بسم الله الرحمن الرحيم

كلية نبتة
NAPATA COLLEGE

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Medical Laboratory Sciences program

Department of Microbiology

Prevalence of Vancomycin Resistance *Staphylococcus aureus* in Khartoum -Sudan

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A graduation project Submitted for the requirement of BSc. Degree (Honor) in medical Microbiology

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BSC of Microbiology

MSC of Microbiology

MSC of Molecular medicine

January 2021

بسم الله الرحمن الرحيم

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

سورة البقرة (32)

Dedication

To the real love who give our life meaning:

Our mother and father

To who had given us dreams to look forward to:

Our brothers and sisters

To the smiles and stars in our dark:

Our friends and families

To the beloved memories of missed souls

To all suffering people and to the beautiful

Sudanese who creating beautiful Sudan

Alamen, Ahmed, Enas, Badr.

Acknowledgement

We are grateful to the god for the good health and wellbeing that were necessary to complete these theses.

We wish to express our sincere thanks to **Us. Amna AbuElgasim** (department of medical microbiology) for her continuous supervision, discussion and guidance.

We are also grateful to our teachers, in the department of medical microbiology we are extremely thankful and indebted to them for sharing expertise, and sincere and valuable guidance and encouragement extended to us.

We place on record, our sincere thanks to **Dr. Mona Omer Ahmed** for the continuous encouragement, helping and golden advices.

Extended appreciation to **Us. Tanzeel Ahmed** for helping us with calculation and data analysis.

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Abbreviations

S. aureus	***Staphylococcus aureus***
CNA	**Colistin Nalidixic Acid**
BSI	**Blood stream infection**
TSST	**Toxic shock syndrome toxin**
PVL	**Panton valentine leukocidin**
CSST	**Complicated of skin and soft tissue infection**
CA-MRSA	**Community associated vancomycin resistant *Staphylococcus aureus***
CSF	**Cerebro spinal fluid**
IV	**Intra venous**
VRSA	**Vancomycin resistant *Staphylococcus aureus***
VISA	**Vancomycin Intermediate *Staphylococcus aureus***
CDC	**Control disease center**
MIC	**Minimum inhibitory concentration**
PCR	**Polymerase Chain Reaction**

Abstract

Background: This study conducted to detect Vancomycin resistance *Staphylococcus aureus* in patients with different clinical manifestations in Sudan - Khartoum state. From October 2020**.** A bio let Cross sectional study were done, (100) samples from different clinical isolates (Urine, Wound, semen and nasal swab) all samples were randomly selected, demographic data was recorded for each sample include age, gender and antibiotic administration.

Material and method: All samples were confirmed by Gram stain, biochemical tests. Overall, 100 samples (77) male, (33) female, age range (19-37) years were included in this study.

Result: The antimicrobial susceptibility of the isolates was determined using Kerpy –Bour disk diffusion method. result showed *Staphylococcus aureus* sensitive for77%, 22% showed intermediate and 1% for vancomycin resistant, this only sample was isolated from female urine culture, intermediate present in 10 females ,12 in male while sensitive 22 female and 65 male.

Conclusion: The result of this study confirm that the Vancomycin is the drug of choice until far So that the situation continues as it is to avoid resistance , 22% intermediate these percentage borderline show sign and alarm to educate population ,should follow the antibiotic administration policies and avoid (misuse and overdose) and Susceptibility test must be done for clinical isolate to avoid antimicrobial drug resistance (ADR).

الخلاصة

اجريت هذه الدراسة للكشف عن بكتيريا المقاومة للفانكومايسين في المرضى ذوي المظاهر السريرية المختلفة في السودان - ولاية الخرطوم.اعتباراً من اكتوبر 2020 تم اجراء دراسة مقطعية بيولوجية ، (100) عينة من عزلات سريرية مختلفة (بول ، جروح ، سائل منوي و مسحات الانف) اختيرت بشكل عشوائي، وتم تسجيل البيانات الديموغرافية لكل عينة والتي شملت العمر والجنس و استعمال المضادات الحيوية. تم تأكيد جمع العينات عن طريق اختبارات صبغة الجرام والاختبارات الحيوكيميائية.

بشكل عام تم تضمين 100 عينة (70) ذكور، (33) إناث ، الفئة العمرية بين (19-37) سنة في هذه الدراسة.

تم تحديد حساسية العزلات لمضادات الميكروبات باستخدام طريقة (كيربي -باور);

, واظهرت النتائج حساسة المكورات العنقودية الذهبية بنسبة 77% ، 22% وسيطة و 1% مقاومة للفانكومايسين ، هذه العينة الوحيدة تم عزلها من عينة بول أنثوية .وكانت الوسيطة في 12 عينة من الذكور و 10 من الاناث ، بينما الحساسة كانت 22 أناث و 65 ذكور .

الخلاصة: تؤكد نتيجة هذه الدراسة أن الفانكومايسين هو الدواء المختار حتى الآن ولكي يستمر الوضع كما هو لتجنب المقاومة. وحيث ان متوسط هذه النسبة هو 22% تظهر اشارة وانذار يجب توعية السكان باتباع سياسات ادارة المضادات الحيوية وتجنب سوء الاستخدام والجرعة الزائدة ويجب اجراء اختبار الحساسية للعزلة السريرية لتجنب مقاومة الادوية المضادة للمكروبات.

CHAPTER ONE

INTRODUCTION& LITERATURE REVIEW

1. Staphylococcus aureus

Staphylococcus aureus is a Gram-positive coccus about 1 µm in diameter, arranged in grape-like clusters [1]. The organisms are non-sporing, non-motile and usually non-capsulate. When grown on many types of agar for 24 h at 37°C, colonies appear opaque and are often pigmented (golden-yellow, hence the 'aureus'). [2]. Commonly used selective media include mannitol salt agar, lipase salt mannitol agar, Columbia colistin–nalidixic acid (CNA) agar and Baird–Parker agar base supplemented with egg yolk tellurite enrichment. [3] *Staphylococcus aureus* was the most prevalent organism causing infections in all the hospitals studied. The majority of *S. aureus* isolates were resistant to Methicillin/oxacillin (MRSA), which indicates their multi-resistant phenotype. [4]

VRSA was identified in two hospitals. The emergence of VRSA emphasizes the high need for programs to prevent the spread of antimicrobial-resistant microorganisms and Control the use of antimicrobial drugs in healthcare settings. [5] The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semisynthetic penicillin's (methicillin, oxacillin) has made the therapy of staphylococcal disease a global challenge. The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA [6]

Vancomycin continues to be used as a first-line antimicrobial agent for the treatment of infection with MRSA [5, 6,7]

cases. The Kirby- Bauer disk diffusion method indicated that MRSA were isolated from 34/49 (69.4%), while VRSA were also detected among enrolled subjects when referring to NCCL and CDC criteria with a significant frequency 10/49 (20.4%) [8].

1.1.2 Virulence factors

Recent years have seen a greater understanding of the pathogenic interaction between the host and *Staph. aureus.* Most strains possess a large number of cell-associated and extracellular factors [10] some of which contribute to the ability of the organism to overcome the body's defenses and to invade, survive in and colonize the tissues. Although the role of each factor is not fully understood individually, it is likely that they are responsible for the establishment of infection, enabling the organism to bind to connective tissue, opposing destruction by the

bactericidal activities of humoral factors such as complement, and overcoming uptake and intracellular killing by phagocytes. [4]

Extracellular enzymes and toxins produced by strains of S. aureus that contribute to its invasiveness and pathogenicity. [11]

Coagulase Clots plasma, interferes with phagocytosis, facilitates spread in the tissues, Haemolysins Leukocidin, Fibrinolysin: Hyaluronidase Facilitates spread in tissues by destroying hyaluronic acid (component of connective tissue). Protein A: Antiphagocytic (prevents complement activation). [12]

Enterotoxins (heat stable): Cause food-poisoning (particularly vomiting) Toxic shock syndrome toxin-1: Shock, rash, desquamation of skin. Epidermolytic toxins A and B: Generalized peeling of the skin, Chemotaxis inhibitory protein: Inhibits migration and activation of neutrophils. [3]

Enterotoxins (heat stable): types A–E, G, H, I and J, are commonly produced by up to 65% of strains of *Staph. aureus*, sometimes singly and sometimes in combination. These toxic proteins withstand exposure to 100°C for several minutes. When ingested as preformed toxins in contaminated food, microgram amounts of toxin can, within a few hours, induce the symptoms of staphylococcal food poisoning: nausea, vomiting and diarrhea. However, enterotoxins, which are superantigens (see below) probably also play an important role in other serious staphylococcal infections, e.g. bloodstream infection (BSI), especially when accompanied by septic shock [13]

● Toxic shock syndrome toxin-1:

TSST-1 and the enterotoxins are now recognized as superantigens, that is, they are potent activators of T lymphocytes resulting in the liberation of cytokines such as tumour necrosis factor, and they bind with high affinity to mononuclear cells. These characteristics partly explain the florid and multi-system nature of the clinical conditions associated with these toxins. [14]

● Epidermolytic toxins A and B:

Two kinds of epidermolytic toxin (types A and B) are commonly produced by strains that cause blistering diseases. These toxins induce intraepidermal blisters at the granular cell layer. Such blisters range in severity from the trivial to the distended blisters of pemphigus neonatorum. The

most dramatic manifestation of epidermolytic toxin is the scalded skin syndrome in small children, where the toxin spreads systemically in individuals who lack neutralizing antitoxin. Extensive areas of skin are affected, which, after the development of a painful rash, slough off; the skin surface resembles scalding [15]

● Panton-Valentine leukocidin (PVL)

This toxin was recognized some decades back but its potential contribution to the clinical manifestations and outcome have been increasingly described in the context of community-acquired MRSA (CA-MRSA) As the name suggests PVL can adversely affect cells, resulting in leucopenia, but animal studies do not suggest high virulence[17] epidemiological data in many countries reveal an association between necrotizing pneumonia and some complicated skin and soft tissue infections (cSSTI) caused by PVL-positive strains of CA-MRSA. [16]

1.1.3Epidemiology:

More than a century after its description, *Staphylococcus* aureus is still a dangerous pathogen for humans. Associated with considerable morbidity and mortality, both in hospitals and in the community. [17]

The clinical and molecular epidemiology of *S. aureus* infections has changed dramatically over the past two decades, with the emergence of community-associated methicillin-resistant *S. aureus* (CA-MRSA). [18]

identified several distinctive traits of *S. aureus* epidemiology, both in developed urban populations and in remote rural communities. [19] By far the most striking feature of this epidemiology is the very high prevalence, ranging from 17% to 74%, of the genes encoding the potent leukotoxin Panton-Valentine leukocidin (PVL) in *S. aureus* isolates. Most of these PVL-positive isolates are methicillin-susceptible, which makes PVL epidemiology in Africa different from that in both Europe, where the prevalence of PVL-positive *S. aureus* is <2%, and North America, where the prevalence is very high but is mostly attributable to the exceptional diffusion of a single CA-MRSA clone. [20]

Overall, the landscape of *S. aureus* infections has evolved worldwide at a fast pace since the turn of the millenium. However, most of these changes had gone largely undetected until recently in non-western countries, probably because research efforts have been mainly focused on the

catastrophic rise of CA-MRSA in North America and on the threat of a similar epidemic in Europe. In the increasingly connected world of the years to come, a global view of *S. aureus* dynamics will probably be necessary to comprehend and anticipate the potentially rapid dissemination of newly emerged clones. Surveillance efforts should be sustained, and more resources have to be committed to investigating and controlling *S. aureus* infections in areas such as Africa, India, and the Far East. [4]

1.1.4 Pathogenicity:

Staphylococcus aureus causes a wide range of infections that may be broadly divided into community and hospital acquired Community-acquired infections include toxin-mediated diseases such asTSS and food poisoning, infections affecting the skin and soft tissues, infection of bones and joints, infection relating to other deep sites (e.g. endocarditis, abscess formation in liver and spleen) and infection of lung and urinary tract. [2]

Toxin-Mediated *Staphylococcal* Diseases:

TSS is a severe disease characterized by high fever, hypotension, diffuse erythematous rash with subsequent desquamation 1–2 weeks later and involvement of three or more organ systems. Clinical features may include mucous membrane hyperemia, myalgia, vomiting and diarrhea, renal and hepatic impairment, altered level of consciousness, coagulopathy and low platelet count. [21]

Food Poisoning:

Staphylococcus food poisoning results from ingestion of preformed SEs (Dinges, Orwin and Schlievert 2000). The mechanism of action of the toxin is still under study. Nausea and vomiting occur after an incubation period of 2 and 6 h. abdominal pain and diarrhea are also common features. [22]

Bacteraemia and Endocarditis:

Around two-thirds of *S. aureus* bacteremia cases are attributable to nosocomial sepsis, most of which relate to an intravenous device. [23]

Bone and Joint Sepsis:

Staphylococcus aureus is the leading cause of primary septic arthritis and osteomyelitis in all ages except neonates.

Pulmonary Infections:

Infection arises because of inhalation of the organism from a site of colonization (community acquired), via an endotracheal tube in ventilated patients or, more rarely, because of haematogenous spread in a bacteraemic patient. [24]

Nosocomial Infection:

The range of nosocomial or hospital-acquired infections includes surgical wound infection, ventilator-associated pneumonia, bacteraemia associated with intravenous devices and infection associated with other types of prosthetic materials such as cerebrospinal fluid (CSF) shunts, prosthetic joints and vascular grafts. However, any disease manifestation may occur in the hospital setting. Investigation will depend on the nature and severity of infection and requires individual assessment for a given patient. [25]

Other Clinical Manifestations:

As a generalization, *S. aureus* may infect any organ or region of the body. Additional deep-site infections such as abscess formation in liver, spleen and kidney usually occur in the setting of a bacteraemic patient who develops seeding to multiple sites. But is reported to be common in the tropical setting. *Staphylococcus aureus* may cause acute otitis media and urinary tract infection.[2]

1.1.5 Prevention and Control:

Hospital infection control measures may prevent a proportion of nosocomial infections. Hand washing plays a central role, reducing transmission of pathogens between individuals and from the hands of a given individual to vulnerable sites such as wounds and dialysis catheters. [26]

Perioperative antibiotic prophylaxis is also important in preventing surgical sepsis, together with good skin preparation before surgery and aseptic and surgical techniques. [27]

Affluent nations can implement hospital infection control through an infection control team who devise policies, monitor hospital infections from a diagnostic microbiology laboratory or by

active ward-based surveillance and implement outbreak procedures where necessary. Medical staff are educated and actively encouraged to follow guidelines that reduce infection rates in given settings. [2]

1.1.6 Treatment:

S. aureus and other staphylococci are inherently susceptible to many antimicrobial agents. About 90% of strains found in hospitals are now resistant to benzylpenicillin due to the production of the enzyme penicillinase [28] a β-lactamase that opens the β-lactam ring. Methicillin, oxacillin, cloxacillin and flucloxacillin, are stable to the enzyme. Cephalosporins and β-lactamase inhibitors are also stable to penicillinase. [29]

MRSA strains are resistant to all β-lactam agents, and often to other agents such as the aminoglycosides and fluoroquinolones. [30]

Glycopeptides (vancomycin or teicoplanin) are the agents of choice in the treatment of systemic infection with MRSA, but these agents are relatively expensive and may be toxic. [31] Isolates of MRSA with reduced susceptibility or full resistance to glycopeptide antibiotics are uncommon, but have been detected sporadically. [32]

These isolates have either thickened cell walls (reduced susceptibility) or the vanA gene (fully resistant), and can be difficult to detect in the routine diagnostic laboratory. [1]

1.1.7 Vaccination:

A considerable body of experimental vaccine-related work has been published, although this has not yet translated into a licensed vaccine preparation [33] The most significant human anti-staphylococcal vaccine study published to date evaluated a single dose of a conjugate vaccine comprising *S. aureus* types 5 and 8 capsular polysaccharides conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A. Evaluation by randomized trial of 1804 adult patients at 73 hemodialysis centres demonstrated partial immunity against *S. aureus* bacteremia for approximately 40 weeks, after which protection waned as antibody levels decreased. [1]

1.2 Antimicrobial agents:

Antimicrobial agents include naturally occurring antibiotics, synthetic derivatives of naturally occurring antibiotics (semi-synthetic antibiotics) and chemical antimicrobial compounds (chemotherapeutic agents). [34]

Generally, the term ‘antibiotic’ is used to describe antimicrobial agents (usually antibacterial) that can be used to treat infection. Compared with antibacterial agents, fewer antiviral and antifungal agents have been developed.

Antibacterial agents can be grouped according to:

1-Their mode of action:

Bacteristatic and bactericidal agents:

Antibacterial agents are generally described as bacteristatic when, at usual dosages, they prevent the active multiplication of bacteria, e.g. chloramphenicol, tetracycline, and erythromycin. Antibacterial agents are described as bactericidal when, at usual dosages, they kill bacteria, e.g. the penicillins, cephalosporins, glycopeptides and aminoglycosides. Some bacteristatic agents become bactericidal when used at higher concentrations, e.g. erythromycin and tetracycline.

2-Antimicrobial spectrum:

Broad spectrum is activity against a wide range of Gram positive and Gram-negative organisms. They include some lactam antibiotics and the tetracyclines, aminoglycosides, sulphonamides, and chloramphenicol. [35]

Narrow spectrum antibiotics are those with activity against one or few types of bacteria, e.g. vancomycin against *staphylococci* and *enterococci*.

3- Mode of action:

According to their ability to inhibit the synthesis of the cell wall, cell membrane, proteins, and nucleic acids of bacteria. [3]

1.2.2 Vancomycin:

Vancomycin is a glycopeptides, Bactericidal I.V. It’s used to treat serious infections such as endocarditis and septicaemia caused by Gram positive bacteria, particularly multi-resistant strains. Treatment requires monitoring due to ototoxicity (damage to hearing) and nephrotoxicity (kidney damage). [3]

Mode of action: In living cells, it inhibits the incorporation of subunits into the mucopeptide and so causes the accumulation of UPD-N-acetyl-muramic acid peptides in Gram positive organism.

It also affects the synthesis and functioning of the protoplast membrane although most of these effects are not apparent until much later than the immediate inhibition of cell wall synthesis, and so inhibition of synthesis of the membrane is considered to be secondary.

Bacteria lack resistance to vancomycin because the antibiotic inhibits the binding of subunits to acceptor because it inhibits the enzyme responsible for this which is called mucopeptide synthetase. [36] Vancomycin blocks subunit subunit transfer from the carrier lipid to the acceptor in vivo and in vitro. It's produced by Nocardia. [5]

2.Literature Review:

The resistance of *S. aureus* to Methicillin is caused
by the *mecA* gene which codes the low affinity
78-Kda penicillin-binding protein (PBP2a). Betalactam antibiotic normally binds to PBPs in the cell wall, resulting in the disruption of synthesis of the peptidoglycan layer and death of bacterium. Since the beta-lactam antibiotics cannot bind to low affinity PBP2a, synthesis of peptidoglycan layer and cell wall are able to continue. [6]

MRSA infections often require systematic antibiotic therapy. Following the spread of MRSA, glycopeptides (usually Vancomycin and more recently Teicoplanin) have become the mainstay of treatment for MRSA infections. [7]

Vancomycin is the choice of drug for MRSA isolates. Patients unable to tolerate vancomycin have Minocycline. [8]

As Vancomycin is commonly used for the treatment of MRSA infections, which has resulted into development of Vancomycin-Intermediate *S. aureus* (VISA) and Vancomycin-Resistant *S. aureus* (VRSA).

Cultures, Gram staining and other biochemical tests were performed for conventional identification. Modified Kirbry-Bauer disk diffusion method was applied and DNA was extracted from MRSA and VRSA isolates and PCR then performed for amplification of mecA, VanA and VanB genes. The results confirmed the existence of Staph. aureus in 49/426 (11.5%) cases among which MRSA were isolated from 34/49 (69.4%) when modified Kirbry-Bauer disk diffusion method was applied. Ten out of these 34 MRSA were confirmed as VRSA by cultures on BHI agar containing 6µg/ml vancomycin according to NCCLS criteria. PCR revealed that out of the 34 MRSA isolates, 26 were mecA positive (76.5%) while 8 (23.5%) were arcC positive. No vanA or VanB genes were detected. Molecular method confirmed the results for MRSA through the presence of either arcC or mecA genes while it failed to approve the occurrence of VRSA since neither VanA or VanB genes were detected. Thus, VRSA may be attributed to other factors. [9]

In March 2015 study in Shendi City for association between *Staphylococcus aureus* with different infections ranging. The study target 123 Staphylococci isolates were collected from patients attending various hospitals and medical centers at Shendi City, Northern Sudan. Clinical samples which included wound swabs, urine, nasal secretions and ear swabs identified primarily

by routine laboratory procedures which included microscopic morphology and biochemical tests. The antibiotic-resistance profile was determined by the disc agar diffusion (DAD) technique use vancomycin with 30 μg (in Mueller-Hinton agar). DNA was extracted from pure *S. aureus* culture using the standard method of phenol chloroform. This study demonstrates that only vanB can be used as diagnostic tool for VRSA strains. This finding has important implications for the management and controlling outbreak and emerges of VRSA in Shendi community. On the basis of this finding, attention should also be given when using conventional disk diffusion method when evaluating resistant *S. aureus* isolates. [10]

In 2019 there is study for determine the prevalence of antibiotic resistance and the prevalent bacterial isolates in hospitalized patients in Khartoum hospitals. Two hundred and twenty-eight isolates were selected by a systematic random stratified method from hospitals' microbiology laboratories. Isolates were recovered from different clinical samples, collected from hospitalized patients with clinical symptoms and signs of infection. Eight bacterial species were isolated: *Staphylococcus aureus, Enterococcus faecalis, Streptococcus spp., Klebsiella pneumoniae, Pseudomonas spp., Escherichia coli, Proteus spp., and Acinetobacter spp.* The result of the study shows Methicillin-resistant *S. aureus* was the most prevalent organism. Gram negative isolates showed good susceptibilities to aminoglycosides and ciprofloxacin. There were high resistance rates to cefuroxime, ceftazidime, and meropenem. Five Vancomycin-resistant *S. aureus* were identified. [11]

3.Rationale:

The infection caused by methicillin -resistant *S. aureus* is global threat to public health. [38]

Vancomycin remains one of the first line drugs for the treatment of MRSA infections. However, S. aureus isolates with complete resistance to vancomycin have emerged in recent years Vancomycin resistant *S. aureus* (VRSA) is mediated by van gene cluster which is transfer from vancomycin -resistant enterococcus. Since the first VRSA isolate was recovered from Michigan, USA in 2002, 52 VRSA strain have been isolated worldwide. In this study the aim is to identify the emergence of vancomycin resistant S. aureus (VRSA) isolates from patients attending will decide the vancomycin can be used as drug of choice for *S. aureus* infection. [39]

4. Objectives:

4.1. General objective:

To determine the prevalence of Vancomycin resistance *staphylococcus aureus* (VRSA) in patients with different clinical manifestations in Khartoum state.

4.2. Specific objectives:

1-To Isolate *Staphylococcus aureus* from various clinical specimens.

2-To determine the susceptibility of *Staphylococcus aureus* to vancomycin by using disc diffusion method.

3-To determine the percentage of vancomycin resistance among *Staphylococcus aureus* isolates.

CHAPTER TWO

MATERIALS AND METHODS

2.1.1. Study Design:

Cross-sectional study design, carried out in Khartoum in time between October to December 2020.

2.1.2. Study Area:

Khartoum state (Military Hospital, Al-Amal Hospital and Napata college students).

2.1.3. Study Population:

S. aureus isolated samples and nasal swabs from *S. aureus* carriers.

2.1.4. Inclusion Criteria:

Isolated *S. aureus* samples from Military Hospital, Al-Amal Hospital samples and

S. aureus isolated from nasal swab *S. aureus* carriers.

2.1.5. Exclusion Criteria:

All *S. aureus* carrier with antibiotic administration.

2.1.6. Sampling Technique and Sample Size:

Non-probability purposeful sample technique.

2.1.7. Ethical approval:

All samples will be taken from authorized hospital and students for using this samples for research purposes. Ethical approval for this study obtained from the faculty of Medical Laboratory Science, Napata College.

2.1.8. Sample procedure:

Clinical isolates a total of 150 *Staphylococci* isolates were collected from patients attending various hospitals and medical centers at Khartoum City after obtaining their informed consent. Clinical samples which included wound swabs, urine and nasal secretions were collected from October 2020 to December 2020. Swabs samples were added in sterile tubes of Brain Heart Infusion Broth while urine samples were inoculated Blood Agars and then all primary cultures were subculture on Mannitol Salt Agar, and identified primarily by routine laboratory procedures which included microscopic morphology and biochemical tests including β-hemolysis on blood agar, 3%, catalase, coagulase and DNase. [6]

The samples were inoculated onto blood overnight aerobically at 37°C. CLED agar was also used for urine samples. All isolates were identified as S. aureus based on morphology, positive catalase, positive coagulase and fermentation mannitol. [7]

Antibiotic Susceptibility Test:

Antibiotic susceptibility test was done for all isolates of *Staphylococcus aureus* using Kirbry-Bauer disk diffusion method. Discs of Vancomycin (VA) 5 µg were used. [7]

2.2 Materials:

2.2.1 Gram staining technique Required: Crystal violet stain Reagent – Lugol's iodine Reagent– Acetone–alcohol decolorize Reagent – Neutral red, 1 g/l (0.1% w/v) c Reagent.

2.2.2. Culture:

Mannitol salt agar, Müeller Hinton agar, Nutrient agar, Nutrient broth and DNAase Agar media were prepared in laboratory depending on the manufacturers' instruction.

Gram technique:

The Gram staining reaction is used to help identify pathogens in specimens and cultures by their Gram reaction (Gram positive or Gram negative) and morphology. Pus cells can also be identified in Gram smears.

Gram positive bacteria: Stain dark purple with crystal violet (or methyl violet) and are not decolorized by acetone or ethanol.

Gram negative bacteria: Stain red because after being stained with crystal violet (or methyl violet) they are decolorized by acetone or ethanol and take up the red counterstain.

2.2.3 Biochemical:

2.2.3.1. Catalase test:

This test is used to differentiate those bacteria that produce the enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as streptococci.

Principle: Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Required: Hydrogen peroxide, 3% H2O2 (10 volume solution).

Method: Pour 2–3 ml of the hydrogen peroxide solution into a test tube. Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution. Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any

of the blood agar is removed with the organism, a false positive reaction may occur. Look for immediate bubbling. [4]

2.2.3.2. Coagulase test:

This test is used to identify *S. aureus* which produces the enzyme coagulase.

Principle: Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus:* Free coagulase which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test. Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase ereacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test.

A tube test must always be performed when the result of a slide test is not clear, or when the slide test is negative and *Staphylococcus* has been isolated from a serious infection. A tube test may be required to detect some MRSA (methicillin resistant *S. aureus*) strains although some commercially available latex test kits to differentiate coagulase positive and coagulase negative *staphylococci*, overcome this. Before performing a coagulase test, examine a Gram stained smear to confirm that the organism is a Gram positive coccus.

Required: EDTA anticoagulated human plasma (preferably pooled and previously HIV and hepatitis tested) or rabbit plasma. The plasma should be allowed to warm to room temperature before being used.

Slide test method (detects bound coagulase).

2.3. Method:

Smear was fixed by heat, genteelly 3 times pass through the flame, and then covered by crystal violet stain for 30-60 seconds.

Rapidly wash of the stain with clean water, then cover with iodine for 30-60 seconds.

Wash of the iodine with clean water.

Rapidly decolorized (few seconds) with acetone-alcohol, then immediately with clean water.

The smear covered with neutral red stain for 2 minutes, and wash of the stain with clean water.

The back was wipe of the slide clean, and place it in a draining rack to air-dry.

The smear was examined microscopically, first with 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cell. [4]

2.3.1. Inoculation of media in petri dishes:

The technique used to inoculate media in petri dishes (plates) should provide single colonies for identification. It must also show whether a culture is pure or mixed, i.e. consisting of a single type of organism or several different organisms. A pathogen isolated in pure culture before it can be identified and tested for antimicrobial sensitivity. The inoculation of media in petri dishes is referred to as 'plating out' or 'looping out'. It is not necessary to use whole plates of media for every specimen. Considerable savings can be made by using a half or even a third of a plate (especially when the medium is a selective one). The area of medium used must be sufficient to give separate colonies. Before inoculating a plate of culture medium, the surface of the medium must be dried, otherwise single colonies will not be formed. To do this, remove the lid of the plate and place this face upwards on an incubator shelf. Invert the base containing the medium and let it rest at an angle on the lid. Usually 30–40 minutes incubation at 35–37°C is sufficient time to dry the surface of an agar plate.

Inoculating technique

Using a sterile loop or swab of the specimen, apply the inoculum to a small area of the plate (the 'well') then spread by using a second sterile loop by streaking method to obtain single colony.

2.3.2. Mannitol salt agar:

A useful selective medium used to screen for nasal carriers. Mannitol Salt Agar contains beef extract and proteose peptone, which makes it very nutritious as they provide essential growth factors and trace nutrients such as nitrogen, vitamins, minerals and amino acids essential for growth. The medium contains 7.5% concentration of sodium chloride which results in the partial or complete inhibition of bacterial organisms other than staphylococci.

Principle: *Staphylococcus aureus* grows on this medium and ferments mannitol to produce yellow colonies. The color of the colonies and the medium is due to the reactivity of phenol red (indicator) to the pH of the medium.

Method of inoculation:

By using wire loop under aseptic technique make streak on plate and incubate overnight aerobically to detect fermentation of mannitol salt. [10]

2.3.3. DNAse test:

Deoxyribonuclease (DNase) Test Definition Deoxyribonuclease (DNase) Test is a biochemical test performed to differentiate organisms on the basis of their ability to produce the DNase enzyme.

Principle:

- DNases are enzymes that hydrolyze DNA and release free nucleotides and phosphate.
- The deoxyribonuclease enzyme produced by bacteria is extracellular endonucleases that break down DNA, yielding a high concentration of oligonucleotides.
- The media used to detect these enzymes can be made by using various indicators (toluidine blue or methyl green) or no indicators to detect the hydrolysis of DNA.
- The first method is performed with no indicator. The hydrolysis of DNA is indicated by the clearing of the agar after the addition of HCl (the oligonucleotides dissolve in acid causing a clear zone, but DNA salts are insoluble). [10]

CHAPTER THREE

RESULTS

3. Results:

A total of 150 participant were collected from October to December 2020. In Khartoum City after obtaining their informed consent.

All data were collected in master sheet paper. Analyzed by Microsoft office excel statistical analysis using statistical professional for social science (SPSS) show in bellow tables and figures

Of the 100 participants were 77 males and 33 were females, ages ranged from (19_ 37) with Mean age± 24 years.

Isolated clinical samples which included (11) wound swabs, (4) urine and (2) semen samples.

	AlAmal Hospital	Military Hospital	Napata College Student	Total
Wound	4	7	0	11
Urine	1	3	0	4
Semen	2	0	0	2
Nasal Swab	0	0	83	83
Total				100

Table (1) show Number of isolated clinical specimens

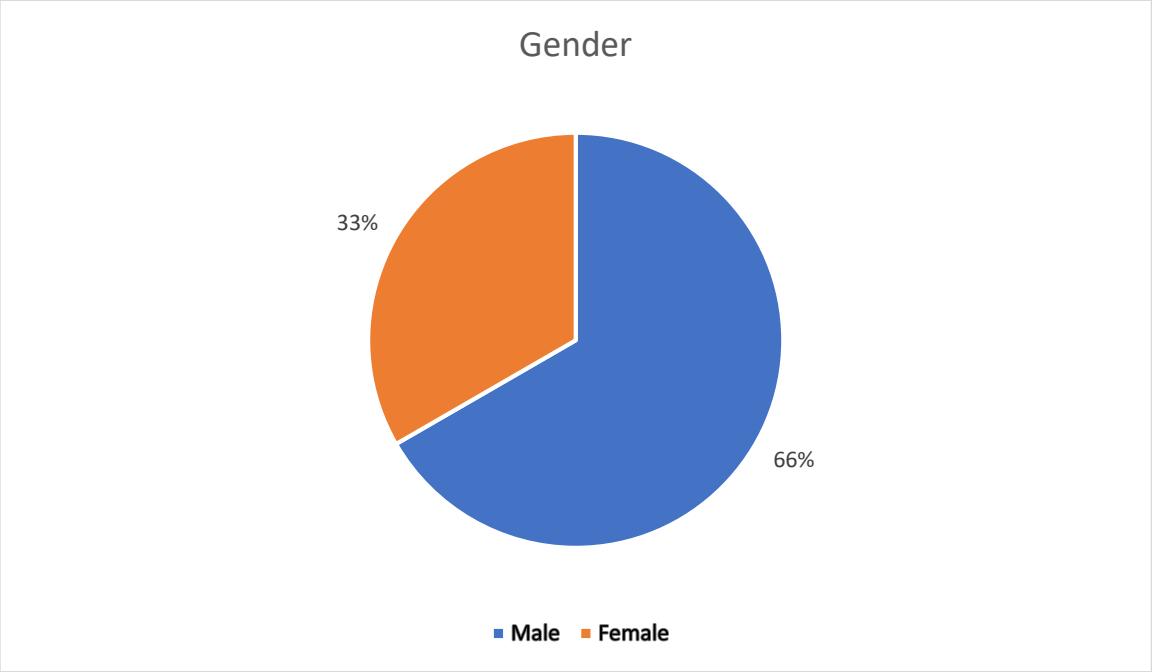

Figure (1) shows: demographic percentage of gender.

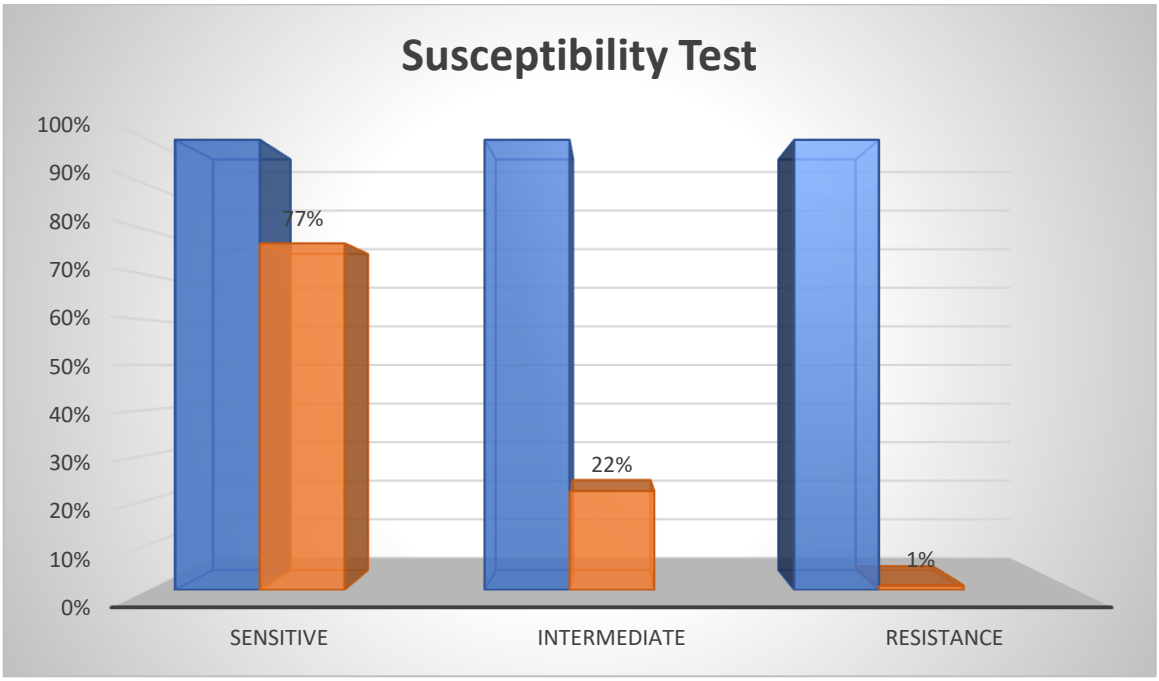

Figure (2) shows: Susceptibility test result.

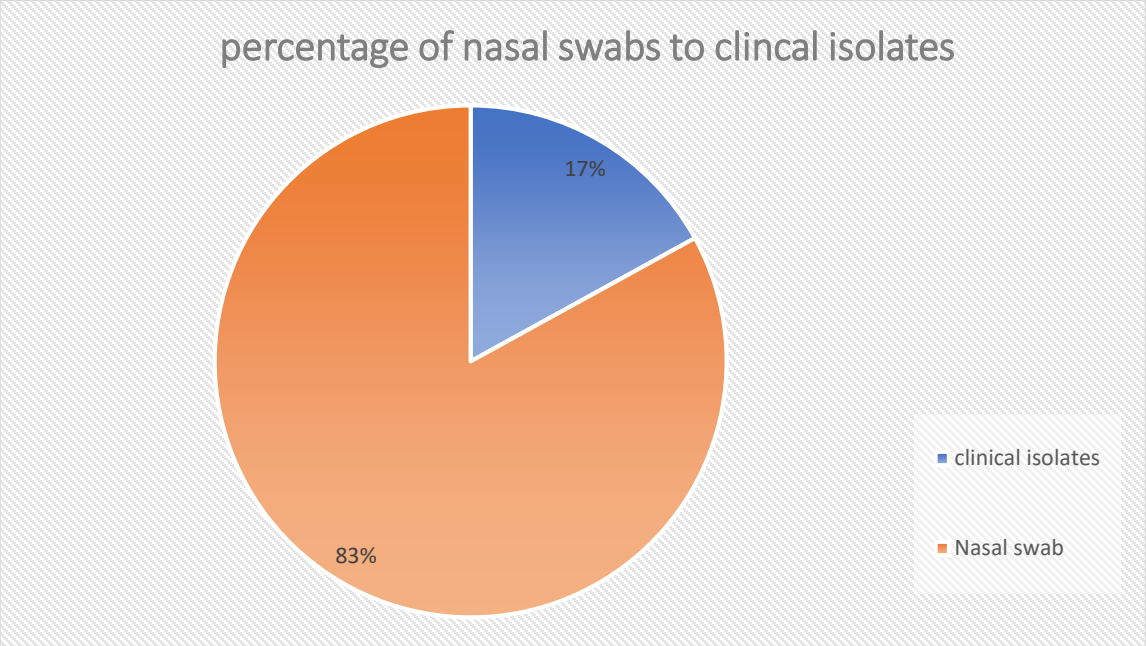

Figure (3) shows: percentage of nasal swabs to clinical isolates.

CHAPTER FOUR

DISCUSSION

4.1. Discussion:

S. aureus has remained a versatile and potent pathogen in humans, since it is one of the most common causes of nosocomial and community acquired infections. [41]

S. aureus is a major cause of infectious morbidity and mortality around the world, causing a wide variety of clinical manifestations ranging from localized infection to toxin mediated diseases and invasive blood stream infections. [42]

In this study, the rate of VRSA is higher when compare to study done by Marwa Mohamed Osman *et a*l " three intermediate resistant isolate MIC between 4-8 µg/ml". The percentage of the VISA strains was 12.0%. The result of this study demonstrates that the unprevalence of resistance to vancomycin may be related to the low usage of this antibiotic in this study area. The majority MRSA isolates were multidrug resistant (MDR) to different classes of antibiotics, so Vancomycin might be used as a drug of choice for the treatment of MRSA infections. [43]

This study was carried out to investigate the prevalence of Vancomycin-Resistant *Staphylococcus aureus* and antibiotic sensitivity pattern in clinical isolates. This study was conducted in October 2020, a total of 150 subject with variable *S.aureus* infections and nasal carriers, all sample randomly selected demographic data was recorded from patients including antibiotics administration and gender. *S.aureus* was to be 77% sensitive and 22% show intermediate sensitivity and 1% show resistant to vancomycin.

Result of study showed significance association for vancomycin sensitivity to *S.aureus* 77 of total 100 patients.The isolates was sensitive to Vancomycin which was examined by vancomycin when compare to other study done by Nada Elsayed which include 200 *s.aureus* isolates 80(40%) from clinical samples and 120 (60%) from nasal carriage samples, which show The prevalence of VRSA was only detect in clinical samples (13.8%).[44]

The findings of this study agree with some previous study which demonstrate the susceptibility of *S.aureus* to Vancomycin antibiotic so Vancomycin might be used as a drug of choice for the treatment of MRSA infection.

4.2. Conclusion:

The conclusion of this study is 1% of *S.aureus* was resist to Vancomycin.

4.3. Recommendations:

1- Vancomycin is drug of choice and must be used by antimicrobial policies (avoid overdose and misuse).

2- Other studies should be conducted including large sample size.

3- Further studies should be done on molecular level, Polymerase Chain Reaction (PCR) and sequencing.

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Appendix

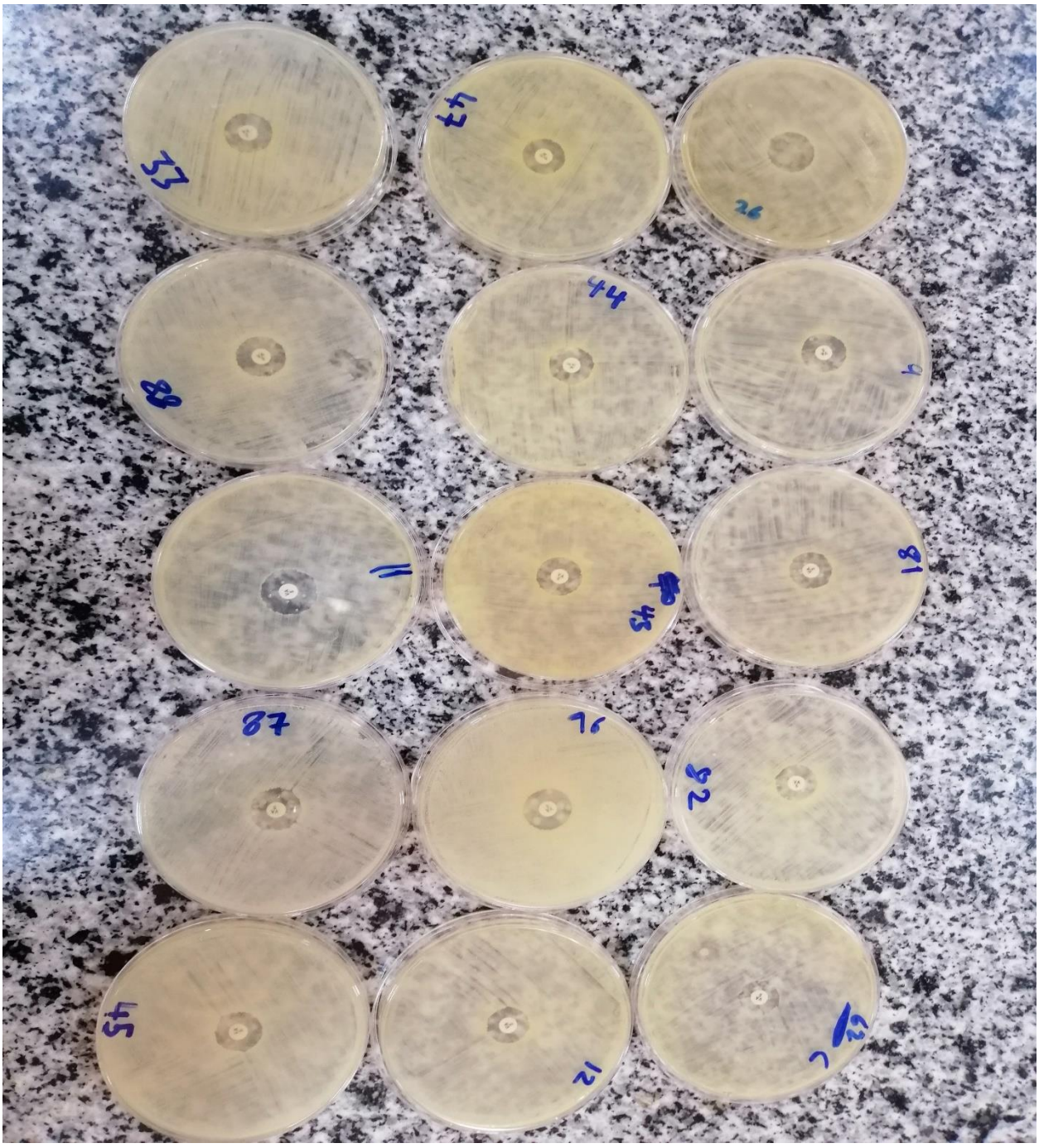

Figure 1: antimicrobial susceptibility

Figure 2: DNAse Test.

Figure 3: Mannitol Salt Agar

Questionnaire

Program: Medical laboratory science **Department: Microbiology**
Semester: 8 **Batch: 1** **Academic Year: 2020-2021**
Targeted degree:

MBBS ☐ B. Pharm. ☐ B.Sc. ☑

Age: ……

Gender: Male ☐ Female ☐

Antibiotic Administration: Yes ☐ No ☐

Sample: Clinical spacemen (From Hospitals) ☐

Nasal Swab (Napata Students) ☐

Informed Consent

Program: Medical laboratory science **Department: Microbiology**
Semester: 8 **Batch: 1** **Academic Year: 2020-2021**
Targeted degree:

MBBS ☐ B. Pharm. ☐ B.Sc. ☑

إقرار موافقة للمشاركة في بحث

نجري بحث لدراسة بعنوان الكشف عن وجود سلالة المكورات العنقودية الذهبية المقاومة لعلاج الفانكوميسين في ولاية الخرطوم وقد تم اختياركم في البحث لآخذ المعلومات والعينات اللازمة لغرض البحث.
أنت مدعو(ة) للمشاركة فى هذا البحث :

- يتم الكشف داخل المعمل باستخدام الطرق المعملية المتخصصة.

- لا توجد اى اثأر جانبيه من الاشتراك في هذا البحث.

- هذا البحث يساعد فى أهمية وجود سلالات بكتريا المقاومة لعلاج الفانكوميسين.

في حال الموافقة على المشاركة في هذه الدراسة، نتعهد بسرية هذه المعلومات وألا يتم استخدامها الا في العلم وسيبقى أسمك سري جدا.

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. وبناءاً عليه فأنني أوافق على الإشتراك فيه. كما أعرف تمام المعرفة بأنني حر في الإنسحاب من هذا البحث متى شئت حتى بعد التوقيع على الموافقة دون أن يؤثر ذلك على العناية الطبية المقدمة لي.

_____ _____

توقيع المشترك إسم المشترك