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Sudan University of Science and Technology
College of Graduate Studies

Frequency of Vancomycin-resistant *Staphylococcus aureus* amongst Bacteria Isolated from Patients with Wound Infection in khartoum State hospitals

تواتر المكورات العنقودية الذهبية المقاومة للفانكومايسين بين البكتيريا المعزولة من المرضى المصابين بعدوى الجروح في مستشفيات ولاية الخرطوم

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الآيـــــة

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
قال تعالى:

{ وَإِذَا مَرِضْتُ فَهُوَ يَشْفِيْن } سورة الشعراء الآية (٨٠) .

Dedication

My parents

To my father whose memory continues to inspire me
To my mother whose love has sustained me through life's rough times

Our teachers

For the people who share us our memories, laughs and our life, the people who draw the best part of our life, the people who know to make us happy

My friends

To my friends and all people who have positive impact in my life

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Abstract

This is a descriptive cross-sectional study carried out in Khartoum State, during the period from February 2022 to May 2022. The study was designed to investigate the frequency of vancomycin-resistant *Staphylococcus aureus* amongst bacteria isolated from patients with wound infection.

A total of 100 specimens were collected from patients who attended Hospitals in Khartoum. The specimens were collected from both males and females. The age of patients ranged from 3 to 75 years old. The specimens were cultured on blood agar and mannitol salt agar then incubated aerobically.

The isolated bacteria were identified according to their colonial morphology, Gram reaction and biochemical reaction. These isolates were tested for susceptibility for several antibiotics by disc diffusion technique.

A total of 48 S. aureus were identified.

The result revealed that antibiotic susceptibility patterns of 48 *S. aureus* were analyzed for 6 types of antimicrobial agents by Kirby-Bauer disc diffusion method.

The results revealed that *S. aureus* was resistant to Amoxicillin 68 %, Ciprofloxacin 57%, Ceftriaxone 42%, Amikacin 34%, Methicillin 19%, vancomycin 6.3 %. Interestingly, 54.2% isolates were multi drugs resistance. However, 5 isolates were sensitive to all antibiotics tested, and only 3 isolates were resistant to vancomycin.

This study concluded that there is high rate of resistance to many drugs and developing little resistance to vancomycin, so appropriate drug prescription is based on susceptibility testing. To avoid resistance antibiotic such Vancomycin should be used judiciously, as the resistance rate is increased. Further studies with large sample size is required to validate the result of the present study.

الملخص

هذه دراسة مقطعية وصفية أجريت في ولاية الخرطوم خلال الفترة من فبراير 2022 إلى مايو 2022. صممت الدراسة للتحقق من تواتر المكورات العنقودية الذهبية المقاومة للفانكومايسين بين البكتيريا المعزولة من مرضى الجرح.

تم جمع ما مجموعه 100 عينة من المرضى الذين حضروا إلى مستشفيات الخرطوم ، وكانت العينات عبارة عن مسحات جروح. تم جمع هذه العينات من كل من الذكور والإناث. وكان النطاق العمري من 3 إلى 75 سنة. تم استزراع العينات واحتضانها هوائيًا بالطريقة التقليدية للحصول على عزلات إكلينيكية نقية ، ثم تم تحديدها وفقًا لتشكلها الاستعماري وتفاعل الجرام والتفاعل الكيميائي الحيوي. تم اختبار قابلية هذه العزلات للتأثر بالعديد من المضادات الحيوية بتقنية الانتشار القرصي. أظهرت هذه العينات عزل 48 من المكورات العنقودية الذهبية ، تم تحليل أنماط الحساسية للمضادات الحيوية لـ 48 من المكورات العنقودية الذهبية لـ 6 أنواع من العوامل المضادة للميكروبات بواسطة طريقة الانتشار القرصي. أظهرت النتائج أن بكتيريا المكورات العنقودية الذهبية كانت شديدة المقاومة للأموكسيسيلين 68٪ ، سيبروفلوكساسين 57٪ ، سيفترياكسون 42٪ ، أميكاسين 34٪ ، ميثيسيلين 19٪ ، فانكومايسين 6.3٪. ومن المثير للاهتمام أن 54.2٪ عزلات كانت مقاومة للأدوية المتعددة. ومع ذلك ، كانت 5 عزلات حساسة لجميع المضادات الحيوية المختبرة ، و 3 عزلات فقط كانت مقاومة للفانكومايسين.

خلصت هذه الدراسة إلى أن هناك نسبة عالية من المقاومة للعديد من الأدوية وتطور مقاومة قليلة للفانكومايسين ، لذا فإن الوصفة الطبية المناسبة تعتمد على اختبار الحساسية. لتجنب مقاومة المضادات الحيوية ، يجب استخدام الفانكومايسين بحكمة ، حيث يزداد معدل المقاومة. مطلوب مزيد من الدراسات مع حجم عينة كبير للتحقق من صحة نتيجة الدراسة الحالية.

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CHAPTER I

INTRODUCTION

1.1 Background

Staphylococcus aureus (*S. aureus)* is a major human nosocomial and community acquired pathogen the bacterium causes infections of the skin and soft tissues, and life threatening systemic diseases and is associated with high rate of morbidity and mortality worldwide (Cong *et al*., 2020; Amini *et al*., 2013). Since the beginning of the antibiotics era, *S. aureus* has been a privileged target of therapeutics research, and numerous antimicrobial agents, as well as Vaccine attempts (Lowy, 2003; Proctor, 2012).

Over the past few decades, there has been an alarming increase in the prevalence of antibiotic resistant strains in serious infections (Brown *et al*., 2005; Khan *et al*., 2013). The occurrence of bacterial infection had decreased with the discovery of penicillin in 1940 until *S. aureus* began producing B-lactamase, which destroys the penicillin b-lactam core ring (Khan *et al*., 2013; Peacock, 2006) This increase in resistance towards penicillin drove the development of methicillin drugs, which are virtually resistant against many genetic variations of the beta-lactamase enzyme. Infection by *S. aureus* was well controlled using methicillin until the isolation of the first strain of methicillin resistant *S. aureus* (MRSA) in 1961 (Lowy, 2005; Brown *et al*., 2005). The glycopeptides vancomycin has been regarded as one the last therapeutic agent for the treatment of infections due to severe MRSA and other resistant Gram-positive strains (McGuinness *et al*., 2017). It remains a challenging global public health crisis due to the emergence and spread of vancomycin-resistant *S. aureus* (VRSA) (Gajdács, 2019) in addition, VRSA tends to be multi-drug resistant (MDR) against a diversity of currently available antimicrobial agents. Therefore, the selection of appropriate antimicrobial therapy requires active

surveillance of emerging resistance trends and continuing education among the health care providers and institutions (El Solh AA and Alhajhusain A, 2009).

1.2 Rationale

Wound infection of *S. aureus* is increased which leads to acquisition of genes of resistance, Inappropriate initial or delay of appropriate antimicrobial therapy for serious *S. aureus* infections is associated with increased mortality and longer lengths of hospital stay (Lowy, 2005; Brown *et al*., 2005). In Sudan there is significant high frequency of MRSA in hospitals which my posses vancomycin resistant genes and most isolates were from wounds (Osman *et al*., 2018) Therefore, isolation, identification and treatment of resistant VRSA strains are urgently required. The present study was undertaken to find out the frequency of vancomycin-resistant *S. aureus* amongst bacteria isolated from patients with wound infection in Royal Care International Hospital.

1.3 Objectives of the study

1.3.1 General Objective

To investigate the frequency of vancomycin-resistant *S. aureus* amongst bacteria isolated from patients with wound infection In Royal Care International Hospital.

1.3.2 Specific Objectives

1. To isolate and identify *S. aureus* from wound infection by phenotypic characters.

2. To detect the susceptibility of *S. aureus* to vancomycin antibiotic using disc diffusion method.

3. To determine the frequency of vancomycin-resistant *S. aureus* strains**.**

CHAPTER II

LITERATURE REVIEW

2.1 Biology of *S. aureus*

S. aureus is Gram-positive cocci, measuring around 1µ in diameter, nonmotile, nonsporing and noncapsulated. They, however, contain a microcapsule, which can be visualized by electron microscope only, but not by a light microscope**.** The cocci are typically arranged in irregular grape-like clusters. This appearance is due to incomplete separation of daughter cells during successive divisions of bacteria, which takes place in perpendicular planes. The grape-like clustering is seen when the bacteria are grown in solid media, but usually short chains are seen when grown in liquid media. In smears taken from pus, the cocci are present either singly or in pairs, in clusters, or in short chains of three or four cells (Parija, 2012).

S. aureus is an important human pathogen that causes a spectrum of clinical diseases. These range from superficial skin lesions like folliculitis to deep-seated abscess and various pyogenic infections like endocarditis, osteomyelitis, etc. The bacterium also causes toxin-mediated diseases, such as food poisoning, toxic shock syndrome (TSS), and staphylococcal scalded skin syndrome (SSSS) (Levinson, 2012). Staphylococci are aerobes and facultative anaerobes but can grow in the absence of oxygen also. They grow at a temperature range of 10–42°C (optimum temperature 37°C) and a pH range of 7.4–7.6 (optimum pH 7) (Parija, 2012). Culture on solid media: Staphylococci can grow on a wide range of media including Mueller–Hinton agar, nutrient agar, blood agar, and MacConkey agar. Primary isolation can be made on nutrient agar and blood agar (Geo *et al*., 2013).

2.2 Ecology and transmission

S. aureus has minimal nutritional requirements which enables it to survive in many different environments, Human cases and carriers are the important reservoir of infection (Knox *et al*., 2015). Human cases of cutaneous and respiratory infections shed large numbers of staphylococci into the environment for a prolonged period of time. Staphylococci colonize the skin very early in life (neonates on the umbilical stump) Staphylococci shed by the patients and carriers contaminate handkerchiefs, bed linens, blankets, and other inanimate fomites and persist in them for weeks. *S. aureus* found in the nose and sometimes on the skin, especially in hospital staff and patients is the main source of infection in hospitals. Domestic animals, such as cows, can also be reservoirs of staphylococcal infection (Nimmo, 2012; Parija, 2012). The squamous epithelium of the anterior nares is the primary natural reservoir of *S. aureus* in human beings. Historically, three patterns of colonization have been described, persistent-carriers (roughly 20% of the general population), intermittent-carriers (30%), and non -carriers (50%) (Denis, 2017). There is a dynamic interaction between community-based sources of MRSA and the introduction of these clones into the household. This dynamic is perhaps best illustrated by the numerous reported outbreaks of MRSA infections that have occurred in a variety of community-based reservoirs including sports clubs, day care facilities, jails, schools, and places of work (Campbell et al., 2002; Bancroft, 2007).

2.3 Pathogenesis of *S. aureus*

S. aureus has evolved a comprehensive strategy to address the challenges posed by the human immune system. The emergence of community-associated methicillin resistant *S. aureus* (CA-MRSA) infections in individuals with no predisposing conditions suggests an increased pathogenicity of the bacterium, which may be related to acquisition of novel genetic elements (Liu, 2009). Colonizing nares, skin,

and the gastrointestinal tract, frequently invades the skin, soft tissues, and blood-streams of humans. Even with surgical and antibiotic therapy, bloodstream infections are associated with significant mortality (Thomer *et al*., 2016).

2.4 Virulence determinants

S. aureus produce a variety of molecules that contribute in tissue invasion and destruction.

2.4.1 Cell wall associated proteins and polymers

Capsular polysaccharide: Few strains of *S. aureus* are capsulated. These strains are more virulent than the noncapsulated ones. Protein A: Protein A is an important virulence factor since it has non-specific interaction with Fc portion of the immune globulin G (IgG) leaving the Fab portions free to combine with specific antigen. Peptidoglycan: It activates the complement, stimulates production of antibodies, and inhibits chemotaxis by inflammatory cells.Teichoic acid: It mediates attachment of staphycocci to mucosal cell. (Parija, 2012).

2.4.2 Extracellular enzymes

2.4.2.1 Coagulase

S. aureus has a unique ability to clot a variety of mammalian plasma. Clotting of plasma is brought about by the action of the enzyme coagulase secreted by the pathogenic strains of *S. aureus*. The enzyme coagulase is of two types: (a) free coagulase and (b) bound coagulase (Geo *et al*., 2013)**.**

2.4.2.2 Catalase

The enzyme catalase reduces H_2O_2 to nascent oxygen and water. This nascent oxygen causes oxidative damage of host tissue. This enzyme is produced after phagocytosis or during metabolism of the bacteria. All strains of staphylococci produce catalase unlike streptococci (Parija, 2012; Levinson, 2012).

2.4.2.3 Hyaluronidase

The enzyme hyaluronidase hydrolyzes the acidic mucopolysaccharides present in the matrix of the connective tissues, thereby facilitating the spread of bacteria in tissues (Parija, 2012; Levinson, 2012).

2.4.2.4 Penicillinase

More than 90% of *S. aureus* produce enzyme penicillinase. The enzyme inactivates penicillin group of antibiotics, hence is responsible for widespread occurrence of penicillin-resistant staphylococci. The gene for this enzyme is acquired through plasmids. Other enzymes: These include phosphatase, deoxyribonucleases, nucleases, proteases, phospholipase, and lipases (Parija, 2012; Levinson, 2012).

2.4.3 Toxins

2.4.3.1 Toxic shock syndrome toxin

Toxic shock syndrome toxin (TSST) is a protein with a molecular weight of 22,000 Da and resembles enterotoxin F and exotoxin C. It is antigenic. Production of toxin is pH dependent and occurs at pH 7–8. The toxin causes toxic shock syndrome (TSS) (Liu, 2009; Parija, 2012).

2.4.3.2 Enterotoxin

Is a heat-stable protein, the toxin is produced by nearly one-third of all the strains of *S. aureus* (Liu, 2009; Parija, 2012).

2.4.3.3 Exfoliative toxin

The toxin breaks intercellular bridges in the stratum granulosum of epidermis and causes its separation from the underlying tissue, resulting in a blistering and exfoliating disease of the skin. Leukocidins, Hemolysins and other toxins (Liu, 2009; Parija, 2012).

2.5 Epidemiology

S. aureus is both a human skin and mucosa commensal but also a frequent cause of serious infections with high morbidity, mortality, and healthcare-associated costs. *S. aureus* is the most frequently occurring bacterial pathogen among clinical isolates from hospital inpatients in the United States and is the second most prevalent bacterial pathogen among clinical isolates from outpatients **(**Styers *et al*., 2006) longitudinal trends. In the industrialized world, the population incidence of ranges from 10 to 30 per 100,000 person-years (Laupland *et al*., 2013). Staphylococcal infections are found throughout the world. Nearly one-third of the adult population is asymptomatic carrier of staphylococci (Parija, 2012). Up to 30% of the human population are asymptomatically and permanently colonized with nasal *S. aureus*. To successfully colonize human nares, *S. aureus* needs to establish solid interactions with human nasal epithelial cells and overcome defense mechanisms. However, some factors like bacterial interactions in the human nose can influence S. aureus colonization and sometimes prevent colonization. On the other hand, certain characteristics and environmental factors can predispose to colonization. Nasal colonization can cause opportunistic and sometimes life-threatening infections such as surgical site infections or other infections in non-surgical patients that increase morbidity, mortality as well as healthcare costs (Sakr *et al*., 2018). The advent and use of antibiotics such as penicillin and methicillin in the mid-20th century initially proved effective against *S. aureus*. However, *S. aureus* rapidly acquired resistance to these antibiotics and infections with penicillin-resistant *S. aureus* (PRSA) (Dantes *et al*., 2013; CDC, 2014). *S. aureus* during the modern antibiotic era has been delineated by distinct strain emergence events, many of which include acquisition of antibiotic resistance. The relative high burden of methicillin-resistant *S. aureus* (MRSA) in healthcare and community settings is a major concern worldwide

(McGuinness 2017). Vancomycin, a glycopeptide antibiotic that inhibits cell wall biosynthesis, remains a drug of choice for treatment of severe MRSA infections. *S. aureus* strains exhibiting increased resistance to vancomycin, known as vancomycin intermediate-resistant *S. aureus* (VISA), were discovered in the 1990s. The molecular basis of resistance in VISA is polygenic and involves (Khatib *et al* 2011). Stepwise mutations in genes encoding molecules predominantly involved in cell envelope biosynthesis. *S. aureus* isolates with complete resistance to vancomycin are termed vancomycin- resistant *S. aureus* (VRSA) they were first reported in the U.S. 2002. Resistance in VRSA is conferred by the vanA gene and operon, which is present on a plasmid. The treatment of VRSA infections is challenging, the burden of VISA is relatively high and the molecular mechanisms of resistance are less well-defined. VISA are associated with persistent infections, vancomycin treatment failure, and poor clinical outcomes (Sievert *et al.*, 2008; McGuinness, 2017).

2.6 Previous Studies

Study Conducted by Dilnessa and Prevalence, (2016). of 1360 clinical specimens analyzed, *S. aureus* was recovered from 194 (14.3%). Rate of isolation of *S. aureus* with regard to clinical specimens was the highest in pus 118 (55.4%). No *S. aureus* was isolated from CSF and urethral discharge. Out of 194 *S. aureus* isolates, 34 (17.5%) were found out to be MRSA and the remaining 160 (82.5%) were MSSA. A total of 98 (50.5%) *S. aureus* isolates were multidrug resistant, and the highest isolates were resistant to penicillin 187 (96.4%) and least resistant for clindamycin 23 (11.9%) and vancomycin 10 (5.1%). MRSA strains were 100% resistant to penicillin G, erythromycin, and trimethoprim-sulfamethoxazole and least resistant to vancomycin 10 (29.4%). Out of 194 *S. aureus* isolates, 153 (79.0%) were beta-lactamase producers.

Olowe *et al*., (2007). In country, Nigeria, depicted that out of 67 *S. aureus* isolates, 32(47.8%) were resistant to methicillin. High prevalence of MRSA, 13 (19.4%), was isolated from wound, while urine sample had the least, 1(1.5%). High resistance levels (87.5%) were detected against penicillin and tetracycline, while gentamicin and vancomycin recorded the least resistance levels of 62.5 and 6.3%, respectively

In Sudan a study conducted to determine the frequency and the antibiogram of MRSA among different clinical isolates. The overall result show that (45.7 %) were MRSA mostly recovered from wounds and blood stream. High percentage was detected in hospital (64.2%) rather than community (Osman *et al*., 2018)

In country Sudan a study conducted by in Khartoum hospitals as One hundred and thirty three various samples were collected from some hospitals in Khartoum over a period of 5 months. The samples were cultured on bacteriological media for the isolation of *Staphylococcus aureus* using standard methods of isolation and identification of bacteria. The *Staphylococcus aureus* were tested for Methicillin susceptibility using 5 µg Oxacillin disc and Oxacillin E-test, with Resistance defined as an MIC of ≥ 4µg/ ml. Results: In this study all MRSA isolates displayed an Oxacillin MIC of ≥256µg/ml. The MRSA strains were 41.0% while the Resistance to vancomycin was examined by vancomycin E-test, with resistance defined as an MIC of ≥16 µg ml. In this study all VSSA isolates displayed vancomycin MIC of ≤ 2µg/ml "except three intermediate resistant isolate MIC between 4-8 µg/ml". The percentage of the VISA strains was 12.0%. With 0.0 % VRSA.((Osman *et al*., 2016).

A total of 200 post-operative surgical specimens were collected from patients hospitalized in gastrointestinal tract (GIT) surgical ward in Ibn Sina hospital, Khartoum, Sudan and were subjected to MRSA screening and sensitivity test. Key Findings: Out of 35 strains of Staphylococcus aureus isolated from surgical samples,

25 (71.4%) were found to be MRSA. Almost all MRSA strains were resistant to Methicillin, 96% to Ofloxacin, 92% to Pencillin G, 24% to Amikacin and 4% to Vancomycin. Cross-resistance was obviously detected. Conclusion: The present study detected alarming levels of S. aureus (MRSA) isolates, at the same time presence of high cross-resistance to other antibiotics.(Kheder, 2012)

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

This study was carried out at Royal Care International Hospital

3.2 Study subjects

Patients who attended to Royal Care International Hospital, with suspected wound infection, and hospitalized during the study period were included in this study. However, other patients without signs and symptoms of infections, with mild injury, non hospitalized or receive antibiotics were excluded.

3.3 Study Variables

Frequency of *S .aureus* infections and antimicrobial susceptibility

3.4 Study design

Hospital based descriptive cross-sectional study was carried out during the period February 2022 to May 2022. To determine Vancomycin-resistant *Staphylococcus aureus* amongst bacteria ịsolated from patients with wound Infection in Royal Care International Hospital, Sudan

3.5 Sample size

100 clinical specimens were collected during the period February 2022 to May 2022. Verbal consent was taken from all participants.

3.6 Data collection

Sociodemographic and clinical data was gathered by direct interviewing questionnaire.

3.7 Culture media

Different types of culture media were used for isolation and identification of

S .aureus. All culture media were prepared according to the instruction of manufacturer's. Blood agar, Nutrient agar, Mueller Hinton Agar, DNASE agar, Mannitol salt agar.

3.8 Reagents and stains

All reagents were kept in well-closed glass stoppered bottles and protected from sun light. Distilled water, Normal saline, Absolute Alcohol, Kovact's reagent, Crystal violet, Lugol's iodine, Safranine Methyl Red solution were used (Barrow and Feltham, 1993).

3.9 Instruments

The instruments have been used in this study include: Autoclave, Bunsen flame, refrigerator, centrifuge, hot air oven, incubator, microscope and sensitive balance (Barrow and Feltham, 1993).

3.10 Equipments

The equipment that have been used in this study include: Beaker, filter paper, flasks, measuring cylinder, oil emersion, petri dishes, bacteriological wire loop, straight wire, forceps, slides, sterile urine containers, bijous bottles, sterile swabs and wooden sticks, antibiotic discs.

3.11 Antibiotic discs

These include: Vancomycin (30 μg), Methicillin (5 μg), Ceftriaxone (30 μg), Amoxicillin (25 μg), Ciprofloxacin (5 μg), Amikacin (30μg)

3.12 Collection of clinical specimens:

3.13 Pus/swab

Sterile cotton-wool swab was used to collect pus from inflamed wound and then, the swab was being immersed in a container of Amies transport medium, when required. And immediately was being transferred to the laboratory.

3.14 Isolation and identification

S. aureus was identified by colonial morphology and pigmentation in blood agar, mannital salt agar and nutrient agar. In addition, microscopic inspection and biochemical tests were done according to Barrow and Feltham (1993).

3.15 Primary identification

3.15.1 Colonial morphology

The inoculated media were morphologically examined for size, color, fermentation of lactose and pigment production and hemolysis of blood.

On blood agar colonies appeared grey white vary in size from small to medium some isolates show Beta hemolysis while other show no hemolysis

Mannitol Salt Agar (MSA) medium was also used as a selective medium.

Gram's staining technique was performed to see the shape, arrangement and Gram's reaction. Bacterial smears were prepared by emulsifying a small inoculum of the suspected colonies in a drop of normal saline and

spread it onto a clean glass slide (15-20 mm). The smears were allowed to dry on air and then heat fixed by passing it two to three times gently over the Bunsen flame with the smear side up.

3.15.2 Gram's stain

The prepared smears (slides) were placed on a staining rack and covered with crystal violet (primary and basic) stain for 30-60 seconds. The stain rapidly washed by tap water and the slide was tipped. Stained smear then covered with Lugol's iodine (mordant) for 30-60 seconds. Iodine immediately washed off and the smear was decolorized with 95% ethanol for few seconds. 0.5% Sufranin (counter stain) was added to the smear for 2 minutes. The red stain then washed off with tap water and smear preparation subsequently air dried and microscopically examined using high resolution objective power. Violet-colored bacteria were labelled Gram positive (Barrow and Feltham, 1993).

3.16 Secondary identification of isolated bacteria

This was done by using the biochemical tests, which were performed according to Barrow and Feltham (1993).

3.16.1 Catalase Test

The test was performed to detect the ability of the isolated bacteria to produce catalase enzyme and differentiate the *Staphylococcus* spp from the *Streptococcus* spp.

Using wooden stick apart of 24 hours growth colony have been taken and put in test tube contain 3% H_2O_2 and observe the air babble indicate positive result.

3.16.2 Dnase Test

Freshly prepared DNA agar was inoculated by the isolated bacteria by making spot middle the plate, then incubated overnight aerobicly at 37 c^{o}

Diluted hydrochloric acid has been add after the incubation, the appearing of clear zone around the spot indicate positive result.

3.16.3 Novobiocin Sensitivity test

Suspension of isolated bacteria has been made equivalent to 0.5 McFarland .then using sterile cotton swab Mueller Hinton agar has been sealed, and 5 ug disk of novobiocin has been applied than incubated over night at 37 c^{o}

Zone of inhibition greater than 16 mm indicated that organism is sensitive,

3.16.4 Mannitol fermentation in MSA

A freshly prepared mannitol salt agar media has been inoculated with the isolated bacteria and incubated at 37C for 24 hours the change in color to yellow indicated mannitol fermentation.

3.17Antibiotics susceptibility test.

All isolated *S. aureus* were examined for their susceptibility to : Vancomycin (30 μg), Methicillin (5 μg), Ceftriaxone (30 μg), Amoxicillin (25 μg), Ciprofloxacin (5 μg), Amikacin (30μg) by disk diffusion technique (Kirby-Bauer method). 2 to 3 of freshly 24 hours grown colonies were emulsified in sterile normal saline then adjusted to reach the McFarland standard turbidity, sterile cotton swab was immersed in the suspension and inoculated on plate of Mueller-Hinton agar. The inoculation was eventually distributed all over the plate surface. Commercially prepared antibiotic discs of Bioanalyse Ltd., were placed on the surface of the medium using sterile forceps. Discs

were gently pressed to ensure full contact with the medium. Plates were incubated at 37c^{o} for 24 hours. The zone of growth inhibition around each disc was measured in millimeters and the result was compared against the chart which was provided by the manufacturer and reported as sensitive, intermediate or resistant, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

CHAPTER IV

RESULTS

During the study period from February 2022 to June 2022 a total of 100 clinical specimens were aerobically cultured, out of which 48 *S. aureus* isolates were obtained.

Tabe 4.1: results of biochemical tests

Isolate	Catalase test	DNASE test	Novobiocin sensitivity	Mannital fermtation
1	+ve	+ve	S	+ve
2	+ve	+ve	S	+ve
3	+ve	+ve	S	+ve
4	+ve	+ve	S	+ve
5	+ve	+ve	S	+ve
6	+ve	+ve	S	+ve
7	+ve	+ve	S	+ve
8	+ve	+ve	S	+ve
9	+ve	+ve	S	+ve
10	+ve	+ve	S	+ve
11	+ve	+ve	S	+ve
12	+ve	+ve	S	+ve
13	+ve	+ve	S	+ve
14	+ve	+ve	S	+ve
15	+ve	+ve	S	+ve
16	+ve	+ve	S	+ve
17	+ve	+ve	S	+ve
18	+ve	+ve	S	+ve
19	+ve	+ve	S	+ve
20	+ve	+ve	S	+ve
21	+ve	+ve	S	+ve
22	+ve	+ve	S	+ve
23	+ve	+ve	S	+ve
24	+ve	+ve	S	+ve

S; sensitive +ve;positive

The mean age of patients infected with *S. aureus* was 41 years old (Mean 41, minimum 3 and maximum 75 years old). Most of the strains were isolated from older patients (>45 years) (46 %) followed by (<15 years) patients (16.2%) (Table 4.1).

Table 4.2: Frequency of *S. aureus* among different age groups.

Age groups (years)	*S. aureus Distribution*	
	Number of Isolates	Percentage (%)
<15	7.7	16%
15-25	6.2	13%
26-35	5.4	11%
36-45	6.7	14%
>45	22	46%
Total	48	

On the other hand; the proportion of *S. aureus* positive cultures is very high among males 70% (34/48) as compared to females 30% (14/48). The gender (male: female) ratio was 2.3:1 (Figure 4.1).

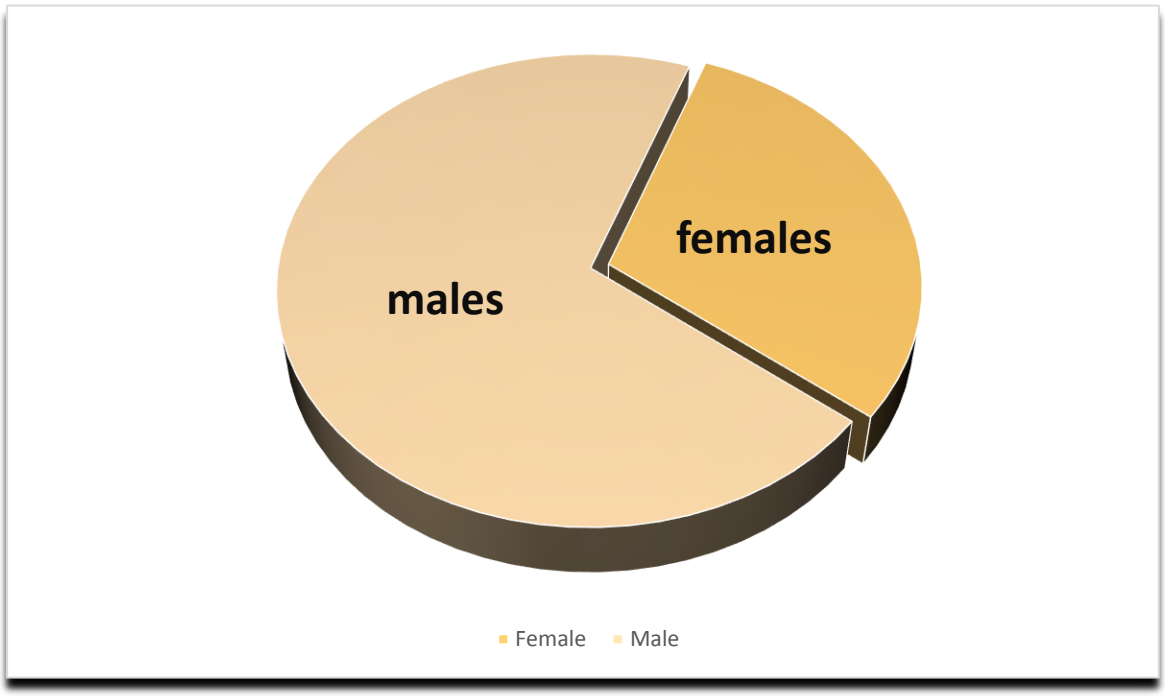

Figure 4.1 Distribution of *S. aureus* among gender.

4.2 Antimicrobial susceptibility patterns

The antibiotic susceptibility patterns of 48 *S. aureus* were carried out for 6 types of antimicrobial agents by Kirby-Bauer disc diffusion technique. The result revealed that *S. aureus* was highly resistant to Amoxicillin 68 % (32/48) followed by Ciprofloxacin 57% (27/48), Ceftriaxone 42% (20/48), Amikacin 34% (16/48), Methicillin 19% (9/48), Vancomycin 6.3 % (3/48). Interestingly, 54.2% (26/48) isolates were multi drugs resistance (Figure 4.2). However, 5 (10.5%) isolates were sensitive to all antibiotics tested. However only 3 isolates were rresistant to vancomycin.

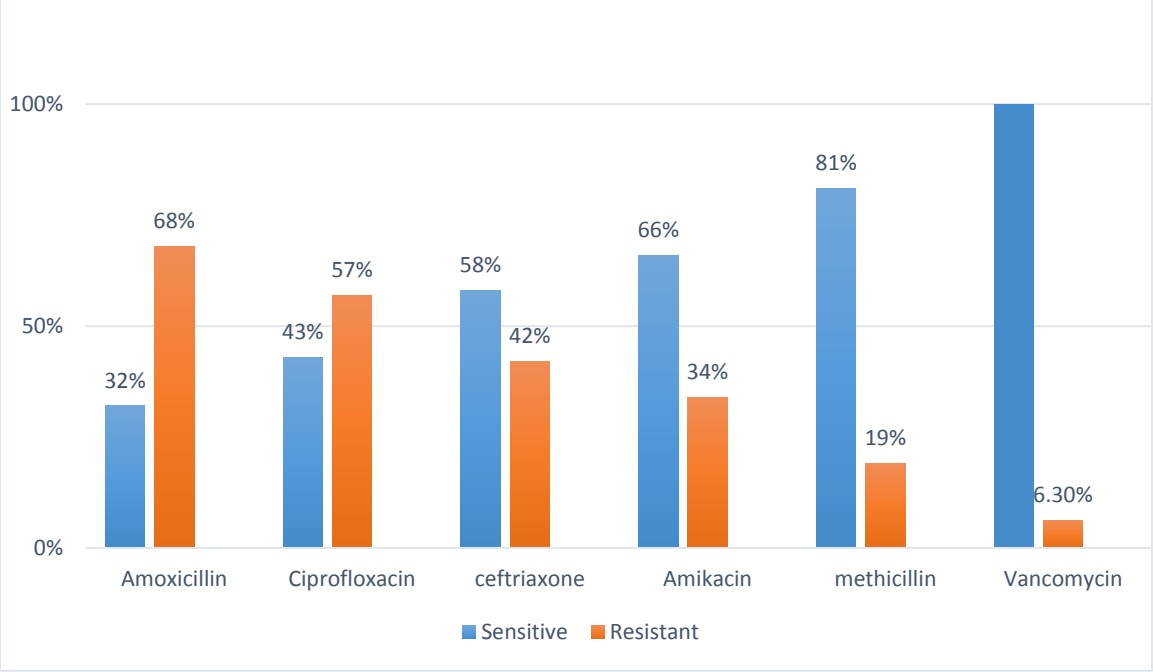

Figure 4.2 Resistance rates (%) of *S. aureus* against Antibiotics.

CHAPTER IV

DISCUSSION

S. aureus presents a serious therapeutic challenge for the treatment of both MRSA and VRSA, increased numbers of MSRS in sudan in contract to reduction in vancomycin sensitivity as vancomycin considered one of the reserve drugs and last line of MRSA treatment, so selection of appropriate antibiotic to initiate therapy is essential for optimizing the clinical outcome. The main objective of the present study was detection the frequency of vancomycin-resistant *S. aureus*.

In this study, out of 100 clinical specimens investigated, 48 *S. aureus* were isolated with a prevalence rate of (48%) which is high in comparing to (39.5%) prevalence rate conducting in hospitals in sudan by Osman *et al*., (2016) and higher that reported by Dilnessa *et al*, (2016) with a prevalence rate o (14.3%). And lower than (73%) prevalence rate reported in Bangladesh by Rashedul *et al,* (2012). difference in prevalence rates within sudan and outside are probably due to the difference in sample size or the source of the infection whether there are MRSA carrier or contamination of hospital items, At hospital setting other factors have a role in prevalence, at the role of causative agent the virulence, the ability to resist antimicrobial and disinfectant and biofilm formation are major factors distinguish hospital *S. aureus* prevalence.

The study showed that the majority (68%) of isolates were resistant to Amoxicillin, followed by ciprofloxacin (57%), Ceftriaxone 42%, Amikacin 34%. The study showed marked variability in antimicrobial sensitivity.

In this study prevalence of MRSA is (19%) which is high in comparing to MRSA (17%) reported in Ghana by Saba *et al.*, (2017), and lower than (45.7%) reported in Sudan by Osman *et al*., (2018). Also low in comparing to (41%) MRSA reported in

Khartoum state, Sudan by Osman *et al*., (2016). The 3 result above are in Khartoum state, Sudan the geographical area, the difference in resistance rate of MRSA probably due to difference in site of infection, some hospitals have MRSA nasal carrier staff.

The prevalence of Vancomycin is (6.3%). The frequency of VRSA is high than the frequency reported in Ephiopia (5.1%) by Dilnessa *et a*l, (2016), and similar to (6.3%) reported by Olowe *et al*, (2007). Lower than the frequency (39%) reported in Bangladesh (Rashedul *et a*l, 2012). In Sudan study conducted by Osman *et al*., (2016) show (0.0 %) VRSA. And low in comparing to study conducted in Sudanese patients in Khartoum which show (40 %) VRSA by Alboshra *et al*., (2020). The difference in VRSA is probably due to different geographical area and the misused of drugs and the presence of gene of resistance. And the unrestricted used of vancomycin.

Conclusion

The present study concluded that the rate of *S. aureus* drug resistance is high to routinely used antibiotics. But still the frequency of VRS is low. The emergence of MRSA organisms with reduced susceptibility to Vancomycin is a serious and ongoing concern, detection of VRSA is difficult and require further development and appropriate screening methods and definitive assays than can be readily adopted by clinical laboratories.

Recommendations

1- Culture and susceptibility technique must be performed to determine the appropriate treatment.

2- Performing PCR technique to determine the responsible gene for VRS as routine tool.

3- Using more sensitive test to detect VRS, and MICs should be determined.

4. Continuous studies about drug resistance is highly recommended

5- Community education about the danger of using antibiotics without prescription.

5. Further studies with new antibiotics

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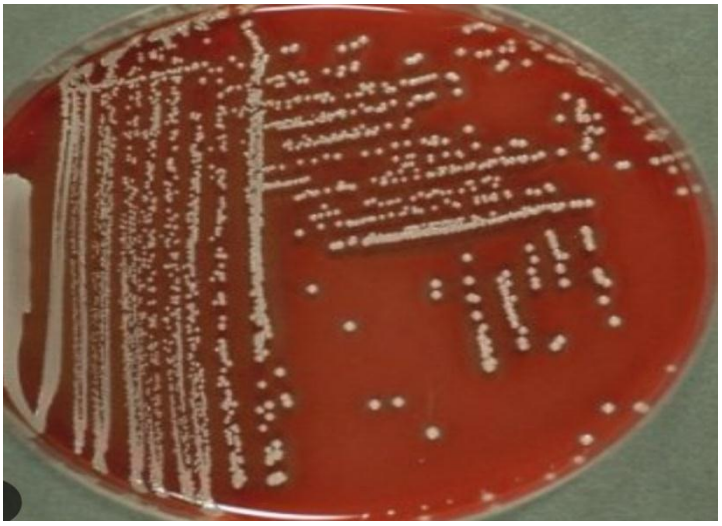
Appendixes



Blood agar

Nutrient agar

MSA

Mueller Hinton agar