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Methicillin-Resistant Staphylococcus Aureus on Environmental Surface Antimicrobial Susceptibility Profile and Identification in Faculty of Life Sciences, University of Maiduguri

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Abstract: ***The paper examines the occurrence of Staphylococcus species and Staphylococcus aureus and Staphylococcus epidermidis on door and toilet handles within the Faculty of Life Sciences, University of Maiduguri. Sterile swabs were used to collect 20 samples with office and toilet handles and analyze them by means of microbiological and biochemical analyses. The findings showed that there was high contamination with S. aureus and S. epidermidis as the overwhelming isolates. The resistance to penicillin and oxacillin was high based on the antibiotic susceptibility tests with the high efficacy of linezolid and vancomycin towards these isolates. The results bring to the fore the possibility of such surfaces as reservoirs of antimicrobial-resistant pathogens, which can be dangerous to the population. The paper highlights the significance of rigorous sanitation, frequent disinfection and effective infection c2ontrol in the learning institutions. The findings also support the use of antibiotic stewardship programs to monitor the trend of resistance and encourage the use of antibiotics.***

I. INTRODUCTION

A. Background to the Study

The Staphylococcus spp. are catalase positive, coagulase positive, and non-motile Gram-positive cocci (Schaumburg et al., 2016). The bacterium is a normal microflora that may be found on the surface of the environment, in the nasal nares of domestic pets, like dogs, cats, and horses, and in the human body surface (Akinrotoye et al., 2019). Staphylococcus spp. can, on the other hand, lead to diverse infections in animals such as mastitis in dairy cows, septicaemia and arthritis in poultry, and genital tract infections in animals. Largely polysaccharide capsule shields the organism against being identified by the immune system of the cow (Yu et al., 2021).

Methicillin-Resistant Staphylococcus aureus (hereafter referred to as MRSA) is a kind of S. aureus bacteria that is resistant to beta-lactam antibiotics. S. aureus is a resilient bacterium and is easy to develop antimicrobial resistance (Moreillon, 2008). These drugs resistant to the beta-lactam antibiotics are the penicillins, cephalosporins, aztreonam, imipenem, meropenem, and ertapenem (Lehne, 2007, p. 962). All these anti-microbials have a beta-lactam ring within their structure. The mechanism through which these drugs act on the bacteria is by attacking the cell walls. These types of antibiotics are bactericidal- that is, their effect is to kill bacteria (Lehne, 2007, p.962 and 972). MRSA strain of S. aureus has developed to an extent that bactericidal effect of such antibiotics is no longer effective. They appeared as mucus membranes in humans of approximately a third of the population, including nasal passages and human skin, and they are highly adaptive to antibiotic pressure, thus they were capable of colonizing healthy individuals, which can act as an origin of infections and transmit among people (Al-Abdli and Baiu, 2016). Immunosuppressed individuals due to the use of suppressive drugs or other diseases that induce immune diseases are more susceptible to infection by Staphylococcus spp. Staphylococcus spp. may employ numerous bacterial immunoevasive mechanisms to infect humans such as the synthesis of virulence factors such as the carotenoid pigment Staphyloxanthine that provide Staphylococcus spp. colonies their golden hue on culture media. This aspect can help the bacteria to escape the reactive oxygen spp. (ROS), which is utilized by the host to kill the pathogens (Bhatta et al., 2018).

Staphylococcus spp. can cause three types of infection that include Pimples, impetigo, boils, cellulitis, scalded skin syndrome (SSS), and abscess; toxicosis, including food poisoning and toxic shock syndrome (TSS); systemic infection, including endocarditis, brain abscesses, and meningitis, osteomyelitis, bacteremia, and sepsis (Roberts and No, 2014).

There are reports of the presence of an antibiotic resistance gene in Staphylococcus spp. such as the mecA gene, which is responsible for methicillin resistance (Hammuel et al., 2014). Staphylococcus spp. was the first to develop resistance against penicillin, and this was identified in 1947, four years after the drug was marketed. Several antibiotics are resistant to Staphylococcus spp., among them being beta-lactam antibiotics like penicillin, amoxylin and oxacillin. The Staphylococcus spp. bacteria have made it hard to treat the infection because it is resistant to the most frequently used antibiotics in hospitals, although methicillin was the preferred drug, it has been substituted by oxacillin (Colin et al., 2018). Around the world half of all Staphylococcus infections are resistant to penicillin, methicillin, tetracycline and erythromycin, with vancomycin as the only possible solution. According to the recent studies, vancomycin-resistant strains have been discovered and are referred to as vancomycin intermediate resistance Staphylococcus spp. and vancomycin resistant Staphylococcus spp. (Safdari et al., 2020). Hand washing, disposable gloves in schools setting to minimize skin contacts may all be used to prevent Staphylococcus spp. infection. Infection prevention agents, like ethanol, quaternary compounds, and sodium hypochlorite, are used to disinfect surfaces (Popovich et al., 2021).

B. Statement of Problem

The fact that Staphylococcus spp. are present on door and toilet handles is a major threat of infection to the community and the potential of the Staphylococcus spp. to gain access into health care facilities. Limited studies have been carried out to determine the prevalence of Multiple Antibiotics Resistant Staphylococcus spp. in doorknobs. The spread of multiple Antibiotics Resistant Staphylococcus spp. is a severe threat to human beings and a significant financial burden to health care resource.

C. Significance of the Study

When contracted in the schools, infectious disease may be fatal to the health of the students. The multi-drug-resistant organisms have been associated with prolonged hospital stay, high cost and increased mortality rate. This study is important because it will aim at examining the presence of Staphylococcus spp on door and toilet handles within the University of Maiduguri in the Faculty of life Sciences. The results of the study will be used in the comprehension of the role played by the fomites in the spread of Staphylococcus spp. in the academic setting. Moreover, information on the determination of the antibiotic sensitivity profile of the isolates will be useful in treatment and management of the Staphylococcus spp. infections. The study will increase awareness of the students, staff and the university administration on the significance of good hygiene practices, cleaning and disinfection of high-touch surfaces and the necessity of infection control methods to avoid the propagation of Staphylococcus spp. and other pathogens. The study in question will eventually be used to make evidence-based policies and practices that prevent the spread of Staphylococcus spp. and ensure a healthier academic setting.

D. Aim and Objectives

1) Aim

This study was done to establish the existence of Staphylococcus spp. on the door and toilet handles of the Faculty of Life Sciences, University of Maiduguri.

2) Objectives of the Study

The desired outcomes were to:

a) Determine the existence of Staphylococcus species on door handles of toilets and staff offices at the faculty of life sciences at the University of Maiduguri.

b) Determine the presence of Staphylococcus species on the door handle of the female toilet of the Faculty of Life Sciences at the University of Maiduguri.

c) Determine the susceptibility of the isolates to antibiotics.

II. LITERATURE REVIEW

A. Methicillin-Resistant Staphylococcus aureus (MRSA).

MRSA is either directly or indirectly transmitted, and most commonly through hands, particularly the hands of health care workers (CDC, 2007). This occurs when the healthcare worker becomes exposed to a colonized or infected patient, contact with a contaminated device or contaminated surface that contains the MRSA-containing body fluids, and the healthcare worker is colonized or infected (CDC, 2007). MRSA may be a result of fomites, and also vectors, in the sense that healthcare workers may transmit MRSA between patients without necessarily getting infected.

B. Staphylococcus spp.

Staphylococcus is a gram-positive bacterial genus of the family of Micrococcaceae. They are non-sporing anaerobics. They are non-motile, spherical (cocci) and are formed in grapelike clusters under the microscope. The association of Staphylococcus genus to human infections was first made in 1880 when the Scottish surgeon Sir Alexander Ogston discovered grape-like clusters of cocci in a knee purulent abscess (Moller et al., 2019). The morphology was the name Staphylococcus was coined, the Greek staphyl (bunch of grapes) and kokkos(grain) being the origin of the name. Thanks to their colour of colonies S. aureus (from Latin, aurum, gold) and S, albus (from Latin, albus, white) now called S. epidermidis due to their presence in human skin, the doctor Friedrich Julius Rosenbach distinguished two spp. in 1884 (Carolus et al., 2019).

There are 51 spp. and 28 subspp. of Staphylococcus genus fitting in two categories, Coagulase-positive Staphylococci (CoPS) (including the most pathogenic spp. S. aureus) and Coagulase-negative Staphylococci (CoNS). They are categorized based on the ability to produce the coagulase enzyme that causes blood coagulation, which is the converting fibrinogen to fibrine. The staphylococcal species have been grouped based on the ability to produce coagulase (Becker et al, 2014; Altunbulakli et al., 2018) (Table2.1)

Table 2.1: The species of the genus Staphylococcus in terms of their ability 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)

The bacteria that belong to the genus of Staphylococcus are mesophilic and also halotolerant. They are commonly isolated and identified using specific growth media like Mannitol salt agar (MSA) and Oxacillin Resistance Screening Agar Base (ORSAB (when isolates are methicillin-resistant)). The medium MSA has a high sodium chloride level (7.5 percent) (selection of halotolerant isolates), phenol red (PH indicator), and mannitol. As the fermentation of the mannitol occurs, the acid pH is denoted by the change of red colour of phenol to yellow (CoPS). A high concentration of salt (6.5%), also constitutes the ORSAB medium. The blue colour of aniline in an acid medium is shown to ferment the mannitol in the isolates CoPS, in this case. Oxacillin supplement placed in the media prevents the growth of methicillin sensitive isolates. Otherwise, staphylococcal spp. can be identified using API Staph gallery, MicroScan, VITEK identification cards or through mass spectrometry MALDI-TOF and others (Madsen et al., 2018, Jia et al., 2021).

C. The Staphylococcus spp. as Opportunistic Pathogens.

The Staphylococcal spp. belongs to the natural microbiota of the human and the majority of mammals and birds' skin and mucous membrane. But there are others of the spp. called as pathogens in the opportunistic infections of both the animals and the human beings, because S. aureus is the most significant of them (Otto, 2013; Rowe et al., 2020).

1) S. aureus

Staphylococcus aureus is an antagonistic human and animal commensal. Fifty to thirty per cent of healthy adults are colonized, and 10 to 20 per cent persistently colonized (Grundmann et al., 2010). But it is also widely known to cause hospital-acquired and community acquired skin and lung infections. It plays a significant role in endocarditis, osteomyelitis, septicaemia and toxic shock syndrome. S. aureus infection is a significant cause of mortality of hospital-associated infectious diseases, especially in the case of patients with underlying infections (immune deficiencies or prime infections by other pathogens) (Otto, 2013). Moreover, it may lead to foodborne diseases and food poisoning, Staphylococcal food poisoning results because of consumption of sufficient doses of staphylococcal enterotoxin (GEs) in contaminated food (Argudin et al., 2010). In other animals, S. aureus is commonly a cause of mastitis in dairy animals, exudative dermatitis in swine and pets and arthritis in poultry (Baba et al., 2012).

2) Other Species of Staphylococcal.

Another spp. of CoPS, which is a pet colonizer, is S. pseudintermedius (Perreten et al., 2010; Paul et al., 2012; Gemez-Sanz et al., 2013; Stull et al., 2014). S. pseudintermedius causes skin infections, urine infections and post-operative lesions in pets and causes some cases of human infections (Berjesson et al., 2015; Grinthal et al., 2015; Somayaji et al., 2016; Lozano et al., 2017). It is recently identified because it has been confused with S. intermedius in the past 30 years until 2005 (Barjesson et al., 2015). CoNS are considered harmless in contrast to CoPS, yet among the most important nosocomial pathogens that are most frequently present in neonatal intensive care units (NICU) and may also be able to colonize the implanted foreign bodies (Becker et al., 2014; Patel and Saiman, 2015; Osman et al., 2016). The bacterium in question are mostly S. epidermidis and S. haemolyticus. Moreover, other spp. are biologically related to human genitourinary tract infection and endocarditis (S. saprophyticus and S. lugdunensis), and bovine mastitis in animals (S. chromogenes, S. epidermidis or S. simulans; Pyerali and Taponen, 2009; Taponen and Pydrala, 2009; Becker et al., 2014) adaptation Capacity and Virulence Factors.

D. Virulence Factor and Adaptation Capacity.

The adaptation ability is dependent upon virulence factors in the plasmid and transposons in the plasmid (Hiramatsu 260). Virulence factors and transposons that have been discovered in the plasmid determine the adaptive ability of the plasmids (Hiramatsu 260). The ability of the microorganism to kill diseases or damage (colonization and cellular invasion, destruction of host cell tissues, proliferation of host immune defense etc.) leading to virulence factors can be defined as the pathogenicity (Gordon and Lowy, 2008). Viralulence profile of staphylococcus spp. relates to cell wall (mucoid capsule, adhesin, protein A, teichoic acid), enzyme (coagulase, hyaluronidase, catalase and nuclease) and multiple extracellular toxin (Gordon and Lowy, 2008; Kong et al., 2016). The capacity to colonize and release extracellular enzymes was assessed at 4, 24, and 48 hrs of incubation (24 and 48 hrs of incubation were chosen to determine the presence of extracellular enzymes (colonization capacity).

1) The Capacity of Colonization Property and Extracellular Enzymes

The capacity to colonize and release extracellular enzymes was evaluated at 4, 24, and 48 hrs of incubation (24 and One of the capacities of the colonization step was established through the ratio of the number of the colonies growing on the positive nutrient plate and the negative nutrient plate, in a 1:1 ratio (see Table 1, Figure 2). One of the capabilities of the colonization step was decided by the count of colonies cultivated on the positive nutrient plate and the count of the pre-culture units on the negative nutrient plate in a 1:1 ratio (see). It is the recognition of collagen, fibronectin, fibrinogen and elastin that causes the Staphylococci to adhere to the host tissues through the help of surface proteins of Staphylococci called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). MSCRAMMs have been implicated in the occurrence of endovascular infection, bone and joint infection and prosthetic-device infection (Wertheim et al., 2005; Gordon and Lowy, 2008). Also, the staphylococci produce various enzymes such as proteases, lipase, and hyaluronidases that destroy tissues and enable the infection to spread to the adjacent tissues. It is also possible that these enzymes are involved in the formation of biofilms, or the destruction of proteins of immune response (Gordon and Lowy, 2008; Kong et al., 2016).

2) Toxins

During the post exponential and the early stationary phases, there is secretion of toxins that are proteins released into the extracellular matrix by the microorganisms. Cytotoxins staphylococcus secreted (hemolysins and leukocidins), exfoliative toxins (Ets) and pyrogenic superantigen toxins (SEs and toxic-shock syndrome toxin-1 (TSST-1)) are among the most frequent toxins produced by staphylococci (Gordon and Lowy, 2008; Kong et al., 2016).

3) Hemolysins

The discovery of this family of peptides was in the late 19% Century in a culture of Staphylococcus which exhibited hemolytic activity. The toxins that lyse red blood cells are hemolysins. They are grouped into five categories, alpha hemolysin (article hla encoded), beta-hemolysin, delta hemolysin, gamma hemolysin and gamma variant hemolysin. The best-investigated one is alpha hemolysin which is linked to dermonecrotic and neurotoxic activity. Its synergistic effect on platelets and myeloid cells in sepsis has been demonstrated to cause the death of a broad host animal collection. Following the binding of the toxin with its own receptor, the pore formation on the cell membranes will result in necrotic cell death (Dinges et al., 2000; Kong et al., 2016). Beta hemolysin is not pore-forming, disintegrates sphingomyelin and destroys monocytes. Although its target cells are known, the mechanism of action of the toxin remains unclear (Dinges et al., 2000; Kong et al., 2016). The delta hemolysin is made by a number of staphylococcal strains including S. intermedius and S. epidermidis. It lyses erythrocyte and a vast variety of cells and organelles (Verdon et al., 2009). The hemolytic properties of gamma hemolysin on rabbit erythrocytes and the membrane harmful effect are also evident in leukocytes. This range of hemolysins are bi-component and they can lyse blood red cells (Dinges et al., 2000).

4) Leukotoxins

The most virulent toxin produced by S. aureus is the Panton-Valentine Leukocidin (PVL) that is only secreted by 2-4 of the strains (Prevost et al., 1995). This toxin belongs to a bi-component Luk-Family. PVL is made up of two protein subunits F and S which are synthesized independently yet act in synergy on the human cell membranes causing pore formation, changes in permeability and eventual destruction of the cell. It is typically related to the presence of skin and soft tissues infections (SSTIs), necrotizing pneumonia, septicaemia or endocarditis (Prevost et al., 1995; Lina et al., 1999; Balachandra et al., 2015).

Other minor leukotoxins of S. aureus which have minor clinical implications are produced. The leukocidin M (kM and lukF) relate to the destruction of the polymorph nuclear leucocytes in ruminants and typically cause bovine and ovine mastitis (Kaneko and Kamio, 2004).

LukED (lukE and IukD) is known to induce dermonecrose but not an hemolytic activity (Gravet et al., 1998). Lastly, LukPQ (lukP and lukQ) is a novel phage protein of leukocidin targeting horses and donkeys which selectively kills neutrophils in equine (Koop et al., 2017).

S. pseudintermedius also expresses a bi-component leukocidin containing F and S subunits, the same as S. aureus PVL. It is abbreviated Luk-I and it kills polymorph nuclear cells (Prevost et al.,1995; Gravet et al., 1998). It is exuded in 90 percent of veterinarian isolates.

5) Exfoliative toxin and pyogenic toxins superantigen.

Staphylococcal exfoliative toxins are serine protease that can be of five types which are: EtA, EXB, EtD, EtC and EtD2: EtA, EtB and EtD cause the staphylococcal scaled skin syndrome (SSSS) predominantly in neonates and infants, although also in adults with immunodeficiency or renal dysfunction. The SSSS leads to the blistering of the skin and shedding of the superficial layers of the skin, dehydration and secondary infections (Dinges et al., 2000; Kong et al., 2016). EtC and EtD2 are mostly animal oriented.

The TSST causes the toxic-shock syndrome, which was initially described in 1978 in pediatrics. It is marked by headache, disorientation, hypotension, fever, eruption of skin, diarrhoea etc. and can result to coma. The S. aureus strains secrete SES which are among the most prevalent causes of food-borne diseases. Over 18 SEs have been identified as heat-stable and belonging to low pH, as such that they do not undergo degradation by cooking processes (Jarraud et al., 2001; Argudin et al., 2010).

E. Immune Evasion Mechanism

The discrimination between self and foreign molecules, pathogen or not, is the prerogative of the human innate immune system. It predominantly consists of the phagocytes and the complement system. S. aureus has a human innate immune system evasion response, which facilitates its adaptation and survival in the initial phases of infection and/or colonization. The innate immune evasion cluster (IEC) of S. aureus consists of 5 genes (sen, chp, sak, sea, sep) to generate seven types of IEC (Van Wamel et al., 2006).

The bacteriophage of the family 3 otherwise referred to as beta hemolysinconverter carries these genes. They are incorporated at the 3'terminal of hlb, and truncate lb expression. The most significant part of IEC is staphylococcal complement inhibitor (SCIN) (encoded by scn gene). SCIN inhibits S. aureus phagocytosis by human neutrophils. CHIPS (encoded by chp gen) is a chemokine receptor inhibitor of neutrophil chemotaxis that is produced by the bacterium. SCIN and CHIP are virulence factors which are significant in the resistance of S. aureus to the innate immune defence (Van Wamel et al., 2006).

Staphylokinase (sak gene) is an anti-opsonic active directly destroying defensins. SEA and SEP (coded by sea and sep, respectively) are enterotoxins, which may exert a synergic effect on the other IEC mechanisms (Rahimpour et al., 1999). It is necessary to add that 90 percent of S. aureus of human origin contain the phage ph3 that has the gene scn, thus scn is regarded as a human origin marker (Van Wamel et al., 2006).

In addition, an equine form of SCIN, eqSCIN (coded by scneq) has recently been described and is an effective inhibitor of equine complement system activation and subsequent phagocytosis (of bacteria) by phagocytes (De Jong et al., 2018). Unlike human S. aureus isolates which only inhibit human complement, eqSCIN is the first animaladapted SCIN variant that can work across a wider host (horses, humans, and pigs); it is expressed by the phage phSaeq] (De Jong et al., 2018).

F. Host-related Risk Factors

Host risk factors also affect the pathogenicity of a microorganism. Individuals who are colonized by Staphylococcus are susceptible to further infections. Actually, the hosts that are vulnerable to staphylococcal infections are weakened either by a chronic illness or an immunodeficiency. Relevant host-related risk factors include i) the absence of primary barriers integrity including the skin and mucous which is regarded as the primary defence mechanism against staphylococcal infections; i.) Clinical factors that encompass an immune deficiency system ii.) The foreign presence of intravenous catheters whose surface is quickly covered with serum constituents (Gibeinogen or fibronectin) and allows staphylococci to adhere with the help of the previous. MSCRAMMs, which enable colonization, etc. (Lowe, 1998; Gordon and Lowy, 2008).

G. Antibiotic Resistance

Antibiotics are designed to kill the bacteria by disrupting key cellular activities causing cell death (bactericide) or preventing the growth of the microorganism (bacteriostatic). Nevertheless, bacteria have proved to adapt swiftly concerning the antibiotics through the evolution of various resistance mechanisms. Staphylococcal resistance mechanisms entail enzyme-based inactivation of the antibiotic, target modification with low affinity with the antibiotic, entrapment of antibiotic and efflux pumps. The mechanisms entail the various cell structures or metabolic pathways.

They may be natural (natural resistance of the spp.), or they may be acquired (spontaneous mutation of horizontal genetic transference) (Pantosti et al., 2007). It is necessary to emphasize that the most significant type of resistance is the acquired one as the resistance encoding genes can be exchanged between commensal, pathogenic and environmental bacteria.

1) Resistance to Beta lactams

The most commonly used group of antibacterial agents in the infectious disease is beta lactam which incorporates penicillin, cephalosporins, monobactams and carbapenems. The earliest natural Beta Lactam in clinical use is penicillin, and in treating infections of staphylococcus. However, a few years after its discovery, it was found that the gene hlaZ (coding a) mediates resistance.

Beta lactamase enzyme) by hydrolytic excision of the penicillin Beta Lactam ring. This had to be followed by the search of additional Beta lactam antibiotics, including methicillin and oxacillin, which are semi-synthetic.

These antibiotics disrupt the formation of cell-wall in bacteria due to the covalent attachment to essential penicillin-binding proteins (PBPs, transpeptidase), enzymes that play a role in the terminal stages of peptidoglycan cross-linking in Gram-negative and Gram-positive bacteria (Bush and Bradford, 2016). Each of the species of bacteria possesses a unique array of PBPs which number can be between three and eight enzymes per specimen. Four intrinsic PBP's (PBPI-PBP4) are present in the case of Staptylococcus spp.

Beta Lactam antibiotics primarily target the PBP2 as it is the sole PBP that has transglucosylase activity in addition to transpeptidase. Actually, the other PBPs do not have the glycosyltransferase that is required in the production of the peptidoglycan. The resistance of Staphylococcal to methicilin is the result of an altered PBP2, named PBP2a that does not have high affinity to Beta Lactam antibiotics as opposed to the other PBPs (Sauvage et al., 2008).

PBP2a is a product of the gene mecA and the pair of regulatory genes, mecl and mecR1. The mecA complex is contained within a 30-60 kb element, staphylococcal chromosomal cassette mec (SCCmec). SCCmec is a mobile genetic element of horizontal transference and it is most frequently found in CoNS, and can be denoted as an antibiotic resistance island in that it may incorporate other mobile elements or resistance genes (Pantosti et al., 2007). Staphylococcus sciuri and Staphylococcus vituinus spp. contained two homologues of mecA gene with homologies of 80 and 91 percent, respectively. The homologue of S. sciuri mecA gene is thought to be widely distributed among spp. group (S. sciuri S. fleureti S. lentus S. vitulinus) and is believed to be the ancestral form of the mecA gene (Tsubakishita et al., 2010; Becker et al., 2014).

Recent reports have shown that in staphylococci the resistance to methicillin was not only mediate by mecA but also by homologous genes, mecC and mecB. Was Was identified in 2011 in England in a S. aureus (LGA251) genome in a novel staphylococcal cassette chromosome element of mec known as mecXI SCCmec.

It is believed to have its origin in CoNS (S. sciuri and S. stepanovici) (Paterson et al., 2014). The mecC was 69 percent homologous on the DNA level to its homologue mecA and protein PBP2c encoded by it was 63 percent homologous to PBP2a. It was noted that the PBP2c has a greater binding affinity of oxacillin. Furthermore, the mecC-positive strains do not get identified through existing mecA laboratories detection systems hence they were deemed methicillin susceptible (Ballhausen et al., 2014; Skov et al., 2014). Even though the etiology of mecC MRSA remains unclear, it is sufficiently demonstrated that zoonotic infection by animals and interspp. transmission of mecC MRSA can occur (Patterson et al., 2014). The mecB gene on the other hand was originally characterized in 2009 in a Macrococcus caseolyticus (Becker et al., 2014). Nonetheless, it was recently documented that a plasmid-encoded and therefore transferable, methicillin resistance was encoded by mec in one of the representatives of the genus Staphylococcus (Becker et al., 2018).

2) Resistance to non-beta lactams

In the case of staphylococcal infections, there are also other substitutes of beta lactam and they are composed of various family of antibiotics including aminoglycosides, macrolide-lincosamide-streptogramines (MLS), tetracyclines glycopeptides, mupirocins, fucidins, diaminopyrimidines, phenicols, oxazolidinones and fluoroquinolones. Sadly enough, the strains that are less susceptible (or high-level resistance) to antibiotic of last resort (resistant to SARM severe infections) that include vancomycin have been reported (Gardete and Tomasz, 2014). Moreover, the other antibiotics such as the last generation are becoming less and less effective. The case is that of linezolid, daptomycin, clindamycin etc. (Chatterjee and Otto, 2013). The phenotype of some bacteria including MRSA are multi-drug resistant (MDR) which implies that the antibiotics of at least three different groups can combat staphylococcal; it complicates the treatment of the bacteria.

H. Environmental Decontamination

It is the critical idea and activity of disinfecting patient-care products and equipment that was developed more than 4 decades ago (Rutala and Weber, 2011). This method involves the use of three classes on patient-care items and equipment. The former are highly dangerous items that pose a major threat of disease in case of contamination. Since they are applied in the sterile parts of the body, then they should be sterilized. Semi-critical things that touch mucous membranes or non-intact skin have to be cleaned properly. On the other hand, there are non-critical goods, which can be in contact with healthy skin and require only to be disinfected on a low level. Environmental surfaces are non-critical items to be disinfected with a low rate daily. Centers for Disease Control and Prevention (CDC) have also published the disinfection and sterilizing guidelines on the medical facilities (Egege et al., 2020). Although Multiple Antibiotics Resistant Staphylococcus spp. has evolved resistance to most of the drugs, this paper highlights that it can easily be eliminated by using detergents and disinfectants. The authors state that to kill bacteria, cleaning solutions should be applied to all the surfaces, used at the right concentration, and be in contact with the surfaces long enough to be acceptable. Most cleaners require a minimum of 10 minutes of contact time (Egege et al., 2020). Nevertheless, these processes are not as frequently adhered to, and this may lead to Multiple being active. Antibiotics Resistant Staphylococcus spp. which persists on the surfaces.

1) Multiple Antibiotics resistant Staphylococcus spp. on the Environmental Surfaces.

When germs are released into the environment by sick and colonized individuals, Multiple antibiotics resistant Staphylococcus spp. can contaminate the environmental surfaces. This can be caused by sneezing, coughing, talking, eating or getting simple medical assistance? It is already proven that infected or colonized patients may spread their own strain of Multiple Antibiotics Resistant Staphylococcus spp. to the environment, leading to the high rates of contamination of surfaces and items around the patient (Igbinosa et al., 2016; Aliyu et al., 2020; Ajoke et al., 2021). As Igbinosa et al. (2016) Multiple Antibiotics Resistant Staphylococcus spp. has occurred in over half of the floor samples, bed linen samples, and patient gown samples. They also found that Multiple Antibiotics Resistant Staphylococcus spp. contamination of the environment was indicated in 85 percent of patients with Multiple Antibiotics Resistant Staphylococcus spp-infected wounds or urine as compared to only 36 percent of patients with Multiple Antibiotics Resistant Staphylococcus spp. infection of the sputum, blood, or conjunctivae. The rate of contamination was increasing with the increase in the position of body that was positive to cultures. The rooms of infected patients were also found to have 32 percent of contaminated surfaces as opposed to just 20% in colonized patient rooms. These germs will stay on the environmental surfaces until they perish or they are wiped off. Several strains of Antibiotics Resistant Staphylococcus spp. were found to survive up to 318 days in the environment when not removed or washed (Igbinosa et al., 2016). There are a number of factors, which affect

the persistence of Multiple Antibiotics Resistant Staphylococcus spp. to persist on environmental surfaces. Recent research found that lower temperatures, decreased humidity and availability of organic material, bovine serum albumin (implemented to replicate organic material) among others enhanced the duration of survival (Coughenour et al., 2011). Moreover, the material used to create surfaces also influences the durability, as plastic and vinyl are more resistant than wood ones (Coughenour et al., 2011). Inclusion of copper in surfaces can lower the life span (Nworie et al., 2017). Comparing Multiple Antibiotics Resistant Staphylococcus spp. strains with non-resistant ones, there is no significant difference in the time of survival (Macori et al., 2017), whereas Multiple Antibiotics Resistant Staphylococcus spp. strains, which are more likely to induce an epidemic, endure longer than Multiple Antibiotics Resistant Staphylococcus spp. strains (Ike et al., 2016). It is controversial whether dirty ambient surfaces play a role in the spread of infections. Several levels of Antibiotics Resistant Staphylococcus spp have been detected on surfaces in the environment and are supposed to be high to transmit (Otter et al., 2011).

One study reported the average Multiple Antibiotics Resistant Staphylococcus spp. concentration on surfaces between 1 and 100 Colony Forming Units (CFU)/cm 2, higher than the infectious dose of fewer than 15 S. aureus cells that were demonstrated to cause infection in experimental lesions (Joke et al., 2021). Multiplex Multiple antibiotics Resistant Staphylococcus spp. was also found to propagate new infections on the surfaces of a London surgical facility in an outbreak of Multiple antibiotics Resistance Staphylococcus spp. (Aliyu et al., 2020).

Moreover, one of the studies was capable of correlating the types of environmental samples strains with patients, suggesting that three out of 26 patients were infected by the environment (Mustapha et al., 2016). Numerous tests have been done to assess the existence of Multiple hueds of antibiotics Resistant Staphylococcus spp. on the numerous surfaces but the findings have been incongruous (Mustapha et al. 2016).

There were several samples of the Multiple Antibiotics Resistant Staphylococcus spp. in 21.8% of hospital samples, with the region under the bed containing the greatest degree of infection. Nworie et al. (2017) found out that in a hospital, Multiple Antibiotics Resistant Staphylococcus spp were positive in 27 percent of the ambient samples, with the floor being the contaminant. Igbinosa et al. (2016) found that contamination rates were significantly higher at a 720-bed hospital with two intensive care units in which 56.3 percent of surface samples were contaminated with Multiple Antibiotics Resistant Staphylococcus spp. 19 percent of Multiple Antibiotics Resistant Staphylococcus sp. patient rooms and 8.7 percent of all patient rooms were contaminated with Multiple Antibiotics Resistant Staphylococcus sp. door knobs (Sutter *et al.*, 2016).

III. MATERIALS AND METHOD

A. *Materials*

1) *Equipment and Apparatus used.*

The equipment used was: 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.

2) *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

B. *Methods*

1) *Sample Collection*

Twenty samples of office and toilet door handles of the University of Maiduguri, Borno state were picked randomly. The collection of the samples was done using sterile swab-sticks. The swab sticks were then dipped in normal saline solution and then the door handle was swabbed and the sample were immediately submitted to Caleb university microbiology laboratory.

C. *Laboratory Procedures*

Isolating and enumerating bacteria involves four distinct processes: isolating, cultivating bacteria, staining, and observing the bacteria to identify them.

1) Isolation and Enumeration of bacteria

Bacteria are isolated and enumerated in four different processes, which include isolating, growing bacteria, staining, and observing bacteria to identify them.

Proper labeling of the samples was done by coding and using reference numbers to inoculate the samples on Nutrient agar and mannitol salt agar where the samples were incubated at 37o C and the count of bacteria was done. Other bacterial spp. are potentially or entirely inhibited at 7.5% sodium chloride concentration that causes MSA to be useful in staphylococci growth. The appearance of a shift of the phenol red signal, which contributes to the differentiation of Staphylococcal spp., indicates fermentation of Mannitol. The isolates were tested using standard laboratory tests like colony morphology, gram staining, and catalase test, and coagulase test in order to validate them.

D. Biochemical Tests and Identification of the Isolates.

To determine the isolates, biochemical tests, including Gram staining reaction, Catalase Test, Sugar Fermentation Test, Citrate Utilization Test, Motility test were run.

1) Gram Staining Reaction

The culture of 24-hours old of the organism was taken on a sterile clean grease free slide as a thin smear film, which was then air dried and heat fixed. The smear was dried and stained with crystal violet, a minute and washed off with water, iodine added then washed off one minute. It was then rinsed with decolourizer 70% ethanol 5s. A minute later Safranin was added, and rinsed off. This slide was then left to dry and immersion oil was put into the slide and examined using X40 objective lens and X100 oil immersion objective lens.

2) Catalase Test

Aseptically, a colony of the isolates was picked using an inoculating loop, and then transferred on a clean grease free slide. One colony is dropped with a drop of 6% hydrogen peroxide.

3) Coagulase Test

Coagulase test Coagulase test on all samples of staphylococcus by using drop normal saline or physiological saline on a clean glass slide. As added and mixed a drop of rabbit plasma. Clumping or agglutination occur instantly on Coagulase positive. This assay is done to distinguish between pathogenic staphylococcus aureus and non-pathogenic staphylococci.

IV. RESULTS

The isolates of Staphylococcus species will be identified through morphological features and tested by biochemical identification tests and speciation.

Table 1: Cultural, Biochemical, and Gram Reaction Characteristics of the Isolates

Isolated Oganisms		Cultural growth characteristics and biochemical test inferences				
Distributions		BA Growth	MSA Growth	Gram Stain	Catalase Test	Coagulase Test
S. aureus		Golden yellow	Yellow Colonies	Cocci/ Purple	+	+
Office Handle	11					
Toilet Handle	7					
S. epidermidis		White colonies	Pink Colonies	Cocci/ Purple	+	-
Office Handle	7					
Toilet Handle	8					

Key: BA = Blood Agar; MSA = Mannitol Salt Agar; + = Positive; - = Negative

A. Antibiotic Susceptibility of Staphylococcus Species of Sample Surfaces.

The resistance and susceptibility of the commonly used antibiotics against Staphylococcus aureus and Staphylococcus epidermidis isolates obtained in the staff door and toilet handles showed a range of resistance and susceptibility to these commonly used antibiotics.

Table 2: Antibiotic Susceptibility of Isolates from Staff Door and Toilet Handles.

Antibiotics	Disc Code	Disc Content	*Staphylococcus aureus* n=18		*Staphylococcus epidermidis* n=15	
		(ug)	R (%)	S (%)	R (%)	S (%)
Penicillin	PEN	10	18 (100)	0(0)	15(100)	0(0)
Oxacillin	OX	1	18(100)	0(0)	13(86.7)	2(13.3)
Gentamicin	G	30	10(55.6)	8(44.4)	6(40)	9(60)
Levofloxacin	LEV	15	3(16.7)	15(83.3)	3(20)	12(80)
Linezolid	LZD	30	2(11.1)	16(88.9)	0(0)	15(100)
Vancomycin	VA	30	0(0)	18(100)	1(6.7)	14(93.3)

Key: ug = microgram, S = Staphylococcus, R = resistance, S = susceptible, % = percentage, n = number, PEN = penicillin, G = gentamicin, LEV = levofloxacin, LZD = linezolid, VA = vancomycin.

V. DISCUSSION, CONCLUSION AND RECOMMENDATION.

A. Discussion

The Staphylococcus spp. are Gram-positive non-motile cocci which are catalase positive, coagulase positive. Being a constituent of the normal microflora, they may be situated on surfaces of the environment, in the nasal passages of the domesticated creatures, including dogs, cats, and horses, and on the surfaces of the human body. Cases of infectious disease acquisition in learning institutions may be fatal to the health of students. The morphological and cultural features of the isolates were studied. The isolates were purple, bluish in colour; all coccobacillus in form, and all Gram-positive. The outcomes of the biochemical tests showed that the gas bubbles evolved, due to the presence of free oxygen, which is the presence of catalase enzymes and, therefore, the presence of a positive result. On the other hand, negative reaction can be relevant by the lack of bubbles. On completion of the tests, all the isolates were discovered to be catalase positive, and produced the catalase enzyme. Also, the isolates were positive on coagulase as this was indicated by the presence of either clumping or agglutination.

The isolates were determined as Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (S. epidermidis). S. aureus grew as golden yellow colonies on Blood Agar (BA) and yellow colonies on Mannitol Salt Agar (MSA). S. epidermidis revealed white and pink colonies on BA and MSA, respectively. The two species are gram-positive cocci. S. aureus positive in catalase and coagulase whereas S. epidermidis positive in catalase and negative in coagulase. S. aureus (11) and S. epidermidis (7) were obtained off of office handles. Toilet handles were the sources of S. aureus (7) and S. epidermidis (8). The fact that coagulase-positive S. aureus was present on the office and toilet handles indicates that there is a possibility of infection since the organism is identified to cause various diseases. Though S. epidermidis is usually regarded as a commensal organism, its presence on office and toilet handles can be a risk of infection especially to people with compromised immune systems. The fact that these species are frequently isolated in offices and toilet handles reminds of the need to ensure the right hygiene and cleaning procedures to avoid microorganism spreading.

The outcomes of the antibiotic susceptibility testing of the Staphylococcus aureus and Staphylococcus epidermidis isolates of the staff door and toilet handle showed a variety of resistance and susceptibility to the most often used antibiotics. The levels of resistance in both S. aureus (100% and 86.7% respectively) and S. epidermidis (100% and 86.7% respectively) were high which shows that the two had high levels of beta-lactamase activity and prevalence of MRSA/MRSE. The S. aureus (55.6) and S. epidermidis (40%), showed resistance indicating low effectiveness of this antibiotic. S. aureus had a susceptibility rate of 83.3 and S. epidermidis had a susceptibility of 80, which means that it has potential as a treatment option. Linezolid also exhibited a high susceptibility (88.9% against S. aureus and 100% against S. epidermidis making it an effective agent against S. aureus and S. epidermidis). The susceptibility of vancomycin to S. aureus and S. epidermidis was found to be 100 and 93.3 respectively which confirmed the importance of vancomycin in the treatment of resistant staphylococcal infections. These results are in agreement with previous studies that show that Staphylococcus aureus on surfaces have high antibiotic resistance rate with the resistance rate of

60.4 percent on methicillin (Adriano et al., 2011). This is an argument that in situations dealing with infections that have started with door handlers, the aforementioned antibiotics are not to be treated as a last resort.

This resistance to penicillin and high resistance rates to oxacillin indicate the prevalence of resistant staphylococcal strains in these settings. The reasons why linezolid and vancomycin are of importance as first-choice agents in the treatment of resistant infections can be summed up by the fact that it is necessary to continuously monitor the development of resistance. The intermediate resistance to gentamicin and high activity of levofloxacin offers other alternatives to the treatment of less drug-resistant infections.

B. Conclusion

The research involved the examination of the occurrence of staphylococcus species and their susceptibility to antibiotics on staff door and toilet handles. The findings revealed that both surfaces had Staphylococcus aureus and Staphylococcus epidermidis, with S. aureus being more prevalent. Staphylococcus aureus was the most common bacteria isolate. Both species were very resistant to penicillin and oxacillin. They were however vulnerable to levofloxacin, linezolid and vancomycin. The results suggest the necessity of better hygiene practices and antibiotic stewardship.

C. Recommendations

The following are the recommendations based on the results of this paper to prevent the proliferation of Staphylococcus spp. and make the Faculty of Life Sciences, University of Maiduguri a healthier place:

Door and toilet handle cleaning/disinfection should be introduced regularly.

The university ought to come up with and implement rigorous policies on infection control.

The health center in the university should have an antibiotic stewardship program, which is being monitored on a regular basis by tracking the trend of antibiotic resistance in Staphylococcus spp.

Staphylococcus spp. and other infectious disease spread should be prevented by instituting hygiene education and awareness programs in schools.

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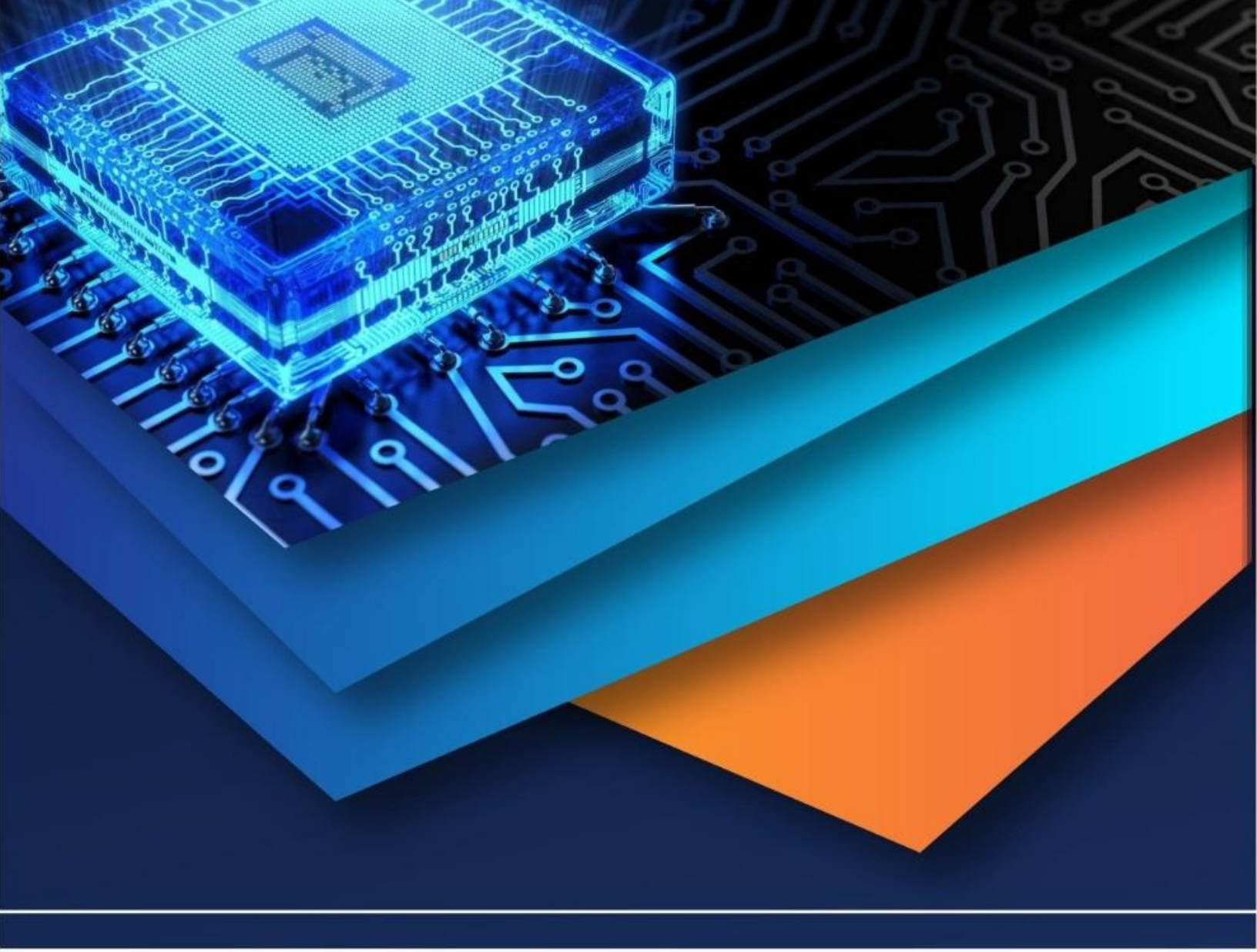

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