* spp concentrations on surfaces in the environment have supposedly been found to be high enough to allow transmission (Otter *et al*., 2011).

According to one study, the average concentration of Multiple Antibiotics Resistant *Staphylococcus* spp. on surfaces was between 1 and 100 Colony Forming Units (CFU)/cm^2, which is higher than the infectious dose of less than 15 *S. aureus* cells that was shown to cause infection in experimental lesions (Joke *et al*., 2021). Surfaces contaminated with Multiple Antibiotics Resistant *Staphylococcus* spp. were also involved in the propagation of new infections during an MRSA outbreak in a London surgical facility (Aliyu *et al*., 2020).

Furthermore, a study was able to link environmental sample strain types to patients, implying that the environment infected three of the 26 patients (Mustapha *et al*., 2016). Many examinations into the presence of Multiple Antibiotics Resistant *Staphylococcus* spp. on various surfaces have been conducted, however the results have been inconsistent. According to Mustapha et al. (2016) Multiple Antibiotics Resistant *Staphylococcus* spp. was found in 21.8 percent of hospital samples, with the area beneath the bed having the highest level of infection. Nworie *et al.* (2017) discovered that 27 percent of ambient samples in a hospital tested positive for Multiple Antibiotics Resistant *Staphylococcus* spp, with the floor being the most contaminated. Igbinosa *et al*. (2016) discovered much higher rates of contamination at a 720-bed hospital with two intensive care units, with 56.3 percent of surface samples contaminated with Multiple Antibiotics Resistant *Staphylococcus* spp. Door knobs were found to be contaminated in 19 percent of Multiple Antibiotics Resistant *Staphylococcus* sp. patient rooms and 8.7% of all patient rooms (Sutter *et al.*, 2016).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

3.1.1 Equipment and Apparatus Used

The apparatus used included: Microscope, Incubator, water bath, autoclave, refrigerator, weighing balance, antibiotic disc, forceps, inoculating loop, swab stick, petri dishes, foil paper, universal bottle, needle and syringe, cotton wool, gloves, nose mask, glass slide, matches, spirit lamp, test tube, conical flask, beaker, durham tubes, spatula, measuring cylinder, test tube rack, marker, masking tape, sanitizer, MacCartney bottle.

3.1.1 Reagents and Media used

Nutrient agar, MacConkey agar(Hi media laboratories Pvt Ltd, June 2024), Mueller Hinton Agar(Hi media laboratories Pvt Ltd, June 2024), Triple Sugar Iron Test, sucrose, maltose, fructose, lactose, glucose, Simmons Citrate agar(Hi media laboratories Pvt Ltd, February 2025) peptone water, urea, urea agar base(Hi media laboratories Pvt Ltd, February 2025) starch soluble, potassium hydroxide pellet, hydrogen peroxide, Crystal violet, Iodine, decolourizer, Safranin, ethanol, Kovac's reagent.

3.2 Methods

3.2.1 Sample Collection

Twenty samples were randomly obtained from hostel door handles across Caleb University, Lagos state. Sterile swab-sticks was used for the collection of samples. The swab sticks were briefly immersed in normal saline solution prior to swabbing of the door handle, and the sample were taken to Caleb university microbiology laboratory immediately for analysis.

3.3 Laboratory Procedures

3.3.1 Isolation and Enumeration of Bacteria

The sample was inoculated onto Nutrient agar and mannitol salt agar to isolate and count *Staphylococcus* spp; these were incubated for 18 hours at 37° C. Reference numbers or coding was used to appropriately label the samples. Bacterial spp. other than *staphylococci* are partially or completely inhibited at a sodium chloride concentration of 7.5% which makes MSA suitable for *staphylococci* growth. A shift in the phenol red signal, which aids in the distinction of *Staphylococcal* spp., suggest Mannitol fermentation. Standard laboratory methods such as colony morphology, gram staining, and catalase test, and coagulase test was used to validate the isolates' identities.

3.4 Biochemical Tests and Identification of the Isolates

To identify the isolates, biochemical tests such as Gram staining reaction, Catalase Test, Sugar Fermentation Test, Citrate Utilization Test, Motility test were carried out**.**

3.4.1 Gram Staining Reaction

A thin smear film of 24-hours old culture of the organism was prepared on a sterile clean grease free slide, air dried and heat fixed. The dried smear was then stained with crystal violet for a minute and rinsed off with water, the iodine was added after for one minute and rinsed off. Decolourizer (70% ethanol) was then added for 5 seconds and rinsed off. Safranin was finally added for a minute and then rinsed off. The slide was then allowed to dry and immersion oil was added to the slide and viewed under X40 objective lens and X100 oil immersion objective lens.

3.4.2 Catalase Test

Colonies of the isolates were aseptically picked with an inoculating loop and placed on a clean grease free slide. A drop of 6% hydrogen peroxide is dropped on the colonies.

3.4.3 Sugar Fermentation Test

Peptone water was prepared and divided into four beakers, each sugars: maltose, glucose, sucrose and galactose were added to each beaker and labeled accordingly. Phenol was added to each. They were put in test tubes except glucose, which was put in test tubes with inverted Durham tubes placed in them. They were sterilized and inoculated with colonies of the isolates and were incubated at 37^0C for 48 hours.

3.4.4 Citrate Utilization Test

The Citrate test was done in order to determine the ability of the isolates to utilize citrate as their sole source of carbon and ammonia as their sole source of nitrogen. Simmons citrate was prepared and homogenized in a water bath. It was then put in test tubes and autoclaved. After sterilization, it was slanted and allowed to solidify. It was then inoculated with the isolates and incubated at 37^0C for 48 hours.

3.4.5 Motility test

Half strength of Nutrient agar was prepared and homogenized in a water bath. It was then put in test tubes and sterilized. The agar was allowed to partially solidify and the isolates were stabbed into the agar and incubated at 37^0C for 48 hours. Motility is indicated by the spreading of the organisms outside the line of stab.

3.4.6 Urease Test

Urea agar base and urea solution were prepared. They were then autoclaved in conical flasks together with empty test tubes. After sterilization, the urea agar base was mixed with the urea

solution and dispensed into the test tubes and slanted. After it was solidified, the isolates were aseptically taken with inoculating loops and inoculated on the slants. The tubes where then incubated at 37^0C for 72hours.

3.5 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done by use of Kirby Bauer disk diffusion method under Clinical Laboratory Standards Institute (CLSI) 2011 guide lines. Five colonies of the organism was emulsified in 5 mls of sterile normal saline and mixed well; the turbidity was compared to 0.5 Mac Farland standard. A sterile cotton swab was used to inoculate the sample into Mueller-Hinton agar plates and allowed to dry. The following antibiotics were used: 30 µg cefoxitin, 20/10 µg amoxicillin/clavulanic acid,10 µg gentamycin, 30 µg ceftazidime, 30 µg vancomycin, 5 µg Levofloxacin, 10 µg ampicillin, 30 µg tetracycline, 1.25/ 23.75 µg trimethoprim-sulfamethoxazole and 15 µg erythromycin. Zone of inhibitions were determined by measuring the size of clear zones and compared to the CLSI. The reporting was done by indicating Resistant, Intermediate or Sensitive.

CHAPTER FOUR

RESULTS

The cultural and morphological characteristics of the isolates. The colours were either white or yellow, some isolates are flat while some are raised in elevation, some isolates are moist, some are dry in texture, all the isolates are round in form. All the isolates are opaque. All isolates were entire while.

The results for biochemical tests. Evolution of gas bubbles caused by free oxygen indicated presence of catalase enzymes which shows positive result, while absence of bubbles indicates a negative reaction. After the test conducted all isolates were catalase positive i.e. produce the enzyme, catalase. The isolates were also positive to urease test after the test was carried out. Colour change from yellow to pink indicated positive result while no colour change indicated negative result for the urease test. The motility and citrate test are negative for all isolates. Colour change from green to deep blue indicated positive results while no colour change indicates negative result for the citrate utilization test. All the isolate are gram negative organisms.

All the isolates are positive to glucose, sucrose, maltose and galactose colour change from red to yellow indicates positive result while negative result has no colour change. In glucose, colour change from red to yellow shows positive result for presence of acid for the sugar fermentation test i.e. all the isolates can ferment sugar glucose. The sensitivity of the Isolate and their percentage. Some of the isolates are resistance to OFX, ZEM, LBC, CIP, AZN, AUG, IMP and GN. While all the isolates are resistance to CXM, AUG, and CTX. The Percentage of the Isolates. *Staphylococcus epidermidis* (20%) and *Staphylococcus aureus* (80%) and the percentage of the sensitivity of the Isolate was well presented.

TABLE 4.1 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF THE ISOLATES

Isolate code	Size	Colour	Elevation	Texture	Form	Margin	Opacity
RE(I) 14	Medium	White	Flat	Moist	Round	Entire	Opaque
RE(ii)14	Medium	Yellow	Raised	Moist	Round	Entire	Opaque
RJ(I) 4	Medium	White	Flat	Moist	Round	Entire	Opaque
RJ(ii)4	Medium	Yellow	Raised	Moist	Round	Entire	Opaque
RE 4	Medium	Yellow	Raised	Dry	Round	Entire	Opaque
ET	Medium	Yellow	Raised	Dry	Round	Entire	Opaque
RJ41	Medium	Yellow	Raised	Moist	Round	Entire	Opaque
RJ 115	Medium	Yellow	Raised	Moist	Round	Entire	Opaque
RJ2	Medium	Yellow	Flat	Moist	Round	Entire	Opaque
RJ26	Medium	Yellow	Flat	Moist	Round	Entire	Opaque
RJ56	Medium	Yellow	Flat	Moist	Round	Entire	Opaque
RE141	Medium	Yellow	Flat	Moist	Round	Entire	Opaque

Key: RE: Elisha Hall room, RJ: Joseph Hall room, ET: Elisha Hall toilet, RJ (ii): Joshua Hall room.

TABLE 4.2 RESULTS FOR BIOCHEMICAL TESTS

ISOLATE CODE	COLONY COUNT (x10^4)	CATALASE	UREASE TEST	MOTILITY	CITRATE TEST	GRAM STAINING STAINING	GLUCOSE	SUCROSE	MALTOSE	GALACTOSE	PROBABLE ORGANISMS
RE14	40	+	+	-	-	+	+	+	+	+	*Staphylococcus epidermidis*
RJ4	28	+	+	-	-	+	+	+	+	+	*Staphylococcus epidermidis*
RE 4	15	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
ET	10	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
RJ41	15	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
RJ 115	18	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
RJ2	12	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
RJ26	16	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
RJ56	7	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
RE141	18	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*

TABLE 4.3: ANTIBIOTICS SENSITIVITY OF THE ISOLATES

S/N	Isolate code	OFX	CTX	CRO	ZEM	LBC	CIP	AZN	CXM	AUG	ERY	IMP	GN
1	RE14	6 (R)	6 (R)	6 (R)	6 (R)	23 (S)	6(R)	6(R)	6(R)	6(R)	6(R)	6 (R)	6 (R)
2	RJ4	18 (I)	6(R)	10(R)	6(R)	23 (S)	6(R)	6(R)	6(R)	6(R)	6(R)	6 (R)	6 (R)
3	RE4	20 (S)	6 (R)	38 (S)	6(R)	26 (S)	6 (R)	22 (S)	6 (R)	6 (R)	6 (R)	6 (R)	6 (R)
4	ET	16(R)	6(R)	25 (S)	6(R)	8(R)	6(R)	12(R)	6(R)	6(R)	6(R)	24(S)	14 (R)
5	RJ41	50 (S)	6(R)	22 (S)	6(R)	5 (R)	6(R)	27 (S)	6(R)	6(R)	18 (I)	6(R)	30 (S)
6	RJ115	25 (S)	6(R)	28 (S)	6(R)	6(R)	6(R)	29 (S)	6(R)	6(R)	6(R)	26(S)	10(R)
7	RJ2	13 (R)	6(R)	30 (S)	6(R)	26 (S)	6(R)	20 (S)	6(R)	6(R)	6(R)	6(R)	16(I)
8	RJ26	15 (R)	6(R)	25 (S)	6(R)	27 (S)	6(R)	14 (R)	6(R)	6(R)	16(I)	6(R)	6(R)
9	RJ56	20 (S)	6 (R)	29 (S)	6 (R)	28 (S)	6 (R)	6 (R)	6 (R)	6 (R)	6 (R)	22(S)	22 (S)
10	RE141	23 (S)	6(R)	16(R)	6(R)	25 (S)	6(R)	22 (S)	6(R)	6(R)	6(R)	6(R)	26(S)

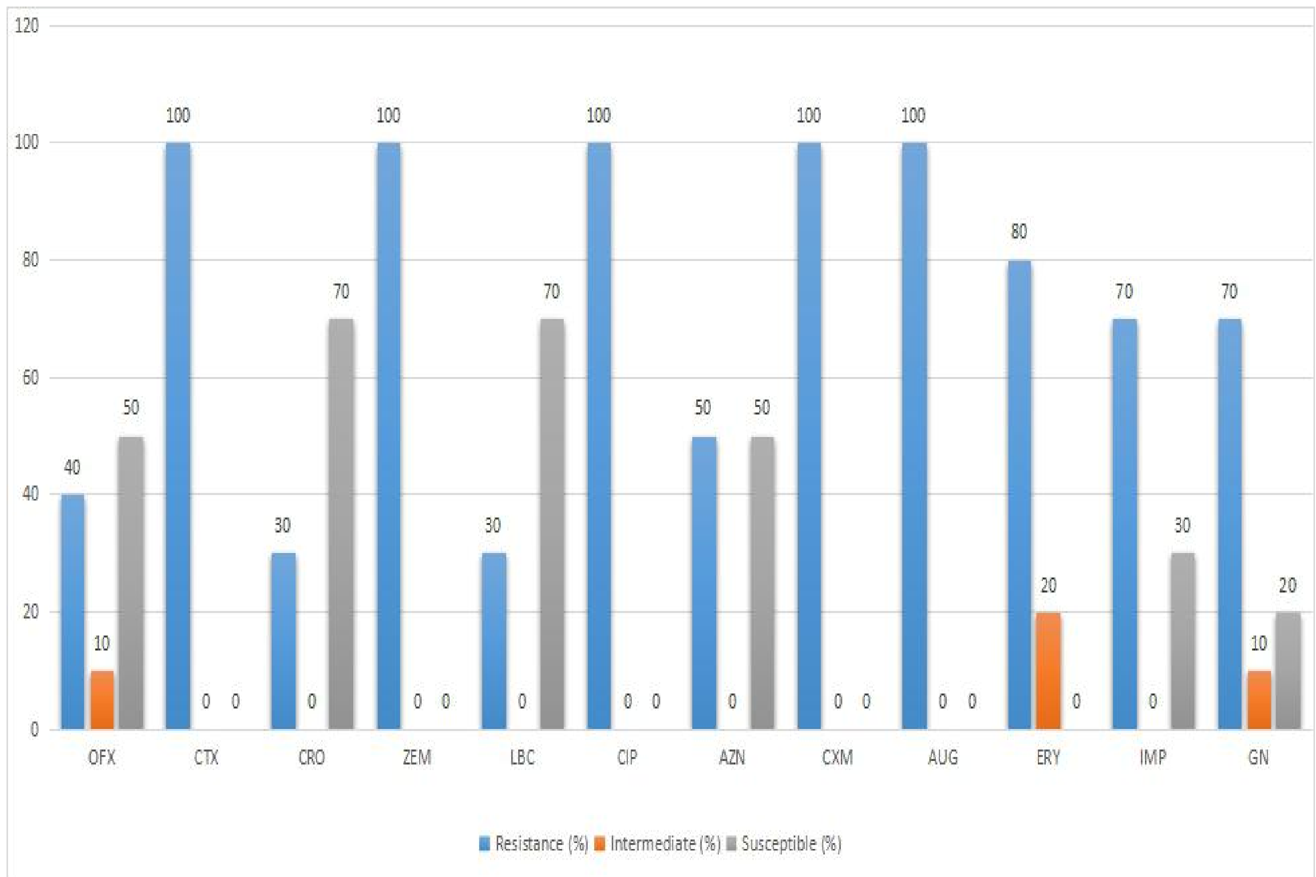

FIGURE 4.1 PERCENTAGE OF THE SUSCEPTIBILITY OF THE ISOLATES

Key: OFX- Ofloxacin, CTX- Cefotaxime, CRO- Ceftriaxone, ZEM- Cefixime, LBC- Levofloxacin, CIP- Ciprofloxacin, AZN- Azithromycin, CXM- Cefuroxime, AUG- Augmentin, ERY- Erythromycin, IMP- Imipenem, GN- Gentamicin.

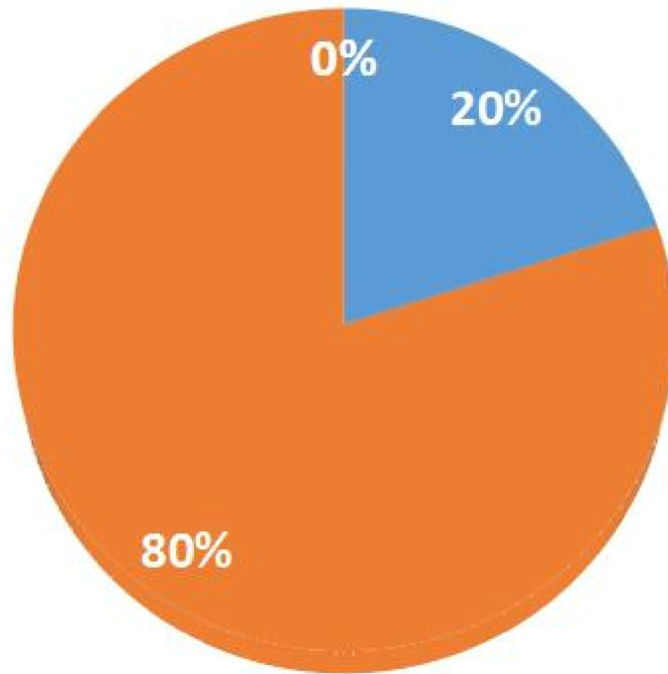

FIGURE 4.2: PERCENTAGE OF THE ISOLATES. *STAPHYLOCOCCUS EPIDERMIDIS* (20%) AND *Staphylococcus aureus* (80%)

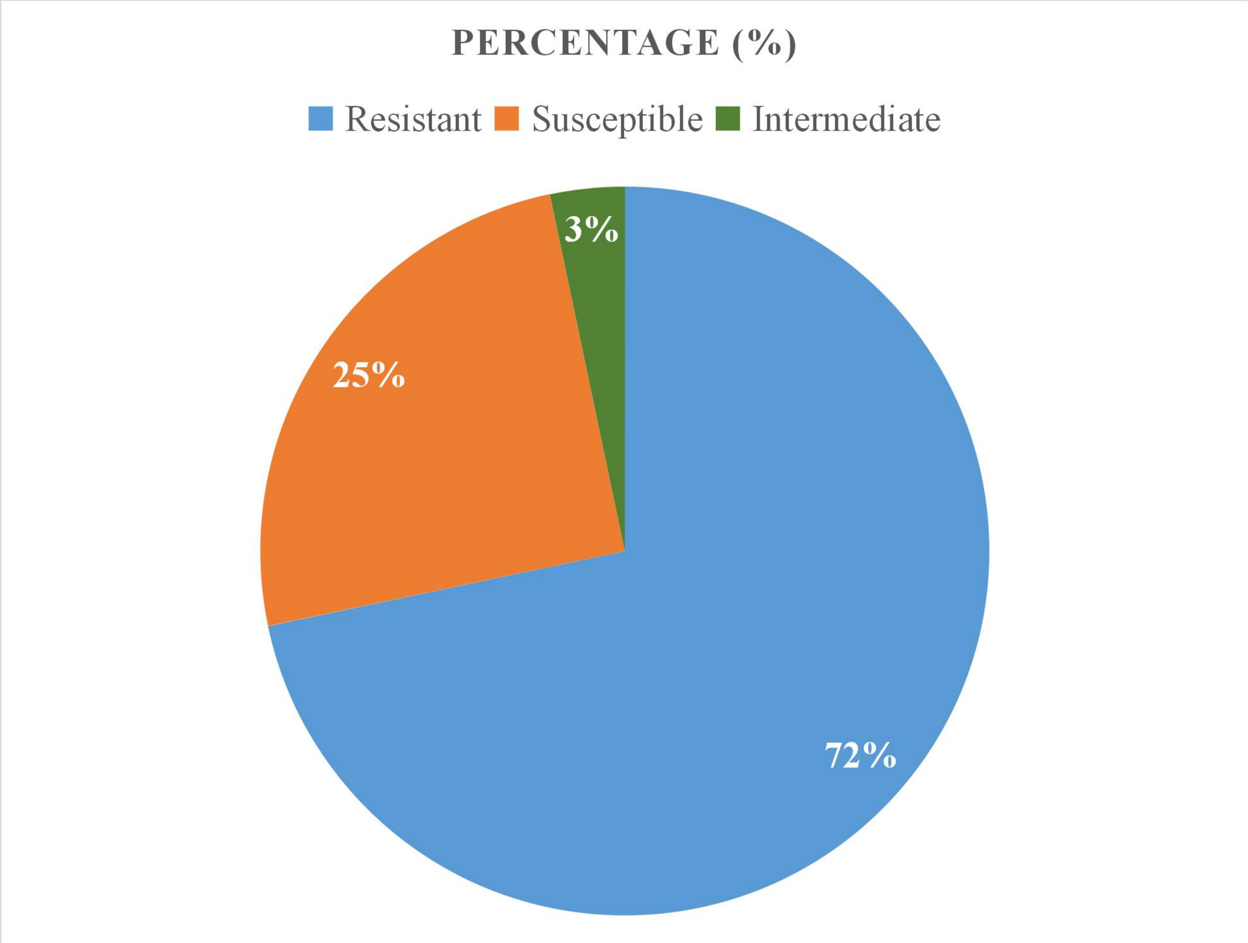

FIGURE 4.3: PERCENTAGE SENSITIVITY OF THE ISOLATES TO MULTIPLE ANTBIOTICS

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSON

5.1 DISCUSSION

The sanitation of the hostel environment plays a crucial role in spreading pathogenic organisms such as *Staphylococcus* spp. (Mitchell *et al.,* 2014). *Staphylococcus* spp. can be transferred from person to person or from person to frequently touch objects in the hostel environment, and vice versa (Nwankwo *et al.*, 2014).

5.1.1 Isolation of *Staphylococcus* spp.

The prevalence of *Staphylococcus* spp. obtained comprised 80% isolates of *S. aureus* and 20% isolates of *S. epidermidis*. In Lagos state, a lower prevalence rate of 2.7% (1/37) was reported by Adekunle *et al*. (2019), who had studied environmental isolates collected from a general hospital in Nigeria. In addition, a prevalence of 17% (8/47), mainly from door handles, was reported in a study conducted in three government hospitals in Ghana (Saba *et al.*, 2017). The prevalence rate obtained in this study was lower than a survey conducted by Mukhiya *et al*. (2012), who obtained 40.7% (11/27) from environmental isolates collected from hospitals in Nepal, a developing country. The level of healthcare provided by these hospitals had not been specified.

The prevalence in this present study was also higher than that of Ekrami *et al.* (2011), who obtained *Staphylococcus* spp. prevalence of 60.0% from hospital environmental isolates collected from hospitals in Iran which is also a developing country. However, students may act as vectors that translocate hostel-acquired pathogens between hostels (Donker *et al*., 2012). This was evident in a study conducted by Donker et al. (2012), who reported a positive correlation

(33.0%) between patient referrals and the incidence of hospital-acquired pathogens such as *Staphylococcus* spp. in hospitals in England and the Netherlands.

One of the transmission routes of *Staphylococcus* spp. is through direct skin contact and shedding of epidermal skin cells (Stiefel *et al*., 2011). Infected students or students that are carriers of *Staphylococcus* spp. may shed their skin onto the door handles. A possible reason may be attributed to the survival abilities of *Staphylococcus* spp. for one to 90 days or more on different materials or surfaces (Neely and Maley, 2000).

Staphylococcus spp. has been associated with a low infectious dosage indicating that *Staphylococcus* spp. is highly contagious even in small amounts (Otter *et al.,* 2014). Therefore, students through direct contact with the surface may be exposed to the pathogen, thus suggesting that contaminated surfaces may be an essential and underappreciated source of *Staphylococcus* spp. transmission (Donker *et al*., 2012). Transmission of pathogens is dependent on a range of factors which are but not limited to the viability of the pathogen on that environmental site, relative humidity, the frequency of contact between students, and contaminated surfaces, ambient temperature, and the dose of the transmitted pathogen (Otter *et al*., 2014).

5.1.2 ANTIBIOTICS SENSITIVITY TEST

All the isolates were resistant to all the antibiotics used. The percentage of isolates resistant to Ofloxacin, Cefotaxime, Ceftriaxone, cefixime, levofloxacin, Ciprofloxacin, Azithromycin, cefuroxime, Augmentin, Erythromycin, Imipenem, and Gentamicin were 40%, 100%, 30%, 30%, 50%, 100%, 100%, 80%, 70%, and 70% respectively. This finding supports previous findings that *Staphylococcus* aureus from surfaces has high percentages of antibiotic resistance, including

methicillin (60.4 percent) (Adriano et al., 2011); Gentamicin, Amoxicillin-clavulanic acid, and Cotrimoxazole (Ajayi and Ekozien, 2014). This shows that in cases of door handle infections, these antibiotics should only be used as a last choice for treatment. However, Ceftriaxone and cefixime appear to be viable therapies, as the isolates in this investigation demonstrated great susceptibility to these antibiotics. This finding is corroborated by the research of Ajayi and Ekozien (2014) and Ogbonna and Azuonwu (2019).

5.2 CONCLUSION

This study has revealed that door handles of different hostels are contaminated by a variety of *Staphylococcus* aureus. The most frequent bacterial isolates was *Staphylococcus aureus* and *Staphylococcus epidermidis* being the least. Isolates of *Staphylococcus* spp. have high rates of resistance to the antibiotics used in this study. However, Ceftriaxone and cefixime appear to be viable drugs, as the isolates in this investigation demonstrated great susceptibility to these antibiotics.

5.3 RECOMMENDATION

Surveillance programs and researches should be done regularly to monitor the antibiotic patterns of *Staphylococcus* spp. This will help curb the problem of developing resistance to antibiotics and more so resistance to antibiotics. Hygiene measures and education should be done in schools to prevent the spread of *Staphylococcus* spp. and other infectious diseases.

REFERENCES

Abdulgader, S. M., Shittu, A. O., Nicol, M. P., & Kaba, M. (2015). Molecular epidemiology of Methicillin-resistant *Staphylococcus* aureus in Africa: a systematic review. *Frontiers in microbiology*, *6*, 348.

Agostino, J. W., Ferguson, J. K., Eastwood, K., & Kirk, M. D. (2017). The increasing importance of community-acquired methicillin-resistant *Staphylococcus* aureus infections. *Medical Journal of Australia*, *207*(9), 388-393.

Aires-de-Sousa, M. (2017). Methicillin-resistant *Staphylococcus* aureus among animals: current overview. *Clinical Microbiology and Infection*, *23*(6), 373-380.

Akinrotoye, K. P., Bankole, M. O., Akinduti, P. A., & Lanlokun, O. A. (2019). Antibiotic resistance profiles of *Staphylococcus* aureus isolated from fomites in community schools within Abeokuta environs leading to detection of MRSA. *Bioscience Methods*, *10*.

Al-Abdli, N. E., & Baiu, S. H. (2016). Isolation of MRSA Strains from hospital environment in Benghazi city, Libya. *Am J Infect Dis Microbiol*, *4*(2), 41-43.

Almeida, S. T., Nunes, S., Paulo, A. C. S., Faria, N. A., de Lencastre, H., & Sá-Leão, R. (2015). Prevalence, risk factors, and epidemiology of methicillin-resistant*Staphylococcus* aureus carried by adults over 60 years of age. *European Journal of Clinical Microbiology & Infectious Diseases*, *34*(3), 593-600.

Altunbulakli, C., Reiger, M., Neumann, A. U., Garzorz-Stark, N., Fleming, M., Huelpuesch, C., & Traidl-Hoffmann, C. (2018). Relations between epidermal barrier dysregulation and *Staphylococcus* spp.–dominated microbiome dysbiosis in patients with atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *142*(5), 1643-1647.

Bhatta, D. R., Hamal, D., Shrestha, R., Hosuru Subramanya, S., Baral, N., Singh, R. K., & Gokhale, S. (2018). Bacterial contamination of frequently touched objects in a tertiary care hospital of Pokhara, Nepal: how safe are our hands?. *Antimicrobial Resistance & Infection Control*, *7*(1), 1-6.

Carolus, H., Van Dyck, K., & Van Dijck, P. (2019). Candida albicans and *Staphylococcus* spp.: a threatening twosome. *Frontiers in microbiology*, *10*, 2162.

Challagundla, L., Luo, X., Tickler, I. A., Didelot, X., Coleman, D. C., Shore, A. C., & Robinson, D. A. (2018). Range expansion and the origin of USA300 North American epidemic methicillin-resistant *Staphylococcus* aureus. *MBio*, *9*(1), e02016-17.

Chen, C. J., & Huang, Y. C. (2014). New epidemiology of *Staphylococcus* aureus infection in Asia. *Clinical Microbiology and Infection*, *20*(7), 605-623.

Colin, M., Klingelschmitt, F., Charpentier, É., Josse, J., Kanagaratnam, L., De Champs, C., & Gangloff, S. C. (2018). Copper alloy touch surfaces in healthcare facilities: An effective solution to prevent bacterial spreading. *Materials*, *11*(12), 2479.

Egea, A. L., Gagetti, P., Lamberghini, R., Faccone, D., Lucero, C., Vindel, A., & Sola, C. (2014). New patterns of methicillin-resistant *Staphylococcus* aureus (MRSA) clones, community-associated MRSA genotypes behave like healthcare-associated MRSA genotypes within hospitals, Argentina. *International Journal of Medical Microbiology*, *304*(8), 1086-1099.

Fan, R., Li, D., Wang, Y., He, T., Feßler, A. T., Schwarz, S., & Wu, C. (2016). Presence of the optrA gene in methicillin-resistant *Staphylococcus* sciuri of porcine origin. *Antimicrobial agents and chemotherapy*, *60*(12), 7200-7205.

Grema, H. A., Geidam, Y. A., Gadzama, G. B., Ameh, J. A., & Suleiman, A. J. A. A. V. S. (2015). Methicillin resistant *Staphylococcus* aureus (MRSA): a review. *Adv Anim Vet Sci*, *3*(2), 79-98.

Guo, D., Liu, Y., Han, C., Chen, Z., & Ye, X. (2018). Phenotypic and molecular characteristics of methicillin-resistant and methicillin-susceptible *Staphylococcus* aureus isolated from pigs: implication for livestock-association markers and vaccine strategies. *Infection and drug resistance*, *11*, 1299.

Hamdy, R. F., Hsu, A. J., Stockmann, C., Olson, J. A., Bryan, M., Hersh, A. L., & Gerber, J. S. (2017). Epidemiology of methicillin-resistant *Staphylococcus* aureus bacteremia in children. *Pediatrics*, *139*(6).

Hammuel, C., Jatau, E. D., & Whong, C. M. (2014). Prevalence and antibiogram pattern of some nosocomial pathogens isolated from Hospital Environment in Zaria, Nigeria. *Aceh International Journal of Science and Technology*, *3*(3), 131-139.

Harkins, C. P., Pichon, B., Doumith, M., Parkhill, J., Westh, H., Tomasz, A., & Holden, M. T. (2017). Methicillin-resistant *Staphylococcus* aureus emerged long before the introduction of methicillin into clinical practice. *Genome biology*, *18*(1), 1-11.

Huh, K., & Chung, D. R. (2016). Changing epidemiology of community-associated methicillin-resistant *Staphylococcus* aureus in the Asia-Pacific region. *Expert review of anti-infective therapy*, *14*(11), 1007-1022.

Jia, Y., Niu, C. T., Xu, X., Zheng, F. Y., Liu, C. F., Wang, J. J. & Li, Q. (2021). Metabolic potential of microbial community and distribution mechanism of *Staphylococcus* spp. during broad bean paste fermentation. *Food Research International*, *148*, 110533.

Junnila, J., Hirvioja, T., Rintala, E., Auranen, K., Rantakokko-Jalava, K., Silvola, J., & Vuopio, J. (2020). Changing epidemiology of methicillin-resistant *Staphylococcus* aureus in a low endemicity area—new challenges for MRSA control. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*(12), 2299-2307.

Kale, P., & Dhawan, B. (2016). The changing face of community-acquired methicillin-resistant *Staphylococcus* aureus. *Indian journal of medical microbiology*, *34*(3), 275-285.

Kuntaman, K., Hadi, U., Setiawan, F., Koendori, E. B., Rusli, M., Santosaningsih, D., & Verbrugh, H. A. (2016). Prevalence of methicillin resistant *Staphylococcus* aureus from nose and throat of patients on admission to medical wards of DR Soetomo Hospital, Surabaya, Indonesia. *Southeast Asian J Trop Med Public Health*, *47*(1), 66-70.

Lakhundi, S., & Zhang, K. (2018). Methicillin-resistant *Staphylococcus* aureus: molecular characterization, evolution, and epidemiology. *Clinical microbiology reviews*, *31*(4), e00020-18.

Lazarus, G., & Audrey, J. (2019). Comprehensive assessment of risk factors associated with methicillin-resistant *Staphylococcus* aureus (MRSA) infection in Asia: a systematic review. *Journal of Asian Medical Students' Association*, *7*(1).

Lee, A. S., De Lencastre, H., Garau, J., Kluytmans, J., Malhotra-Kumar, S., Peschel, A., & Harbarth, S. (2018). Methicillin-resistant *Staphylococcus* aureus. *Nature reviews Disease primers*, *4*(1), 1-23.

Li, H., Andersen, P. S., Stegger, M., Sieber, R. N., Ingmer, H., Staubrand, N., & Leisner, J. J. (2019). Antimicrobial resistance and virulence gene profiles of methicillin-resistant and-susceptible *Staphylococcus* aureus from food products in Denmark. *Frontiers in microbiology*, *10*, 2681.

Lim, W. W., Wu, P., Bond, H. S., Wong, J. Y., Ni, K., Seto, W. H., ... & Cowling, B. J. (2019). Determinants of methicillin-resistant *Staphylococcus* aureus (MRSA) prevalence in the Asia-Pacific region: A systematic review and meta-analysis. *Journal of global antimicrobial resistance*, *16*, 17-27.

Madsen, A. M., Moslehi-Jenabian, S., Islam, M. Z., Frankel, M., Spilak, M., & Frederiksen, M. W. (2018). Concentrations of *Staphylococcus* spp. in indoor air as associated with other bacteria, season, relative humidity, air change rate, and S. aureus-positive occupants. *Environmental research*, *160*, 282-291.

Moller, A. G., Lindsay, J. A., & Read, T. D. (2019). Determinants of phage host range in *Staphylococcus* spp.. *Applied and environmental microbiology*, *85*(11), e00209-19.

Moodley, A., Damborg, P., & Nielsen, S. S. (2014). Antimicrobial resistance in methicillin susceptible and methicillin resistant *Staphylococcus* pseudintermedius of canine origin: literature review from 1980 to 2013. *Veterinary microbiology*, *171*(3-4), 337-341.

Moon, D. C., Jeong, S. K., Hyun, B. H., & Lim, S. K. (2019). Prevalence and characteristics of methicillin-resistant *Staphylococcus* aureus isolates in pigs and pig farmers in Korea. *Foodborne pathogens and disease*, *16*(4), 256-261.

Narvaez-Bravo, C., Toufeer, M., Weese, S. J., Diarra, M. S., Deckert, A. E., Reid-Smith, R., & Aslam, M. (2016). Prevalence of methicillin-resistant *Staphylococcus* aureus in

Canadian commercial pork processing plants. *Journal of applied microbiology*, *120*(3), 770-780.

Nelson, R. E., Slayton, R. B., Stevens, V. W., Jones, M. M., Khader, K., Rubin, M. A., ... & Samore, M. H. (2017). Attributable mortality of healthcare-associated infections due to multidrug-resistant gram-negative bacteria and methicillin-resistant *Staphylococcus* aureus. *infection control & hospital epidemiology*, *38*(7), 848-856.

Nurjadi, D., Fleck, R., Lindner, A., Schäfer, J., Gertler, M., Mueller, A., & Stich, A. (2019). Import of community-associated, methicillin-resistant *Staphylococcus* aureus to Europe through skin and soft-tissue infection in intercontinental travellers, 2011–2016. *Clinical Microbiology and Infection*, *25*(6), 739-746.

Peng, H., Liu, D., Ma, Y., & Gao, W. (2018). Comparison of community-and healthcare-associated methicillin-resistant *Staphylococcus* aureus isolates at a Chinese tertiary hospital, 2012–2017. *Scientific reports*, *8*(1), 1-8.

Popovich, K. J., Green, S. J., Okamoto, K., Rhee, Y., Hayden, M. K., Schoeny, M., & Weinstein, R. A. (2021). MRSA transmission in intensive care units: Genomic analysis of patients, their environments, and healthcare workers. *Clinical Infectious Diseases*, *72*(11), 1879-1887.

Roberts, M. C., & No, D. B. (2014). Environment surface sampling in 33 Washington State fire stations for methicillin-resistant and methicillin-susceptible *Staphylococcus* aureus. *American journal of infection control*, *42*(6), 591-596.

Rowe, S. E., Wagner, N. J., Li, L., Beam, J. E., Wilkinson, A. D., Radlinski, L. C., ... & Conlon, B. P. (2020). Reactive oxygen spp. induce antibiotic tolerance during systemic *Staphylococcus* aureus infection. *Nature microbiology*, *5*(2), 282-290.

Safdari, H., Aryan, E., Sadeghian, H., Shams, S. F., & Aganj, M. (2020). Frequency of methicillin-resistant *Staphylococcus* aureus (MRSA) in nose and cellular phone of medical and non-medical personnel of emergency departments of Ghaem hospital in Mashhad city. *Clinical Epidemiology and Global Health*, *8*(4), 1043-1046.

Schaumburg, F., Köck, R., Leendertz, F. H., & Becker, K. (2016). Airport door handles and the global spread of antimicrobial-resistant bacteria: a cross sectional study. *Clinical Microbiology and Infection*, *22*(12), 1010-1011.

Sit, P. S., Teh, C. S. J., Idris, N., Sam, I., Syed Omar, S. F., Sulaiman, H., ... & Ponnampalavanar, S. (2017). Prevalence of methicillin-resistant *Staphylococcus* aureus (MRSA) infection and the molecular characteristics of MRSA bacteraemia over a two-year period in a tertiary teaching hospital in Malaysia. *BMC infectious diseases*, *17*(1), 1-14.

Stegger, M., Wirth, T., Andersen, P. S., Skov, R. L., De Grassi, A., Simões, P. M., & Laurent, F. (2014). Origin and evolution of European community-acquired methicillin-resistant *Staphylococcus* aureus. *MBio*, *5*(5), e01044-14.

Szabó, J. (2014). Molecular methods in epidemiology of Methicillin resistant *Staphylococcus* aureus (MRSA): advantages, disadvantages of different techniques. *Journal of Medical Microbiology & Diagnosis*, *3*(3), 1.

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., ... & Fowler, V. G. (2019). Methicillin-resistant *Staphylococcus* aureus: an overview of basic and clinical research. *Nature Reviews Microbiology*, *17*(4), 203-218.

Uhlemann, A. C., Otto, M., Lowy, F. D., & DeLeo, F. R. (2014). Evolution of community-and healthcare-associated methicillin-resistant *Staphylococcus* aureus. *Infection, genetics and evolution*, *21*, 563-574.

Wang, H. K., Huang, C. Y., & Huang, Y. C. (2017). Clinical features and molecular characteristics of childhood community-associated methicillin-resistant *Staphylococcus* aureus infection in a medical center in northern Taiwan, 2012. *BMC Infectious Diseases*, *17*(1), 1-6.

Wong, J. W., Ip, M., Tang, A., Wei, V. W., Wong, S. Y., Riley, S., & Kwok, K. O. (2018). Prevalence and risk factors of community-associated methicillin-resistant *Staphylococcus* aureus carriage in Asia-Pacific region from 2000 to 2016: a systematic review and meta-analysis. *Clinical Epidemiology*, *10*, 1489.

Ye, X., Wang, X., Fan, Y., Peng, Y., Li, L., Li, S., & Chen, S. (2016). Genotypic and phenotypic markers of livestock-associated methicillin-resistant *Staphylococcus* aureus CC9 in humans. *Applied and Environmental Microbiology*, *82*(13), 3892-3899.

Yu, C. H., Shen, S., Huang, K. Y. A., & Huang, Y. C. (2021). The trend of environmental and clinical methicillin-resistant *Staphylococcus* aureus in a hospital in Taiwan: Impact of USA300. *Journal of Microbiology, Immunology and Infection*.