

Microbial assessment of wound infection among patients in hospitals within Enugu metropolis

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Abstract

Wound infection is a major health problem which is associated with chronicity and delay in wound healing. It is difficult to manage and treat particularly in developing countries and this can lead to morbidity and mortality of the patient. The aim of this study was to assess the microorganisms that are associated with wound infection. A total of forty (40) wound samples were collected from the lesions of 40 persons, with infected wounds from various hospitals within Enugu metropolis using sterile moist cotton swabs. Each of the sample swab sticks were streaked on Blood agar, MacConkey agar, Mannitol salt agar and Sabouraud dextrose agar (SDA). All the inoculated plates except SDA plates were incubated aerobically at 37°C for 24 hours and SDA plates were incubated aerobically at 37°C for 4 to 5 days. The isolates were identified based on their cultural characteristics, morphological and biochemical reactions. The result shows that out of the 40 samples that were analyzed, 4 fungal isolates and 5 bacterial isolates were identified. They include: *Aspergillus fumigatus* 32 (80%), *Aspergillus terreus* 28(70%), *Candida albicans* 31(78%), *Fusarium spp.* 20(50%), *Pseudomonas aeruginosa* 23 (58%), *Staphylococcus aureus* 29 (72%), *Escherichia coli* 13(33%), *Proteus mirabilis* 21 (53%) and *Klebsiella pneumonia* 18(45%). All the bacterial isolates were tested for antimicrobial susceptibility and the result shows that all *Proteus mirabilis* isolates were sensitive to Ciprofloxacin and Ofloxacin. 35 isolates were sensitive to Erythromycin while 34 isolates were sensitive to Gentamycin and 30 isolates were sensitive to Chloramphenicol. Also, 18 isolates were sensitive to Amoxicillin and all the isolates were resistant to Streptomycin and Tetracycline. It was also observed that 36 *Escherichia coli* isolates were sensitive to Ciprofloxacin while 34 isolates were sensitive to Ofloxacin. Also, 30 isolates were sensitive to Gentamycin, Amoxicillin and Chloramphenicol while 20 isolates were also sensitive to Streptomycin and Tetracycline. All the isolates were resistant to Erythromycin. All *Pseudomonas aeruginosa* isolates were sensitive to Ofloxacin while 35 isolates were sensitive to Gentamicin and 19 isolates were also sensitive to Streptomycin and Ciprofloxacin. All the isolates were resistant to Erythromycin, Chloramphenicol, Tetracycline and Amoxicillin. It was also observed that all *Klebsiella pneumonia* isolates were sensitive to Ciprofloxacin while 36 isolates were sensitive to Streptomycin and Tetracycline. Also, 31 isolates were also sensitive to Gentamycin and Ofloxacin while 24 isolates were sensitive to Amoxicillin. 20 isolates were also sensitive to Chloramphenicol while 18 isolates were sensitive to Erythromycin. Thirty-five (35) isolates of *Staphylococcus aureus* were sensitive to Levofloxacin and Ofloxacin. 34 isolates were sensitive to Gentamycin while 33 isolates were sensitive to Vancomycin. Also, 32 isolates were sensitive to Cefotaxime and Cloxacillin while 31 isolates were also sensitive to Chloramphenicol and 28 isolates were sensitive to Clindamycin. All the isolates were resistant to Tetracycline. These microorganisms were associated with wound infection and they can be so detrimental to human health. These microorganisms may have invaded the wound sites through direct contact with the wound or through iatrogenic transmission. Infection occurs when the virulence factors expressed by the microorganisms in the wound outweighs the host's immunity and if viable tissues are infected, it leads to local and systemic host response. If the wounds are not properly managed the microorganisms thrive in them and this can lead to long term disabilities, trauma, treatment cost rise and chronic wound or bone infection that can result to amputation as well as death. Therefore, identifying and managing wound infection early can contribute to faster wound healing, thus reducing the risk of negative outcomes.

Keywords: Wound; infection; Bacteria; Fungi; Antimicrobial susceptibility.

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1. Introduction

Skin protects the body from microbial populations that live on the skin surfaces and also prevents underlying tissues from becoming colonized and invaded by potential pathogens. Exposure of subcutaneous tissue following a loss of skin integrity (eg. wound) provides moist, warm and nutritious environment that is

conducive to microbial colonization and proliferation⁴. Breaks in the skin can occur through punctures, abrasions, or lacerations¹¹. They often occur after an accident or surgery. Wound can be said to be infected when there are pathogenic microorganisms within the wound sites which lead to tissue injury and if purulent materials drain from it^{8,4}. However, the abundance and diversity of microorganisms in any wound will be influenced by factors such as wound type, depth, location, and quality, the level of tissue perfusion, poor preoperative preparation, wound contamination, improper antibiotic selection, or the lack of ability of an immunocompromised patient to fight against infection⁴. The presence of microorganisms within the wound causes local tissue damage and impedes wound healing, wound chronicity, loss of limb or digits and increased health costs^{7,10}. Infections show a manifestation of organisms successfully colonized by entering the body, which reproduces and initiate an inflammatory response which are characterized by the classic signs of redness, rise in temperature of the body, pains, fever, warmth, pain, swelling, tenderness in the area of the wound, swollen nodes, presence of pus in the wound and inability of the wound to heal within 10 days after the injury⁹. Wound can be infected by a variety of microorganisms ranging from bacteria to fungus and parasites¹⁸. Risk factors associated with wound infection include older age, diabetes, immune system disorders, cancer, human immunodeficiency virus infection, and malnutrition¹⁷. Nosocomial infections are much pronounce in patients with wounds, and this is due to the nature of the wound itself, immune-compromise, time and depth of the wound in combination with type and dose of virulence of the infecting organism⁵. It is a major challenge to patients, health care staff and the general health care system in terms of costs and management¹⁵. An infected wound affects the quality of life, and compromises the wound's healing rate. Wound infections represent one third of nosocomial infections among surgical patients and are responsible for 70–80% of mortality^{16,19}. It is important in the morbidity and mortality of patients irrespective of the cause of the wound and are regarded as one of the most common nosocomial infections⁸. It is also important because it can delay healing and cause wound breakdown. If the wounds are not properly managed both fungal and bacterial pathogens can thrive in it and this can lead to long term disabilities, trauma, treatment cost rise and chronic wound or bone infection that can result to amputation as well as death.

II. Materials And Methods

Sample Collection

A total of forty (40) wound samples were collected from the lesions of 40 persons (both male and female), with infected wounds from various hospitals within Enugu metropolis using sterile moist cotton swabs under aseptic conditions. The wounds were cleaned with sterile 0.9% saline. Thereafter, the active part of the wound below the necrotic tissue at the edge of the wound and the wound base were swabbed with premoistened swab stick. The swab was rolled deep into the wound in a zig-zag motion. When there was more than one wound at the same location, the largest wound was sampled. Then, the swab was placed back into the tube and within one hour, the samples were transported in a cool box to the microbiology laboratory of Enugu State University of Science and Technology for analysis.

Microbiological Analysis

Isolation of Microorganisms

Each of the sample swab sticks were streaked on four different culture media namely: Blood agar, MacConkey agar, Mannitol salt agar and Sabouraud dextrose agar (SDA). All the inoculated plates except SDA plates were incubated aerobically at 37°C for 24 hours. SDA plates were also incubated aerobically at 37°C for 4 to 5 days. The isolates were identified based on their cultural characteristics, morphological and biochemical reactions.

Identification of Bacterial Isolates

The isolates were identified based on their gram staining and biochemical tests⁶.

Gram staining: A smear of each bacteria isolate was made on different clean grease free slides with a sterile wire loop and left to dry and after they were heat fixed and allowed to cool. Then the different smears were stained with crystal violet for 30-60 seconds and rapidly washed off with clean water. Then the smears were stained with lugol's iodine for 30-60 seconds and rapidly washed off with clean water. The smears were decolourized with 75% alcohol for 30 seconds and washed out immediately with clean water. Then the smears were stained with safaranine for 30-60 seconds and washed off immediately with clean water. The stained slides were then allowed to air dry. After drying, a few drops of oil immersion were dropped on the stained smears and viewed under microscope (100 oil objective lens) to check for the microscopic properties of the organism. The gram negative cells appeared red pink in colour while gram positive cells appear purple or blue.

Biochemical Tests

Several biochemical tests were carried out in order to have a presumptive and further identification of the potential bacteria.

Catalase test: Two mls (2mls) of hydrogen peroxide solution was poured into a clean test tube and using a wire loop, a good growth of the test organism was removed and immersed into the hydrogen peroxide solution, active bubbling indicated a positive result while no release of bubble indicated a negative test.

Coagulase test: A drop of physiological saline was placed on a clean slide and a loopful of the isolate was emulsified into it then a loopful of plasma was placed on it. It was rocked and clumping indicated a positive result while no clumping indicated negative result.

Indole test: The little portion of each of the isolate was inoculated into 5mls of sterile peptone water which was added in different test tubes using wire loop and then the test tubes containing the organisms were left to incubate at 37°C for 48 hours. After incubation, 3-4 drops of indole reagent were added and it was mixed gently. A red surface layer after 10 minutes gave a positive result while no red surface layer after 10 minutes gave a negative result.

Citrate utilization test: The test organisms were inoculated into Simmon citrate agar slant and incubated for 24 hours at room temperature. The appearance of growth with blue colour indicated positive result while green colour indicated negative result.

Urease test: A 24 hours culture of each of the isolates was streaked into the surface of urea agar slant medium contained in bijou bottle; they were incubated at 27°C for 24 hours. Purple pink colour indicated positive test.

Sugar fermentation test: The ability of an organism to ferment various sugars or digest carbohydrate is indicated by the production of acid and gas.

- The test organism was incubated in peptone water both containing 1% solution of desired sugar
- Phenol red was added as an indicator
- An inverted durham tube was inserted in the culture tube and was incubated at 37°C for 24 hours. Acid production was indicated by the change of colour of the medium to yellow. If gas is produced, it collects in durham tubes, which rise up the culture tubes.

Antibiotic susceptibility test: This was performed using the standardized disc diffusion method ⁶.

The 0.5 Macfarland standard was used to adjust the turbidity of the inocula for the antimicrobial susceptibility test. The 0.5 Macfarland was prepared by adding 0.5ml of a 1.1775% (wt/vol) barium chloride dehydrate (BaCl₂ 2H₂O) solution into 99.5ml of 1% (vol/vol) sulphuric acid (H₂SO₄). The turbidity standard was then aliquoted into screw capped test tubes identical to those used to prepare the inoculum suspensions. The test tubes were then sealed with wax to avoid evaporation. Inoculating needle was used to pick the isolated colonies and these were transferred into test tubes containing sterile saline. They were vortexed thoroughly. The test tube containing the turbidity standard was also vortexed so that white precipitates of barium could be mixed well. The bacteria suspensions were then compared with 0.5 Macfarland turbidity standard. Those test tubes with inoculum that did not appear to be of the same density as the 0.5 Macfarland turbidity were either added more sterile saline or increased by adding more organisms. Within 15 minutes after adjusting the turbidity of the inoculum suspension, they were inoculated on plates containing Muller Hinton agar and sterile glass spreader was used to streak the inoculum for even distribution of the organisms. Gram negative discs were placed on the inoculated plates using sterile forceps and they were incubated at 37°C for 24 hours. Clear zones of inhibition produced by the organisms were observed and measured.

Identification of fungal isolates

The fungal isolates were identified using cultural and morphological features with reference to the Manual of Fungal Atlas.

III. Result

The result shows that out of the 40 samples that were collected from 40 different patients, 23(57%) were females while 17 (42%) were males (table 1).

The isolates were identified on the basis of cultural, morphological and biochemical characteristics and the antibiotic sensitivity pattern of the bacterial isolates were determined. The isolates identified were 4 fungal isolates and 5 bacterial isolates. They include: *Aspergillus fumigatus* 32(80%), *Aspergillus terreus* 28(70%), *Candida albicans* 31(78%), *Fusarium spp.* 20(50%), *Pseudomonas aeruginosa* 23 (58%), *Staphylococcus aureus* 29 (72%), *Escherichia coli* 13(33%), *Proteus mirabilis* 21 (53%) and *Klebsiella pneumoniae* 18(45%)(table 2).

All the bacterial isolates were tested for antimicrobial susceptibility and the result shows that all *Proteus mirabilis* isolates were sensitive to Ciprofloxacin and Ofloxacin. 35 isolates were sensitive to Erythromycin while 34 isolates were sensitive to Gentamycin and 30 isolates were sensitive to Chloramphenicol. Also, 18 isolates were sensitive to Amoxicillin and all the isolates were resistant to Streptomycin and Tetracycline (table 3).

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It was also observed that 36 *Escherichia coli* isolates were sensitive to Ciprofloxacin while 34 isolates were sensitive to Ofloxacin. Also, 30 isolates were sensitive to Gentamycin, Amoxicillin and Chloramphenicol while 20 isolates were also sensitive to Streptomycin and Tetracycline. All the isolates were resistant to Erythromycin (table 4).

All *Pseudomonas aeruginosa* isolates were sensitive to Ofloxacin while 35 isolates were sensitive to Gentamicin and 19 isolates were also sensitive to Streptomycin and Ciprofloxacin. All the isolates were resistant to Erythromycin, Chloramphenicol, Tetracycline and Amoxicillin (table 5).

It was also observed that all *Klebsiella pneumonia* isolates were sensitive to Ciprofloxacin while 36 isolates were sensitive to Streptomycin and Tetracycline. Also, 31 isolates were also sensitive to Gentamycin and Ofloxacin while 24 isolates were sensitive to Amoxicillin. 20 isolates were also sensitive to Chloramphenicol while 18 isolates were sensitive to Erythromycin (table 6).

Thirty-five (35) isolates of *Staphylococcus aureus* were sensitive to Levofloxacin and Ofloxacin. 34 isolates were sensitive to Gentamycin while 33 isolates were sensitive to Vancomycin. Also, 32 isolates were sensitive to Cefotaxime and Cloxacillin while 31 isolates were also sensitive to Chloramphenicol and 28 isolates were sensitive to Clindamycin. All the isolates were resistant to Tetracycline (table 7).

Table 1: Sampled patients according to gender

Gender	Number of samples	Number of positive samples(%)
Male	40	17 (42%)
Female	40	23(57%)

Table 2: Organisms that were isolated from the samples

Organism isolated	Number of samples	Number of positive Samples(%)
<i>Escherichia coli</i>	40	13 (33%)
<i>Proteus mirabilis</i>	40	21 (53%)
<i>Pseudomonas aeruginosa</i>	40	23 (58%)
<i>Klebsiella pneumonia</i>	40	18 (45%)
<i>Staphylococcus aureus</i>	40	29 (72%)
<i>Aspergillus fumigatus</i>	40	32 (80%)
<i>Aspergillus terrus</i>	40	28 (70%)
<i>Candida albicans</i>	40	31 (78%)
<i>Fusarium</i>	40	20 (50%)

Table 3: Antimicrobial susceptibility pattern of *Proteus mirabilis* isolates

Antibiotics	No of isolates	No sensitive	No resistant	Disc Potency (µg)	Zone of inhibition (mm)
Erythromycin	40	35	5	10	25
Streptomycin	40	0	40	10	0
Ciprofloxacin	40	40	0	5	25
Gentamycin	40	34	6	10	21
Ofloxacin	40	40	0	5	24
Choramphenicol	40	30	10	10	20
Tetracycline	40	0	40	10	0
Amoxicillin	40	18	22	10	19

Table 4: Antimicrobial susceptibility pattern of *Escherichia coli* isolates

Antibiotics	No of isolates	No sensitive	No resistant	Disc Potency (µg)	Zone of inhibition (mm)
Erythromycin	40	0	40	10	0
Streptomycin	40	20	20	10	20
Ciprofloxacin	40	36	4	5	23
Gentamycin	40	30	8	10	22
Ofloxacin	40	34	6	5	22
Choramphenicol	40	30	10	10	24
Tetracycline	40	20	20	10	20
Amoxicillin	40	30	10	10	21

Table 5: Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolates

Antibiotics	No of isolates	No sensitive	No resistant	Disc Potency (µg)	Zone of inhibition (mm)
Erythromycin	40	0	40	10	0
Streptomycin	40	19	21	10	20
Ciprofloxacin	40	19	22	5	22
Gentamycin	40	35	5	10	24
Ofloxacin	40	40	0	5	25
Choramphenicol	40	0	40	10	0
Tetracycline	40	0	40	10	0
Amoxycillin	40	0	40	10	0

Table 6: Antimicrobial susceptibility pattern of *Klebsiella pneumoniae* isolates

Antibiotics	No of isolates	No sensitive	No resistant	Disc Potency (µg)	Zone of inhibition (mm)
Erythromycin	40	18	22	10	17
Streptomycin	40	36	4	10	23
Ciprofloxacin	40	40	0	5	25
Gentamycin	40	31	10	10	21
Ofloxacin	40	31	9	5	22
Choramphenicol	40	20	20	10	20
Tetracycline	40	36	4	10	23
Amoxycillin	40	24	16	10	19

Table 7: Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates

Antibiotics	No of isolates	No sensitive	No resistant	Disc Potency (µg)	Zone of inhibition (mm)
Vancomycin	40	33	7	10	25
Gentamycin	40	34	6	10	20
Chloramphenicol	40	31	9	10	22
Clindamycin	40	28	12	5	18
Cloxacillin	40	32	8	10	21
Levofloxacin	40	35	5	5	24
Ofloxacin	40	35	5	5	25
Cefotaxime	40	32	8	5	22
Tetracycline	40	0	40	10	0

IV. Discussion

In this study, the isolates identified were 4 fungal isolates and 5 bacterial isolates. They include: *Aspergillus fumigatus* 32(80%), *Aspergillus terreus* 28(70%), *Candida albicans* 31(78%), *Fusarium spp.* 20(50%), *Pseudomonas aeruginosa* 23 (58%), *Staphylococcus aureus* 29 (72%), *Escherichia coli* 13(33%), *Proteus mirabilis* 21 (53%) and *Klebsiella pneumoniae* 18(45%). The bacterial species that were isolated is in conformity with the work of Lucinda¹⁴ who isolated *Pseudomonas aeruginosa* (17%), *Staphylococcus aureus* (37%), *Escherichia coli* (6%), and *Proteus mirabilis* (10%) from wound infection and^{15,1,16} who also isolated similar organisms from infected wound. The fungal isolates that were isolated also conforms with the work of²¹ who isolated *Aspergillus terreus* and¹² who also isolated *Candida albicans*, all from wound infections. It also agrees with² who isolated *Aspergillus fumigatus* from infected wound and²⁰ who also isolated *Fusarium solani* from wound infection.

Antimicrobial susceptibility test was carried out on the bacterial isolates and it was observed that *Klebsiella pneumoniae* and *Staphylococcus aureus* were more susceptible to antibiotics when compared to other test organisms. *Klebsiella pneumoniae* was susceptible to all the test antibiotics (Erythromycin (45%), Streptomycin (90%), Ciprofloxacin (100%), Gentamycin (77.5%), Ofloxacin (77.5%), Chloramphenicol (50%), Tetracycline (90%) and Amoxycillin (60%)). *Staphylococcus aureus* was also susceptible to all the antibiotics but was highly susceptible to Levofloxacin (87.5%), Ofloxacin (87.5%), Gentamycin (85%) and Vancomycin (82.5%) when compared to other antibiotics. It was resistant to only tetracycline. This agrees with the work of¹³ who reported that *Staphylococcus aureus* was sensitive to Gentamycin (50%) and Vancomycin (30%). It also conforms to the work of¹⁴ who observed that *Staphylococcus aureus* was susceptible to Vancomycin. *Escherichia coli* was highly susceptible to Ciprofloxacin (90%) and Ofloxacin (85%) than other antibiotics but showed resistant to Erythromycin. *Pseudomonas aeruginosa* was also highly susceptible to Ofloxacin (100%), Gentamycin (87.5%) and Ciprofloxacin (47.5%). It was resistant to Tetracycline, Erythromycin, Chloramphenicol and Amoxycillin. It is in line with the work of¹³ who reported sensitivity of *Pseudomonas aeruginosa* to Gentamycin (71%) and Ciprofloxacin (47%). It also conforms with³ who opined that the organism was sensitive to gentamycin(62.5%) and ciprofloxacin.

Also, *Proteus mirabilis* was highly susceptible to Ciprofloxacin (100%), Ofloxacin (100%) and Erythromycin (87.5%) but was resistant to Tetracycline and Streptomycin. This agrees with the work of ¹⁸ who opined that isolated *Proteus species* were sensitive to Ofloxacin and Ciprofloxacin and ³ who agrees that *Proteus* spp. were resistant to tetracycline.

These organisms have been reported as common contaminants of wound. Exposed subcutaneous tissue provides a favourable substratum for a wide variety of microorganisms to contaminate and colonize, and if the involved tissue is devitalized and the immune response is compromised, the conditions become optimal for microbial growth. Wound contaminants are likely to originate from three main sources: (i) the environment (exogenous microorganisms in the air or those introduced by traumatic injury), (ii) the surrounding skin (involving members of the normal skin microflora) and (iii) endogenous sources involving mucous membranes (primarily the gastrointestinal, oropharyngeal, and genitourinary mucosa). These supply the vast majority of microorganisms that colonize wounds. The progression of a wound to an infected state is likely to involve a multitude of microbial and host factors including the type, site, size, and depth of the wound, the extent of nonviable exogenous contamination, the level of blood perfusion of the wound and the general health and immune status of the host, the microbial load and the combined level of virulence expressed by the types of microorganisms involved ⁴. Therefore, identifying and managing wound infection early can contribute to faster wound healing, thus reducing the risk of negative outcomes.

V. Conclusion

It was observed that the isolated microorganisms were associated with the wound infections and it is imperative that early detection and adequate management of the infected wound will help in reducing the negative outcomes of the infection.

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