

# **1. Introduction**

## **1.1. Overview**

Bed sores commonly known as pressure sores, skin ulcers or decubitus are cuts, lesions or swelling caused due to prolonged pressure on any part of body and usually affect parts of the body over bony areas. Bed sores commonly occur among handicapped, disabled, paralyzed or bed ridden patients as they are unable to change their position frequently and continuous pressure on specific parts of body lead to bed sores. Bed sores can be treated effectively if found early but can become a life threatening disease as it cuts off blood circulation to parts of the body (**Smith *et al*, 2010**).

Bed sores commonly occur around the buttocks, heels, lower back and the hip bone area among bed ridden individuals while spine, ankles, knees, head , shoulders are also susceptible to pressure sores depending on the fixed position of the patient. It is important to note that bedsores, if small and detected early can be treated by regular dressings while large or deep bedsores need plastic surgery techniques ,it one of type of wound infection and one of nosocomial infection (**Smith *et al*, 2010**).

## **Symptoms of Bed Sores**

Based on the severity of the condition, bed sores go through four different stages and the symptoms seen during each stage vary.

## **1.2. Problem statement and Justification**

Bed sores can trigger other ailments, cause patients considerable suffering, and be expensive to treat. Some complications include autonomic dysreflexia, bladder distension, osteomyelitis, pyarthroses, sepsis, amyloidosis, anemia, urethral fistula, gangrene and very rarely malignant transformation . Bed sores are also painful, with patients of all ages and all stages of bed sores reporting pain.

### **1.3. Objectives:**

#### **1.3.1. General objectives**

To measure the effects of the bacteria that causes the Bed sore infection on the patients

#### **1.3.2. Specific objectives**

1. To isolate bacteria that causes the Bed sore infection
2. To identify bacteria that causes the Bed sore infection
3. To perform susceptibility testing for bacteria that causes the Bed sore infection

### **1.4. Research Questions**

What the type of the bacteria that causes the Bed sore infection?

Can we treat the Bed sore infection with antibiotic?

## CHAPTER TWO

### 2. Literature review

#### 2.1. Nosocomial infection

Nosocomial infection is infection which develops during hospitalization and was not incubate or present at the time of admission to the hospital. It includes incidence in which a single microorganism spreads from person to person or from a common source in the hospital environment. This infection may present as sporadic which draw the attention and end endemic situations present far greater challenge in terms of mortality and morbidity as well as in control or reduction of incidence. Person at the risk the discharged, inpatient, outpatient, hospital staff and subsequently it may spill over in the community. Predisposing factors for nosocomial infection the hospital environment heavily laden with a variety of pathogen, hospital microbial flora is usually multi-drug resistance, instrumentation in hospitals may introduce infection, blood, blood products and IV fluids may transmit many infections and accidental inoculation of infectious material. Reliable data regarding prevalence of nosocomial infections in developing countries is very scanty. (Murray *et al*, 2002)

Murray *et al* (2002) explain the common agents of nosocomial infections: Gram positive: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Clostridium difficile*, *Clostridium perfringens*, and *Clostridium tetani*  
Gram negative: *Escherichia coli*, *Citrobacter*, *Klebsiella*, *Serratia*, *Enterobater*  
*Proteus* , *Pseudomonas* and *Legionella*.

Viruses: Hepatitis D, Hepatitis B, HepatitisC,HIV,Herpes virus,Cytomegalo virus,Influenza virus,Enteroviruses,SARS CoV.

Fungi: *Aspergillus* and *Candida albicans*.

Parasites: *Toxoplasma gondii*,*Entamoeba histolytica*,*Pneumocystis carinii*  
And *Cryptosporidium*.

Common types of hospital infections:

These include: Wound infection, Urinary tract infections, Respiratory tract infection, Skin infection, bacteraemia, septicemia, gastrointestinal infections. (Murray *et al*, 2002)

## 2.2. Wound types

Wounds can be broadly categorized as having either an acute or a chronic etiology. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries. (Davis *et al*, 1992).

Irrespective of the nature of the cutaneous injury, acute wounds are expected to heal within a predictable time frame, although the treatment required to facilitate healing will vary according to the type, site, and depth of a wound. The primary closure of a clean, surgical wound would be expected to require minimal intervention to enable healing to progress naturally and quickly. However, in a more severe traumatic injury such as a burn wound or gunshot wound, the presence of devitalized tissue and contamination with viable (e.g., bacterial) and nonviable foreign material is likely to require surgical debridement and antimicrobial therapy to enable healing to progress through a natural series of processes, including inflammation and granulation, to final re epithelialization and remodeling. (Davis *et al*, 1992).

In marked contrast, chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue. (Davis *et al*, 1992).

Pathophysiological abnormalities that may predispose to the formation of chronic wounds such as leg ulcers, foot ulcers, and pressure sores include compromised tissue perfusion as a consequence of impaired arterial supply (peripheral vascular disease) or impaired venous drainage (venous hypertension) and metabolic diseases such as diabetes mellitus. Advancing age, obesity, smoking, poor nutrition, and immunosuppression associated with disease (e.g., AIDS) or drugs (e.g., chemotherapy or radiation therapy) may also exacerbate chronic ulceration. Pressure or decubitus ulcers have a different etiology from other chronic wounds

in that they are caused by sustained external skin pressure, most commonly on the buttocks, sacrum, and heels. However, the underlying pathology often contributes to chronicity, and in this situation, pressure sores, like all chronic wound types, heal slowly and in an unpredictable manner. (**Davis *et al*, 1992**).

## 2.3. Wound Infection

**Peel** (1992) describe that wound infection occurs when virulence factors expressed by one or more microorganisms in a wound outcompete the host natural immune system and subsequent invasion and dissemination. The overall average percent frequencies of anaerobic bacteria in non infected and infected wounds are 38 and 48%, respectively. These numbers compare very closely with those observed by **Bowler and Davies** (1999), specifically in non infected and infected leg ulcers (36 and 49%, respectively). A correlation between the incidence of anaerobic bacteria and wound infection is thus evident.

**2.3.1. Surgical wound infections:** The risk of infection is generally based on the susceptibility of a surgical wound to microbial contamination (**Raahave et al**, 1986). Further **Mangram et al** (1999) has made more details that surgical site infection has recognized *S. aureus*, coagulase-negative staphylococci, *Enterococcus* spp , *Escherichia coli*, *P. aeruginosa*, and *Enterobacter* spp. as the most frequently isolated pathogens.

**2.3.2. Acute soft tissue infections:** **Meislin et al** (1977) and **Brook and Finegold** (1981) agreed that acute soft tissue infections include cutaneous abscesses, traumatic wounds, and necrotizing infection. Microbiological investigations have shown that *S. aureus* is the single causative bacterium in approximately 25 to 30% of cutaneous abscesses. **Pallua et al** (1999) added that the same organism has also been recognized as being the most frequent isolate in superficial infections seen in hospital, Accident and Emergency Departments. However, other studies have demonstrated that approximately 30 to 50% of cutaneous abscesses and 50% of traumatic injuries of varied etiology. **Brook and Frazier** (1990), **Sweeney et al** (1984) and **Elliot, Kufera and Myers** (1996) pointed that 47% of necrotizing soft tissue infections have a poly microbial aerobic-anaerobic micro flora.

Necrotizing soft tissue infections occur with different degrees of severity and speed of progression; they involve the skin (e.g., clostridial and non clostridial anaerobic cellulitis), subcutaneous tissue to the muscle fascia (necrotizing fasciitis), and muscle tissue (streptococcal myositis and clostridial myonecrosis). *S. aureus* has been described as being the single pathogen in two patients with rapidly progressing necrotizing fasciitis of the lower extremity (**Robson**, 1997). **Brook** (1998) studied necrotizing fasciitis in eight children, and reported the presence of pure *Streptococcus pyogenes* in two patients and a mixed predominance of *Peptostreptococcus* spp., *S. pyogenes*, *B. fragilis*, *C. perfringens*, *E. coli*, and *Prevotella* spp. in the others. Potentiation of infection by microbial synergistic partnerships between aerobes, such as *S. aureus* and *S. pyogenes*, and nonsporing anaerobes

has been recognized in various types of non clostridial cellulitis and necrotizing fasciitis, which agreed by (**Kingston and Seal**, 1990).

**2.3.3. Bite wound infections: Griego *et al*** (1995)The reported infection rate for human bite wounds ranges from 10 to 50% depending on the severity and location of the bite, and up to 20% of dog bites and 30 to 50% of cat bites become infected. **Brook** (1987) reported that 74% of 39 human and animal bite wounds contained a poly microbial aerobic-anaerobic micro flora, with *S. aureus* , *Peptostreptococcus* spp and *Bacteroides* spp. being the predominant isolates in both wound types .Due to the complex nature of the oral micro flora in humans and animals, the majority of bite wounds harbor potential pathogens, many of which are anaerobes. As well as the common anaerobes in both human and animal bite wounds, such as *Bacteroides* , *Prevotella* , *Porphyromonas* and *Peptostreptococcus* spp ( **Goldstein** ,1989). Less common potential pathogens such as *Pasteurella multocida*, *Capnocytophaga canimorsus*, *Bartonella henselae*, and *Eikenella corrodens* may also be involved (**Fleischer**, 1999).

**2.3.4. burn wound infections: Robson** (1999) and **Walker, Helm and Pulliam** (1987)reported infection is a major complication in burn wounds, and it is estimated that up to 75% of deaths following burn injury are related to infection and burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts. Many studies have reported the prevalence of aerobes such as *P. aeruginosa*, *S. aureus*, *E. coli*, *Klebsiella* spp ,*Enterococcus* spp and *Candida*spp (**Mayhall** ,1993), (**Robson**, 1999) and (**Bariar *et al***, 1997).

In other studies involving more stringent microbiological techniques, anaerobic bacteria have been shown to represent between 11 and 31% of the total number of microbial isolates from burn wounds ( **Randolph**, 1981) (**Ramakrishnan *et al***, 1986) and (**Mousa** , 1997).

**Brook and Randolph** (1981) and **Mousa** (1997) reported aerobes isolated in the latter studies were similar to those reported previously, predominant anaerobic burn wound isolates were *Peptostreptococcus* spp., *Bacteroides* spp and *Propionibacterium acnes*. And the

presence of *Bacteroides* spp in the wounds of 82% of patients who developed septic shock and concluded that such microorganisms may play a significant role in burn wound sepsis. Management of infection in burn wounds involves the use of topical and systemic antimicrobial agents, aggressive debridement of dead tissue, maximization of the immune response, and provision of adequate nutrition. (MacMillan , 1980) `

**2.3.5. Diabetic foot ulcer infections:** Plantar ulcers associated with diabetes mellitus are susceptible to infection due to the high incidence of mixed wound microflora (Diamantopoulos *et al* ,1998). And the inability of the PMNs to deal with invading microorganisms effectively (Armstrong, Liswood and Todd, 1995). **Pathare *et al*** (1998), **Karchmer** (1994) and **Armstrong , Liswood and Todd** (1995) describe that the *S. aureus* is a prevalent isolate in diabetic foot ulcers, together with other aerobes including *S. epidermidis*, *Streptococcus* spp, *Ps. aeruginosa*, *Enterococcus* spp and coliform bacteria. With good microbiological techniques, anaerobes have been isolated from up to 95% of diabetic wound. **Gerding** (1995), **Karckmer and Gibbons** (1994), **Johnson *et al*** (1995), **Diamantopoulos *et al*** (1998) and **Wheat *et al*** (1986) all agreed that the predominant isolates being *Peptostreptococcus*, *Bacteroides* , and ` *Prevotella* spp

### **2.3.6. Leg and decubitus (pressure) ulcer infections :**

**Bowler and Davies** (1999), **Brook and Frazier** (1990) and **Hansson *et al*** (1995) all agreed in chronic venous leg ulcers is frequently poly microbial, and anaerobes have been reported to constitute approximately 30% of the total number of isolates in non infected wounds . And *S. aureus* is the most prevalent potential pathogen in leg ulcers reported a significantly greater frequency of anaerobes (particularly *Peptostreptococcus* spp). and pigmented and non pigmented gram-negative bacilli) in clinically infected leg ulcers than in noninfected leg ulcers (49 versus 36% of the total numbers of microbial isolates, respectively). The same investigators also suggested that aerobic-anaerobic synergistic interactions are likely to be more important than specific microorganisms in the pathogenesis of leg ulcer infection; this mechanism is not widely recognized in the management of surgical (Rotstein, Pruett and Simmons,1985) and chronic wound infections.



**Decubitus ulcers** develop as a consequence of continued skin pressure over bony prominences; they lead to skin erosion, local tissue ischemia, and necrosis, and those in the sacral region are particularly susceptible to fecal contamination. Approximately 25% of decubitus ulcers have underlying osteomyelitis (**Brown and Smith**, 1999) and bacteremia is also common (**Lance George**, 1989). One of the few reported acknowledgments of the role of poly microbial synergy in chronic wound infection was made by **Kingston and Seal** (1990), who commented that since the bacteriology of decubitus ulcers is similar to that of some of the acute necrotizing soft tissue infections, the anaerobic and aerobic bacteria involved are likely to contribute to the deterioration of a lesion. The opportunity for microbial synergy in many decubitus ulcers was demonstrated by **Brook** (1991). Who reported mixed aerobic and anaerobic micro flora in 41% of 58 ulcers in children. *S. aureus*, *Peptostreptococcus* spp and *Bacteroides* spp. (formerly members of the *B.fragile* group and *P.aeruginosa* were the predominant isolates. Although localized wound care is normally sufficient to facilitate primary healing in decubitus ulcers, occasional necrosis of adjacent soft tissues leading to necrotizing fasciitis has been reported (**Brook**, 1991). Initial management of infected decubitus ulcers normally involves aggressive surgical debridement and broad-spectrum antimicrobial coverage (**Lance George**, 1989). Although leg ulcers frequently display a dense microflora, the occurrence of infection is relatively low (<5%) (**Hutchinson and Lawrence**, 1991). However again treatment normally includes topical and systemic antimicrobial agents and surgical debridement as necessary.

#### **2.4. Bed sore:**

Bed sores, also known as pressure ulcers, pressure sores or decubitus ulcers are skin lesions which can be caused by friction, humidity, temperature, continence, medication, shearing forces, age and unrelieved pressure. Any part of the body may be affected; bony or cartilaginous areas, such as the elbows, knees, ankles and sacrum are most commonly affected. The sacrum is a triangular bone at the base of the spine and the upper and back part of the pelvic cavity (like a wedge between the two hip bones).

If discovered early, bed sores are treatable. However, they may sometimes be fatal.

According to health authorities in the UK and USA, bed sores are the second iatrogenic cause

of death, after adverse drug reactions. Iatrogenic cause of death means unexpected death caused by medical treatment - death caused by the action of a physician or a therapy the doctor prescribed.

In the 1950s, Doreen Norton (1922-2007), a British nurse, used research to demonstrate that the best treatment and prevention of bedsores was removing the pressure by turning the patient every two hours. Norton is seen as instrumental in changing nursing practices to effectively treat pressure ulcers, which was a major killer of hospital patients.

*An acute decubitus ulcer is " a severe form of bedsore, of neurotrophic origin, occurring in hemiplegia or paraplegia."* (Hemiplegia = paralysis on one side of the body. Paraplegia = paralysis of the lower part of the body, including limbs. Pressure ulcers (bed sores) develop when the skin and the tissue below it becomes damaged. In severe cases the muscle and the bone may be damaged too. Pressure ulcers are much more common among patients who are unable to move because of paralysis, illness or old age. Sustained pressure can cut off circulation to vulnerable parts of the body, especially the skin of the buttocks, hips and heels - the affected tissue dies if it does not receive an adequate flow of blood (Smith *et al*, 2010).

**Figure 2.1: Photo of Bed sore**



Source: (Brendan Healy and Andrew Freedman, 2006)

## **2.4.1. Causative agents**

### **2.4.1.1. Staphylococci**

They are Gram positive spherical bacteria and form clumps. *Staphylococcus aureus* (*S.aureus*) is a bacterium that frequently colonized the human skin and is present in the nose of 40% of healthy people.

### **2.4.1.2. Bacteroides**

They are non sporing, strict anaerobes which colonize the large bowel, vagina and oropharynx. *Bacteroides fragilis* (*B.fragilis*) causes appendicitis, abdominal injuries and wound infection after colorectal or gynecological surgery.

### **2.4.1.3. Aerobic Gram negative bacilli**

They are normal inhabitants of large bowel. *Escherichia coli* (*E.coli*) and *Klebsiella* species are lactose fermenting. Proteus species are non lactose fermenting. Most organisms in this group act in synergy with *Bacteroides* to cause wound infections after bowel operations .The Pseudomonas tends to colonize burns and tracheostomy wounds as well as the urinary tract. *Pseudomonas aureginosa* (*P.aureginosa*) may be regarded as markers and colonize wards and intensive care units.

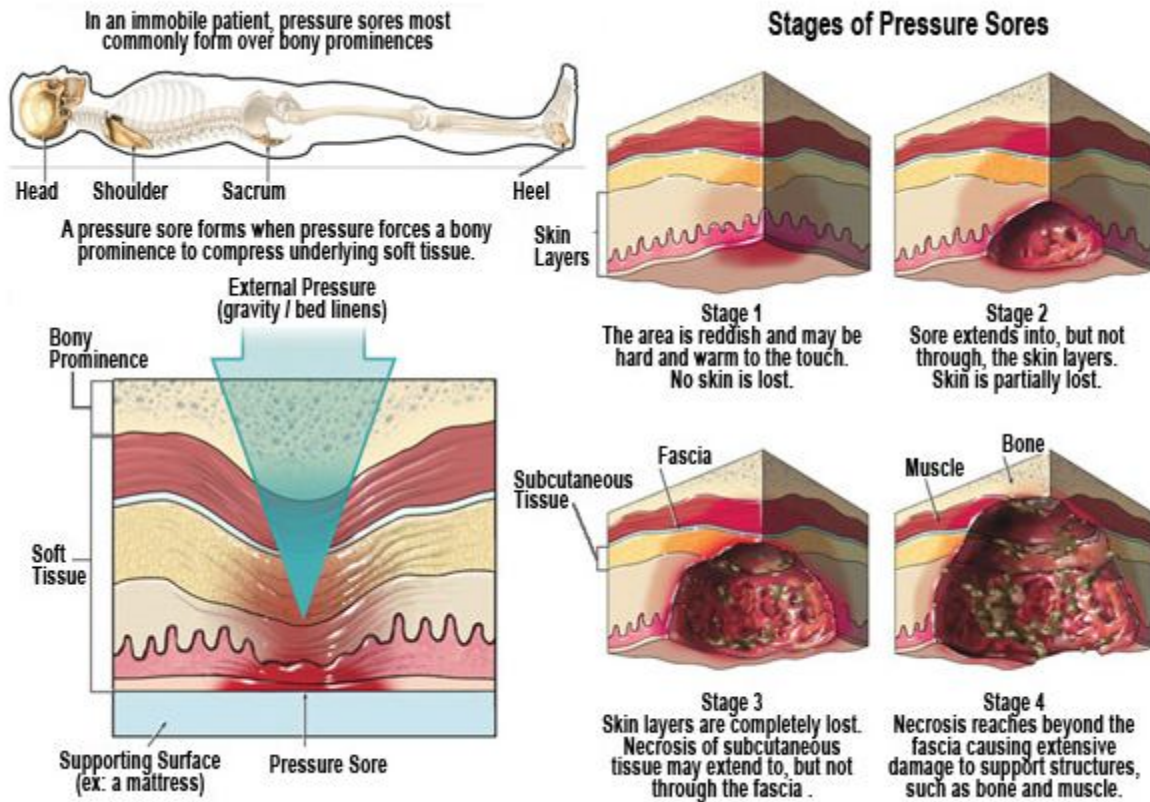
**2.4.2. The major risks that lead to pressure sores** include: stress, duration of stress, paralysis or spasticity, infections, edema, moisture, lack of nerve supply, poor nutrition. (Smith *et al*, 2010).

### **2.4.3. Stages of bedsore:**

Stage I Non blanchable erythema of intact skin (heralding lesion of skin ulceration). Stage II Partial-thickness skin loss involving epidermis or dermis. The ulcer is superficial, and presents clinically as an abrasion, blister, or shallow crater. Stage III Full-thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to, but not through, underlying fascia. The sore presents clinically as a deep crater with or without undermining of adjacent tissue. Stage IV Full-thickness skin loss with extensive destruction,

tissue necrosis, or damage in muscle, bone, or supporting structures (for example, tendon or joint capsule) (Smith *et al*, 2010).

**Figure 2.2: Photo shows the stages of bed sores**



**Source:** (Smith *et al*, 2010).

**2.4.5. Medical Complications as a Result of Bedsores** Cancer: Chronic pressure sores can develop into squamous cell carcinoma, Joint/Bone Infections: Bone and joint infections develop after burying themselves deep into pressure sores, Sepsis, Cellulitis (Smith *et al*, 2010).

**2.4.6. Bed Sore Treatment** Stage I pressure sores, a combination of antibiotics and cleaning solutions are usually used. Victims should also be repositioned regularly. For the

advanced stages, victims may need to undergo surgery via mechanical, autolytic, or enzymatic debridement in order to successfully remove the damaged tissues. Along with pain medication and antibiotics, victims are encouraged to eat a balanced, healthy diet as nutrition and hydration will help the healing process (**Smith et al** ,2010). Hyperbaric oxygen therapy for pressure sores is proven very successful and safe adjunctive treatment to daily wound dressing, administration of antibiotics and surgical debridement, Electrical stimulation (**Stone**, 1998).

## **CHAPER THREE**

### **3. MATERIALS AND METHODS**

#### **3.1. Study area:**

This study was conducted at Omdurman Teaching Hospital, Khartoum North Teaching Hospital, Police Hospital, Military Hospital and microbiology laboratory of Sudan University for Sciences and Technology.

#### **3.2. The Materials**

The materials used in this study include several types of media and reagents.

#### **3.3. The Methodology**

##### **3.3.1. Study sample**

60 patients has been planned to investigated, but the number proposed cannot be covered because of time and money limitation beside lack of bed sores' patients. So only a total of thirty wound swab (bed sore) specimens were collected from immobilized patients.

##### **3.3.2. Period of the research**

The period of the research has been from February to May, 2014.

##### **3.3.3. Collection of specimens**

A 30 specimens were collected from immobilize hospitalized patients, the specimens collected by sterile Cotton wool swabs (Cheesbrough, 2007).

##### **3.3.4. Sample processing**

###### **3.3.4.1. Inoculation**

Under aseptic condition near Bunsen burner the specimens were inoculated on blood agar and MacConkey agar (Cheesbrough, 2007).

### **3.3.4.2. Incubation**

The inoculated plate were incubated aerobically at 37°C for 24 hours (Cheesbough,2007).

### **3.3.4.3. Identification**

After incubation periods, the plates were examined for significant growth. The morphological characteristics (size -shape-coloures-pigment-and odours) were studies (Cheesbuogh, 2007).

#### **- Gram 's reaction**

Smear was prepared by emulsification apportion of colony of normal saline then spread evenly covering an area of about 15-20 mm diameter on slide smear was left in safe place to dry .Then fixed by rabidly passed the slide three time through the flame and allowed to cool the slide was covered with crystal violet stain for 30-60 seconds, washed then covered with lougo's iodine for 30-60 seconds and washed decolourization was done by adding acetone alcohol for few seconds and then suffranine was added for 2 minutes and finally washed .Then the back of slide was wiped, The film was air dried and examined under microscope firstly by 40x objective to check the stain then examined by using oil immersion objective (Cheesbrough,2007).

#### **- Method of biochemical tests used in identification of Gram negative bacteria**

- **Indole test**

Under aseptic conditions the tested organism was inoculated in the test tube containing 3 ml of sterile tryptone water, and then incubated at 35-37°C for to 24 hours, then Kovač's reagent was added and checking with 5 minutes. The positive result was indicated by presence of red ring, negative result showed no change of colour (Cheesbrough, 2007).

- **Urease test**

Test was used to identify some bacteria that produce urease enzyme which break down the urea ( $2\text{HN}_2\text{CONH}_2$ ) into ammonia ( $\text{NH}_3$ ) and  $\text{CO}_2$  which change the ph to alkaline this change the color of indicator (phenol red) to pink colour . The test was done by inoculating urea agar with test organism and incubated at 37°C for 24hours the change in the colour to pink colour indicates positive test (Cheesbrough, 2007).

- **Citrate utilization test**

Under aseptic conditions the tested organism was cultured in the medium which contains sodium citrate, and ammonium salt and bromothymol blue as indicator incubated at the 37°C for 24 hours and the medium was examined for turbidity and change of colour from green to blue which indicates positive result, negative result no change in colour (Cheesbrough, 2007).

- **Kligler's iron agar (KIA)**

The test based on the ability of bacteria to ferment sugar (lactose and glucose), production of gas and H<sub>2</sub>S. Under aseptic condition KIA was incubated with organism under test by using straight wire loop. Then incubated at 37°C for overnight. Then change in colour crack and production of H<sub>2</sub>S was observed (Cheesbrough, 2007).

**- Method of biochemical tests used in identification of gram positive bacteria:**

- **Catalase test**

The test is used to differentiate those bacteria that produce the enzyme catalase which breaks down of hydrogen peroxide to H<sub>2</sub>O and O<sub>2</sub> which give air bubble, such as Staphylococcus, from non catalase producing bacteria such as Streptococci (Cheesbrough). Under aseptic conditions remove a good growth of test organism by using sterile wooden stick and immersion in the hydrogen peroxide (3%) solution, immediate bubbling was produced (Cheesbrough, 2007).

- **Mannitol salt agar (MSA)**

A useful medium was for identifying Staphylococci species which are able to grow on agar contain 70-100g/l sodium chloride. Some species of Staphylococci are able to ferment mannitol and other cannot ferment. The test done by inoculating the organism under test in MSA medium (which contain phenol red as indicator). And then incubate the plate at 37°C for 24 hours and then the colour was observed (Cheesbrough, 2007).



- **DNase agar**

A useful medium for identification of Staphylococci species which are able to . The test done by inoculating plates by spotting a heavy inoculum of test organism then incubate the plate at  $35\pm 2$  °C for 18-24 hours, then flood plates with 1 N HCL and then observed clearing around the spot(Cheesbrough,2007).

- **Disc diffusion method for antibiotic:**

Muller Hinton agar medium was used as culture medium, sterilized by autoclaving at 37°C for 15minutes, allowed to cool and then poured in sterile petridishes.

- **Preparation of suspension:**

Using sterile wire loop, touch 3-5 well isolated colonies of similar appearance to the test organism and emulsifying 3-4 ml physiological saline.In a good light match the turbidity of the suspension with the turbidity of standard when comparing turbidities. Immersion the swab in the bacterial suspension and remove the excess of suspension by pressing and rotating the swab against the side of the tube (Cheesbrough, 2007).

- **Seeding**

Muller Hinton agar medium was seeded by using the swab and streak the swab evenly over the surface of the medium in three direction, allow 3-5 minutes for the surface of agar to dry (Cheesbrough, 2007).

- **Disc application**

Using sterile forceps, needle mounted in holder or multi disc, place the antimicrobial disk on the inoculation plate and incubate it aerobically at 35°C for 16-18hours (Cheesbrough,2007).

- **Reading**

Using a ruler on the underside of plate measure the diameter of each zone of inhibition per mm (Cheesbrough, 2007).

- **Interpretation of result**

NCCLs chart was used for interpretation of zone of inhibition.

### **3.4. Statistical analysis**

The data were analyzed using SPSS 11.5 BY frequency, graph, and chi-square test.

## CHAPTER FOUR

### 4. RESULTS

This study was carried out at Omdurman Teaching Hospital, Khartoum North Teaching Hospital, Ribat University Hospital, Military Hospital, Khartoum state during the period from February to May 2014. A total of 30 cotton swab specimens were collected and isolated on macConkey agar and blood agar.

#### 4.1. Isolation

Isolation of 36 organisms from 28 samples and 2 samples gave no growth 5(16.7%) of thirty samples are mixed (contain 2 or 3 bacteria).

#### 4.2. Identification

##### 4.2.1. Gram's reaction

Found Gram negative bacilli and Gram positive cocci.

##### 4.2.2. Biochemical tests used in identification of Gram negative bacteria

Table (1) shows identification of Gram negative bacteria.

- **Indole test**

Found indole positive bacteria (*E.coli*) and indole negative bacteria (*P.mirabilis*, *Ps.aeruginosa*, *K.pneumonia*, *C.freundii*, *S.marcescens*).

- **Urease test**

Found urease positive bacteria (*K. pneumonia*, *P. mirabilis*) and urease negative bacteria (*E. coli*, *Ps. aeruginosa*, *C. freundii*, *S. marcescens*).

- **Citrate utilization test**

Found citrate positive bacteria (*P. mirabilis*, *Ps. aeruginosa*, *K. pneumonia*, *C. freundii*, *S. marcescens*) and citrate negative bacteria (*E. coli*).

- **Kligar 's iron agar (KIA)**

Found glucose and lactose fermented bacteria (*E. coli*, *K. pneumonia* and glucose fermented and non lactose fermented (*P. mirabilis*, *S. marcescens* ). And found bacteria production H<sub>2</sub>S (*P. mirabilis* , *C. freundii* ) and bacteria not production H<sub>2</sub>S (*E. coli* , *K. pneumonia* *Ps. aeruginosa* and *S. marcescens* ).And found bacteria production gas (*P. mirabilis* , *C. freundii* , *E. coli* , *K. pneumonia* and *S. marcescens* ) and bacteria not production gas (*Ps. aeruginosa* ).

**Table (1): Shows Identification of Gram negative bacteria**

Bacteria	Biochemical tests			KIA medium				Frequency	Percent
	Indole	urease	Citrate	Slope	Butt	H <sub>2</sub> S	Gas		
<b>E. coli</b>	+ve	-ve	-ve	Y	Y	-	+	2	5.6
<b>Ps.aeruginosa</b>	-ve	-ve	+ve	R	R	-	-	8	22.2
<b>K.pneumonia</b>	-ve	+ve	+ve	Y	Y	-	+	4	11.1
<b>P.mirabilis</b>	-ve	+ve	+ve	R	Y	+	+	3	8.3
<b>C.freundii</b>	-ve	-ve	+ve	Y	Y	+	+	9	25
<b>S.marcescens</b>	-ve	-ve	+ve	R	Y	-	+	1	2.8

Source: Researcher Work

#### 4.2.3. Biochemical tests used in identification of Gram positive bacteria:

Table (2) shows identification of gram positive bacteria

- **Catalase test**

Found catalase positive bacteria (*S. aureus*).

- **Mannitol salt agar (MSA)**

Found mannitol fermented bacteria (*S. aureus*).

- **DNase agar**

Found DNase production bacteria (*S. aureus*).

**Table (2): Shows Identification of Gram positive bacteria**

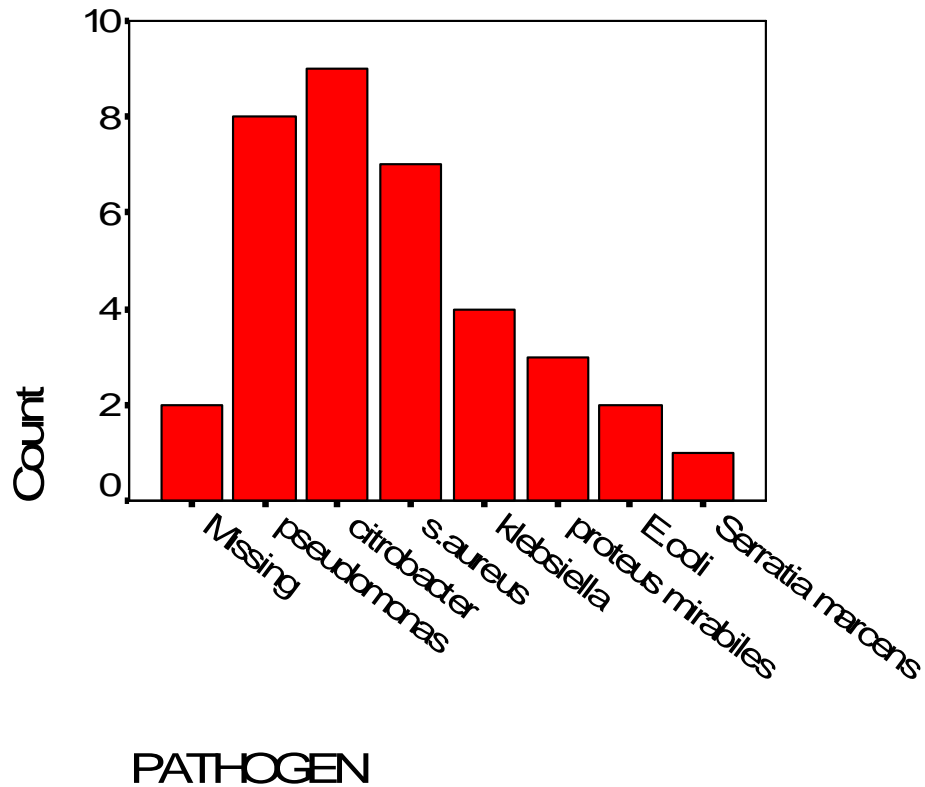
<b>Bacteria</b>	<b>Biochemical tests</b>			<b>Frequency</b>	<b>Percent</b>
	<b>Catalase</b>	<b>DNase</b>	<b>MSA</b>		
<i>S. aureus</i>	+ve	+ve	+ve	7	19.4

Source: Researcher Work

### The statistical analyses result

9 sample identified (25%) as *Citrobacter freundii* , 8 samples of clinical isolates were identified (22.2%) as *Pseudomonas aeruginosa* , 7 samples of clinical isolates were identified(19.4%) as *Staphylococcus aureus*, 4 samples identified(11.1%) as *Klebsiella pneumonia* , 3 samples identified (8.3%) as *Proteus mirabilis* , 2 sample identified (5.6%) as *Escherichia coli* , 1 sample identified as (2.8%) *serratia marcescens* , 2 samples (5.6%) no growth (show as missing in figure(4.1)).

Figure (4.1)



Source: Researcher Work

#### 4.2.4. Disc diffusion for antibiotic

12 of antibiotics were examined against clinical isolated bacteria (Ciprofloxacin, Gentamicin, Amoxclav, Tetracycline, Ceftriaxone, Chloramphenicol for G-ve and Vancomycin, Oxacillin, Ciprofloxacin and Gentamicin for *S. aureus* and Ceftaxin, Ambicacin, Nerobinin and Streptomycin for *Pseudomonas aureginosa*).

Ciprofloxacin inhibit 33.3% of *Citrobacter freundii*, 100% of *Klebsiella pneumonia*, 66.7% of *Proteus mirabilis*, 100% of *E. coli*, 100% of *Serratia marcescens*, 71.4% of *S. aureus*.

While Gentamicin inhibit 33.3% of *Citrobacter freundii*, 100% of *klebsiella pneumonia*, 66.7% of *Proteus mirabilis*, 100% of *E. coli*, 100% of *Serratia marcescens*, 71.4% of *S. aureus*

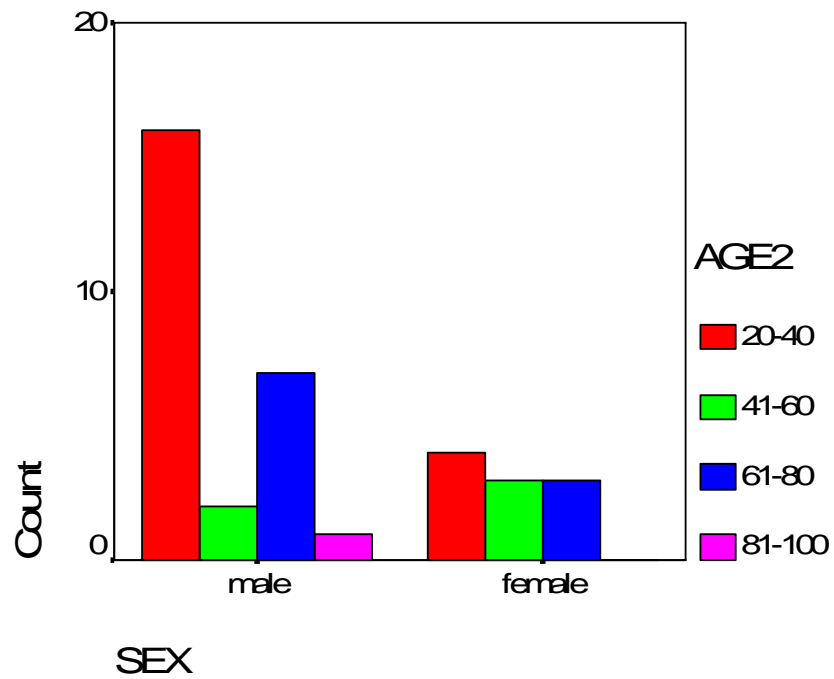
Chloramphenicol inhibit 22.2% of *Citrobacter freundii*, 100% of *Klebsiella pneumonia*, 66.7% of *Proteus mirabilis*, 100% of *E. coli*, 100% of *Serratia marcescens*. While ceftriaxone inhibit 11.1 % of *Citrobacter*, 75% of *Klebsiella pneumonia*, 66.7% of *proteus mirabilis*, 100% of *E.coli*, 100% of *Serratia marcescens*.

Tetracyclin and Amoxclav not inhibit *Citrobacter freundii*, *Klebsiella pneumonia*, *Proteus mirabilis*, *E. coli*, and *Serratia marcescens*. *Pseudomonas aeruginosa* inhibition by Streptomycin, Nerobinin, Amikacin, and not inhibition by Ceftaxine.

### 4.3. Demographic characteristics

- Gender: The male were more susceptible to bed sore infection 24 (80%) than female 6 (20%)
- Age Group: Within the sample of the study the infection of bed sore has been in the age group of 20-40.

Figure (4.2)



Source: Researcher Work

## CHAPTER FIVE

### 5. Discussion

This study was carried out in Omdurman, Khartoum North, , Military and Police hospital at Khartoum state during the period from February to May 2014 . A total of 30 cotton swab specimens were collected and isolated on MacConkey agar and blood agar. 36 organisms were isolated from 30 samples and 2 samples no growth. 5 (16.7%) of thirty samples are mixed (contain 2 or 3 bacteria).

The 36 organisms were isolated. 7 (19.4%) samples of clinical isolates were identified as *Staphylococcus aureus*, 8 (22.2%) samples of clinical isolates were identified as *Pseudomonas aeruginosa*, 9 (25%) samples identified as *Citrobacter freundii*, 4 (11.1%) samples identified as *Klebsiella pneumoniae*, 3 (8.3%) samples identified as *Proteus mirabilis*, 2 (5.6%) samples identified as *Escherichia coli*, 1 (2.8%) samples identified as *Serratia marcescens*, 2 (5.6%) samples no growth.

Study agrees with Anita Khanafari (2013) in the most common causes of bed sores: *S. aureus* and *P. aeruginosa* but does not agree that the very most common cause is *Citrobacter freundii*.

Does not agree with Ghaly (2013), Shalaby (2013), Shash (2013), Baraka (2013) and Aly (2013) for the most common causes.

The organism was sensitive to broad spectrum antibiotics. The effective antibiotics for Gram-negative bacilli are Ciprofloxacin and Gentamicin and followed by Chloramphenicol and Ceftriaxone and resistance to Tetracycline and Amoxiclav.

*S. aureus* is sensitive to Vancomycin, Gentamicin and Ciprofloxacin and resistance to Oxacillin.

This study showed that males were more susceptible to bed sore infection (24 (80%)) than females (6 (20%)) and showed that surgery ward patients were more susceptible to bed sore infection (13 (43.3%)), followed by orthopedics (7 (23.3%)), medicine (5 (16.7%)) and ICU (3 (10%)).



HDU(6.7%).And showed the high individual infection with bed sore between 20-40 showed in figure no(4) due to this is years of active and exposure to accident and immobilization.

## CHAPTER SIX

### 6. CONCLUSIONS AND RECOMMENDATIONS

#### 6.1. Conclusions:

- In this study pathogens frequently associated with bed sore infection in patients in order frequency were *Staphylococcus aureus* ,*Citrobacter freundii*, *Pseudomonas aeruginosa* , *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *serratia marcescens*.
- From this study it can be demonstrated that *citrobacter freundii* is the prime cause of bedsore in hospitalized patients.
- This study showed the ciprofloxacin and gentamicin effective for G-ve bacilli and *S.aureus*, and streptomycin, ambikacin and nerobinin is effective for *Ps.aeruginosa* .
- This study shows that the male were more susceptible to bed sore.
- Also highlights that surgery ward patients were more susceptible to bed sore infection.
- The study reflects within the sample of the study the infection of bed sore has been in the age group of 20-40 years.

#### 6.2. Recommendations

- Further in depth investigations of anaerobic bacteria has to be taken in bed sore. Consider large number of samples.
- Best dressing of the bedsore is by disinfection. The treatments of the bed sores in the early stage before it reach the late stage and require surgery.
- The best treatment for bedsore infection is broad spectrum antibiotic.

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## Appendix (1)

All media are prepared from Hi media laboratories pot,itd,made in India

### Kilgler iron agar:

<b>Ingredients:</b>	<b>Gram/Liter</b>
Peptic digest of animal tissue	15.0
Beef extracts	3.00
Yeast extract	3.00
Protose peptone	5.00
Lactose	10.0
Dextrose	1.00
Ferrous sulphate	0.20
Sodium chloride	0.50
Sodium thiosulphate	0.30
Phenol red	0.025
Agar	15.0

#### Preparation:

From Kilgler iron agar powder 57.5g dissolve in one liter of distilled water, and then sterilize by autoclaving at 121°C for 15 minute .Then cooled to about 50°C and poured in to sterile test tube and allowed to solidify in a slope position to give abutt 25-30mm deep and aslope 20-25mm final ph(at 25 °c)7.4±2

## Appendix (2)

### Blood agar:

<b>Ingredients:</b>	<b>Gram/liter</b>
Special nutrient substrate	23.0g
Starch	1.0g
Sodium chloride	5.0g
Agar	15.0g

#### Preparation:

Dissolve 42g in 1 liter of D.W and sterilize by autoclave (Gritten and George Ltd, England)at 121°C for 15minutes then cool to 50 °C add the blood then pour in plates.

### **Appendix (3)**

#### **MacConkey agar:**

<b>Ingredients:</b>	<b>Gram/liter</b>
Peptone	17.0g
Protease peptone	3.00g
Lactose	10.0g
Bile salt	1.50g
Sodium chloride	5.00g
Neutral red	0.03g
Agar	15.0g

#### **Preparation:**

51.53g powder dissolve in 500 ml D.W. and sterilize by auto clave (Gritten and George Ltd , England) at 121°C for 15 minutes then cool and pour in petridishes Final ph 7.1.

### **Appendix (4)**

#### **Nutrient agar:**

<b>Ingredients:</b>	<b>Gram/liter</b>
Lab-Iemco	10.0g
Yeast extracts	20.0g
Peptone	50.0g
Sodium chloride	50.0g

#### **Preparation:**

From nutrient agar powder 23g were suspended in one liter of distilled water, then sterilize by autoclave at121°C for 15minutes.Then cooled to about 50°C and poured in to sterile petridishes in 15ml amount .The poured plates were left to solidify at room temperature.



## **Appendix (5)**

### **Urea agar:**

<b>Ingredients:</b>	<b>Gram/liter</b>
Peptic digest of animal tissue	1.00
Dextrose	1.00
Sodium chloride	5.00
Di sodium phosphate	1.20
Mono potassium phosphate	0.80
Phenol red	0.012
Agar	15.0

#### **Preparation:**

From urea agar powder 24g dissolve in one liter of distilled water ,then sterilize by autoclaving at 121°C for 15minutes.Then cool to about 50°C and add aseptically 50 ml of urea extract is add to the media and mix well, then poured in to sterile test tubes and allowed to solidify in vertical position final ph6.8±0.2

## **Appendix (6)**

### **Mannitol salt agar:**

<b>Ingredients:</b>	<b>Gram/liter</b>
Meat extracts	1.00
Casein peptone	5.00
Meats peptone	5.00
Sodium chloride	75.0
D-Mannitol	10.0
Phenol red	0.025
Agar	15.0

#### **Preparation:**

From mannitol salt agar powder 108g dissolve in one liter of distilled water ,then sterilize by autoclaving at 121°C for 15 minutes .Then cooled to about 50°C and poured in to sterile

petridishes in 15ml amount .The poured plates were left to solidify at room temperature final pH 7.4±0.2.

### **Appendix (7)**

**Acetone alcohol**(It prepares by mixing ethanol (475ml) and distilled water(25ml) then acetone(500ml) measure and added to alcohol solution).

**Carbol fuchsin** (It was prepares by dissolve basic fuchsin (10g) in ethanol (100ml) then add 50g phenol and the volume complete with distilled water until one liter).

**Crystal violet** (Dissolve crystal violet (20g)in ethanol (95ml) then add (9g) ammonium oxalate ,the volume complete with distilled water until one liter).

**Lugol's iodine solution** (Dissolve potassium iodide (20g) and iodine (10g) in one liter distilled water).

**Hydrogen peroxide 3%** (Hydrogen peroxide 3ml in 100ml distilled water).

**Kovac's reagent** (dissolve the para-dimethylaminobenzaldehyde (2g) in the amyl alcohol (30ml) reagent store at 4C°).

**McFarland 0.5%** (barium sulphate)standard (0.5 ml of barium sulphate solution adds to 99.5 ml of sulphuric acid solution and mixed together).

**Absolute methanol.**

**Absolute petroleum ether.**

**Normal saline** (Dissolve 0.85gm of sodium chloride in 100 ml distilled water).

### **Appendix (8)**

#### **Ingredient:**

#### **Simmon's citrate agar:**

	<b>Gram/liter</b>
Magnesium sulphate	0.02g
Ammonium di hydrogen phosphate	1.0g
Di potassium phosphate	7.0g
Sodium citrate	2.0g
Sodium chloride	5.0g

Bromothymol blue	0.08g
Agar	15.0g
D.W	100ml

### **Appendix (9)**

<b>Dnase agar</b>	<b>Gram/liter</b>
Tryptose	20g
Deoxyribonucleic acid	2.0g
Sodium chloride	5.0g
Agar	15g

Preparation:

Suspended 42g in 1 liter of distilled water and heat to boiling and constant stirring to dissolve completely. Sterilize by autoclaving at 121 °C for 15 minutes. Cool to 50 °C and pour it in to the plates.

### **Appendix (10)**

Auto clave (Griffin and Italy George Ltd).

Hot air oven (Leader Engineering U.K).

Incubator (Leader Engineering U.K).

Water bath (science U.K).