



Infection of Wounds by Potential Bacterial Pathogens and Their Resistogram

Isyaka M. Tom^{1*}, Muhammad M. Ibrahim², Askira M. Umoru¹, Jidda B. Umar³, Musa A. Bukar¹, Ali B. Haruna¹, Abdullahi Aliyu⁴

¹Department of Medical Laboratory Sciences, University of Maiduguri, Maiduguri, Nigeria

²Department of Microbiology, University of Maiduguri, Maiduguri, Nigeria

³Department of Medical Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria

⁴Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria

Email: Isykatom77@gmail.com

How to cite this paper: Tom, I.M., Ibrahim, M.M., Umoru, A.M., Umar, J.B., Bukar, M.A., Haruna, A.B. and Aliyu, A. (2019) Infection of Wounds by Potential Bacterial Pathogens and Their Resistogram. *Open Access Library Journal*, 6: e5528. <https://doi.org/10.4236/oalib.1105528>

Received: June 8, 2019

Accepted: July 2, 2019

Published: July 5, 2019

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Abstract

The major innate barrier to the establishment of infections in internal tissues is the skin, the disruption of which leads to wound formation. Such wounds can be contaminated by bacterial pathogens thereby hampering the healing process and its management becomes resource demanding. Here, we assess the diversity of potential bacterial pathogens in the infection of different types of wounds among hospitalized patients. Three hundred and twenty wound swab samples were collected and processed via microscopy, and cultured on Blood, MacConkey and Chocolate Agar. Isolates were further confirmed using biochemical tests and Kirby Bauer disc diffusion test was used to determine their antimicrobial susceptibility pattern. 87.5% of samples collected yielded bacterial growth comprising of single bacterial isolates (52.17%) and polymicrobial/mixed growth (47.82%). *Staphylococcus aureus* (32.61%) was the most prevalent bacterial specie identified. Gram-negative bacteria (62.33%) were the most pervasive group, chief among which were *E. coli* (23.64%) and *Pseudomonas aeruginosa* (17.13%). Rate of infection was highest among Wound sepsis and Burns wound where *Enterobacter* spp. and *Streptococcus* spp. were the most prevalent respectively. Differences in wound type in relation to rate of infection with Gram-negative bacteria was statistically significant ($f = 5.9592$; $df = 29$; $p\text{-value} = 0.001645$; $p < 0.01$; Mean \pm SD = 7.633 ± 6.3706). Resistivity profile of isolates has shown that the most significant resistance rate was against Amoxicillin and Ampicillin, among Gram-positive and Gram-negative bacteria identified respectively. This suggests that wounds can be infected by potential bacterial pathogens which can exacerbate the progression of the wound and complicate the healing process.

Subject Areas

Infectious Diseases, Microbiology, Pathology

Keywords

Wound, Infection, Bacterial Pathogens, Drug Resistance

1. Introduction

Wound infection is the invasion of a wound by proliferating microorganisms to a level that invokes a local and/or systemic response in the host. The presence of microorganisms within the wound causes local tissue damage and impedes wound healing [1]. Breach in intact skin surface whether it is caused by trauma, accident, surgical operation, or burn provides an open door for bacterial infections [2]. The risk of wound infection increases with the degree of contamination and it has been estimated that about 50% of wounds contaminated with bacteria become clinically infected [3] [4]. Wound infections are classified on a continuum; contaminated, colonized, local infection, spreading infection, and systemic infection (sepsis). Infections of the skin and soft tissue either due to trauma, surgery, or burns may result in the generation of exudates composed of dead leucocytes, cellular debris, and necrotic tissues [5].

Chronic wounds can be colonized on the surface by a wide range of organisms [6]. Common bacterial pathogens associated with wound infection include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus* spp., *Streptococcus* spp., and *Enterococcus* spp. [7]. These organisms exhibit natural resistance to many antibiotics and antiseptics in which they may survive for long periods, and may even multiply in the presence of minimal nutrients and have the ability to colonize traumatised skin [8] [9].

The likelihood of a wound becoming infected is related to the number and virulence of the infecting microorganism and the ability of the host to resist infection [10]. Most of these opportunistic pathogens form part of the host's normal microbiota, or are found in damp environmental sites or on hospital equipments and medicament [9]. Once they gain access into the body, they develop mechanisms to exploit the host for continuous survival and dissemination [11]. Patients with wound complication arising from the dissemination of pathogenic microorganisms tend to be associated with bacteraemia, septicaemia, shock and prolonged hospital stay with an increasing chance of developing drugs resistant infections. Drug resistance leads to prolonged epidemics [12], and consequently, an unattended wound-site, being the most vulnerable point of entry of pathogenic bacteria could be difficult to treat if a multi-drug resistant strain is implicated.

This development is worrisome with a resultant increase in morbidity, mor-

tality and cost not only to patients and their relatives but including hospital management. This study was designed to evaluate the diversity of pathogenic bacteria in infected wounds and their resistivity pattern.

2. Materials and Methods

2.1. Study Area

The study was conducted in Maiduguri, the capital of Borno state. The city is located in the north-eastern part of Nigeria which lies within latitude 11.15°N and longitude 30.05°E in the Sudano-Sahelian savanna zone with a dense population that are mostly crop farmers, fishermen, herdsman and traders [13]. Based on the national census conducted in 2006, Borno state has a population of 4 million [14].

2.2. Sample Population

The target population for the study include in-patients and out-patients attending four selected hospitals in Maiduguri namely: University of Maiduguri Teaching Hospital (UMTH), State Specialist Hospital (SSH), MammanShuwa Memorial Hospital (MSMH) and Umaru Shehu Ultra-Modern Hospital (USUMH). UMTH is a tertiary-care hospital that serves a population of over 20 million in the North-eastern sub-region of Nigeria, comprising six states (Borno, Bauchi, Yobe, Adamawa, Taraba and Gombe) as well as a sizeable number across the borders of Cameroon, Chad and Niger Republic [15].

2.3. Sample Collection and Processing

Three hundred and twenty (320) wound swab samples were collected from consented patients in both inpatient and outpatient departments. Wound bed was prepared before sample collection by using Levine's technique, where the wound surface exudates and contaminants were cleaned off with a moistened sterile gauze and sterile normal saline solution. Aseptically the swab stick was rotated over 1 cm² area for 5 seconds with sufficient pressure to express fluid and bacteria to surface from within the wound tissue [16]. The wound swab samples were transported to Microbiology Laboratory after collection in 0.5 ml sterile normal saline solution for bacterial preservation.

2.4. Microbiological Analysis

The wound swab samples were cultured by plating onto 5% Blood agar, Chocolate agar and MacConkey agar plates and incubated aerobically at 37°C for 18 - 24 hours. Suspected colonies were further subcultured to obtain discrete colonies. Gram stain was conducted as a preliminary test. Pure culture was isolated and identified based on morphological appearance on enriched (Blood agar) and differential media (MacConkey agar), motility, Gram stain reaction and reaction to biochemical tests which include phenylalanine deaminase, urease, hydrogen sulphite production, indole, methyl red, vogesproskauer, citrate, maltose fer-

mentation and ornithine decarboxylase test [17] [18].

2.5. Antibiotic Susceptibility Test

2.5.1. Preparation of Bacterial Inoculum

Pure isolate of bacteria was inoculated into Nutrient broth and incubated at 37°C for up to 5 hours until turbidity equals to 0.5 McFarland standard on the turbidity scale. This turbidity scale was adjusted by adding 9.6 ml of 1% aqueous solution of barium chloride in 0.4 ml of 1% sulphuric acid to give an approximate bacterial density of 1.2×10^9 CFU/ml [17].

2.5.2. Antimicrobial Susceptibility Testing

Pure bacterial isolates were tested against selected antibiotics using Gram-negative and Gram-positive multidiscs. Gram-negative multidisc consist of the following antibiotics:- Cephalexin (10 µg/ml), Gentamicin (10 µg/ml), Augmentin (30 µg/ml), Nalidixic acid (30 µg/ml), Streptomycin (30 µg/ml), Ampicillin (30 µg/ml), Ofloxacin (30 µg/ml), Pefloxacin (10 µg/ml), Ciprofloxacin (10 µg/ml), Sulphamethoxazole-Trimethoprim (30 µg/ml); whereas the Gram-positive multidisc consist of the following antimicrobial drugs:- Levofloxacin (20 µg/ml), Norfloxacin (10 µg/ml), Ampiclox (20 µg/ml), Amoxicillin (20 µg/ml), Chloramphenicol (30 µg/ml), Rifampicin (20 µg/ml), Erythromycin (30 µg/ml), Gentamicin (10 µg/ml), Streptomycin (30 µg/ml), and Ciprofloxacin (10 µg/ml).

Prepared bacterial inoculum (1.2×10^9 CFU/ml) was seeded onto prepared Mueller Hinton agar (MHA) plate under aseptic condition and the surface was allowed to absorb. Gram-negative and Gram-positive multidisc was then carefully placed onto the surface of the seeded plate with the aid of sterile forceps and incubated at 37°C for 18 - 24 hours. After 24 hours, the zones of inhibition were measured in millimetres. Results were interpreted in accordance with CLSI interpretation chart for antimicrobials susceptibility testing [19]. The percentage frequencies of sensitivity and resistance were recorded.

2.6. Data Analysis

Data generated were analysed using Microsoft Excel Software and Socstatistics Statistical calculators [20]. Data were presented as frequencies and percentages. One-way Anova and Chi-square were calculated and evaluations were carried out at 99% confidence level and $P < 0.01$ was considered as statistically significant.

3. Results

3.1. Recovery of Potential Bacterial Pathogens in Wounds of Patients Examined

280 (87.50%) out of the 300 samples processed yielded bacterial growth of 368 isolates, comprising of single and polymicrobial infections. Single bacterial species were the most frequently isolated 192 (52.17%) compared to polymicrobial

infections which was observed among 88 samples (consisting of two or more bacterial species per sample totalling 176 (47.82%). The predominant species found in the polymicrobial infections was *E. coli* 44 (11.96%), followed by *Staph. aureus* 36 (9.78%) and the least was *Enterobacter* spp. 13 (3.53%). Gram-negative bacteria constitute the most pervasive group (229; 62.33%) followed by Gram-positive bacteria (139; 37.77%). Among Gram-negative organisms, *E. coli* (87; 23.64%) was the most prevalent followed by *Pseudomonas* spp. (63; 17.13%), and the least was *Enterobacter* spp. (13; 3.53%). *Staphylococcus aureus* and *Streptococcus* spp. were the Gram-positive organisms identified (32.61% and 5.16% respectively) (Table 1, Table 2).

3.2. Distribution of Potential Bacterial Pathogens in Relation to Age and Sex of Patients

The infection rate in relation to sex and age of patients was highest among male patients (57.07%) and those within the age group of 21-30years (24.45%). On age versus sex basis, infection was most significant among male patients within the age category of 21 - 30 years (15.76%) (Table 3).

Table 1. Distribution of potential bacterial pathogens in the wounds of patients attending selected hospitals in Maiduguri.

Name of Hospital	No of Samples examined (%)	No of Samples that yielded bacterial Growth (%)	No of Samples that yielded no bacterial growth (%)
UMTH	115 (35.94)	105 (32.81)	10 (3.13)
SSH	100 (31.25)	88 (27.50)	12 (3.75)
MSMH	60 (18.75)	49 (15.31)	11 (3.44)
USUMH	45 (14.06)	38 (11.88)	7 (2.19)
Total (%)	320 (100)	280 (87.50)	40 (12.50)

Key: **USUMH**: Umaru Shehu Ultramodern Hospital; $X^2 = 3.7954$; p-value = 0.284423; p < 0.01: *not significant. **MSMH**: Mohammed Shuwa Memorial Hospital; **SSH**: State Specialist Hospital; **UMTH**: University of Maiduguri Teaching Hospital.

Table 2. Isolation of single and mixed potentially pathogenic bacteria from wound of patients attending selected hospitals in Maiduguri.

Bacterial spp Isolated	Single Isolates (%)	Double Isolates (%)	Total Isolates (%)	
Gram-positive	<i>Staph. aureus</i>	84 (22.83)	36 (9.78)	120 (32.61)
	<i>Streptococcus</i> spp.	0 (0.00)	19 (5.16)	19 (5.16)
	<i>Escherichia coli</i>	43 (11.68)	44 (11.96)	87 (23.64)
	<i>Pseudomonas</i> spp.	35 (9.51)	28 (7.61)	63 (17.12)
Gram-negative	<i>Klebsiella</i> spp.	22 (5.98)	16 (4.35)	38 (10.33)
	<i>Proteus</i> spp.	8 (2.17)	20 (5.44)	28 (7.61)
	<i>Enterobacter</i> spp.	0 (0.00)	13 (3.53)	13 (3.53)
Total (%)	192 (52.17)	176 (47.83)	368 (100)	

Table 3. Frequency of bacterial isolation among patients with wound infection on the basis of their age and sex.

Age Group (years)	Sex		Total Number of Bacteria Isolated (%)
	Male (%)	Female (%)	
0 - 10	29 (7.88)	23 (6.25)	52 (14.13)
11 - 20	37 (10.05)	19 (5.16)	56 (15.21)
21 - 30	58 (15.76)	32 (8.69)	90 (24.45)
31 - 40	41 (11.14)	34 (9.24)	75 (20.38)
41 - 50	23 (6.25)	22 (5.98)	45 (12.23)
51 - 60	12 (3.26)	14 (3.80)	26 (7.06)
61 and Above	10 (2.72)	14 (3.80)	24 (6.54)
Total (%)	210 (57.07)	158 (42.93)	368 (100)

3.3. Distribution of Potential Bacterial Pathogens in Patients Based on Hospitals Attended

Among the various hospitals examined, patients attending UMTM yielded the highest bacterial recovery rate of 32.07%. The most significant bacterial spp identified among these patients was *Staphylococcus aureus* (11.14%) and the least prevalent was *Streptococcus* spp. (0.09%). The least rate of isolation was observed among patients attending USUMH (10.33%) where the most significant bacterial spp identified was *Staphylococcus aureus* (5.43%) and the least prevalent was *Proteus* spp. and *Enterobacter* (0.54%). The organism with the highest rate of occurrence was *Staphylococcus aureus* (32.61%) followed by *Escherichia coli* (23.64%), *Pseudomonas* spp. (19.12%), *Klebsiella* spp. (10.33%), *Proteus* spp. (7.61%), *Streptococcus* spp. (5.16%), and the least prevalent was *Enterobacter* spp. (3.53%) (Figure 1).

3.4. Rate of Recovery of Potential Bacterial Pathogens from the Wound Types Examined

Infection rate among the various wound types examined has shown that Wound Sepsis was the most-infected, followed by Burns Wound and the least was observed in Gunshot Wounds. *Enterobacter* spp. was the most significant bacteria identified in Wound Sepsis; *P. aeruginosa* in Wound Ulcer; *Streptococcus* spp. in Diabetic Wounds, Burns and Gunshot wounds while *E. coli* was the most incriminated in Post-operative Wounds (Figure 2).

3.5. Resistogram Profile of Potential Bacterial Pathogens Recovered from the Wounds of Patients Attending Selected Hospitals

The resistivity profile of the isolates has shown that *Staphylococcus aureus* and *Streptococcus* spp. were highly resistant to Amoxicillin (91.7% and 73.7% re-

spectively); *P. aeruginosa*, *Klebsiella* spp., and *Enterobacter* spp. were also resistant to Ampicillin (100%, 73.7% and 84.6% respectively); *Proteus* spp. isolates were resistant to Augmentin (89.3%); and *E. coli* was highly resistant to Nalidixic Acid (75.9%) (Table 4, Table 5).

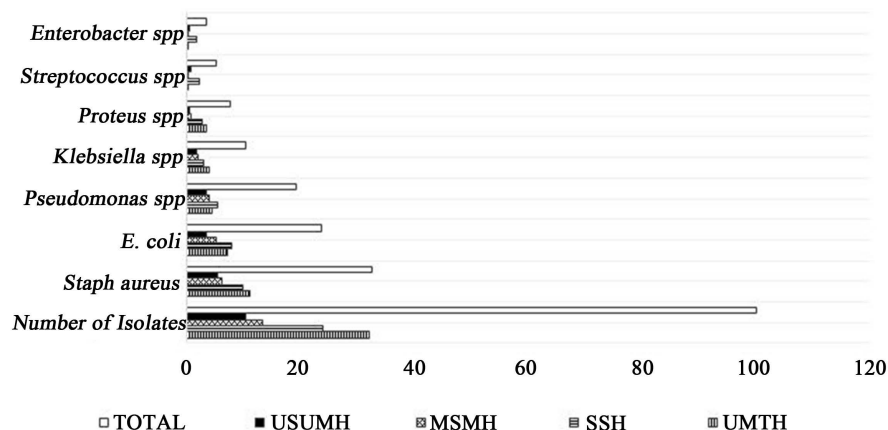


Figure 1. Isolation of potentially pathogenic bacteria among patients attending selected hospitals in Maiduguri. *Staphylococcus aureus* was the most significant bacterial pathogen isolated from all hospitals, whereas UMTH was the hospital with the most significant rate of infection. Key: USUMH: Umaru Shehu Ultramodern Hospital; MSMH: Mohammed Shuwa Memorial Hospital; SSH: State Specialist Hospital; UMTH: University of Maiduguri Teaching Hospital.

Table 4. Percentage susceptibility profile of gram-negative bacteria isolated from infected wounds of patients attending selected hospitals in Maiduguri Metropolis.

Antibiotic	Susceptibility Profile									
	<i>E. coli</i> n = 87		<i>P. aeruginosa</i> n = 63		<i>Klebsiella</i> spp. n = 38		<i>Proteus</i> spp. n = 28		<i>Enterobacter</i> spp. n = 13	
	Sens.	Rest.	Sens.	Rest.	Sens.	Rest.	Sens.	Rest.	Sens.	Rest.
CPX	81.6	18.6	76.2	23.8	79.0	21.1	85.7	14.3	61.5	38.5
SXT	34.1	52.9	31.8	68.3	42.1	57.9	60.7	39.3	46.2	53.9
S	63.2	36.8	55.6	44.4	65.8	34.2	78.6	21.4	76.9	23.1
PN	27.6	72.4	0.0	100.0	26.3	73.7	25.0	75.0	15.4	84.6
CEP	36.8	63.2	23.8	76.2	34.2	65.8	25.0	75.0	30.8	69.2
OFX	59.8	40.2	47.6	52.4	55.3	44.7	71.4	28.6	69.2	30.8
NA	24.1	75.9	14.3	85.7	29.0	71.1	14.3	85.7	23.1	76.9
PEF	64.4	35.6	60.3	39.7	63.2	36.8	64.3	35.7	76.9	23.1
CN	50.6	49.4	27.0	73.0	47.4	52.6	64.3	35.7	53.9	46.2
AU	34.5	65.5	7.9	92.1	34.2	65.8	10.7	89.3	38.5	61.5

Key: CPX: Ciprofloxacin; SXT: Sulphamethoxazole-Trimethoprim; S: Streptomycin; PN: Ampicillin; CEP: Cephalexin; OFX: Ofloxacin; NA: Nalidixic Acid; PEF: Pefloxacin; CN: Gentamicin; AU: Augmentin. Sens: Sensitive Rest: Resistant.

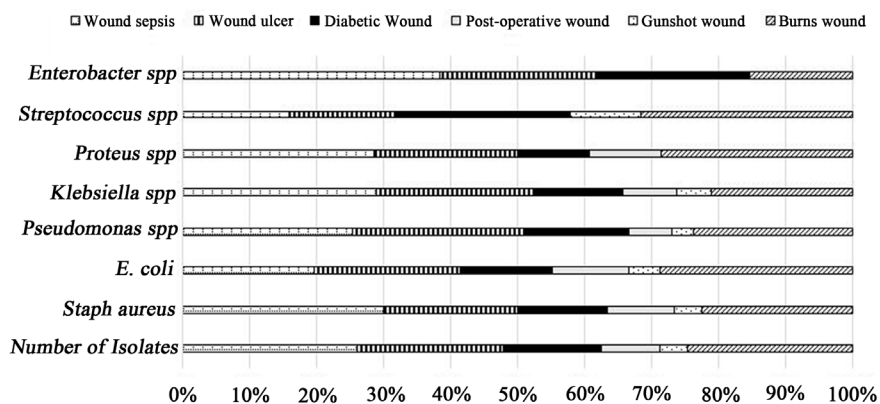


Figure 2. 100% Stacked bar representing the rate of infection of wounds by potential bacterial pathogens.

Table 5. Percentage susceptibility profile of gram-positive bacteria isolated from infected wounds of patients attending selected hospitals in Maiduguri metropolis.

Antibiotic	<i>Staph. aureus</i> n = 120		<i>Streptococcus</i> spp. n = 19	
	Sensitive	Resistant	Sensitive	Resistant
CH	37.5	62.5	63.2	36.8
LEV	73.3	26.7	79.0	21.1
CN	34.2	65.8	47.4	52.6
RD	53.3	46.7	63.2	36.8
NB	46.7	53.3	52.6	47.4
APX	12.5	87.5	36.8	63.2
E	27.5	72.5	31.6	68.4
AMX	8.3	91.7	26.3	73.7
S	51.7	48.3	68.4	31.6
CPX	70.0	30.0	84.2	15.8

Key: CH: Chloramphenicol; LEV: Levofloxacin; CN: Gentamicin; RD: Rifampicin; NB: Norfloxacin; APX: Ampiclox; E: Erythromycin; AMX: Amoxicillin; S: Streptomycin; CPX: Ciprofloxacin.

4. Discussions

The array of bacterial species observed in this study are a mixture of classical and opportunistic pathogens and most of which are thought to possess extra traits that puts them at an advantage or enhances their pathogenic potential. One of these characters is the ability to form biofilm [21]. This is a structured community of microbes with genetic diversity and variable gene expression (phenotype) that creates behaviours and defences used to produce unique wound infections (chronic infection), with a significant tolerance to antibiotics and biocides whilst remaining protected from host immunity [22]. Infection of wounds with such pathogens can potentially lead to challenges in therapy due to antibiotic resistance.

In this study, it was observed that the rate of infection was most pronounced

among male patients that are within the third decade of life. This is similar to the findings of [11] and [23], who asserted that the predominance among patients in this category is most likely due to the fact that male exposure to a possible wound and/or trauma is greater as they represent the majority of the workforce responsible for hard/risky labour. Others suggested that age significantly affects the prevalence of wound infections, since adolescent and active-age adults are usually the ones involved in activities such as sports and farming which may expose them more to injuries and infections [3].

The hospital whose patients had the highest bacterial contamination was UMTN. This reflects the findings of [11] and [24], who observed that over stretched facilities as a result of overcrowding and inadequate infection prevention and control practice was found to be chief among the major problems bedevilling the hospital.

Staphylococcus aureus was the most predominant bacteria spp observed in this study. This is contrary to the finding of [24] but concurred with reports of similar studies conducted from different parts of Nigeria [3] [25] [26] [27] [28]. Some suggested that the sources of most wound infections are endogenous flora of the patient's skin or mucous membrane. *Staphylococcus aureus*, *E. coli* and *Pseudomonas* spp. are among major bacterial species incriminated in nosocomial wound infection and are associated with bacteraemia, septicaemia, shock and prolonged hospital stay [4]. *S. aureus* is the major causative agent of surgical wound infections and epidermal skin diseases in newborn infants [29]. Virulence in *S. aureus* is mediated by the release of several virulence factors like invasins, hyaluronidase, catalase, coagulase, hemolysins, leukotoxin, and leukocidin [30]. These enzymes have invasive and degradative abilities in tissues and can enhance the progression of wound disease.

E. coli has also been isolated in significant numbers, together with *Pseudomonas aeruginosa*. *E. coli* naturally inhabit the gastrointestinal tract and are associated with skin infections in regions of close proximity to the rectum, particularly with incontinent individuals. Individuals undergoing surgical procedures associated with the gastrointestinal tract and lower regions of the spine are also at risk of contracting infection [5]. *Pseudomonas* spp. has been implicated in diverse nosocomial infection likes nosocomial pneumonia, urinary tract infection, surgical site infection, severe burns and infections of patients undergoing either chemotherapy for neoplastic disease or those on antibiotic therapy [31] [32]. The unique feature of *P. aeruginosa* is the resistance to a variety of antibiotics, primarily attributed to low permeability of the cell wall, production of inducible cephalosporinase, active efflux and poor affinity for the target (DNA gyrase) [33].

In this study, wound sepsis and Burns wound were the most colonized. Similar findings were reported elsewhere [11] [24]. Burn wounds and traumatic wounds occurring impromptu, promote multiple infections due to damage to the skin and can induce immune suppression [34] [35].

Infection is a major complication in burn wounds, and is estimated to cause 75% of deaths. Burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts and many studies have indicated the contamination of wounds by aerobes such as *P. aeruginosa*, *S. aureus*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp., and *Candida* spp. [36] [37]. The clinical manifestation of an infected Burns wound is more aggravating especially when it involves dilapidated patients such as diabetic patients, because they are more susceptible to infections due to increased glucose levels and suppressed immune response as well as the neuropathy associated with a decreased blood flow to extremities that lead to slow-healing of the wounds [38].

Infected surgical wounds cause suppurative skin reactions, bacterial fluid lesions and subcutaneous nodules leading to metastasis, when not properly addressed. The risk of infection is generally based on the degree of susceptibility of a surgical wound to microbial contamination [36] [39], clean surgery carries a 1% to 5% risk of postoperative wound infection, and in dirty procedures that are significantly more susceptible to endogenous contamination, a 27% risk of infection has been estimated [36] [40]. The damage in Gunshot wounds in most instances, extends beyond the subcutaneous fat layer of the skin affecting both bone and muscle as well as supporting structures with extensive drainage and tends to be necrotized [10]. The condition may become worse if microbial invasion is involved, the resultant consequences may include prolonged hospital stay associated with difficulties in therapy due to drug resistance, bacteraemia, septicaemia, immune-suppression, shock and even death.

In this study, Ampicillin recorded the highest resistivity rate with an average of 84.7% among Gram-negative bacteria identified. This is in concurrence with the findings of [41] who observed that majority of the organisms isolated among a cohort of hospitalized women suffering from acute antepartum pyelonephritis in Dallas, Texas, were resistant to Ampicillin. Similar findings were reported elsewhere [42]. Quinolones and Aminoglycosides antibiotics were found to be most effective in this study with the exception of Nalidixic acid and Gentamicin. The frequency at which bacterial pathogens acquire resistance properties is quite alarming and patients stand the risk of developing multi-resistant wound infections. However, the prevalence of a particular resistant strain in a particular hospital is related to the frequency of antibiotic usage, and the predominance of a multi-resistant strain may be maintained by the wide spread use of any one of the antibiotics to which it is resistant [43].

5. Conclusion

We found a high infection rate of wounds by potential bacterial pathogens chief among which were *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*. Infection was highest among young adult males. We also reveal that Wound sepsis and Burns wound were the most infected, and isolates identified have shown a marked resistance towards Ampicillin and Amoxicillin.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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