ANTIBIOTIC RESISTANCE OF *Staphylococcus* SPECIES ISOLATED FROM NASAL CAVITIES OF HEATHY PRIMARY AND SECONDARY SCHOOL STUDENTS IN OYE EKITI, EKITI STATE, NIGERIA.

BY

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MARCH, 2019

CERTIFICATION

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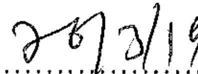

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DEDICATION

My dedication goes to the Almighty God, Father of all universe for the privilege he granted me. I also dedicate it to my amiable parents.

ACKNOWLEGEMENT

Firstly, my sincere thanks to the Lord Almighty for completion of this project work, and for what he will till do in my life.

I also give a big thank you to my parent and siblings for their love and support before, during and after this work. I pray may the good God continue to bless you in all ramifications of life.

I sincerely appreciate my supervisor, Prof. B.O Ogeneh for his fatherly love, directions, correction and care showed throughout the project work. God bless you sir.

I am sincerely grateful to Dr, S.K Ojo for his concern, time, directions, advice, readiness, correction and most importantly care during this project work. God bless you sir.

I appreciate all my lecturers, and the laboratory workers for their support and for impacting knowledge in me, I say thank you.

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ABSTRACT

Staphylococcus aureus is a human commensal colonizing about 30 per cent of the population. Besides, it is a frequent cause of infections such as skin, wound and deep tissue infections and also more life-threatening conditions such as pneumonia, endocarditis and septicaemia *Staphylococcus aureus* may also cause different toxicoses. Moreover, this bacterium is one of the most common causes of nosocomial infections worldwide and an increase in antibiotic resistance. The aims of this study were to isolate and increase the knowledge of *Staphylococcus species* pattern. This study was carried out on seventy five student from primary and secondary school in Oye, Ekiti State. The swab taken from the student were anlysed using conventional bacteriological methods. Sixty four (64) Staphylococcus species were phenotypically identified and the isolates were differentiated into 28(43.7%) *Staphylococcus aureus*,21(26.5%) CONS and 15(29.6%) *Staphylococcus epidemidis*. All the isolate are susceptible to Pefloxacin(PEF), which makes it the most suitable antibiotic that can be used to cure the infection. *Staphylococcus aureus* shows zero resistance to Rocephin(R), Pefloxacin(PEF) and Cirpofloxacin(CPX), slightly resistance to Streptomycin(S),Septrin(SXT), Erytromycin(E),Gentamycin(CN), Ampicox(AX) and Zinnacef(Z). The high rate of antibiotic resistance gene Staphylococci in this study suggested that they have been able to transfer their gene to other infectious bacteria in man and animals.

CHAPTER ONE

1.0 INTRODUCTION

Genus *Staphylococcus* is in the bacterial family Staphylococcaceae, which includes five lesser known genera, *Gemella, Jeotgalicoccus, Macrococcus, Nosocomiicoccus and Salinicoccus.* There are currently 47 recognised species of staphylococci and 21 subspecies most of which are found only in lower mammals (Prax *et al.*, 2013).*Staphylococcus* spp. are a challenge for the modern day medicine due to the complexity of disease process and presence and expression patterns of their respective virulence factors (Harro *et al.*, 2010).

The members of this genus possess many known toxins, multiple immunoavoidance mechanisms and adherence factors, most of which demonstrate transient, timed, and disease specific expression. They cause different types of infections in a host that are either planktonic, biofilm mediated or both. Sepsis and pneumonia are mainly caused by planktonic forms whereas, a whole range of diseases, namely, endophthalmitis, osteomyelitis, endocarditis, chronic skin infections, indwelling medical device infections, chronic rhino-sinusitis, and dental implants are caused by the biofilmic form of the bacteria. Abscess can be caused by both of the forms (Harro *et al.*, 2010).

Staphylococcus aureus is a species of bacteria commonly found on the skin and or in the noses of healthy people (Ajoke *et al.*, 2012). Globally, it is a leading cause of human bacterial infections (DeLeo *et al.*, 2010, Tekalign *et al.*, 2013). *Staphylococcus aureus* has been found to be the most frequently implicated pathogen in bloodstream infections, skin and soft tissue infections, and pneumonia. Infection rate from *Staphylococcus aureus* is high. Recently, the increased recognition of its involvement in community acquired infections has some levels of clinical and pharmacological implications for the health care providers (Malachy *et al.*, 2009). *Staphylococcus aureus* is an important pathogen that causes, septicemia and endocarditis, such that infections involving antibiotic resistant strain may impact on human health (Adegoke and Okoh, 2011; Bashir *et al.*, 2007, Ombui *et al.*,2000,

Yemeen *et al.,2010*). Staphylococcus.*epidemidis* accounts for about 75% of all clinical isolates, probably reflecting its preponderance in the normal skin flora. Other species include S. *capitis* and S. *xylosus*, S.*schleifieri*, S. *saprophyticus* (casual agent of urinary tract infections in immunocompetent women), S. lugdunensis (implicated in sepsis), and S. haemolyticus which has been associated with endocarditis and osteomyelitis (Azih and Enabulele, 2013; Bashir *et al.*, 2007).

Attempts to control these diseases through the use of antimicrobial agents particularly antibiotics have led to increased prevalence of resistance to these agents (Lowy *et al.*, 2003). *Staphylococcus aureus* is a species of bacteria commonly found on the skin and or in the noses of healthy people (Ajoke *et al.*, 2012). The hand carriage and nasal carriage of *Staphylococcus aureus* are strongly correlated (Wertheim *et al.*, 2006) suggesting that contaminated hands most commonly cause the colonization of the nares. Nasal carriers can act as "cloud" individual during rhinitis, dispersing *Staphylococcus. aureus* into the environment (Sherertz *et al.*, 2001).Microorganism have different mechanisms which include production of structure-altering or inactivation enzymes (beta-lactamase or aminoglycoside-modifying enzymes), alteration of penicillin-binding proteins or other cell-wall target sites, altered DNA gyrase targets, permeability mutations, active efflux and ribosomal modification enables them overcome activities of antimicrobial agent (Akinjugunla and Enabulele, 2010; Gad *et al.*, 2010).

1.1 AIMS OF STUDY

The aim of this study would be to identify *Staphylococcus species* from healthy individuals and determine their resistance pattern to commercially used antibiotics.

1.2 SPECIFIC OBJECTIVES

The specific objective of this study is:

- To isolate *Staphylococcus species* from healthy nasal cavity.
- To carry out antibiotic resistance test on the isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DESCRIPTION OF *Staphylococcus species*

The genus Staphylococcus is composed of Gram-positive bacteria with diameters of 0.5-1.5 μm, characterized by individual cocci that divide in more than one plane to form grape-like clusters (Ana *et al.*, 2013). These bacteria are non-motile, nonspore forming facultative anaerobes, featuring a complex nutritional requirement for growth at low G+C content of DNA (in the range of 30-40 mol%), a tolerance to high concentrations of salt and resistance to heat (Plata *et al.*, 2009, Ana *et al.*, 2013).

The genus Staphylococcus is traditionally divided in two groups based on the bacteria ability to produce coagulase, an enzyme that causes blood clotting: the coagulase-positive staphylococci, which includes the most known species *Staphylococcus aureus*; and the coagulase-negative staphylococci (CoNS), which are common commensals of the skin (Ray *et al.*, 2003). *Staphylococcus aureus* belongs to the family *Micrococcaceae* and is part of the genus Staphylococcus, which contains more than 30 species such as *Staphylococcus epidermidis, Staphylococcus saprophyticus and Staphylococcus haemolyticus.*

Among the staphylococcal species, *Staphylococcus aureus* is by far the most virulent and pathogenic for humans. *S. aureus* is a 1 μm, Gram-positive cell that in the laboratory may be observed as single cells, in pairs or as grape-like irregular clusters. It is characterized as coagulase- and catalase positive, non-motile, non-spore-forming and as facultative anaerobe. It often asymptomatically colonizes the skin and mucous membranes of healthy individuals, inparticular the anterior nares (Wertheim *et al.*, 2005, Ana *et al.*, 2013). It grows in yellow colonies on nutrient rich media and is referred to as the yellow staphylococci (Winn Washington *et al.*, 2006). *S. aureus* was discovered in 1880 by the surgeon Sir Alexander Ogston. He observed grape-like clusters of bacteria when examining a purulent discharge from patients with post-operative wounds during microscopy. He named them

staphylé, the Greek expression for a bunch of grapes. In 1884, Rosenbach succeeded in isolating yellow bacterial colonies from abscesses and named them *Staphylococcus aureus*, "aureus" from the Latin word for golden. *Staphylococcus aureus* has the ability to adapt to different environments and it may colonize the human skin, nails, nares and mucus membranes and may thereby disseminate among recipient host populations via physical contact and aerosols. Colonization with *Staphylococcus aureus* is an important risk factor for subsequent *Staphylococcus aureus* infection (Wertheim *et al.*, 2004).

2.2 Pathogenesis of staphylococcal infection

Staphylococcus aureus causes a wide range of infections from a variety of skin, wound and deep tissue infections to more life-threatening conditions such as pneumonia, endocarditis, septic arthritis and septicemia. This bacterium is also one of the most common species in nosocomial infections. However, little is known about the virulence factors behind all these conditions. In addition, *Staphylococcus aureus* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome, through production of different toxins (Winn Washington, 2006). *Staphylococcus aureus* is known for its capacity to cause a broad range of important infections in humans, such capacity is related to the expression of an array of factors that participate in pathogenesis of infection, allowing this bacterium to adhere to surfaces/tissues, avoid or invade the immune system, and cause harmful toxic effects to the host (Ana *et al.*, 2013).

2.3 Virulence factor and strategies

Various virulence factors contribute to the ability of S*taphylococcus* aureus to cause infection; enzymes, toxins, adhesion proteins, cell-surface proteins, factors that help the bacteria to evade the innate immune defense, and antibiotic resistance mediate survival of the bacteria and tissue invasion at the site of infection. Moreover, certain toxins cause specific disease entities (Zecconi and Scali, 2013).

Infection may not be explained by the action of a single virulence factor, and it is likely that a number of different factors operate together in the pathogenic process. This assumption is supported by

studies in animal models where the infection caused by a mutant isolate, deficient in a single virulence determinant, is compared with the infection caused by the wild type strain. These studies have indicated a decrease in severity of the infection (Hienz *et al.*, 1996; Moreillon *et al.*, 1995). The survival of *S. aureus* in the host is important for pathogenesis. The bacteria may be protected by a polysaccharide capsule that inhibits opsonization by complement and thereby escapes phagocytosis (O'Riordan and Lee, 2004). It may also secrete cytolytic toxins and tissue-cleaving enzymes (Dinges *et al.*, 2000). Moreover, *Staphylococcus aureus* may expresse a multitude of adhesion factors that mediate interactions with host cells and extracellular matrix (ECM), allowing efficient colonization (Chavakis *et al.*, 2005). *Staphylococcus aureus* has developed strategies against the antimicrobial peptides, the complement system, and the recruitment and actions of phagocytes (Chavakis *et al.*, 2007) all of which are strategies against the innate immune response of the host (Foster, 2005; Rooijakkers *et al.*, 2005).

Table 1: Common enzymes use by *S. aureus* as virulence factor

Virulence Factor	Enzymatic function	Effect as virulence factor in host	Reference
Catalase	Deactivate free hydrogen peroxide	Has been found to be essential for nasal colonization.	(Chavakis *et al.*, 2007; Cosgrove *et al.*, 2007)
Coagulase	Binds to prothrombin and thereby becomes enzymatically active	Catalyse the conversion of fibrinogen to fibrin. Coating the bacteria with fibrin and make them resistance to opsonization and phagocytosis	(Kawabata *et al.*, 1986)
Hyaluronidase	Degrade hyaluronic acid in connective tissue Hydrolyzes the intracellular matrix of acid mucopolysaccharides in tissue and , thus may act to spread the organism to adjacent areas in tissue	May convert local tissue into nutrient require for bacterial growth	(Dinges *et al.*, 2000; Winn Washington 2006)
Nuclase	Exonuclease and endonuclease activity	Contribute to evasion of neutrophil extrophil extracellular traps May degrade host tissue into nutrient required for bacterial growth	(Berends *et al.*, 2010; Cheung *etal.*, 2004; Dinges *et al.*, 2000)
Protease	Degrade human fibronectin, fibrinogen and kininogen	May contribute to the ability of Staphylococcus aureus to disseminate in host Aid in tissue invasion	(Imamura *et al.*, 2005; Massimi *etal.*, 2002; Potempa *et al.*, 1986; Prokesova *et al.*, 1992)

Staphylokinasa	Plasminogen activator that converts plasminogen to a serine protease, plasma more 67% of S. aureus strains express the gene for staphylokinase	Nutralizes the bactericidal effect by foming complex with -defensin. May cleave complement factor C3 Controls fibrinolysis The bacteria exploit the proteolytic activity of plasmin to degrade components of ECM as well asfibrinogen for dissemination in the host	Chavakis *et al.*, 2007; Koch *et al.*, 2012; Lahteenmaki *et al.*, 2000; Rooijakkers *et al.*, 2005; Zecconi andScali, 2013)

Virulence factor	Function	Virulence effect on host	References
Exfoliative Toxins	Glutamate-specific serine proteases that digest desmoglein 1, a keratinocyte cell-cell adhesion molecule. Exfoliative toxins (ETs) act as "molecular scissors" facilitating bacterial skin invasion Prevalence of eta and/or etb range from 0.5-3 % in MSSA but 10 % of MRSA strains have been found to be eta positive	The ETA and ETB are the two most important isoforms and they are associated with staphylococcal bullous impertigo and staphylococcal scalded skin syndrome ETA ETB ETC (not associated with human disease) and ETD Mediate superantigen activity	(Becker et al., 2003; Kato et al., 2011; Peacock et al., 2002; Sila et al., 2009; Zecconi and Scali, 2013)
Hemolysins	Pore forming toxin with cytolytic effect on erythrocytes and monocytes (α–toxin) Cytolytic activity on cytokine containing cells (β –hemolysin also known as sphingomyelinase C) Neutrophil and monocyte binding (δ –hemolysin)	The vast majority of the hemolysins are hemolytic α-toxin has pro-inflammatory properties on host	(Chavakis et al., 2007; Zecconi and Scali, 2013)
Leukocidines	A bi-component pore-forming leukotoxin. Consists of one class S protein and one class F protein. The subunits form a ring with a central pore, through	Kills leukocytes PVL stimulates and lyses neutrophils and macrophages γ-toxin is hemolytic	(Chavakis et al., 2007; Grumann et al., 2013; Kaneko and Kamio, 2004)

	which cell contents leak Different members of the group are γ-hemolysin (hlg), Panton-Valentine leukocidin (PVL) and Leukocidins D, E, M (LukD, LukE, LukM)		
Staphylococcal Enterotoxins	Gastroenteric toxicity; immunomodulation via superantigen activity	Causes food poisoning At least 20 serologically different staphylococcal superantigens have been described, including SEs A to V	(Chavakis *et al.*, 2007; Pinchuk *et al.*, 2010; Zecconi and Scali, 2013)
Toxic shock syndrome toxin	Toxic for endothelium, direct and cytokinemediated Mediate superantigen activity	The toxin causes the rare condition 'toxic shock syndrome' (TSS) These infections are characterized by a rapid onset with high fever, rash, vomiting, diarrhea and multiorgan failure	Chavakis *et al.*, 2007; Peacock *et al.*, 2002; Zecconi and Scali, 2013)

2.4 Antibiotic resistance of *Staphylococcus aureus*

Penicillin was used initially to treat *Staphylococcus aureus* infections. Soon afterwards, resistance emerged when strains acquired a genetic element coding for β-lactamase production, and today over 80 % of all S*taphylococcus aureus* strains are resistant to penicillins. The next drug to be introduced for treating infections with *Staphylococcus aureus* was the semisynthetic penicillinase-resistant penicillin named oxacillin or methicillin, but shortly after its introduction the first isolate with resistance was detected (Winn Washington 2006).

With the emergence of resistance to the penicillinase-resistant penicillins, the glycopeptide agent vancomycin became the treatment of choice for infections with MRSA, and in 1996 the first isolate with intermediate vancomycin resistance was detected in Japan (Winn Washington 2006). Although resistance to methicillin is considered the most important for *Staphylococcus aureus*, other types of resistance exist. For example, a fusidic acid-resistant impetigo clone has caused infections around Europe. The antibiotic fusidic acid is used to treat superficial skin infections caused by *Staphylococcus aureus*, which include impetigo and atopic dermatitis (Brown and Thomas, 2002), and the substance has been in use since the early 1960s. Despite this, the resistance remained low until the 1990s (Brown and Thomas, 2002).

Through the last decade an increase in prevalence of fusidic acid-resistant *Staphylococcus aureus* has been seen in northern Europe, and this resistance has been primarily associated with strains causing impetigo bullosa (O'Neill *et al.*, 2004; Osterlund *et al.*, 2002; Tveten *et al.*, 2002). The resistance is a consequence of the recruitment of the fusB gene (O'Neill and Chopra, 2006; O'Neill *et al.*, 2004). Since fusidic acid is the primary treatment for impetigo in many countries, this is likely to be the reason for the success of this clone in causing disease. The management with antibiotic-resistant bacteria of infections suffered by the elderly living in nursing homes is something to take into consideration now and in the future. For example, MRSA has become endemic in hospitals as well as in health care settings globally (Chambers and Deleo, 2009; DeLeo and Chambers, 2009).

Many nursing home residents have chronic and multiple diseases, and therefore generally require constant medical care and significant assistance with daily living. This causes the residents to be considered as unintentional vectors disseminating pathogens between hospitals and nursing homes and vice versa (Bonomo, 2000; Chamchod and Ruan, 2012). For *Staphylococcus aureus*, information about resistance to cefoxitin, erytromycin, clindamycin, fusidic acid, gentamicin and norfloxacin has been registered.

2.4.1. Methicillin-resistant *Staphylococcus aureus*

The massive consumption of antibiotics over the past 50 years has led to the selection of drug-resistance among *S. aureus* strains, and by far the most important is the resistance against methicillin. In 1961, methicillin (celbenin) became available for treatment of penicillin-resistant *Staphylococcus aureus* strains. Only six months thereafter, the first methicillin-resistant *Staphylococcus aureus* was detected and nosocomial infections began to increase, and in Sweden efforts to combat the spread were established. In the 1980s the detection of MRSA isolates suddenly increased, and a few strains began to expand worldwide (Chen *etal.*, 2012). MRSA is now a leading cause of nosocomial infections worldwide and has also emerged as a community-associated pathogen (Chambers and Deleo, 2009). MRSA strains are inherently cross-resistant to virtually all beta-lactam antibiotics, the most effective and widely used class of antimicrobials.

Moreover, in many countries clinical strains are quite often multi-resistant, which significantly reduces the therapeutic options for treatment of staphylococcal infections (Oliveira and de Lencastre, 2011). The resistance mechanism against methicillin involves the acquisition of the mecA gene, which is a determinant of a unique penicillin binding protein, (PBP)2a, that has reduced affinity for β-lactams, including cephalosporins (Hartman and Tomasz, 1981; Song *et al.*, 1987). The expression of PBP2a causes resistance to all β-lactam antibiotics as the protein blocks binding at the active site for β-lactams

(Fuda *et al.*, 2005a; Fuda *et al.*, 2005b). The resistance gene mecA is inserted in a large heterologous chromosomal cassette, the SCCmec element (Ito *et al.*, 1999). In the first international molecular epidemiological study of MRSA, it was discovered that only a few MRSA lineages were responsible for MRSA infections in hospitals located in Europe, the USA and the Far East (Oliveira *et al.*, 2002) with confirmatory result from later studies (Enright *et al.*, 2002). In a recent European study, the prevalence of MRSA, in blood stream infections varied between 0.5 and 30.2 % in the different participating countries (ECDC). This was in contrast to the low prevalence of MRSA in the general healthy population, where the rates did not exceed 2.1 % (den Heijer *et al.*, 2013). To prevent further spread of MRSA in Sweden, a nationwide surveillance program was launched. All hospitalized patients at risk of carriage of MRSA (i.e. known carriage of MRSA, hospital care outside the Nordic countries, or hospital care in connection with an ongoing outbreak) are screened for the presence of MRSA and other multi-resistant bacteria. Confirmed carriers of MRSA must be isolated and contact tracing is performed around this individual.

2.5 Treatment for *staphylococcus aureus* infection

2.5.1. Selecting antimicrobial therapy

Selecting antimicrobial therapy for *Staphylococcus aureus* infections can be difficult because MRSA, which cause a high proportion of *Staphylococcus aureus* infections in hospitalized patients, are often resistant to many antimicrobial classes (Archer *et al.*, 2001).Vancomycin is typically prescribed for patients with invasive MRSA infections or for patients suspected of having an MRSA infection because, currently, only a few MRSA isolates are resistant to vancomycin. Nevertheless, researchers have observed a "MIC creep" which could increase the incidence of resistant strains (Dhand *et al.*, 2012).Furthermore, vancomycin may not be an appropriate treatment for all patients with aMRSA infection. Vancomycin has several important limitations: it is not bactericidal, it does not penetrate well into lung tissue, and it can cause serious adverse effects including nephrotoxicity and red man syndrome. Vancomycin was first introduced for the treatment of penicillin-resistant *Staphylococcus*

aureus infections in 1958 (Dhand, *et al.*, 2012). However, it was rarely used until the 1980s when the incidence of MRSA infections began to increase (Peacock, *et al.*, 1980). Today, organizations such as the Infectious Diseases Society of America (IDSA) recommend vancomycin for the treatment of some MRSA infections (Liu, *et al.*, 2011). However, the literature varies regarding which treatment is optimal for patients with invasive MRSA infections.

Linezolid is another antimicrobial agent used to treat complex MRSA infections (Yanagihara,*et al.*, 2009). examined a mouse model of MRSA pulmonary infection and found that mice treated with linezolid had lower concentrations of viable bacteria in their lungs and a lower mortality rate than mice treated with vancomycin (Yanagihara *et al.*, 2009). A randomized controlled study that treated 1,184 patients with either linezolid or vancomycin for hospital-acquiredpneumonia found a significantly higher rate of clinical success in patients treated with linezolid, but the mortality rates were similar in the two treatment groups (Wunderrink, *et al.*, 2012).

Additionally, nephrotoxicity occurred more frequently among patients treated with vancomycin (18.2%) than among those treated with linezolid (8.4%) (Wunderrink, *et al.*, 2012). A lower rate of clinical failure, mortality, microbiologic failure, and reoccurrence is found among bacteremic patients treated with daptomycin compared with patients treated with vancomycin (Moore, *et al.*, 2012). Also, ceftaroline, a newer antimicrobial agent, might be a good option for treating MRSA infections since most MRSA strains are currently susceptible to this drug (Richter, *et al.*, 2009).

Even though clinicians typically prescribe vancomycin for MRSA infections, this agent is not effective for all patients possibly due to pathogenic characteristics of the MRSA strain. For example, vancomycin treatment failure has been associated with *agr*dysfunction. One study identified a dysfunctional *agr* in 58% of heterogenous vancomycin-intermediate *Staphylococcus aureus* (hVISA) isolates compared with only 12.5% forvancomycin-susceptible *Staphylococcus aureus* isolates (P = 0.02) (Harigaya, *et al.*, 2011). In contrast, linezolid inhibits production of virulence factors, such as staphylococcal enterotoxins A and B, protein A, and α-hemolysin (Bernardo,K.J et al.,2004). Similarly, Micek et al. described improved clinical outcomes in patients with MRSA pleuropulmonary infections

who received antimicrobials that inhibit exotoxin expression (linezolid or clindamycin) (Micek, *et al.*,2005).

2.5.2 Phage therapy

The rise of multidrug resistant bacteria has enforced the resurgence of phage therapy in the West, though this mode of therapy is being practiced for several years in Eastern Europe. Some of the success stories on phage therapy are described here. The Eliava Institute in Tbilisi, Republic of Georgia, has developed a highly virulent, monoclonal staphylococcal bacteriophage active against 80-95% of *S. aureus* strains including MRSA. This product was used for local and generalized infections, including neonatal sepsis, osteomyelitis, wound infections, pneumonia etc. (Hanlon, 2007). There are some polyvalent obligate lytic *Staphylococcus aureus* phages e.g. phage phi812, phageK and phage44AHJD which have been successfully tested for their efficacy in killing *Staphylococcus aureus* including MRSA strains (Mann, 2008). Evaluation of phageK showed marked reduction of pathogenic and antibiotic resistant coagulase positive and negative staphylococci associated with bovine and human infections that included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus chromogenes*, *Staphylococcus capitis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus caprae*, and *Staphylococcus hyicus*. The modified phage generated by passing through less susceptible target strain can be used in combination with phageK to increase the host range. This study had also shown the potential of delivering the phage in the form of handwash or antistaphylococcal cream (O'Flaherty *et al.*, 2005b).

Laboratory-based production and quality control of a cocktail was demostrated, currently under evaluation, consisting of exclusively lytic bacteriophages for the treatment of *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections of burn wound (Merabishvili, *et al.*, 2009). phage456, in reducing the biofilm formation and adherence of *S. epidermidis* biofilms on both hydrogel-coated andserum/hydrogel coated silicone catheters (Curtin and Donlan 2006). The presence of divalent cations in the growth medium (Mg++, Ca++) further increased the efficacy of phage456 in reducing biofilm

formation. Polyvalent *Staphylococcus* phage combined with highly efficient *Pseudomonas* T7- like phage (phage phiIBB-PF7A) effectively showed reduction in dual species biofilms, killing and finally removal of bacteria from the host substratum (Sillankorva *et al.*, 2010). There were efforts to engineer bacteriophage by over-expressing proteins to target gene networks, particularly non-essential genes, to enhance bacterial killing by antibiotics. Using this approach, engineered a T7 phage which significantly reduced *Escherichia coli* biofilm (Lu and Collins, 2007). This combinatorial approach may reduce the incidence of antibiotic resistance and enhance bacterial killing.

There are many advantages of using phage in therapeutics.

(i) Dysbiosis can be avoided due to their specificity.

(ii) Multiple administrations are not required because phage replicates at the site of infection.

(iii) Phage could select resistant mutants of the selected bacteria.

(iv) Selection of new phages is rapid compared to the development of new antibiotic which may take several years. However, the disadvantage is that the causal organism needs to be identified before administering the phage (Sulakvelidze *et al.*, 2001).

Moreover, prior to the extensive therapeutic use of phages it is prudent to ensure the safety of therapeutic phages. The phages should not carry out generalized transduction and possess gene sequences having significant homology with known antibiotic resistances, phage-encoded toxins and other bacterial virulence factors (Sulakvelidze *et al.*, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Material used

Analytical weighing balance, incubator, autoclave, microscope, refrigerator, deep freezer, water bath, plastic petri dishes, test tubes, microscopic slides, inoculating loops, Bunsen burner, beakers, cotton wool, aluminum foil paper, hypothermic syringe, conical flask, measuring cylinder, masking tape, test tube rack, McCartney bottles, hand gloves, sterile swab sticks, spatula, magnetic stirrer hot plate and magnetic bar. Ethanol (75%), hand sanitizer, crystal violet, gram's iodine, acetone, safranin, distilled water, standard antibiotic disks , hydrogen peroxide (3%), sodium chloride , peptone water, Mannitol salt agar, Nutrient agar, Mueller Hinton agar, MHA (Himedia).

3.2 Specimen

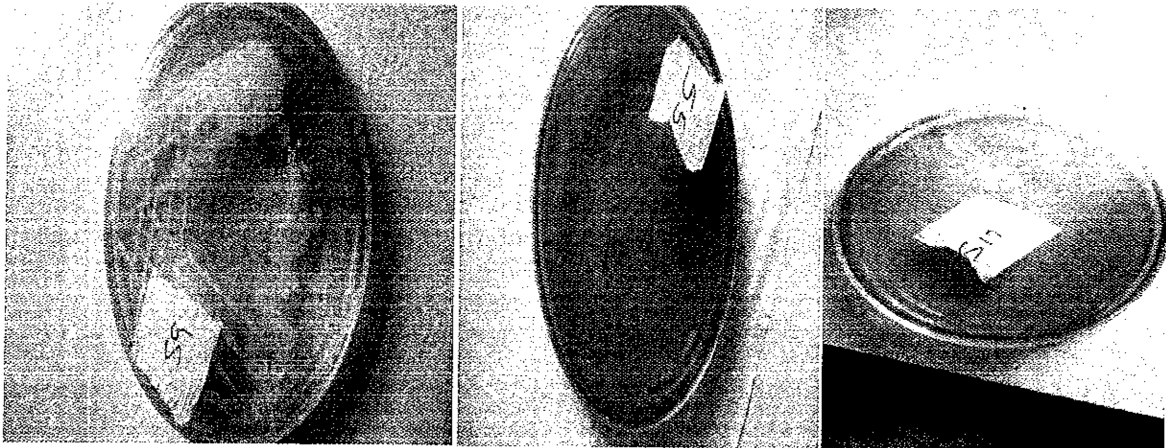
Nasal swab was collected from 75 healthy school children within the age range of 6-12 in Oye-Ekiti, Ekiti State.

Table 2: Distribution of samples

S/N	Sample type	Number of samples
1	St Mary primary school	25
2	Methodist primary school	25
3	Oye-Igbo community school	25

3.3 Isolation of staphylococci from the sample

Sample gotten from nostrils of student of oye community was aseptically inoculated into sterile peptone water microbial enrichment and incubate for 2 hours. Each test tube was subsequently inoculated onto mannitol salt agar and incubated at 30^0C for 24 hours. Isolate that were morphologically distinct, yellow, white, cream and change the colour of the agar to yellow was presumptively considered *Staphylococcus spp.* Single colonies were stored in nutrient agar slants for further assay.



3.4 Gram staining:

It is used to distinguish between gram-positive and gram-negative bacteria, which have distinct and consistent differences in their cell walls. The difference between gram-positive and gram-negative bacteria lies in the ability of the cell wall of the organism to retain the crystal violet.

A loop full of sterile water was dropped on a clean grease free slide. Sterile inoculating loop was used to pick isolate onto drop of water on the slide and spread over a small area (smear).The smear was allowed to air dry and then heat fixed by passing it through bunsen flame two or three times without exposing the dried film directly to the flame. The slide was flooded with crystal violet solution for 60 seconds, washed off with running water for 5 seconds and drained. The slide was flooded with Gram's Iodine solution (mordant) and allowed for 60 seconds then washed off with running water. The slide was flooded with 95% alcohol for 10 seconds and washed off with running water and then drained.

Slide was flooded with safranin solution and allowed to counter stain for 30 seconds, washed off with running water, drained and allowed to air dry. All stained slides were examined under the oil immersion lens (mag 1000).

3.5.0 Biochemical characterisation

3.5.1 Catalase test:

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2. Catalase mediates the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water (Hager, *et. al.*, 1972). Enterococci are known to be catalase negative.

A drop of 3% H_2O_2 was placed on a surface of clean and dry glass slide using a sterile inoculating loop. A pure culture was transferred on it and mixed. A positive result is the rapid evolution of oxygen (5-10 sec.) as evidences by bubbling. A negative result is no bubbles or only a few scattered bubbles.

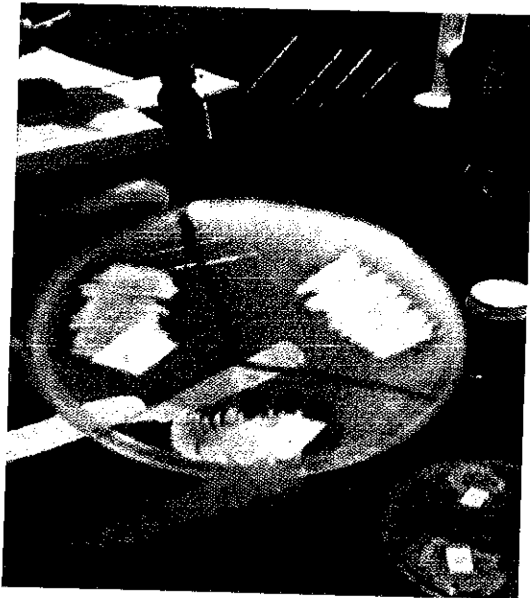
3.5.2 Coagulase test

This test is used to differeniate Staphylococcus aureus (positive) from coagulase negeive Staphylococcus(CONS). Coagulase is an enzyme produced by *Staphylococcus aureus* that convert(soluble) fibrinogen in plasma o (insoluble) fibrin.

3.5.3 DNAse test

DNA Hydrolysis test or Deoxyribonuclease (DNase) test isused to determine the ability of an organism to hydrolyse DNA and utilze it as a source of carbon and energy for growth. An agar, a differential medium is used to test the ability of an organism to produce deoxyribonuclease or DNA. It

contain nutrients for the bacteria. If the organism that grows in the medium produce Deoxyribonuclease, it breaks down DNA ito smaller fragments. When the DNA is broken down, the colony is surrounded by a colourless zone.



3.5.4 Oxidase test

Oxidase test is used to determine if a bacterum produce certain cytochrome C oxidase. Disks impregnated with N,N,N',N'- tetramethyl-P- phenylenediamine (TMPD) or N,N-dimethylo-P-phenylenediamine(DMPD) which is also a redox indicator.

3.5.5 Citrate test

Citrate agar tests determine the ability of organisms to utilize citrate as carbon source. Organism which can utilize citrate as their sole carbon source use the enzyme citrase to transport the citrate into the cell. These organism also convert the ammonium dihydrogen phosphate to ammonia and ammonium hydroxide, which create an alkaline environment in the medium. If the medium turns blue, the organism is citrate positive.

3.5.6 Urase test

Urease broth is a differetial medium that tests the ability of an organism to produce an exoenzyme, called urease, that hydrolyses urease to ammonia and carbon dioxide. The broth contain two pH buffers, urea, a very small amount of nutrients for the bacteria, and the pH indicator phenol red. If the urea in the broth is degraded and ammonia is produced, an alkaline environment is created, and the media turns pink.

3.5.7 Sugar farmentation test

The carbohydrate fermentation test is used to determine whether or not bacteria can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. It test for the presence of acid and/or gas produced from carbohydrate fermentation. Basal medium containing a single carbohydrate source such as glucose, lactose, sucrose or any other carbohydrate is used for this purpose.

Sucrose fermentation-sucrose is fermented to produce acid as end product. The medium is a nutrient broth to which 1.0% sucrose is added. Indicator (phenol red) in the medium changes colour to indicate acid production. If acid is produced the culture will change to yellow.

3.5.8. β-lactamase assay

This test was carried our as described by Ako-Nai et al. (2005). Strips of starch paper measuring 4cm x 7cm were cut and sterilized with 70% ethanol. These strips were then soaked for 10mins in a solution of benzyl penicillin dissolved in phosphate buffer containing 105units. They were spread over an area of 2 to 3mm. Each test paper was then used to test two (2) organisms at a time with the inocula placed at least 2cm apart. The Petri dishes were then incubated for 30mins at 37^0C after which the plate was flooded with Grams iodine solution. This was immediately drained off. This caused the starch paper to turn uniformly black within 30seconds of application. Colonies with decolourized zones

thereafter were indicative of β-lactamase production. Results were read within 5mins as black background tends to decolourize, making interpretations more difficult.

3.6 Antibiotic susceptibility test:

This test was carried out using seven antibiotics (ceftazidime, cefuroxime, ceftraxone, erythromycin, gentamicin, ofloxacin and vancomycin). The medium used was Mueller Hinton agar.This was done to know how resistant the isolates are to the selected antibiotics.

Isolates were picked from the slants and inoculated into tryptone soy broth for enrichment at 37°C for 2 hours. It was transfered onto slanetz and bartley medium plate and incubated for 24 hours at 44°C. Distinct colonies (3) were picked and inoculated into tryptone soy broth and allowed to grow for 2 hours. A sterile swab was dipped into the broth culture of organism and gently squeezed against the side of the test tube in order to remove excess fluid in the swab. The swab was used to make streak on MHA plate for a lawn of growth. Antibiotic disc was placed on the surface of the agar using sterilized forceps and the disc was gently pressed onto the surface of the agar. The plate was incubated at 37°C for 24 hours. After incubation, a metric ruler was used to measure the diameter of the zone of inhibition of each disc, and was compared with measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone. This is done to know if the organism is resistant or intermediate or susceptible to the antibiotics

CHAPTER FOUR

4.0 RESULT

Sixty four isolates gotten from nasal swab were isolated using different biochemical tests and sugar fermentation test. They were observed to compose of 28(43.7%) *Staphylococcus aureus*, Twenty one (26.5%) CONS and fifteen (29.6%) *Staphylococcus epidemidis*. Twenty six (40.6%) showed fermentation on mannitol while Thirty eight (59.4%) showed no fermentation (Table 4). The beta lactamase assay test carried out on samples showed presence of lactamase producing species in 29(45.3%) isolate and 35(54.7%) non beta lactamase producing species (table 5). Susceptibility pattern of staphylococcus species isolated were tested with different commercially available antibiotics (Table 6). Figure 1 shows the percentage resistance of Staphylococcus species to the commercially available antibiotics. Staphylococcus aureus showed zero resistance to Rocephin(R), Pefloxacin(PEF) and Cirpofloxacin(CPX), minor resistance to Streptomycin(S),Septrin(SXT), Erytromycin(E),Gentamycin(CN), Ampicox(AX) and Zinnacef(Z). Figure 2 shows the antibiogram pattern of multiple resistance. There are eighteen different antibiotics multiple resistant pattern. Thirty two (32) of all the isolates showed resistance to only Rocephin (R).

Table 3: Sample and isolate obtained

S/N	Sample type	Number of samples	Number of positive	Number of negative	Total number of isolate
1	St Mary primary school	25	17	8	17
2	Methodist primary school	25	23	2	23
3	Oye-Igbo community school	25	24	1	24

Table 4. Biochemical characterization and identification of nasal swab from healthy student in Oye-Ekiti

Biochemical test	No of staphylococcal isolate (%)	
	Positive	**Negative**
Gram Staining	64 (100%)	0(0%)
Catalase	64(100%)	0(0%)
Coagulase	45(70.3%)	19(29.7%)
DNase	45(70.3%)	19(29.7%)
Mannitol fermenter	26(40.6%)	38(59.4%)
Citrate	64(100%)	0(0%)
Urase	64(100%)	0(0%)
Oxidase	0(0%)	64(100%)
Sucrose	45(70.3%)	19(29.7%)

Table 5. Characterization and occurrence of β-lactamase and non-β-lactamase producing *S. aureus* and CONS

Isolate	No of Isolate	No(%) of BL producer	No(%) of Non-BL producers
S. aureus	28	25 (89.3%)	3 (10.7%)
CONS	36	28 (77.8%)	8 (22.2%)

Table 6. Antibiotic susceptibility profile on isolated *Staphylococcus aureus* of nasal swab from healthy student in Oye-Ekiti.(%)

Sample	S		SXT		E		PEF		CN		APX		Z		AM		R		CPX	
Source	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
St Mary	20	80	0	100	0	100	0	100	10	90	10	90	0	100	60	40	100	0	0	100
Methodist	0	100	0	100	0	100	0	100	10	90	10	90	10	90	54	46	100	0	0	100
Oye-igbo	33	66	25	75	25	75	0	100	9	91	10	90	16	84	25	75	100	0	0	100

Table 7. Antibiotic susceptibility profile on isolated CONS of nasal swab from healthy student in Oye-Ekiti.(%)

Sample	**S**		**SXT**		**E**		**PEF**		**CN**		**APX**		**Z**		**AM**		**R**		**CPX**	
Source	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
St Mary	10	90	10	9	10	90	0	100	10	90	30	70	10	100	60	40	100	0	20	80
Methodist	16.7	83.3	25	75	8.3	91.7	0	100	50	50	41.7	58.3	25	75	50	50	100	0	8.3	91.7
Oye-igbo	7.1	92.9	0	100	0	100	0	100	0	100	0	100	0	100	28.6	71.4	100	0	14.3	85.7

Figure 1

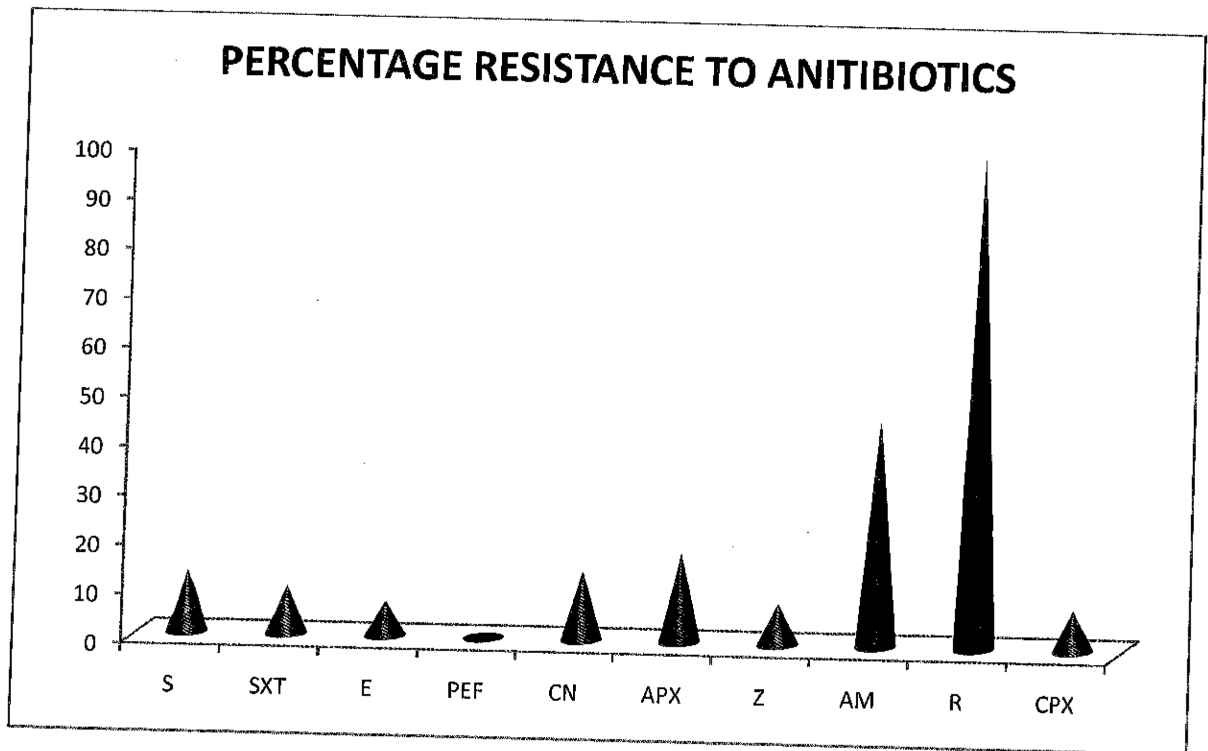
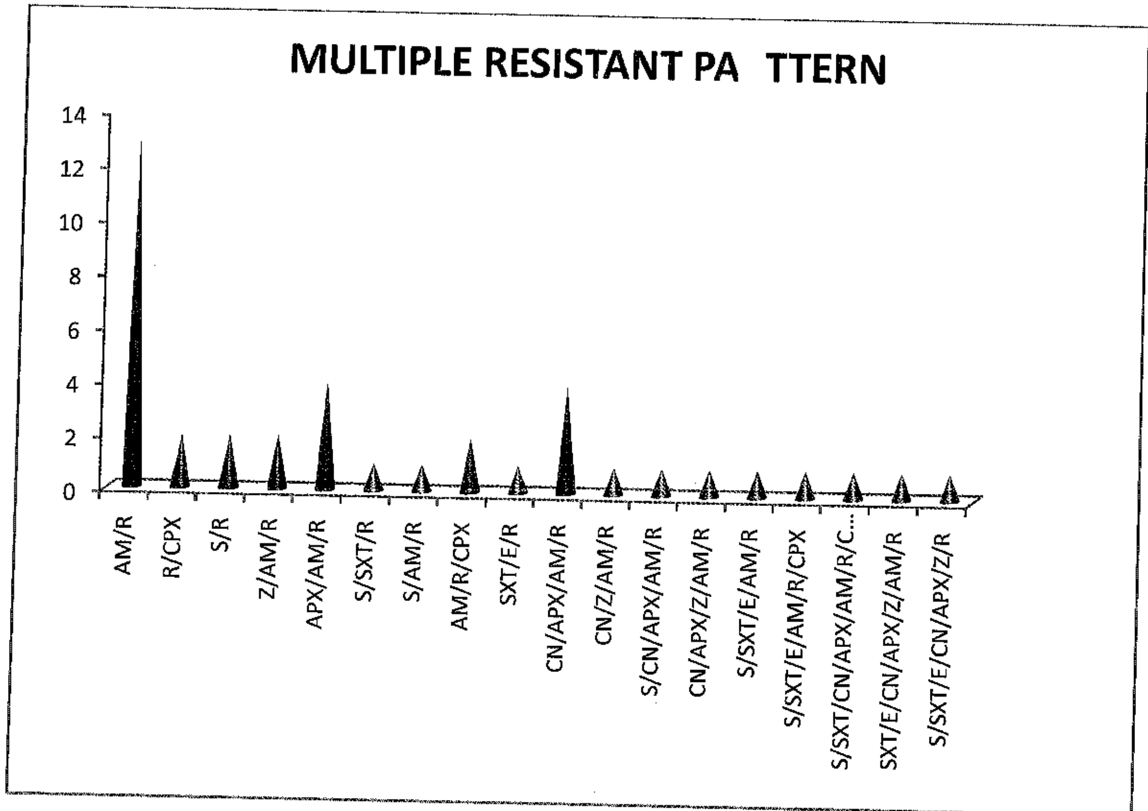


Figure 2



CHAPTER FIVE

5.0 DISCUSSION

The study was conducted to determine the susceptibility, resistance profile of the isolate and to compare the result with sick children. The prevalence of *S. aureus* have the largest percentage (43.7%) of the isolate which is similar to previous findings of (Chiju and Ezeronye 2013) who reported 50% nasal colonization in both hospital and non-hospital subject in Abia, Abia State Nigeria and (Nsofor *et.al.,* 2013) which reported a more higher rate of 62.9% carriage in school children in Elele, Rivers State, Nigeria. In contrary,(Onanuga and Temedia 2011) reported a lower *S.aureus* nasal colonization rate (33.3%) in healthy inhabitants of Amossoma in Niger delta region of Nigeria and (Adesida *et.al* 2007) reported a much lower (14.0%) nasal colonization in medical student in Lagos, Nigeria. These variations maybe attributed to the characteristic of the population under study. A population that is on antibiotics as at the time of sampling may yield a much lower prevalence of *S.aureus* while a population from hospital settings may yield a much higher prevalence because of high prevalence of infectious patients in the environment. Other factors that can cause variations may be sampling and culture techniques and age group of the subjects.

The isolation of CONS and Beta Lactamase producing CONS in this study was in agreement with Akinjojunla and Enabulele (2010), who isolated 9 (42.9%) CONS, (Akinkunmi and Lamikanra 2010) isolated 118(40.3%) CONS, while (Ako-Nai *et.al* 2005) isolated 52 (31%)CONS strain and 26(50%) of Beta lactamase producing CONS, (Ojo *et.al* 2013) isolated 3 (14%) CONS.

This study also isolated B- lactamase producing S.aureus, which conform to earlier studies of (Ako-Nai *et al*,. 2005; Akunjogunla and Enabulele 2010; Bashir *et al.*, 2007; and Ojo *et al.*, 2013).

In this study *S. aureus* group showed a high sensitivity rate pattern to streptomycin(S), septrin(SET),erytromycin(E),pefloxacin(PEF),Gentamycin(CH),Ampiclox(APX),Zinnacef(Z) and Cirpoofloxacin(CPX) and high resistance rate to Rocephin(R) and moderately resistance to Amoxicillin

(AM). (Akinjogunla and Enabulele 2010) reported 50% resistance to Amoxicillin. The high Rocephin resistance in this study do not conform to similar studies by (Adejoke and Okoh 2011) reported a high Erytromycin resistance, 51% Erytromycin resistance by (Bashir *et.al.*, 2007),98% in (Yameen *et.al.*2010),while (Ako-Nai et.al., 2005) reported 54.3%resistance to Erytromycin, Amoxicillin (64.9% and 86.8%).

CONCUSION

Staphylococci are versatile bacteria widely distributed in the environment. As natural inhabitants of the human skin, they are exposed to selective pressure of antibiotic administration and they are known to have developed wide range of resistance to the antibiotics. Staphylococcus species are known to show zero resistance to Rocephin(R), Pefloxacin(PEF) and Cirpofloxacin(CPX). This shows that these antibiotics may not be suitable for the treatment of staphylococcus infection. With the presence of antibiotic resistance gene in Staphylococci, they have been able to transfer their gene to other infectious bacteria in man and animals. Pefloxacin(PEF), is observed to have the most antibiotic ability against *Staphylococcus spp.* among all the antibiotics used in this study.

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APPENDIX

Sample	No of isolates	Slant label	Change in agar colour	Colony colour	Colony Morphology			
					Margin	Size	Shape	Elevati
1	1	1	no change	white	entire	small	round	slightly raised
2	0							
3	1	3	no change	white	entire	small	round	slightly raised
4	1	4	no change	white	entire	small	round	slightly raised
5	1	5	no change	white	entire	small	round	slightly raised
6	0							
7	1	7	no change	white	entire	small	round	slightly raised
8	0							
9	1	9	yellow	yellow	entire	small	round	slightly raised
10	1	10	yellow	yellow	entire	small	round	slightly raised
11	0							
12	0							
13	1	13	yellow	yellow	entire	small	round	slightly raised
14	1	14	yellow	yellow	entire	small	round	slightly raised
15	0							
16	1	16	no change	pink	entire	small	round	slightly raised
17	1	17	yellow	yellow	entire	small	round	slightly raised
18	1	18	no change	white	entire	small	round	slightly raised
19	2	19w	no change	white	entire	small	round	slightly raised
		19p	no change	pink	entire	small	round	slightly raised
20	1	20	yellow	yellow	entire	small	round	slightly raised
21	0							
22	2	22p	no change	pink	entire	small	round	slightly raised
		22y	yellow	yellow	entire	small	round	slightly raised
23	1	23	no change	white	entire	small	round	slightly raised
24	1	24	no change	white	entire	small	round	slightly raised

25	1	25	no change	pink	entire	small	round	slightly raised
26	0							
27	1	27	yellow	yellow	entire	small	round	slightly raised
28	1	28	no change	white	entire	small	round	slightly raised
29	1	29	yellow	yellow	entire	small	round	slightly raised
30	1	30	yellow	yellow	entire	small	round	slightly raised
31	1	31	yellow	yellow	entire	small	round	slightly raised
32	1	32	no change	white	entire	small	round	slightly raised
33	1	33	no change	white	entire	small	round	slightly raised
34	2	34y	yellow	yellow	entire	small	round	slightly raised
		34w	no change	white	entire	small	round	slightly raised
35	0							
36	1	36	no change	white	entire	small	round	slightly raised
37	1	37	yellow	yellow	entire	small	round	slightly raised
38	1	38	no change	white	entire	small	round	slightly raised
39	1	39	no change	white	entire	small	round	slightly raised
40	1	40	yellow	yellow	entire	small	round	slightly raised
41	2	41y	yellow	yellow	entire	small	round	slightly raised
		41w	no change	white	entire	small	round	slightly raised
42	1	42	no change	white	entire	small	round	slightly raised
43	1	43	yellow	yellow	entire	small	round	slightly raised
44	1	44	no change	white	entire	small	round	slightly raised
45	1	45	yellow	yellow	entire	small	round	slightly raised
46	1	46	no change	white	entire	small	round	slightly raised
47	1	47	yellow	yellow	entire	small	round	slightly raised
48	1	48	yellow	yellow	entire	small	round	slightly raised
49	1	49	yellow	yellow	entire	small	round	slightly raised

50	1	50	no change	white	entire	small	round	slightly raised
51	1	51	yellow	yellow	entire	small	round	slightly raised
52	1	52	no change	white	entire	small	round	slightly raised
53	2	53y	yellow	yellow	entire	small	round	slightly raised
		53w	no change	white	entire	small	round	slightly raised
54	2	54y	yellow	yellow	entire	small	round	slightly raised
		54w	no change	white	entire	small	round	slightly raised
55	1	55	no change	cream	entire	small	round	slightly raised
56	1	56	no change	cream	entire	small	round	slightly raised
57	1	57	no change	white	entire	small	round	slightly raised
58	1	58	yellow	yellow	entire	small	round	slightly raised
59	1	59	yellow	yellow	entire	small	round	slightly raised
60	0							
61	1	61	no change	white	entire	small	round	slightly raised
62	1	62	no change	white	entire	small	round	slightly raised
63	2	63y	yellow	yellow	entire	small	round	slightly raised
		63c	no change	cream	entire	small	round	slightly raised
64	1	64	yellow	yellow	entire	small	round	slightly raised
65	1	65	yellow	yellow	entire	small	round	slightly raised
66	1	66	no change	cream	entire	small	round	slightly raised
67	1	67	no change	white	entire	small	round	slightly raised
68	1	68	no change	cream	entire	small	round	slightly raised
69	1	69	no change	cream	entire	small	round	slightly raised
70	1	70	yellow	yellow	entire	small	round	slightly raised
71	1	71	no change	white	entire	small	round	slightly raised
72	1	72	no change	white	entire	small	round	slightly raised
73	1	73	yellow	yellow	entire	small	round	slightly

								raised
74	1	74	no change	white	entire	small	round	slightly raised
75	1	75	no change	white	entire	small	round	slightly raised

Antibiotics used

	No of Isolate	Samle No	S	SXT	E	PEF	CN	APX	Z	AM	R	CPX
1	1	1	25-S	25-S	25-S	25-S	25-S	18-S	15-R	14-R	25-R	25-S
2	0											
3	1	3	25-S	25-S	25-S	25-S	25-S	25-S	25-S	10-R	18-R	25-S
4	1	4	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
5	1	5	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	15-R
6	0											
7	0											
8	0											
9	1	9	15-R	25-S	25-S	20-S	6-R	11-R	20-S	10-R	10-R	25-S
10	1	10	25-S	24-S	25-S	25-S	18-S	20-S	25-S	18-R	25-R	25-S
11	0											
12	0											
13	0											
14	0	16	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
15	1	17	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
16	1	18	25-S	25-S	25-S	25-S	13-R	15-R	23-S	13-R	14-R	25-S
17	2	19w	25-S	25-S	25-S	25-S	25-S	20-S	25-S	25-S	17-R	25-S

18		19p	25-S	25-S	25-S	25-S	15-S	10-R	25-S	10-R	23-R	25-S
19	1	20	25-S	25-S	25-S	25-S	25-S	25-S	20-S	27-S	25-R	26-S
	0											
20												
21	1	22y	11-R	11-R	20-R	25-S	25-S	19-S	22-S	11-R	11-R	20-R
22	1	23	25-S	25-S	25-S	25-S	16-S	10-R	25-S	16-R	25-R	25-S
	1	24	25-S	25-S	25-S	25-S	25-S	21-S	19-S	23-S	13-R	25-S
23	1	25	25-S	25-S	25-S	18-S	25-S	25-S	25-S	16-R	19-R	25-S
24	0											
25	1	27	25-S	25-S	25-S	24-S	24-S	25-S	25-S	25-S	25-R	24-S
26	1	28	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
27	1	29	25-S	25-S	25-S	25-S	25-S	21-S	25-S	11-R	25-R	25-S
28	1	30	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	19-R	25-S
29	1	31	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
30	1	32	16-R	15-R	25-S	25-S	25-S	25-S	25-S	25-S	10-R	25-S
31	1	33	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
32	2	34y	25-S	25-S	25-S	25-S	25-S	21-S	25-S	18-R	25-R	25-S
33		34w	17-S	23-S	25-S	25-S	13-R	11-R	23-S	7-R	15-R	25-S
34	0											

	1	36	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
35	1	37	25-S	25-S	25-S	25-S	18-S	17-R	25-S	18-R	25-R	25-S
36	1	38	10-R	11-R	25-S	25-S	11-R	12-R	24-S	10-R	20-R	15-R
37	1	39	25-S	23-S	25-S	25-S	13-R	10-R	25-S	9-R	17-R	25-S
38	1	40	25-S	25-S	25-S	25-S	19-S	20-S	24-S	19-R	19-R	25-S
39	2	41y	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
40		41c	18-S	10-R	17-R	18-S	10-R	12-R	16-R	17-R	18-R	25-S
41	1	42	25-S	25-S	25-S	25-S	8-R	11-R	25-R	11-R	10-R	25-S
	1	43	25-S	25-S	25-S	25-S	17-R	20-S	16-R	17-R	25-R	25-S
42	1	44	25-S	25-S	25-S	25-S	10-R	11-R	25-S	10-R	20-R	25-S
43	1	45	25-S	25-S	25-S	25-S	25-S	21-S	25-S	25-S	25-R	25-S
44	1	46	25-S	25-S	25-S	25-S	20-S	20-S	25-S	18-R	25-R	25-S
45	1	47	12-R	25-S	25-S	25-S	18-S	20-S	25-S	11-R	20-R	25-S
46	1	48	25-S	25-S	25-S	25-S	17-S	11-R	20-S	11-R	10-R	25-S
47	1	49	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
48	1	50	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
49	1	51	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-S	25-R	25-S
50	1	52	25-S	25-S	25-S	25-S	25-S	25-S	25-S	18-R	25-R	25-S
51	2	53y	25-S	25-S	25-S	25-S	25-S	21-S	25-S	25-S	25-R	25-S

52		53w	25-S	25-S	25-S	25-S	25-S	20-S	25-S	25-S	25-R	25-S
53	2	54y	25-S	20-S	25-S	25-S	23-S	21-S	25-S	25-S	25-R	24-S
		54w	25-S	25-S	25-S	25-S	24-S	20-S	25-S	25-S	25-R	25-S
54	1	55	25-S	24-S	25-S	24-S	24-S	21-S	25-S	20-S	22-R	24-S
	1	56	26-S	22-S	25-S	25-S	25-S	20-S	25-S	23-S	24-R	25-S
55	1	57	23-S	18-S	25-S	18-S	20-S	21-S	25-S	19-R	25-R	24-S
56	1	58	10-R	10-R	17-R	21-S	13-R	12-R	16-R	20-S	25-R	25-S
57	1	59	10-R	15-R	16-R	21-S	19-S	20-S	25-S	10-R	20-R	24-S
58	0											
59	1	61	21-S	21-S	25-S	24-S	24-S	21-S	25-S	20-S	25-R	24-S
60	1	62	22-S	19-S	25-S	19-S	19-S	21-S	25-S	19-R	25-R	24-S
61	2	63y	25-S	25-S	25-S	25-S	18-S	25-S	15-R	15-R	23-R	25-S
62		63c	19-S	20-S	25-S	24-S	20-S	21-S	25-S	11-R	25-R	19-R
63	1	64	11-R	19-S	25-S	23-S	21-S	20-S	25-S	21-S	25-R	22-S
	1	65	25-S	23-S	25-S	24-S	23-S	21-S	25-S	24-S	24-R	23-S
64	1	66	21-S	21-S	25-S	24-S	24-S	21-S	25-S	20-S	25-R	24-S
65	1	67	20-S	20-S	25-S	24-S	20-S	20-S	25-S	24-S	25-R	22-S
66	1	68	21-S	21-S	25-S	24-S	24-S	21-S	25-S	20-S	23-R	24-S
67	1	69	24-S	24-S	25-S	24-S	19-S	20-S	25-S	25-S	25-R	18-R

68	1	70	25-S	11-R	17-R	24-S	23-S	21-S	25-S	22-S	20-R	22-S
69	1	71	21-S	18-S	25-S	25-S	25-S	20-S	25-S	23-S	25-R	23-S
70	1	72	11-R	20-S	25-S	20-S	25-S	21-S	25-S	20-S	25-R	24-S
71	1	73	25-S	24-S	25-S	25-S	20-S	20-S	25-S	25-S	25-R	25-S
72	1	74	22-S	24-S	25-S	17-S	22-S	21-S	25-S	19-R	24-R	16-R
73	1	75	25-S	23-S	25-S	19-S	24-S	21-S	25-S	26-S	25-R	24-S
74		no/% resistant	9/12.5%	7/9.72%	5/6.94%	0/0%	10/13.8%	13/18.06%	6/8.33%	33/45.83%	72/100%	6/8.33%

sample	no of isolate	slant label	gram stain	catalase	coagulase	biochemical test Dnase	mannitol	citrate	urase	oxidase	beta lactamase
1	1	1	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
2	0										
3	1	3	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
4	1	4	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
5	1	5	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
6	0										
7	1	7	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
8	0										
9	1	9	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
10	1	10	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
11	0										
12	0										
13	1	13	Positive	Positive	negative	negative	positive	Positive	Positive	negative	positive
14	1	14	Positive	Positive	negative	negative	positive	Positive	Positive	negative	positive
15	0										
16	1	16	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
17	1	17	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
18	1	18	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
19	2	19w	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
		19p	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
20	1	20	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
21	0										
22	2	22p	Positive	Positive	positive	positive	negative	Positive	Positive	negative	negative
		22y	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
23	1	23	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
24	1	24	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
25	1	25	Positive	Positive	positive	positive	negative	Positive	Positive	negative	negative
26	0										
27	1	27	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
28	1	28	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
29	1	29	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
30	1	30	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
31	1	31	Positive	Positive	negative	negative	positive	Positive	Positive	negative	negative
32	1	32	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
33	1	33	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
34	2	34y	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
		34w	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
35	0										
36	1	36	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
37	1	37	Positive	Positive	positive	positive	positive	Positive	Positive	negative	negative
38	1	38	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
39	1	39	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
40	1	40	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
41	2	41y	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
		41w	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
42	1	42	Positive	Positive	negative	negative	negative	Positive	Positive	negative	negative
43	1	43	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
44	1	44	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
45	1	45	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
46	1	46	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
47	1	47	Positive	Positive	negative	negative	positive	Positive	Positive	negative	positive
48	1	48	Positive	Positive	positive	positive	positive	Positive	Positive	negative	negative
49	1	49	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
50	1	50	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
51	1	51	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
52	1	52	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
53	2	53y	Positive	Positive	negative	negative	positive	Positive	Positive	negative	positive
		53w	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
54	2	54y	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
		54w	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
55	1	55	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
56	1	56	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
57	1	57	Positive	Positive	positive	positive	negative	Positive	Positive	negative	positive
58	1	58	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
59	1	59	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
60	0										
61	1	61	Positive	Positive	negative	negative	negative	Positive	Positive	negative	negative
62	1	62	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
63	2	63y	Positive	Positive	positive	positive	positive	Positive	Positive	negative	positive
		63c	Positive	Positive	negative	negative	negative	Positive	Positive	negative	positive
64	1	64	Positive	Positive	positive	positive	positive	Positive	Positive	negative	negative