Isolation and Identification of Bacteria causing Post-operative Surgical Wound Infection and Their Susceptibility to common Antimicrobial use in Patients attending Wad Medani Teaching Hospital (2014-2015)

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Department of Microbiology Faculty of Medical Laboratory Sciences University of Gezira

August 2015
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(2014-2015)

By:

Rehab Ibrahim Ali Babekir

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<td>External Examine</td>
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August 2015
Dedication

To the soul of my grandfather ......................

To my husband Amar who help and support me with their love

To my mother and father who overwhelmed me with his kindness and patience ..................

To my sisters, brother and uncles who help me with their love ........................................

To my teachers and friends who support me with their confidence ......................................

I
Acknowledgment

First and foremost, great thank to Allah, for giving me the strength and patience to carry out this work.

My thanks and gratitude to my supervisor Dr. Badawi Talha for his continuous help, support and assistance.

I would like to thank my co supervisor Dr. Bakri Yosif Mohamed Nour

My great thank to my teacher Dr. Badr aldeen for helping.

Special thanks to staff of microbiology department
عزل وتعريف البكتيريا المسببة لإلتهاب الجروح بعد العمليات واختبار حساسيتها للمضادات الحيوية الشائعة لدى المرضى المتوردين على مستشفى ود مدني التعليمي (2014-2015)

ملخص الدراسة

أجريت هذه الدراسة في مستشفى ود مدني التعليمي بولاية الجزيرة في الفترة من يوليو 2014 الي يونيو 2015 وشهدت الدراسة لعزل البكتيريا الهوائية التي تسبب التهاب الجروح بعد العمليات واختبار حساسية هذة البكتيريا للمضادات الحيوية. تم جمع 65 عينة وتم عزل البكتيريا باستخدام طريقة التنزير ووجد أن أكثر المسببات شيوعا البكتيريا العنقودية الذهبية 39% (Staphylococcus aureus) والكلبسيلا نموني18% (Klebsiella pneumoniae) والسيدوموناس ايرفينوزا 13% (Pseudomonas aeruginosa) , البكتيريا السرطانية 5% (Streptococcus pyogenes) , البلعوما 2% (P. mirabilis) ووجدت الدراسة أن معظم البكتيريا المعزولة حساسة للجريانايماسين والسبروفوكاسين ومعظم البكتيريا المعزولة مقاومة للإيثروماسين والسبروفوكاسين.

ووجدت الدراسة أن معظم المسببات وهى البكتيريا العنقودية الذهبية والكلبسيلا والسودوموناس ايرفينوزا تتصف بأنها ذات مقاومة عالية للمضادات الحيوية المستخدمة. وأظهرت الدراسة أن المرضى الأكثر عرضة للإصابة هم مرضى السكرى و أصحاب الأوزان الزائدة و الذين أعمارهم (21-30) من النساء.

IV
Isolation and Identification of Bacteria causing Post-operative Surgical Wound Infection and Their Susceptibility to common Antimicrobial use in Patients attending Wad Medani Teaching Hospital (2014-2015)

Abstract

This study was carried out in Wad Medani Teaching Hospital, Gazeera State from July 2014 to June 2015. The study aimed to isolate and identify aerobic bacteria that cause Post-operative surgical wound infections and to assess their susceptibility to common antibacterial used. Sixty-five wound swab were collected from Wad Medani Teaching Hospital. Bacteria isolation was done by using cultural techniques. The most common causative agents were: *Staphylococcus aureus* (39%) followed by *Klebsiella pneumoniae* (18%), *Pseudomonas aeruginosa* (18%) *E.coli* (13%), *Streptococcus pyogenes* (5%), *Enterococcus faecalis* (5%) and *proteus mirabilis* (2%). Most bacteria isolated were sensitive to Gentamicin and Ciprofloxacin while most were resistant to Erythromycin and Cephalexin. The study revealed that the common causative agents *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were highly resistant to the commonly used antibacterial agents. The high-risk group are obesity, dipetic patient Age group (21-30) and female gende
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<td>Center of Disease Control</td>
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<td>NNIS</td>
<td>National Nosocomial Infection Surveillance</td>
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1. Introduction

1.1. General Introduction:
Wound infection means contamination of wound by microorganisms and this occurs when virulence factors expressed by one or more microorganism in wound overcome the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provoke serious local and systemic host responses (1).

Definition of postoperative wound infection:
Post-operative wound infection means wound infection after surgical operation. Infection may occur after accidentally or intentional trauma of the skin or other tissue. It has been shown that surgical wound infections may occur shortly after surgery or several days (may be month) postoperatively, and that the site of infection may be limited to suture line or may extend into the operation site (2)

Post-operative wound infection occurs within 30 days of the operative procedure and involves only the skin and subcutaneous tissue of the incision and at least one of the following:-

-purulent drainage from the superficial incision

-organisms isolated from aseptically obtained culture of fluid or tissue from the superficial incision.

-the surgeon or physician declares the wound infection

-at least one of the following sign or symptoms of infection:

Pain or tenderness, redness, localized swelling (3).
Surgical wound infection is a common post-operative complication and causes significant post-operative morbidity and mortality. Any purulent discharge from a closed surgical incision, together with signs of inflammation of the surrounding tissue should be considered as post operation surgical wound infection. Although the total elimination of wound infection is not possible, must reduce the infection rate to minimal level. The rate of post-operative wound infection vary from one hospital to another (4).

At 1992, the US centres for disease control (CDC) revised its definition of wound infection creating the definition of surgical site infection (SSI) to prevent confusion between the infection of surgical incision and the infection of traumatic wound (5).

Infection of surgical wound by bacteria is either:

- Endogenous infection by normal flora in the skin of patient or

- Exogenous infection by bacteria that contaminate operation room, surgical instruments, patient clothes and bacteria from operation staff (3).

The control of surgical site infection requires knowledge of the source and transmission of the causative organisms. In this days operating environment more than half of clean surgical site, infectious pathogens originate from normal skin flora of patient or staff (3). In the operation theatre, the sources of microorganisms may be the air, equipments and articles coming direct contact with the patients (6).

Bacteria on skin squamous, lint and by turbulent air currents deposit on surfaces they are spread by direct contact between carrier and wound,
but the importance of airborne bacteria as a source of infection remains a subject of debate among professionals in infection control(7).

1.2 Problem identification and Justification

The incidence of post-operative wound infection is becoming more serious because of low quality antiseptic and medical solution for treatments, difficulties to prepare definition of the responsibility among the hospital staff.

Review of medical literature indicated that microbial contamination in hospital and the Resistance of nosocomial infection organism to most available drugs has become a major problem.

The complications of surgical wound infection cause considerable morbidity. It also increase healthcare costs by delaying discharge from hospital and increasing the need for investigation, treatment and nursing care(Surgical site are the second to third most common site of health care).

Therefore, periodic detection of organism’s level and elimination is crucial to prevention of infections acquired from hospital.
1.3 Objectives:

2.2.1 General objective:

To study the aerobic bacteria that cause post-operation wound infection in Wad Madani Teaching Hospital 2015.

2.2.2 Special Objectives:

-To isolate and identify the common bacteria that cause post-operation wound infection.

-To determine antimicrobial susceptibility to isolate bacteria.
2. Literature Review:

A study was done; during the period January 2003 to December 2004, to identify the microorganisms associated with surgical site infections in patient who underwent class 1 and class 2 surgeries at small urban to rural community hospital. 89 surgical site infections were identified. *Staphylococcus aureus* was the most common pathogen (25.8%), *Enterobacteriaceae* were the second most frequently isolated organism (12.4%), followed by *Streptococci species* (11.2), coagulase negative *Staphylococci* (10.1%), *Enterococci species* (7.95); and *Pseudomonas aeruginosa* (6.7%). (8)

In Nigeria, study was done, during the period from July to September 2004; to determine the prevalence of *Pseudomonas aeruginosa* in post-operative wound infection, the result of this study shows that:

*Pseudomonas aeruginosa* (33.3%), *Staphylococcus aureus* (21.7%), *Klebsiella species* (16.7%), *Escherichia coli* (11.7%), *Proteus species* (6.7%), *Streptococcus pyogenes* (1.7%), atypical coliform (6.7%) and *Enterococcus faecalis* (1.7%) were the causative agent (9).

National nosocomial infection surveillance found the frequency of aerobic bacteria in post-operative wound infection to be *Staphylococcus aureus* (17%), *Enterococci* (13%), *Escherichia coli* (10%), *Pseudomonas aeruginosa* (8%), *Enterbacter species* (8%), *Proteus mirabilis* (4%) and *Klebsiella pneumoniae* (3%) (10).

Center For Disease Control and Prevention (CDC) study pathogens that cause post-operative surgical wound infection and found: *Staphylococcus aureus*(20%), coagulase negative *Staphylococci* (14%)
Klebsiella (12%), Escherichia coli(8%), Pseudomonas aeruginosa (8%), Enterococci species (7%), proteus mirabilis (3%) and Klebsiella pneumoniae(3%), group D Streptococci(2%), Bacteroides fragilis(2%), Candida Albican(3%) and other Streptococci (3%)to be the causative agents (11).

Brook in 1989 analysed 89 specimen from post-surgical operative abdominal wounds and found the predominant pathogens to be Escherichia coli, Bacteroides species and Clostridium species (12).

Analysis of pus from gasterectomy site wound infection in 22 children predominant isolates to be: Staphylococcus aureus, Escherichia coli, Enterococcus, Peptostreptococci and Bacteroides species (13).

Most common bacteria that contaminate operation room in Khartoum were reported to be: Staphylococcus aureus (30.7%), Staphylococcus epidermidis (23.8%), Staphylococcus Saprophyticus (10%), Pseudomonas aeruginosa (14%) Klebsiella pneumoniae (9.6%), Escherichia coli (8.7%), proteus mirabilis (1.6%) and Citrobacter (1.6%) (14)

**Epidemiology:**

Post-operation wound infections are the second most common nosocomial infection. In one survey from Switzerland that excluded asymptomatic bacteruria as a cause of nozocomial infection, surgical site infections (SSIs) were actually the most frequent infection documented .While usually localized to the incision site, surgical wound infections can also extend into adjacent deeper structure;The term surgical wound infection has now been replaced with the more suitable name, surgical site infection (15).
Among surgical patients, SSIs are the most common nosocomial infection, accounting for 38 percent of nosocomial infection. It is estimated that SSIs develop in 2 to 5 percent of the million patients undergoing surgical procedures each year (i.e., one out of every 24 patients who have inpatient surgery in the United State has a postoperative wound infection (SSI).

SSI have been estimated to occur in up to (15 %) of elective surgical patients and approximately (30%) of patients whose surgical procedure was classed as contaminated or dirty (15).

SSIs are associated not only with increased morbidity but also with mortality (77%) of the death of surgical patients were related to surgical wound infection (3).

**Surgical site infections are three types:**

1-Superficial incisional SSI: infection involves only skin and subcutaneous tissue incision.

2-Deep incisional SSI: infection involves deep tissue such as muscle layers. This includes infection involving both superficial and deep tissue incision sites.

3-Organ space SSI: infection involves any part of the anatomy in organs and spaces other than the incision, which are opened during operation (10).
**Risk factors:**

There are many factors that are thought to affect the susceptibility of any wound to infection, some of which strongly predispose to wound infection. These factors include pre-existing illness, length of operation, wound class, and wound contamination. Other factors such as extremes of age, malignancy procedures and long duration of post-operative hospitalization (18).

Decreased host resistance can be due to systematic factors affecting the patient healing response, local wound characteristic, or operative characteristic.

- Systematic factors include age, malnutrition, hypovolemia, obesity, diabetes, steroid, and other immunosuppressant.

- Operative characteristic include: poor surgical technique, length operation (more than 2 h), intra-operative contamination, including infected theatre staff and instruments, pre-operative stay in the hospital and hypothermia.

- Wound characteristic: e.g. foreign material (11).

Duration of surgery is one factor that influences the wound infection rate, Procedure that takes longer than 2 hours are associated with higher infection rates (19).

The risk of SSI can be conceptualized according to the relationship between two essential factors: first dose of bacterial contamination and its virulence, secondly, the resistance of the host, both factors are detecting the risk of surgical site infection (20).
Lack of microbiologically safe environment in the theatre results in delay of recovery and they are associated with SSIs, which lead to increase morbidity and mortality. It also increase health care costs by delaying discharge from hospital and increasing the need for investigation, treatment and nursing care (21).

**Pathophysicsology:**

Wound healing is a continuous complex biological processes at the molecular level. Healing is divided into the following phases: inflammatory phase, proliferative phase, and maturation phase (22).

The inflammatory phase begins when tissue is disrupted by injury; this begins the coagulation cascade to limit bleeding. Platelets are the first of the cellularity components that aggregate to the wound, and, as a result of their degranulation (platelet reaction). These cytokines include platelet-derived growth factor (PDGF), insulin like growth factor-1 (IGF-1), epidermal growth factor (EGF), and fibroblast growth factor (FGF). Serotonin is also released, which, together with histamine induces a reversible opening of the junctions between the endothelial cells, allowing the passage of neutrophils and monocytes (which become macrophages) to the site of injury. This large cellular movement to the injury site is induced by cytokines, inflammatory exudates that contains red blood cells, neutrophils, macrophages, and plasma proteins, including coagulation cascade proteins and fibrin strands, fills the wound in a few hours (22).

The proliferative phase begins as the cells that migrate to the site of injury, such as fibroblasts, epithelial cells, and vascular endothelial cells, start to proliferate and the cellular of the wound increases. The
cytokines involved in epithelial cell and fibroblast proliferation, the marginal basal cells at the edge of the wound migrate across the wound, and, within 48 hours, the entire wound is epithelialized. In the depth of the wound, the number of inflammatory cells decreases with the increase in stromal cells, such as fibroblasts and endothelial cells, which in turn continue to secrete cytokines. Cellular proliferation continues with the formation of extracellular matrix proteins, including collagen and new capillaries (angiogenesis). This process is variable in length and may last several weeks (22).

In the maturation phase, the dominant feature is collagen. The dense bundle of fibers, characteristic of collagen, is the predominant constituent of the scar. Wound contraction occurs to some degree in primary closed wounds but is a pronounced feature in wounds left to close by secondary intention. The wound continuously undergoes remodeling to try to achieve a state similar to that prior to injury. The wound has (70-80%) of its original tensile strength at 3-4 months post-operative (22).

**Causative agents (aerobic bacteria):**

In bacteria capable of causing wound infections, structural feature, enzyme production and metabolic products contribute to virulence and pathogenicity. The possession of capsules; presence of fine surface appendage (Pilli), polysaccharide components of the cell wall, production of extra cellular enzymes, production of extracellular toxins (endotoxins and exotoxins) and expression of genes that code for virulence determinants. Distribution patterns of microorganisms are always subject to a combination of chemical, physical and biological factors and every microbial species has specific demand (2).
**Staphylococcus aureus:**

*Staphylococcus aureus* is Gram positive capsulated bacteria has virulence ability, has toxin and enzyme (haemolysin, exofolitase, DNase, coagulase, staphylokinase, lipase, hyluronidase, betalactemase and leukocidin). These pathogenisity determinant help *Staphylococcus aureus* to be the most common cause of post operative surgical wound infection (23).

**Streptococcus pyogenes:**

Gram positive capsulated bacteria, Pathogenisity determinant are (streptokinase, haemolysin, protease, hyluronidase, erethrogenic toxin). *Streptococcus pyogenes* cause wound infection and cellulitis (24).

**Enterococcus faecalis:**

They are Gram positive cocci, multi drug resistant, one of causes of hospital acquired infection and rare may cause post-operative surgical wound infection (23).

**Escherichia coli (E.coli):**

Gram negative bacilli, capsulated, motile bacteria, *E coli* cause considerable number of post operative surgical wound infection (24).

**Klebsiella pneumoniae:**

*Klebsiella pneumoniae* are Gram negative rods, capsulated, motile bacteria, resist chemical, have beta lactemase and associated with hospital-acquired infection (24).
**Proteus mirabilis:**

Gram negative bacilli, capsulated, motile bacteria and multi drug resistant, *Proteus* bacteria is one cause of Hospital-acquired infection and cause post-operative surgical wound infection but in small percentage (3%) (23).

**Pseudomonas aeruginosa:**

*Pseudomonas aeruginosa* is a Gram negative rod, motile bacteria, has low selective permeability in the cell wall so its multi drug resistant and also can be survive in moist places (e.g. wash pelvic, air condition) and resist detergent e.g. detol, all this character give chance to *Pseudomonas aeruginosa* to cause severe infection of post operative surgical wound (24).

**Laboratory diagnosis:**

The diagnosis of wound infection cannot be made without bacteriological examination of the wound swab.

Specimen: wound swab taken from patients suffering from post-operative wound infection, the specimens must reach the laboratory as soon as possible.

Gram film: to identify causative bacteria according to Gram reaction: Gram negative or Gram positive.

Culture: on media such as Blood agar and Schocolate agar and MacConkey agar.

Biochemical test: to identify type of isolated bacteria
Antimicrobial Susceptibility Test:

1-Dilution method (Minimum inhibitory concentration, Minimum bactericidal concentration).

2-Disc diffusion includes:

-Modified Kirby-Bauer method.

-Stocke method.

3-E test

Reduction of wound infection:

The judicious use of antibiotics and the use of an organized system of wound surveillance are the most effective means to reduce the wound infection rate. Tissue level factors such as micro-environment and the presence of white cells and cellular products are important elements of the local immune response; thus, their manipulation may be useful in planning wound management strategies. Other procedures such as preoperative hair removal, use of adhesive drapes, and wound irrigation are of small benefit only (17).

Treatment:

-Classes of Several different antibacterial agents are available and suitable for treatment of post-operative surgical wound infection. These include:

Cefazolin

Erythromycin

Cefoxitin
Gentamicin

Ciprofloxacin

Clindamycin

-Opening the wound, evacuating pus and clearing the wound (25).

**Prevention:**

1- Prevent contamination of operation room by:
   - All hair must be kept covered and nail must be cut.
   - Staff should enter and leave through identified doors.
   - The number of people in the operation room must be kept at minimum.

2- Complete sterilization of operation room and surgical instruments.

3- Laminar air flow in high risk area.

4- Exclusion of staff with infections.

5- Good preparation of patient and use of certain effective antibiotics before operation (3)
3. Material and Methods

3.1. Study design:

This is prospective descriptive study to identify the post-operation wound infection associated with operative patients in Wad Medani Teaching Hospital. The study was conducted during the period from July 2014 to June 2015.

3.2. Study population:

Patients in Wad Medani Teaching Hospital with post-operative wound infection during the study period.

3.3. Sample size:

Sixty five wound swabs were taken from patients with post-operative surgical wound infection selected by simple random sampling.

3.4. Inclusion criteria:

Sample was collected from site that characterized with an anatomic or mechanical problem after operation. Sample by use swab. All ages, tribes and both gender was included in this study.

3.5. Exclusion criteria:

Every wound, which dose not shows any of these characters was excluded.
3.6. Ethical consideration:

Was taken form the ministary of health authartis, permission form the head director of hospital and written consent from the patints.

3.7. Methods of data collection and analysis:

3.7.1. Methods of data collection:

- A well designed questionnaire including personal information and clinical data.

- Laboratory tests.

3.7.2. Method of data analysis:

Data were analyzed by SPSS

3.8. Sample processing:

On day 1: collection and culture of wound swab:

Wound swabs were taken from wound of patients suffering from postoperative wound infection. Appearance of the specimen was described (macroscopic examination) and report on the colour of pus and whether it contains granules or not was performed. The samples were immediately cultured on enriched culture media including blood agar, chocolate agar and macConkey (distribution on dish using wire loop) and DNA containing media to test for DNase enzymes for gram positive cocci. The media were prepared according to manufactures instruction in 500 ml bottle and sterilized by autoclaving at 121 C for 15 minutes.
The plates were incubated at 37 C° for 18 – 24 hours in an incubator aerobically. The Plates were read in the following day but extended to 48 hours if there is no bacterial growth within 24 hours.

**On day2: Identify the isolate:**

**Colonial morphology:** blood agar was examined for haemolysis, and in MacConkey was examined for colour of lactose fermenting, consistency, size and appearance.

**Gram Stain:**

1-Preparation of smear

- Slide was passed on flame three times to remove fat.

- One drop of normal saline was add on slide

- Sterile wire loop was used to take small portion of growth on the culture media and smear made by dissolving in the drop of normal saline until it became homogenous and allow the smear to dry by air.

- The smear was fixed by passing it on the flame three times.

2-Stain the smear by the Gram stain:

- add 5 % methyl violet to smear for 1 min, wash with water.

- add logals iodine to smear for 2 min, wash with water.

- add 70 % alcohol to smear (decolorizer) within few second wash with water immediately.

- add saffranine (counter stain) to smear for 2 min, wash with water

- allow drying by air
- examine under microscope using 100X (Gram positive violet color while Gram negative red color).

**Biochemical tests:**

**Catalase test:**

This test differentiates *Staphylococci* and *streptococci*. Put 2-3 ml of 3% hydrogen peroxide solution on test tube. Then take several colonies of growth using wooden stick and immerse in hydrogen peroxide solution. Appearance of air bubbles indicates positive result (24) (Picture- 1).

**Coagulase test:**

This test differentiate *Staphylococcus aureus from other spp*. On clean slide divided into two sites (test &control), drop of sterile normal saline was placed into two sites, Then by using a sterile wire loop 2-3 from catalase producing colonies were mixed with normal saline to form smooth milky suspension, then a drop of undiluted human plasma was added to Test site only and the control site remain as negative, mix it with the suspension (rotate). A reaction was considered positive when clumping becomes visible to the naked eye within 10 seconds in test side only while the control side remains as negative control (24).

**DNase test:**

This test was used to differentiate *Staphylococci species*. Inoculate organism under test on DNA media using sterile wire loop making heavy spots. Incubate the plate overnight at 37 C° after that execrate plate from incubator and covered with HCL. Present of clear area surrounding the inoculums of the tested organism indicate positive result (24) (Picture- 2).
**Urease test:**

This test was used to differentiate between Gram negative bacteria by inoculate small portion of growth in urea agar media using sterile loop and incubate overnight at 37°C. The change of the colour to pink indicator positive result (24) (Picture- 3).

**Indole test:**

This test was used to differentiate between Gram negative bacteria, inoculate small portion growth in 2ml peptone water and incubate overnight at 37°C, after the end of incubation period add kovac's reagents presence of pink red ring indicate positive result (24)(picture- 4).

**Citrate utilization test:**

This test was used to differentiate Gram negative bacteria, by inoculate small portion of bacteria growth in citrate media and incubated aerobically at 37°C overnight. Change of color to blue indicates positive result(24) (picture 5).

**Oxidase test:**

This test detects oxidizing enzyme in bacteria, which the catalyze transport of electrons donors in the bacteria done by smeared organism colonies in oxidase disc. If the test is positive, a deep blue color appears due to oxidation of phenylene diamine in the disk (24)( Picture -6).

**Motility testy:**

In this test microorganism was inoculated by using straight wire loop in semi solid media, making single stab down the center of the tube,
and then incubated aerobically at 37°C for overnight. Motile bacteria were giving diffused growth; while the non-motile bacteria were give growths in stab line and not diffused (24) (Picture- 7).

**Kiligler iron agar:**

This test was used to differentiate between Gram negative bacteria by inculcating small portion of bacteria growth in kiligler agar (slop and butte). Appearance of yellow color Lactose fermenting bacteria and air bubble and cracks indicate gas production (black color indicate Hydrogen sulfate production (24) (Picture-8)

**On day 3:**

Reading of the above tests to identify causative agent and do sensitivity testing.
Table A: demonstrate characteristic of gram negative bacteria isolated from post operative wound infection patients

<table>
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<tr>
<th>Isolated organism</th>
<th>Indole</th>
<th>Citrate</th>
<th>Ureas</th>
<th>Slope</th>
<th>Butt</th>
<th>gas</th>
<th>Hs</th>
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<tbody>
<tr>
<td>Escherichia coli</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>Y</td>
<td>Y</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>Y</td>
<td>Y</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>R</td>
<td>Y</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas aerugimosa</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Keys:
+ve = Positive result
-ve = Negative result
Y = Yellow colour
R = Red-pink colour
Hs = Hydrogen sulphide

Table B 1The biochemical characteristic of gram Positive bacteria isolated from post -operative wound infection patients

<table>
<thead>
<tr>
<th>Isolated organism</th>
<th>Catalase</th>
<th>Coagulase</th>
<th>DNase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Keys
+ve = Positive result
-ve = Negative result
Table B2: The biochemical characteristic of gram Positive bacteria isolated from post-operative wound infection patients

<table>
<thead>
<tr>
<th>Isolated organism</th>
<th>Catalase</th>
<th>Bacitracin disc</th>
<th>Sculin test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pyogenes</td>
<td>-ve</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Enterococci</td>
<td>-ve</td>
<td>R</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Keys
+ve = Positive result
-ve = Negative result
S = Sensitive
R = Resistant
Antimicrobial Susceptibility Test:

The modified Kirby-Bauer method of antibiotic susceptibility was used in this study.

- Preparation of Inoculums:

Each culture tested was streaked on to Nutrient agar media. After incubation at 37°C overnight, 4 or 5 colonies were selected using wire loop and transferred to a tube of sterile normal saline. The bacteria suspension was then compared to the turbidity standards.

- Inoculation Procedure:

After adjusting the turbidity of the inoculums, a sterile cotton swab was immersed into suspension, pressed firmly against the inside wall of the tube just above the fluid level, rotated to remove the excess fluid. Swab streaked over the entire surface of the Muller-Hinton agar three times plate rotated approximately 60 degrees, after each application to ensure an even distribution of inoculums. Finally, all around the edge of the agar surface was swabbed. Antimicrobial disks were applied to the plate immediately, not longer than 15 minute after inoculation.

On day 4:

Recording and interpreting results:

After incubation at 37°C for 16-18 hours, the diameter of the zones of complete inhibition was measured with ruler under the surface of the plate without opening the lid. The zones of inhibition were compared with the zone size of interpretative table and recorded as sensitive, intermediate or resistant according to the zone diameter.
Results of reading were interpreted according to the manufacturer’s sheet, which was identical to diameters of given in the most recent NCCLS documents.
4.1. Results

The study was conducted during the period from July 2014 to June 2015 to identify the causative agents of post-operative surgical wound infection in 65 patients in Wad Medani Teaching Hospital using ordinary culture technique.

From the total 65 of specimens, isolation was possible in 45 specimen but no bacterial isolates was obtained from 20 cases, (69%) of specimens were growth after overnight incubation at 37 C° while (31%) of specimens were no growth (figure 4.1).

*Staphylococcus aureus* was the predominant microorganism (39%) followed by *Klebsiella pneumoniae* (18%) *P.aeruginosa* (18%), less frequent isolates were *E.coli* (13%), *Streptococcus pyogenes* (5%), *Enterococcus faecalis* (5%) and *Proteus mirabilis* (2%) (table 4.3).

Percentage of bacteria growth was more in females (60%) than in males (40%) (Figure 4.2)

Most of those who developed post-operative wound infection were within the age groups (21-30 year) (table 4.2).

Grams stain was used for primary identification of pathogens in specimens and cultures by their gram reaction (Gram positive or Gram negative) and morphology (cocci or rods), the result obtained in (figure 4.3) show that 49% were Gram-positive cocci and 51% were Gram negative rods.
Antibacterial susceptibility test was done to each isolated bacteria (Table 4.4) which reveals that *Staphylococcus aureus* isolate were resistant to most of the used antimicrobial except Ceprofloxacin and Gentamicin. *Klebsiella pneumoniae* isolate were only sensitive to Co-Trimoxazole, Ceprofloxacin and Gentamicin while resistant to the other antibacterial disc.

*Pseudomonas aeruginosa* isolated were resistant to most of the used antimicrobials.
Figure 4.1: The bacterial growth after overnight incubation at 37°C
Figure 4.2: The distribution of bacterial growth according to sex
Figure 4.3: The Gram stain of the isolated bacteria

Post-operative wound infection person

- Gram –ve rod
- Gram +ve cocci
Table (4.1): Age and sex distribution of the patient who developed Nosocomial infection.

<table>
<thead>
<tr>
<th>Group of age</th>
<th>Male</th>
<th>Male %</th>
<th>Female</th>
<th>Female %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>5</td>
<td>11%</td>
<td>2</td>
<td>5%</td>
</tr>
<tr>
<td>21-30</td>
<td>7</td>
<td>16%</td>
<td>15</td>
<td>33%</td>
</tr>
<tr>
<td>31-40</td>
<td>3</td>
<td>7%</td>
<td>9</td>
<td>20%</td>
</tr>
<tr>
<td>41-50</td>
<td>1</td>
<td>2%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>51-60</td>
<td>2</td>
<td>4%</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>40%</td>
<td>27</td>
<td>60%</td>
</tr>
</tbody>
</table>
Table 4.2: The isolated bacteria from post-operative wound infection patients

<table>
<thead>
<tr>
<th>Isolated organism</th>
<th>Post operative wound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Isolated</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>18</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>8</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>2</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>2</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
</tr>
</tbody>
</table>
Table 4.3: Antimicrobial Susceptibility Test

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Staphylococcus Aureus</th>
<th>Streptococcus pyogenes</th>
<th>Enterococcus fecalis</th>
<th>Proteus Mirabilis</th>
<th>Klebsiella pneumonia</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>R100</td>
<td>S100</td>
<td>S100</td>
<td>R100</td>
<td>R100</td>
<td>S100</td>
<td>R100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Ss90</td>
<td>R100</td>
<td>S100</td>
<td>S100</td>
<td>R100</td>
<td>Rr84</td>
<td>S16</td>
</tr>
<tr>
<td>Co-Trimoxazole</td>
<td>R73</td>
<td>S100</td>
<td>R100</td>
<td>S100</td>
<td>S100</td>
<td>Ss64</td>
<td>R36</td>
</tr>
<tr>
<td></td>
<td>i10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>s17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>S100</td>
<td>R100</td>
<td>S100</td>
<td>S100</td>
<td>Ss54</td>
<td>Ss78</td>
<td>R22</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S100</td>
<td>R100</td>
<td>S100</td>
<td>S100</td>
<td>Ss90</td>
<td>S100</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>R100</td>
<td>R100</td>
<td>R100</td>
<td>Rr74</td>
<td>S26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>R100</td>
<td>S100</td>
<td>R100</td>
<td>Rr54</td>
<td>i19</td>
<td>s27</td>
<td></td>
</tr>
<tr>
<td>Cephalexin</td>
<td>Rr85</td>
<td>R100</td>
<td>Rr70</td>
<td>Rr84</td>
<td>S30</td>
<td>s16</td>
<td>R100</td>
</tr>
<tr>
<td></td>
<td>S15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Keys:

S = Sensitive.  I = Intermediate.  R = Resistance
Figer (1): Represent antibacterial susceptibility of Staphylococcus aureus

Figer (2): Represent antibacterial susceptibility of Streptococcus pyogenes
Figer (3): Represent antibacterial susceptibility of Enterococcus faecalis

Figer (4): Represent antibacterial susceptibility of Proteus mirabilis
Chart (5): Represent antibacterial susceptibility of Klebsiella pneumoniae

Figer (6): Represent antibacterial susceptibility of Escherichia coli
Figer (7): Represent antibacterial susceptibility of Pseudomonas aeruginosa
5.1. Discussion

Surgical wound infections are common and consume considerable portion of health care finances.

A thought surgical wound infection can not be completely eliminated; a reduction of infection rate to a minimal level could have significant benefits, by reducing post-operative morbidity and mortality.

In this study the isolation of bacteria was done by using ordinary culture technique. The study results showed that *Staphylococcus aureus* was (39%), *Klebsiella pneumoniae* (18%), *Pseudomonas aeruginosa* (18%), *E.coli* (13%), *Streptococcus pyogenes* (5%), *Enterococcus faecalis* (5%) and *Protoes mirabilis* (2%) were the aerobic bacteria cause post operative wound infection.

Showing an increase of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as most commonest causative agents of post-operative surgical wound infections. This finding agrees with results obtained by Center for Disease Control and Prevention (1990-1996) in which it was found that *Staphylococcus aureus* (20%), *Klebsiella* species (12%). *Pseudomonas aeruginosa* (10%), *E.coli* (8%), and *Enterococci species* (7%) this may due to similar environmental factor, geographical distribution of bacteria and similar antibacterial use for prophylaxis.

This study found the most common organism that causes post-operative surgical wound infection is *Staphylococcus aureus* and this result agree with result obtained by National nosocomial infection surveillance which found *Staphylococcus aureus* to be the most commonest causative agents of post-operative surgical wound infection.
(17%), followed by Enterococci (13%), E.coli (10%), P.aeruginosa (8%), Enterobacter Spp (8%), Proteus merabilis (4%), Klebsiella pneumoniae (3%) and candida species (2%). (10)

This study showed increase rate of incidence of Staphylococcus aureus in post-operative surgical wound infection due to more geographical distribution of Staphylococcus aureus and more adapted to hospital than others, relaxation of general hygienic measure, mass production of low quality antiseptic and medical solution, Staphylococcus aureus is multi drug resistant and may resist detergents and antiseptic used in hospital. Also Staphylococcus aureus had produce beta lactemase(help to resist antibacterial agents),other enzymes ,toxins and capsule play role in it virulence and pathogenicity and over come from phagocytosis. (10)

The second common causative agent was Klebsiella pneumoniae which had beta lactmase ,acquired gene of resistant to disinfectant and antiseptic and capable survive in moisture place, surgical division and operation room.

This study found considerable percentage of Pseudomonas aeruginosa (18%) because excessive use of antibacterial agents can lead to eliminate normal flora and provided non competitive environment of Pseudomonas aeruginosa also its multi drugs resistant and resist to antiseptic and detergents used.

This study agree with study done in Med west during the period January 2003 to December 2004 in which also found Staphylococci was most causative agent of post operative surgical wound infection because Staphylococcus aureus highly adapted to hospital and due to similar condition ,treatment, antiseptic used and personal hygen (11)
Our study disagrees with result reported by (Opara AA 2004) which show that most causative agents distribution were *Pseudomonas aeruginosa* (33.3 %), *Staphylococcus aureus* (21.7%), *Klebsiella species* (16.7%), *E.coli* (11.7), *Proteus species* (6.7%) *Streptococcus pyogenes* (1.7%), *Enterococcus faecalis* (1.7%) and A typical *coliform* (6.7%). This may due to different geographical distribution of bacteria, different environmental factors, different antibiotic used as prophylaxis; different quality of antiseptic, different hygienic measures and (Opara A A 2014) study report that found *Pseudomonas aeruginosa* carries in hands of nurses working in words of infected patients.

This study results agree with results of study reported by (Ayman A M 2007) in Khartoum State which isolate bacteria that contaminate operation room and found the most common bacteria were *Staphylococcus aureus* (36.1%), followed by *Staphylococcus epidermidis* (23%), *Pseudomonas aeruginosa* (14%), *Staphylococcus saprophyticus* (10%), *Ecoli* (8.7%), *Klebsiella pneumoniae* (3.8%), *Citobacter* (1.6%), *Proteus mirabilis* (2%), this study found that the most common bacteria contaminated operation room is *Staphylococcus aureus* and there is considerable number of *P.aeruginosa*

In this study the antibacterial susceptibility test explain that *Staphylococcus aureus* have Betalactemase which break down beta lactam ring of antibacterial so resist to Amoxycilin, Co-Trimoxazole, Erythromycin ,Chloramphenicol and Cefalexin while it sensitive to Gentamicin ,Tetracycline and Ciprofloxacain.
Streptococcus pyogenes was sensitive to most antibacterial used (Amoxycillin, Tetracycline, Co-Trimoxazole and Chloramphenicol while resist only to Erythromycin, Ciprofloxacin and Cefalexin.

Enterococcus faecalis was sensitive only to Amoxycillin, Tetracycline and resist to other antibacterial discs used because it also has betalactemase.

P. aeruginosa have low selective permeability in the cell wall which prevent entrance of antibacterial agents lead to highly resistant organisms there for only sensitive to Gentamicin and Ciprofloxacin.

Klebsiella pneumoniae was found to be sensitive to Co-Trimoxazole, Gentamicin and Ciprofloxacin and resistant to Amoxycillin, Tetracycline, Erythromycin, Chloramphenicol and Cefalexin because acquired gen of resistant and have betalactemase.

E.coli sensitive to most antibacterial used (Amoxycillin, and Co-Trimoxazole, Gentamycin and Ciprofloxacin) and resist only to Tetracyclin and Cefalexin.

Proteus mirabilis sensitive to Tetracyclin, Co-Trimoxazole, Gentamycin and Ciprofloxacin) while resist to Amoxycillin, Tetracyclin, Chloramphenicol and Erythromycin.

This study agree with study conducted by (Coron J 1999) in which use Erythromycin, Gentamycin and Ciprofloxacin, for treatment of post-operative wound infection but is different from above study in the sense that all isolated bacteria are resistant to Erythromycin.

High risk age group is (21-30) years old. Fat women in obstetric department suffer more from post-operative wound infection than slim ones.
Conclusion

The commonest causative agents for post-operative wound infection in Wad Madania Teaching Hospital were *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* which showed resistance to common antibacterial agents, however most bacterial isolates show high sensitivity to ciprofloxacin and Gentamicin.

Gram negative-bacteria showed high susceptibility to antimicrobial agents compared to Gram positive bacteria.
Recommendation

1- More emphasis should put on sterilization room and all materials used.

2- Use of Gentamicin and ciprofloxacin as prophylaxis.

3- Further studies should be conducted to isolate anaerobic bacteria that may cause post-operative wound infection.

4- More emphasis to cleaning the wound of high risk group and must take strong antibacterial agents.
Reference


8-CarolA cantlon , William R, Michall A. Hoffman and salah s. Qutai shat. significant pathogens isolated from SSIs. at community hospitals, October 2006. Volume 34. Number 8.


Appendix

A-Equipments:

1- Incubator.
2- Microscope.
3- Hot air oven.
4- Refrigerator.
5- Dryer.
6- Sensitive balance.
7- Autoclave.
8- Water path.

B-Reagents:

1- 3% Hydrogen peroxide
2- Hcl
3- Normal saline
4- Crystal violet:

Preparation:

Crystal violet 20g+ammonium oxalate + ethanol 95 ml then complete by D.W to litter.

Weight the crystal violet and transfer to brown bottle, add ethanol weight ammonium oxalate and dissolved in 200ml D.W added to the stain and complete by D.W. up litter.
5. Ethanol 95% V/V.

6. Lugolis iodine solution:

Potassium iodine 20g + iodine 10g + D.W to one liter.

- Weight potassium iodine and transfer to brown bottle.

7. Oxidase reagent(in form of disc):

To make 10 ml.

Tetramethyl -P – phenylene diamine dihydrochloride 0.1 g +

D.W 10 ml.

Preparation:

Dissolve the chemical in water.

8-Kovac's reagent

Contents: to make 40 ml

4Dimethy amino benzaldehyde 2g

Iso amyl alcohol (3-methyle-butanol) 30 ml

Concentrated hydrochloric acid 10 ml

Procedure:

Weight the dimethy amino benzealdehyde, and then dissolve in the

Iso- amy alcohol. Then add concentrated hydrochloric acid and mix

well. Keep in clean brown bottle stored at 2-8 C°.

9-Turbidity standard Equivalent McFarland 0.5 (Barium sulphate):

Contents:
Concentrated sulphuric acid 1 ml
Dehydrated Barium chloride (BaC12.2Ho) 0.5 g
Distilled water 150 ml

Procedure:
Prepare 1% V/V of sulphuric acid solution by adding 1ml of concentrated sulphuric acid to 99 ml of Distilled water, mix. Prepare 1% W/V solutions of Barium chloride by dissolving 0.5 ml of Dehydrated Barium chloride (BaC12.2Ho) in 50 ml of Distilled water. Add 0.6 ml of sulphuric acid then mix well.

C- Glass wars:
- Petri dishes
- Tubes & Slides
- Universal bottles
- Flasks
- Slides

D- Culture media:
1- Mueller Hinton agar

Formula:
38.0 g for liter.

Contents
Agar 12.0g
Starch 1.5 g
Heat extracts 5.0 g
PH 7.4

Direction

Suspend 38.0 g of powder in 1 liter of Distilled water, then boiling to ensure complete dissolving. Autoclaved at 121 °C for 15 minutes, then cool the medium to 45 °C then distributed in sterile Petri dishes.

2-Peptone water

Formula:

15.0 g of 1 liter

Contents:

Peptone 10.0 g
Sodium chloride 5.0 g
PH 7.0 - 7.4

Direction:

Add 15.0 g of powder to 1 liter of distel water. Mix and distributed into tubes and sterilize by autoclave at 121 °C for 15 minutes, then cool the medium to 50°C.

3-Blood agar

Formula:

40.0 g for liter

Contents:
Protease peptone  15 g
Liver digest  2.5 g
Yeast extract  5.0 g
Sodium chloride  5.0 g
Agar  12.0 g
PH  7.4

**Direction:**

Suspend 40.0 g of powder in 1 liter of Distilled water, then boiling to ensure complete dissolving. Autoclaved at 121 C° for 15, then cool the medium to 50 C° then 7% v/v of sterile defibrinated blood was added then mixed and poured into Petri dishes.

**4-Chocolate agar**

**Contents:**

As the same of the blood agar and the same method but here use heated blood.

**5-MacConkey** Formula:

36.2 gram for 1 liter

**Contents:**

Peptone  4.0 g
Lactose  10.0 g
Agar  15.0 g
Tryptone  4.0 g
PH 7.3 g

**Direction:**

Suspend 36.2 g of powder in 1 liter of Distilled water, then boiling to ensure complete dissolving. Autoclaved at 121°C for 15 minutes, then cool the medium to 45°C then distributed in sterile Petri dishes.

**Sterilization:**

By autoclaving at 121°C and 15 Lb/inch for 15 minutes after the temperature fall to (45-50°C), we dispense it in Petri dishes 20 ml in each one under sterile condition.

**E-Biochemical test:**

1- DNA agar:

**Content:**

- Selection peptone.
- NaCL
- DNA
- Agar

**Preparation:**

8g from medium dissolved in 200 ml D.W

**Sterilization:**

By autoclaving at 121°C and 15 Lb/inch for 15 minutes after the temperature fall to (45-50°C), we dispense it in Petri dishes 20 ml in each one under sterile condition.
2- **Citrate broth**

**Content:**

- Magnesium sulphate.
- Potassium di hydrogen phosphate.
- Tri sodium citrate.
- Sodium ammonium phosphate.
- Bromothymol blue.

**Preparation:**

416 mg dissolved in 80 ml then boils, dispense and sterilization by the same method.

3- **Kliger Iron Agar (KIA):**

**Content:**

- Meat extract.
- Dextrose.
- Yeast extract.
- Ferric ammonium citrate.
- Peptone mixture.
- Na-thiosulphate.
- NaCL.
- Phenol red.
- Lactos
- Agar.
- Glucose

**Preparation:**

11.2mg of powder dissolved in 200 ml D.W. then boil and dispense in tube (5ml in each one).

**Sterilization:**

By autoclaving at 121 C° and 15 Lb/inch for 15 minutes after the temperature fall to (45-50) C° the tube put in sloped position until it solidifies.

**4-Urea agar**

**Content:**

- Peptone.
- NaCL.
- Dextrose
- Di-sodiumm hydrogen phosphate.
- Potassium di-hydrogen.
- Phenol red.
- Agar.

**Preparation:**

2.3g urea agar dissolved in ml D.W.
**Sterilization:**

By the same method. After temperature fall to (45-50°C) we added urea solution (4g of urea dissolved in 5 ml sterile D.W.) under sterile condition.

**F- Others:**

- Benzene Burner
- Straight loop
- Wire loop
- Gloves
- Rack tube
- Forceps
- Antimicrobial disc
- Watch, wooden stick, marker and Ruler.
Picture (1): Catalase test negative result

Picture (2): Shows Deoxribonuclease (DNase) test
Picture (3): Urease test; right positive (pink color); left negative

Picture (4): Indole test; Right positive, left negative
Picture (5): Citrate test; Right positive, left negative

Picture (6): Oxidase test; positive test (violet color)
Picture (7): Motility test diffusion indicate motile organism

Picture (8): Kiligler iron agar reaction
Picture 9: *E. coli* in MacConkey agar (lactose fermenter)

Picture 10: *Pseudomonas aeruginosa* in neutral agar with greenish pigmentation
Picture 11: *Proteus mirabilis* swarming growth on blood agar
A questionnaire:-

1/ data: .................................................................

2/ No: .................................................................

3/ Name: ............................................................

4/ Sex: ............................................................... 

5/ Age: ............................................................... 

6/ Type of operation: ..........................................

7/ Post-operative antibacterial agents taken:
   a/ Yes ( ) No ( )
   b/ If yes: what antibacterial ................................

8/ physical statue .............................................

9/ lab result:
   a/ culture: growth ( ) or no growth ( )
   b/ if growth bacteria is: ......................................
   c/ Antibacterial sensitivity: ...............................