Bacterial Isolates from Wound Infections and Their Antibiotic Susceptibility Pattern in Kassala Teaching hospital, Sudan

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Abstract

BACKGROUND: Wound infections are usually caused by the patient's normal flora or by bacteria from the environment or the skin of hospital staff and surgical wound infection which consider as most important causes of morbidity and mortality worldwide. The commonest organism of Gram positive is Staphylococcus aureus, Gram negative bacteria which include E. coli, Proteus spp. Klebsiella spp. and Ps. Aerogenosa. [1].

OBJECTIVES: Isolation and identification of bacteria from wounds and burns infection and antibiotic susceptibility of the isolated bacteria.

MATERIALS AND METHODS: A total of 756 wound swabs were collected from different infected wounds from the outpatient and inpatients admitted in the ward of surgery of Kassala teaching hospital were included in this study. All the swab and pus samples collected were tested for the direct microscopy, culture, biochemical reaction and antibiotic susceptibility tests was applied for all isolated bacteria. Analytical profile index (API system) plus conventional techniques were used in identification of bacterial isolates. The McFarland 0.5 standard was used to adjust the turbidity of the inoculum for the susceptibility test. Antibiotic susceptibility pattern of the isolates was assessed by Modified Kirby Baur disc diffusion technique.

RESULTS: During this period of study (756) samples were collected from different infected wounds from Kassala teaching hospital. (76.7%) were male and (23.3%) were female. Types of wounds observed from seven hundred and fifty six (756) patients were of two groups either non-operative/primary wound (82%) and post operative infection (18%). Positive growth was observed in 92.6% (700) of wound cultures and no bacterial isolates were obtained in 7.4% (76). From the culture materials Staphylococcus aureus was the most frequently isolated microorganism 30% followed by Staphylococcus epidermidis 19%, Escherichia coli (18 %), Pseudomonas aeruginosa (12%), Klebsiella pneumoniae (8%), Proteus mirabilis (7%), and Streptococcus pyogenes (6%). Antibiotic disc were exposed to 385 Gram positive isolates, 239 (62%) were resistant and 146 (38%) were susceptible, (210) Staphylococcus aureus of which 81 (38.6%) were susceptible and 129 (61.4%) were resistant, (133) Staph. epidermidis of which 51 (38.3%) were susceptible and 82 (61.7%) were resistant and (42) Streptococcus pyogenes 14 (33.3%) susceptible and 28 (66.7%) resistant and 223 out of 315 Gram negative isolates (70.8%) were resistant and 92 (29.2%) were susceptible. Antibiotic susceptibilities for (126) E. coli shows 53 (42%) susceptible and 73 (58%) resistant, (84) Pseudomonas shows 14 (16.7%) susceptible and 70 (83.3%) resistant, (56) Klebsiella shows 23 (41%) susceptible and 33 (59%) resistant, and (49) Proteus shows 45 (92%) resistant and 4 (8%) susceptible.

CONCLUSION: Microbiological analysis of the wound specimen and their antibiotic susceptibility testing are recommended that will guide medical practitioners for empirical treatment of wound infection, so as to reduce the spread of resistant bacteria.

Keywords: wound infection, antibiotic susceptibility


1. Introduction

A wound infection happens when germs enter a break in the skin. Wounds can be punctures (holes), lacerations (tears), incisions (cuts), or burns. Deep ulcers (open sores), large burns, or bite wounds are more likely than other wounds to get infected. Wound infection can also happen in small wounds that were not treated. When a large number of bacteria get into a wound, it can get infected. There are different types of bacteria. More than one type may infect the wound at the same time. Normal bacteria that live on their skin often enter a wound first. A break in the skin gives them a chance to enter it and cause infection. Bacteria may also come from the environment, such as...
soil, air, or water. If an object such as a nail caused the wound, bacteria may come from that. If you are bit by an animal or person, their saliva (spit) can also cause infection. Wounds may be grouped according to the cause, the environment in which they occur, their extent, and whether they are clean or contaminated. The microorganisms that typically infect wounds and the skin depend on what is present in the environment, the state of the person’s immune system, and the depth of the wound. Bacteria, fungi, and viruses can cause skin and wound infections [2]. Often involving the subcutaneous and connective tissue of skin causing redness, heat, and swelling. Necrotizing fasciitis—a serious but uncommon infection that can spread rapidly and destroy skin, fat, muscle tissue and fascia, the layer of tissue covering muscle groups. This type of infection often involves Group A streptococci, which are sometimes referred to as “flesh-eating bacteria.” Other common skin infections such as ringworm and athlete’s foot are not caused by bacteria but by fungi. Fungi can be found on thorns, splinters, and dead vegetation and can lead to deep wound infections that require special cultures for detection and identification. Yeast infections cause by Candida species may occur in the mouth (thrush) or on other moist areas of the skin. A variety of warts, such as common and plantar warts, are due to human papilloma virus (HPV). Wound infections due to bites tend to reflect the microorganisms present in the saliva and oral cavity of the human or animal that created the bite wound. They may involve one or more aerobic, microaerophilic, and/or anaerobic microorganisms. Human bites may become infected with a variety of aerobic and anaerobic bacteria that are part of the normal oral flora. The majority of animal bites are from dogs and cats, and the most common bacteria recovered from these cultures is Pasteurella multocida. Although rare, there is a risk of a rabies viral infection with bites from unvaccinated animals. Trauma Is a wide category of injuries caused by physical force. It includes everything from burns to injuries from motor vehicle accidents, crushing injuries, cuts from knives and other sharp instruments, and gunshot wounds. Burns may be caused by scalding or flammable liquids, fires and other sources of heat, chemicals, sunlight, electricity, and very rarely by nuclear radiation. First-degree burns involve the epidermis. Second-degree burns penetrate to the dermis. Third-degree burns penetrate through all of the layers of the skin and frequently damage the tissues below it. The large moist exposed surface of burns becomes colonized by bacteria within 24 hours. Staph. aureus. is the commonest isolate from burns, followed by Ps. aerogenosa and then various enterobacteria. (e.g. acinetobacter spp.) and streptococcus pyogenes groups A, B, C and G. [3]. Initial infections tend to bacterial infection. Fungal infections due to Candida, Aspergillus, Fusarium, and other species may arise later since they are not inhibited by antibacterial treatment. Viral infections, such as those caused by the herpes virus, may also occur. Wound infections are usually caused by the patient’s normal flora or by bacteria from the environment or the skin of hospital staff. The commonest organism is Staphylococcus aureus. Other common causative organisms include other Gram-negative aerobes, Streptococcus spp. and anaerobes. Bacterial causes of surgical wounds infection include Staphylococcus aureus which can infect any wound, Gram negative bacteria which include E. coli, Proteus spp. Klebsiella spp. Ps. Aerogenosa, which can infect any wound but more commonly associated with abdominal, urological and gynecological wounds [2]. For laboratory diagnosis of wound infection swabs are commonly sent for culture. However, pus if available is a better sample than a swab. Other fluids or tissue biopsy samples may also be cultured. Blood culture is recommended in febrile patients. Factors that affect wound healing include Diseases such as diabetes (high blood sugar level), cancer, or liver, kidney or lung conditions slow down healing, Foreign objects dead tissue and foreign objects, such as glass or metal, stuck in the wound may delay wound healing repeated trauma also weak immune system which is the part of the body that fights infection. This may be weakened by radiation, poor nutrition, and certain medicines, such as anti-cancer medicines or steroids. Symptoms of a wound infection are high or low body temperature, low blood pressure, or a fast heart beat. Increased discharge (blood or other fluid) or pus coming out of the wound. The discharge or pus may have an odd color or a bad smell. This may be characterized by redness, pain, swelling, and fever [4].

Wound care include Cleansing which may be done by rinsing the wound with sterile (Clean) water. Germ-killing solutions may also be used to clean wound. Debridement is done to clean and remove objects, dirt, or dead skin and tissues from the wound area. The patient may be given antibiotic treatment to fight infection. He may also be given medicine to decrease pain, swelling, or fever. The aim of this study is to isolate the pyogenic bacteria from pus and swab samples and to determine their antibiotic susceptibilities to various antibiotics commonly used in chemotherapeutic interventions.

2. Materials and Methods

2.1. Specimen Collection

This cross-sectional study was conducted at Kassala teaching hospital, Kassala town, Sudan. during the period October 2014 up to June 2016. A total of 756 pus and wound swabs were collected from different infected wounds (abscess, surgical wounds and diabetic wounds) randomly from the outpatient and inpatients admitted in the ward of surgery of Kassala teaching hospital. Pus was collected during abscess is incise and drained or after it is rupture naturally. Special care was taken to avoid contamination with communal organisms from the skin. As soon as possible, a specimen from a wound was collected before antisepctic dressing was applied. Swab when pus was not being discharged, use a sterile cotton swab to collect a sample from the infected site, immerse the swab in a container of Amies transport medium [5].

2.2. Laboratory Methods

All the swab and pus samples collected were tested for the direct microscopy, culture, biochemical reaction and antimicrobial susceptibility tests. wound specimens carefully taken using sterile cotton-swab it was immediately applied...
onto freshly prepared blood agar plates, streaked and incubated aerobically at 37°C for 24 hours. Specimens were collected aseptically by nurses or technicians before the wound cleaning and before application of an antiseptic solution. At the time of swab collection, standard care was taken to avoid contamination by the normal flora of the surrounding skin. Then the specimens were transported within one hour to the Microbiology laboratory of the hospital to perform the culture and susceptibility tests. Bacterial colonies on blood agar plates and were later Gram stained. Characterization of bacterial isolates was based on standard microbiological methods; these include Gram stain, morphological and cultural characteristics on nutrient agar, motility and carbohydrate fermentation tests, nitrate reduction, catalase, hydrogen sulphide production and indole production. Other tests include citrate utilization, gelatin liquefaction, Methyl Red-Voges Proskaeur test, coagulase, haemolysis on blood agar, morphological and cultural characteristics on mannitol salt and eosin-methylene blue agar. Smear of the specimen was made (wound specimen) on clean, dry slide allowed to air drying, smear was fixed by heat, Stained by the Gram technique. The 756 collecting specimens from wounds were inoculate onto Blood agar and MacConkey’s agar then incubated at 37°C for 18-24 hours. Colony morphology had been done for all the culture plates to study the characteristic features of the colonies like lactose fermentation, mucoid or non mucoid colonies, size of the colonies etc to differentiate between Enterobacteriaceae which affect wounds. And then the presumptive colonies examined under microscope using Gram stain technique. Subculture was conducted on selective media according to the reaction of Gram stain.

2.3. API System

Biochemical reactions which done for all the isolated bacteria of Gram positive and Gram negative manually and then pure culture was inoculated on the API-20E test strip system to differentiate between the Enterobacteriaceae members as confirm test. The biochemical tests are done according to Gram staining reaction. For Gram positive isolates, coagulase, catalase, DNAse, to differentiate between staphaphylococcus and streptococcus. For Gram negative the biochemical tests were sugar fermentation tests, oxidase, and urease, Indol, V.P., citrate and motility test [6].

2.4. Antibiotic Susceptibility Test

Mueller-Hinton agar medium is the only susceptibility test medium that has been validated by NCCLS. Mueller-Hinton agar should always be used for disk diffusion susceptibility testing. Antimicrobial Susceptibility Testing (Agar Disk Diffusion Method). Antimicrobial agents suggested for use in susceptibility testing of Chloramphenicol, Ampicillin, tetracycline and ciprofloxacin etc.

Commonly used antibiotics such as amoxicillin (10 µg), penicillin (10 µg), vancomycin (30 µg), azithromycin (15 µg), cephradine (30 µg), tetracycline (30 µg), cloxacillin (5 µg), co-trimoxazole (23.75 µg), ciprofloxacin (5 µg), ceftime (5 µg), cefuroxime (30 µg), imipenem (10 µg), ceftriaxone (30 µg), and nitrofurantoin (300 µg) using the Kirby Bauer disc diffusion method to confirm and verify that susceptibility test results are accurate, control organism were used (ATCC 25922 is the E. coli control organism) and (ATCC 25923) is the staphylococcus areus control organism. Zone diameters of control organism should compare with the results of kerby –Baur method. Susceptibility tests are affected by variations in media, inoculum size, incubation time, temperature, and other factors. NCCLS 1999.

3. Results

3.1. Results of Sex Distribution of the Patients

During this period of study (756) samples were collected from different infected wounds (Abscess, Trauma, Boils, surgical wounds and diabetic patient’s wounds) from Kassala teaching hospital 580 samples out of 756 (76.7%) were male and 176 (23.3%) were female Figure 1.

![Figure 1. Sex Distribution of the patients](image)

3.2. Results of Non Operative and Post Operative Culture of Wound Infection

Types of wounds observed from seven hundred and fifty six (756) patients were of two groups either non-operative/primary wound infection (Abscess, Trauma, Boils and diabetic wounds) 574 (82%) and post operative infection 126 (18%) (Table 1). Seven hundred and fifty six (756) bacterial isolates were recovered from various infected wounds.

<table>
<thead>
<tr>
<th>Patient type</th>
<th>Growth</th>
<th>No growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Non operative wound infection</td>
<td>613</td>
<td>87.6</td>
</tr>
<tr>
<td>Post operative wound infection</td>
<td>87</td>
<td>12.4</td>
</tr>
<tr>
<td>Total</td>
<td>700</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1. Results of culture in Non operative and post operative wound infection
Table 2. Results of Gender-wise distribution of growth and no growth culture

<table>
<thead>
<tr>
<th>Patient type</th>
<th>Growth</th>
<th>No growth</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Male</td>
<td>406</td>
<td>58</td>
<td>43</td>
</tr>
<tr>
<td>Female</td>
<td>294</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>700</td>
<td>100</td>
<td>56</td>
</tr>
</tbody>
</table>

3.3. Results of Bacterial Growth

Positive growth was observed in 92.6% (700) of wound cultures and no bacterial isolates were obtained in 7.4% (56) from the culture material. *Staphylococcus aureus* was the most frequently isolated microorganism (30%) followed by *Staphylococcus epidermidis*, (19%) and *Streptococcus pyogenes* (6%), these constituted (55%) of Gram positive microorganisms.

Gram positive bacteria constituted (55%) of the total number of the isolates.

Table 4. Results of (315) Gram negative Enterobacteriaceae isolated from different types of infected wounds in Kassala teaching Hospital.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Number</th>
<th>Average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>126</td>
<td>18</td>
</tr>
<tr>
<td><em>Pseudomonas aerogiosa</em></td>
<td>84</td>
<td>12</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>49</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>315</td>
<td>45</td>
</tr>
</tbody>
</table>

Gram negative bacteria constituted (45%) of the total number of the isolates which identified as *Escherichia coli* (18%), *Pseudomonas aerogiosa* (12%), *Klebsiella pneumoniae* (8%) and *Proteus mirabilis* (7%).

Table 5. Bacterial Isolates Recovered from Wounds’ Patients isolated from different types of infected wounds in Kassala Teaching Hospital

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Number</th>
<th>Average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>210</td>
<td>30</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>133</td>
<td>19</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>126</td>
<td>18</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>84</td>
<td>12</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>49</td>
<td>7</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>42</td>
<td>6</td>
</tr>
</tbody>
</table>

Staphylococcus aureus was the most frequently isolated microorganism 30% followed by Staphylococcus epidermidis 19%, *Escherichia coli* (18 %) *Pseudomonas aeruginosa* (12%) *Klebsiella pneumoniae* (8%) *Proteus mirabilis* (7%) and Streptococcus pyogenes (6%).

3.4. Results of Antibiotic Susceptibility Tests of Gram Positive Isolates

Antibiotic disc were exposed to (210) *Staphylococcus aureus* of which 81(38.6%) were susceptible and 129 (61.4%) were resistant, (133) *Staph. epidermidis* of which 51(38.3%) were susceptible and 82(61.7%) were resistant and (42) *Streptococcus pyogenes* 14 (33.3%) susceptible and 28 (66.7%) resistant. Out of 385 Gram positive isolates 239 (62%) were resistant and 146(38%) were susceptible.

Table 6. Antibiotic susceptibility tests (disk diffusion method) for (385) Gram positive isolated from different types of infected wounds in Kassala teaching Hospital according to Kirby –Bauer method

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th><em>Staph. aureus</em> (210)</th>
<th><em>Staph.epidermidis</em> (133)</th>
<th><em>Streptococcus pyogenes</em> (42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic susceptible test</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Results</td>
<td>81(38.6%)</td>
<td>129(61.4%)</td>
<td>51(38.3%)</td>
</tr>
</tbody>
</table>
3.5. Results of Antibiotic Susceptibility Tests of Gram Negative Isolates

223 out of 315 Gram negative isolates (70.8%) were resistant and 92 (29.2%) were susceptible. Antibiotic susceptibilities for (126) E. coli shows 53(42%) susceptible and 73(58%) resistant, (84) Pseudomonas shows 14(16.7%) susceptible and 70(83.3%) resistant, (56) Klebsiella shows 23 (41%) susceptible and 33 (59%) resistant, and (49) Proteus shows 45 (92%) resistant and 4(8%) susceptible.

3.6. Results of Antimicrobial Susceptibly of the Isolates

Antibiotic susceptibility of the isolates were 34% sensitive and 66% were resistance.

3.7. Results of Antibiotic Susceptibility of All the Isolates

Activities of 13 antimicrobial agents (antibiotic discs) against (210) Staph aureus isolates were resistant to Ampicillin/Sulbactam, Cephalexin, Cefotaxime, Pefloxacin, Ofloxacin, Cloxacillin, Roxithromycin, Lincomycin. Susceptible drugs for S.aureus were Co-Trimoxazole 6 (7.4%), Ciprofloxacin 29 (36%), Gentamycin, 27(33%) Tetracycline 3(3.8%) and Vancomycin 23(28%). (126) isolates of E. coli were tested against (12) antimicrobial agents showed resistance to Ampicillin, piperacillin, Cefitoxime, Ofloxacin, Roxithromycin, Gentamycin and ceftaxime, and the isolates were susceptible to Cotrimoxazole 11 (20.7%), chloramphenico 16 (11%) Tetracycline 2(4%) ciprofloxacin, 30 (57%) and pefloxacin 4(7.5%). Eighty four (84) Pseudomonas aeroginosa isolates were tested against (12) antimicrobial agents which were resistant to Ampicillin, Co-Trimoxazole, Cefitoxime, ciprofloxacin piperacillin, Cefoxamine, Ofloxacin, pefloxacin and Chloramphimicol and susceptible to Pseudomonas aerogenosa was susceptible for Gentamyacin 8(58%) and Amikacin (42. %). Fifty six (56) Klebsiella aerogense were tested against (12) antimicrobial agents showed resistance to 7 drugs out of 12 which were pefloxacin Ampicillin, Co-Trimoxazole, piperacillin, Cefaxamine, Ofloxacin, and Chloramphimicol and susceptible to Gentamyacin(26%) and Amikacin (74%). Forty nine (49) Proteus mirabilis isolates were resistance for 11drugs out of 12 and was the only sensitive isolate to Ampicillin. (133) Staphylococcus epidermidis shows resistant for 7 drugs and susceptible to Gentamyacin 31(60.7%), cotrimoxazole 10(19.6%), ciprofloxacin 5(9.8%), Tetracycline 3(5.9%) and vancomycin 2(4%). Forty two (42) Streptococcus pyogenes showed resistant for 9 drugs and was sensitive to Penicillin G(50%), Gentamyacin 4(29%) cotrimoxazoleand 2(14.%),less sensitive for ciprofloxacin 1(7%). The percentage of susceptible microorganisms constitute 238 out of 700 (34%), and resistant isolates were 462 out of 700 (66%).
Results of antibiotic susceptibility test of *Escherichia coli*

![Figure 7](image-url)  
**Figure 7.** Antimicrobial susceptibility of *Escherichia coli*  
(A: Ciprofloxacin, B: Vancomycin, C: Gentamycin, D: Co-Trimoxazole, E: Tetracycline)

Results of antibiotic susceptibility test of *Pseudomonas aeruginosa*

![Figure 8](image-url)  
**Figure 8.** Antimicrobial susceptibility of *Pseudomonas aeruginosa*  
(A, Gentamycin; B, Amikacin)

The results of the resistant antibiotics for the isolates from infected wounds were (66%) and sensitive were 34%.

### 4. Discussion

Wound infection has been a major concern among health care practitioners, sources of infection can be endogenous from the patient's own micro flora from nasopharynx, oral cavity and the skin, or nosocomial infection.

Microbiological results in this study reveals that *Staphylococcus aureus* was the most frequently isolated microorganism of wound infection in Kassala teaching hospital the percentage were (30%) followed by *Staphylococcus epidermidis* (19%), *Escherichia coli* (18 %) *Pseudomonas aeruginosa* (12%) *Klebsiella pneumoniae* (8%) *Proteus mirabilis* (7%) and *Streptococcus pyogenes* (6%). In this study Gram positive organism constituted (45%) and Gram negative the family Enterobacteriaceae constituted 55% of the total number of isolates, on the other studies done by Abdalla O. A. Ahmed, et-al (1998) [7]. Sudan, showed. (42%)of the total number of post surgical wound infections were due to, *Staphylococcus aureus* similar increasing of *S. aureus* reported by Sana E.M.Hamed. (2005) *Staphylococcus aureus* obtained from infected wounds was (93%), which is very high comparing to our results. Other studies of wound infection done in Nigeria, by SHITTU A.O et-al. (2002) [8] in which *S. aureus* was the common etiologic agent of wound infection (25%) followed by *Escherichia coli*, (12%) *Pseudomonas* (10.5%) and *Staphylococcus epidermidis* (9%) and studies done in India showed that (32%) of wound infection was *P. aeruginosa* [9].

The presence study showed differences in antibiotic sensitivity of the isolates to antibiotics used for the treatment of wounds infection, in which 700 isolates were tested. In this study the pattern of susceptibility showed that the best drugs were Gentamycin, (58%), ciprofloxacin 38% Cotrimoxazole (40%), and Vancomycin (28%). Amikacin (42%), On other studies, done by [10]. The study reported that the best active drugs were Ciprofloxacin (56.9%) Amikin (12.3%) and Gentamycin (11.5%). Ampicillin was found to be the most resistant antibiotic for all the isolates of wounds infection.

### References


[2] HemantSinghal. (2006). Wounds infection-surgical site infection (SSI) MD, MBBS, FRCSed, FRCS, FRCSC, Senior Lecturer, Department of Surgery, Imperial College School of Medicine, UK; Consulting Surgeon, Northwick Park and St Marks Hospitals, UK 2006.


