PREVALENCE OF PATHOGENIC BACTERIA ASSOCIATED WITH WOUND INFECTIONS OF PATIENTS ATTENDING OLABISI ONABANJO UNIVERSITY TEACHING HOSPITAL, SAGAMU, NIGERIA

Abstract

Rapidly emerging nosocomial pathogens and the problem of multidrug resistance necessitate periodic review of bacteria pattern isolated in wounds. This study aim to investigate the prevalence of pathogenic bacteria from wound patients in Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Ogun state, Nigeria. Ziehl-Neelsen (ZN) staining techniques, Gram reaction were used to screened Acid fast bacilli and morphology of the bacteria respectively. Biochemical identification of bacteria was done using conventional methods. Gram staining, ZN staining, and 10% KOH mount indicated that 143(36.6%) of the organisms were Gram-negative bacilli, 3(0.97%) acid fast bacilli and 16(4.1%) fungal elements were seen respectively, while 235(60.1%) showed no growth, 388(99.2%) no AFB seen and 375(95.9%) indicated that there was no fungal element seen respectively. Patients in the range of 41-45years old had the highest distribution of 71(18.2%) while 76-80years have the least frequency of 8(2.0%) though it was shown that female constituted the highest percentage 253(61.7%) when compared with male that were 156(38.3%). *Burkhoderia cepacia* 18(12.0%), *Pseudomonas aeruginosa* 17 (11.3%) respectively while *Vibrio choleriae and Vibrio parahaemolyticus* has the least frequency of 2 (0.7%). The present study may be due to the effective diagnostic methods employed in identification of organisms.

Keywords: Gram Reaction, Bacteria, Wounds, Biochemical Identification, Patient

1.0 Introduction

Wound is considered infected when the integrity and protective function of the skin is breached and microorganisms colonize and multiply in the exposed subcutaneous tissue (Oladeinde *et al.,* 2013; Baba *et al.,* 2018). However, abundance and diversity of microorganisms in any wound may be due to factors such, as wound type, depth, location and quality, level of perfusion and the antimicrobials (Onajobi *et al.,* 2020). Hence infections in wound remain a public health problem worldwide (Caflisch *et al.,* 2018).

Rapidly emerging nosocomial pathogens and the problem of multidrug resistance necessitate periodic review of antibiogram patterns of organisms isolated in wounds (Anapurba *et al*., 2003). The widespread use of antibiotics with length of time over which they have been available have also led to major resistant pathogens in wound infections contributing to morbidity and mortality (Nwanchukwu *et al.,* 2009; Baba *et al.,* 2018). In the recent time, the treatment of wound infection is consistently difficult due to increasing rate of resistant bacterial strains (Morrison *et al.,* 2017) and microorganisms growing in a biofilm which are more resistant to antimicrobial agents than planktonic bacteria (Nallapareddy, 2006; Caflisch *et al.,* 2018).

Several authors have reported prevalence of wound infection in some parts of Nigeria including Oladeinde *et al.,* (2013), Akinjogumla *et. al.,* (2009), who reported the prevalence

of 70.1%, 95% and 98.8% from Okada, Uyo and Maiduguri with polymicrobial infection of 24.4%, 65% and 14.5% respectively. Oladeinde *et al.* (2013) reported prevalence of 100% with polymicrobial infection of 29.8% and 70.3% monomicrobial infection. However, their findings are not in agreement, and therefore there is need to resolve. Effective and rapid diagnosis of patients suspected of having wound infection remains a challenge; therefore, it is of public health importance that the available diagnostic facilities be assessed for identification of the microorganisms.

The skin is a complex tissue that consists of various components that effectively protect the body against the external environment due to its multi-layered and conified anatomical barrier and keeps other organs in place (Thibodeau and Patton, 2007). Its role includes homeostatic preservation of body fluid (Thibodeau and Patton, 2007; Guyton and Hall, 2006), thermoregulation (Charkoudian, 2003) and body protection against infection (Fuchs and Horsley, 2008; Fuchs, 2008). The acidity and dryness of the skin, and the secretion of antibodies and antimicrobial inhibitory substances as well as the microbial normal flora on the skin prevent infection (Thibodeau and Patton, 2007). This study aim to investigate the prevalence of pathogenic bacteria from wounds of patient attending Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Ogun state, Nigeria.

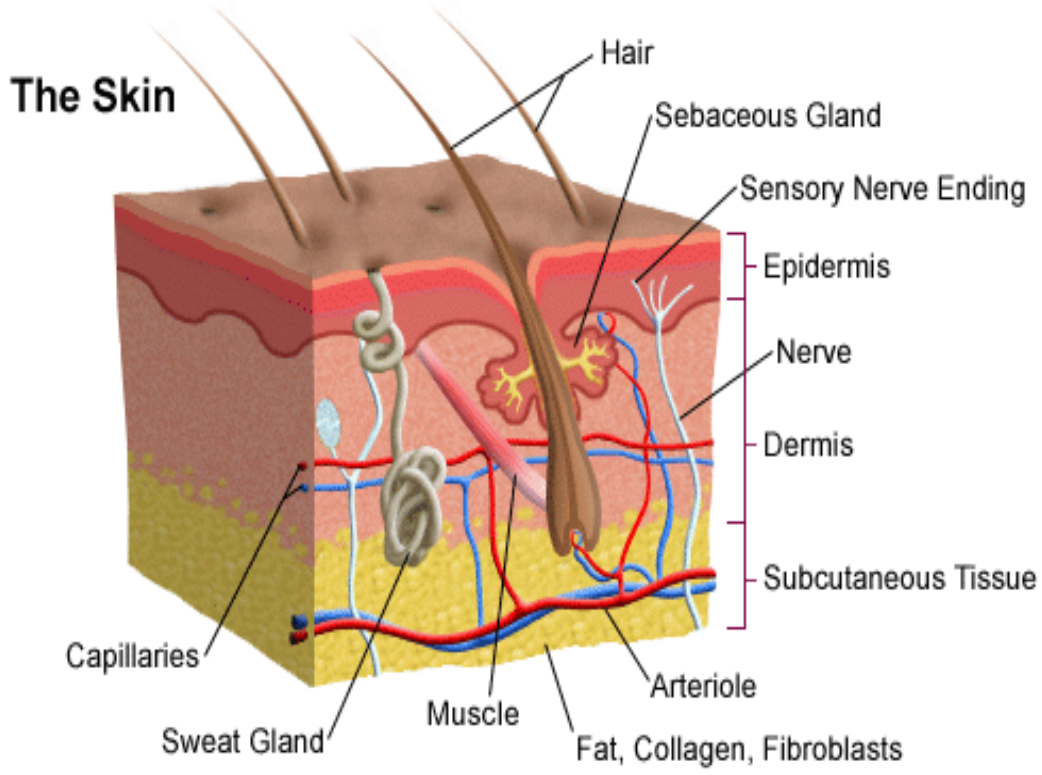

Figure 1: Diagrams of Human skin structure

Source: Thibodeau and Patton (2007)

2.0 Material and Methods

2.1 Study Area

This study was conducted in Olabisi Onabanjo University Teaching Hospital, Sagamu , Ogun state, Nigeria. Sagamu in the Remo division of Ogun State, South west, Nigeria lies on latitude $6^0$50" North and longitude $3^0$39" East with total area of 614km^2 with estimated population of 253,425 (Vanguard News, 2020). Ogun state lies approximately between longitude $2^0$30' E and $4^0$30' E, latitude 6^o30' and 8^0N, and it borders Lagos state to the South.,Oyo and Osun State to the North, Ondo State to the East and Republic of Benin to the West. It has a land mass of 16,762km^2 and a population of 3,751,409 (THISDAYLIVE, 2019).

Source:
https://en.wikipedia.org/wiki/Olabisi_Onabanjo_University_Teaching_Hospital#/map/0

Figure 2: The geographical location of Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Nigeria

2.2 Study Population

A purposive sampling method was used for selecting the patients with wound infection among patients attending Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state, Nigeria between 2016 to 2018.

2.3 Sampling size determination

Sample size was determined using the formula;

$$N = \frac{t \times t \times p\,(1-P)}{m}$$

Where: N = number of sample to be analyzed

t = confidence level at 95% (standard level or normal deviation of 1.96)
p = Estimated prevalence rate
m = margin of error at 5% (standard value or consent deviation of 0.05).

Considering similar study by Mofikoya *et.al*. (2011) the minimum number of 310 sample size was calculated. Therefore, a minimum number of 391 samples were examined.

2.3.1 Inclusion Criteria

Patients recruited into this study were those that had clinical lesions of wound on their body and sign or thumbprint consent form.

2.3.2 Exclusion Criteria

Patients that were excluded from this study were those without clinical lesions of wound and patients that refused to sign or thumbprint the consent form were exempted from the study.

2.3.3 Ethical Consideration

Prior to the commencement of this study, ethical approval for this study was obtained from Hospital Research Ethical Committee (HREC), Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state, Nigeria.

2.4 Sample collection

2.4.1 Wound Sample

The wounds were judged as infected by the presence of purulent material and the cleansing of wound was done aseptically using 60-120ml of sterile normal saline. A visible $1cm^2$ area of viable wound bed tissue was swabbed by rotating the sterile swab stick (Sterilin, UK) for a period of 5 seconds using gentle pressure to release tissue exudates. The wound swabs collected were transported to the laboratory at room temperature within one hour.

2.5 Sample Processing

2.5.1 Preparation of wound smears

A frosted end slide was labelled with laboratory serial number on the swab stick using grease pencil. The first swab received was used for direct microscopy. The smear was prepared by rolling the swab stick on a clean, grease-free glass slide over a 1x2cm area of the unfrosted area of the slide. The smear was allowed to air dry and then heat-fixed by passing the slide over a flame 2- 3 times for about 2-3 seconds each. Smears were separately stained by Gram and Ziehl-Neelsen staining techniques and screened for pus cells, Gram reaction, morphology, acid fast bacilli, number and arrangement of the organisms were noted respectively.

2.5.2 Gram staining technique

The prepared smears were thinly covered with crystal violet (primary stain) solution for 30-60seconds.The stain was washed off by rinsing the smears with sterile distilled water. Lugol s iodine solution (mordant) was applied to the surface of the smear for 45-60seconds and the smears were later rinsed with distilled water. The smears were then decolourized with acetone until the purple dye no longer flow from the smear and rinse with distilled water to prevent excessive decolourization after which the slides were covered with Safranin solution (secondary stain) and left for 60 seconds. The slides were rinsed again with distilled water and allowed to air dried. A drop of immersion oil was applied onto each smear and they were examined under oil immersion (x100) objective (Olutayo *et al.,* 2017).

2.5.3 Ziehl-Neelsen -Staining Technique.

The smear was covered with filtered strong carbol fucshin and heat was applied underneath the slide with flame intermittently to steam without boiling. The heated stain remained on the slide for 10minutes.The slide was rinsed with water and decolourised using 3%v/v acid alcohol for 3 minutes. Counterstaining was done using 0.3% methylene blue for 1 minute and allowed to drain and then air dried. It was then examined under the microscope using oil immersion objective (x100) according to CLSI (2017).

2.5.4 Direct KOH Mount

Wound swabs were emulsified with two drops of 15% Potassium hydroxide solution on a clean grease free slide, the mixture was covered with cover slip Thereafter, it was examined microscopically using x10, x 40 objective lens of the microscope to search for the presence of fungal elements according to CLSI (2017).

2.5.5 Bacteria Culture and isolation

Swab samples were inoculated into various culture media including McConkey Agar, Blood Agar, and Chocolate Agar by streaking method. Cultures were incubated for 18-24 hours at 37^0C and growing colonies were identified to CLSI (2017).

2.5.6 Biochemical Characterization of the bacterial Isolates

The following biochemical tests; Catalase, Indole, Kliger Iron Agar, Urease and Sugar fermentation tests were done to confirm and identify the bacteria isolates from quarry effluent and domestic water sampled according to CLSI (2017) and Olutayo et al. (2017).

2.5.6.1 Catalase Test

Pure isolates were transferred from nutrient agar slope using a sterile loop onto a clean grease free slide. This was air- dried and inserted into hydrogen peroxide solution held in small clean tube. Positive result produce bubbles while negative result produces none (Olutayo et al., 2017).

2.5.6.2 Sugar Fermentation Tests

Peptone water was supplemented using 0.015g of bacto-brews-cressol purple (DIFCO laboratories) as an indicator. This served as a base medium. Then 1% of the respective carbohydrate such as glucose, lactose, maltose and fructose were added. This was stirred to dissolve completely over a bunsen burner. It was then dispensed into test tubes in 5ml aliquots and Durham tubes added in inverted position. This was then plugged with cotton wool and sterilized at 121oC for 15 minutes. About 5ml of the sugar medium were inoculated with the test organism and incubated at 37oC for 3-5 days. The production of yellow color in the medium indicated positive carbohydrate fermentation reaction. Lack of yellow color in the medium indicated negative carbohydrate fermentation reaction. Gas formation is indicated by the appearance of gas bubbles in the durham tube (CLSI, 2017; Olutayo *et al.*, 2017).

2.5.6.3 Indole Test

The test illustrates the ability of specific bacterial organisms to degrade the amino acid called tryptophan to indole which accumulates in the medium. The medium were sterilized by autoclaving at 121^{o}C for 15 minutes. The peptone water broth tubes were inoculated with the pure cultures of the isolates and incubated at 37^{o}C for 24- 48 hours. After incubation, 0.5 ml of Kovac's reagent was added to the broth culture down the side of the tube and shaken gently. Observe color change at meniscus. The development of a brown-red ring within 20 seconds indicated positive indole reaction while negative test is colorless or slightly yellow (Olutayo *et al.*, 2017).

2.5.6.4 Urease Test

The Christensen's urea medium was used for the test. The medium was sterilized by autoclaving at 121oC for 15 minutes. Then inoculate the broth with inoculum from an 18-24 hour pure culture. Shake the tube gently to suspend the bacteria. Incubate the tubes at 37^{o}C and observe for color changes. Positive urea enzyme production changes the slant from light orange to pink while negative urea produces no color change (Olutayo *et al.*, 2017).

2.6 Statistical Data analysis

Analysis of variance (ANOVA) was used to test for the significance of the wound location samples, Age of patients attend OOUTH taking p value >0.05 at 95% confidence interval according Zhang and Liang (2014).

3.0 Results and Discussion

The figure 3 showed the frequency distribution of patients with wound infections in various wards and clinics at OOUTH. The figure 3 also shows that patients attending GOPD has the highest frequency of 113 (33.3%) while patients attending orthopaedic clinic has the least frequency of 34 (8.7%).

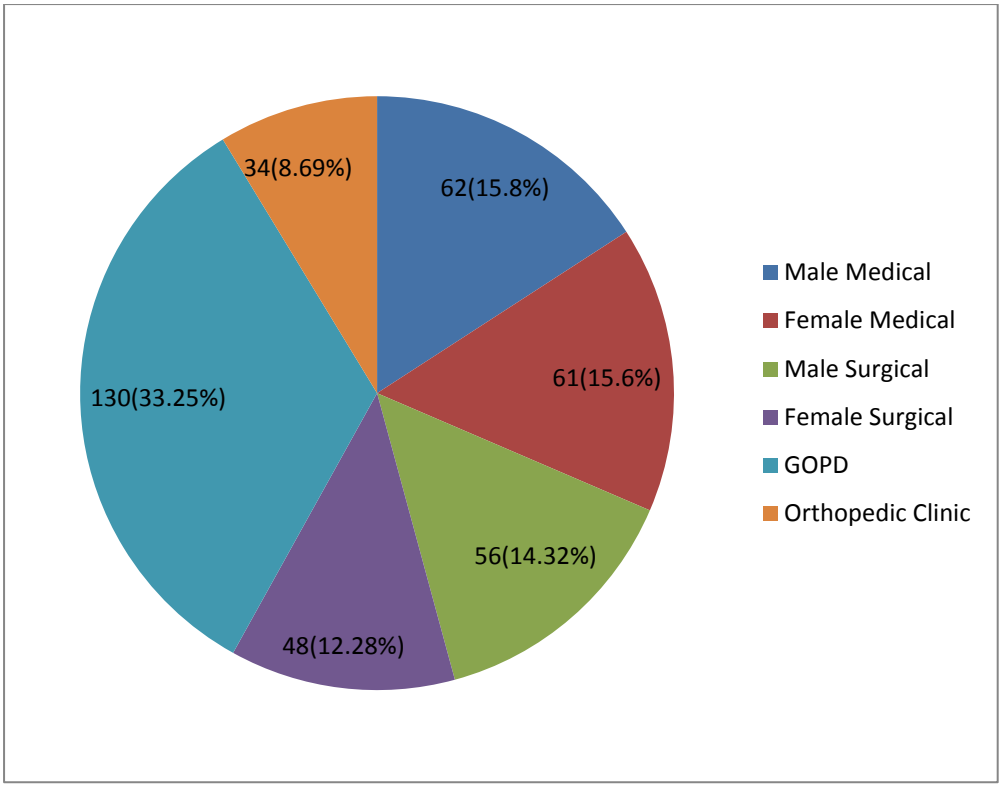

Figure 3: Frequency distribution of patients with wound infections among various Wards / Clinic at OOUTH

Table 1: Distribution of wound sample sites among various ward /clinics at OOUTH, Sagamu

Site of Wound	Wards/clinics at OOUTH Sagamu												Total	
	Male Medical		Female Medical		Male Surgical		Female Surgical		GOPD		Orthopedic Clinic			
	N	(%)	n	(%)	n	(%)	N	(%)	n	(%)	n	(%)	n	(%)
LEG	12	(10.9)	15	(13.6)	12	(10.9)	12	(10.9)	50	(45.5)	9	(8.2)	110	(28.0)
KNEE	2	(20)	2	(20)	1	(10)	1	(10)	3	(30)	1	(10)	10	(2.6)
OPERATION	3	(15.8)	2	(10.5)	3	(15.8)	2	(10.5)	7	(36.8)	2	(10.5)	19	(4.9)
BONE	4	(14.3)	6	(21.4)	5	(17.9)	4	(14.3)	8	(28.6)	1	(3.6)	28	(7.2)
BURN ULCER	1	(10)	1	(10)	2	(20)	2	(20)	3	(30)	1	(10)	10	(2.6)
SHOULDER	2	(22.2)	1	(11.1)	1	(11.1)	1	(11.1)	3	(33.3)	1	(11.1)	9	(2.3)
FOOT	12	(16.4)	13	(17.8)	12	(16.4)	10	(13.7)	19	(26.0)	7	(9.6)	73	(18.7)
ABDOMEN	14	(19.7)	12	(16.9)	12	(16.9)	10	(14.1)	16	(22.5)	7	(9.9)	71	(18.2)
CHEST	4	(22.2)	3	(16.7)	3	(16.7)	2	(11.1)	5	(27.8)	1	(5.6)	18	(4.6)
HAND	4	(17.4)	3	(13.0)	2	(8.7)	2	(8.7)	9	(39.1)	3	(13.0)	23	(5.9)
SKIN	4	(20)	3	(15)	3	(15)	2	(10)	7	(35)	1	(5)	20	(5.1)
TOTAL	62	(15.9)	61	(15.6)	56	(14.3)	48	(12.3)	130	(33.3)	34	(8.7)	391	(100)

$\chi^2 = 21.489$, $P>0.05$

Key: GOPD = General out-patients department

Table 1 showed the distribution of wound from various anatomical sites in the ward/clinic. This table 1 showed that wound from leg has the highest frequency 110 (28.1%). It was revealed that wound from shoulder has the least frequency of 9 (2.3%) and there was no significant association between site of wound and ward/clinic, that is, not significantly associated. $\chi^2 = 21.49$, $p>0.05$, This was not similar to the study carried out in Niger Delta area of Nigeria where non-traumatic wound (including diabetic foot ulcers) accounted for more than a third of all infected wounds (Kemebradikumo *et al.*, 2013). The present study corresponds to the work carried out in India (Shashikala *et. al*., 2016) where diabetic foot ulcers had high prevalence of 25%.

Table 2: Age and Gender distribution of patients with wound infections

Age Range	Gender				TOTAL	
	Male		Female			
	N	(%)	N	(%)	N	(%)
<20	0	(0)	5	(100)	5	(1.3)
21-25	8	(44.4)	10	(55.6)	18	(4.6)
26-30	20	(36.4)	35	(63.6)	55	(14.1)
31-35	16	(28.6)	40	(71.4)	56	(14.3)
36-40	16	(39)	25	(61)	41	(10.5)
41-45	16	(22.5)	55	(77.5)	71	(18.2)
46-50	24	(61.5)	15	(38.5)	39	(10)
51-55	20	(44.4)	25	(55.6)	45	(11.5)
56-60	4	(44.4)	5	(55.6)	9	(2.3)
61-65	8	(100)	0	(0)	8	(2.0)
66-70	8	(44.4)	10	(55.6)	18	(4.6)
71-75	8	(44.4)	10	(55.6)	18	(4.6)
76-80	8	(100)	0	(0)	8	(2.0)
Total	156	(38.3)	235	(61.7)	391	(100)

χ^2=48.191, P<0.05

Table 2 showed the distribution of age and gender among patients presenting with wound infection. The table indicated that patients in the range of 41-45years old had the highest distribution of 71(18.2%) while 76-80years have the least frequency of 8(2.0%) respectively though it was shown that female constituted the highest percentage 253(61.7%) when compared with male that were 156 (38.3%). In a study carried out in Niger Delta by Pondei *et al.* (2013), the age group related to wound was between 21-30 years old. The age of a patient seems likely to have a bearing on wound infection and healing, people at the extremes of life being more prone to wound infections. In the present study, the wound infection is more associated with age range 41- 45years old among female patients. χ^2 =48.191, p <0.05, this was not in agreement with the study carried out in Northern Nigeria where it was reported that male were more prevalent than female due to the fact that males spend most of their time outdoors doing hard jobs that could exposed them to more risk of amputation than female (Baba *et al.,* 2018), but in this study the more prevalence in female might be borne out of the fact that female encountered occupational hazards like their male counterparts and frequent visitation to the hospital. The present study was in disagreement with a study conducted in United State of America with male gender associated with wound infection (75.2%) and female (24.8%) (Williams, 2017) and a study in a tertiary hospital, Benin city, Nigeria conducted by Egbe *et al*. (2011) which was non-significantly affected by gender but affected by age, $p< 0.001$ where minimum age of prevalence of wound infection was $< 5 years\ old$ and maximum age group was 36-40years old.

Table 3: Distribution of isolated microorganisms from wound of patients attending OOUTH using Microscopy

Microscopy	Reaction Frequency N	(%)
Gram's staining :		
Gram-positive bacteria		
Cocci	13	(3.3)
Bacilli	0	(0)
Gram-negative bacteria		
Cocci	0	(0)
Bacilli	143	(36.6)
No Growth	235	(60.1)
Total	391	(100)
Total bacterial Growth	156	39.9
Ziehl Nelseen Staining:		
AFB seen	3	(0.8)
No AFB seen	388	(99.2)
Total	391	(100)
10 % KOH mount:		
Fungal element	16	(4.1)
No Fungal element seen	375	(95.9)
Total	391	(100)

Key: AFB = Acid fast bacilli, KOH = Potassium hydoxide

Table 3 showed the micro-organisms obtained from wound of patients attending OOUTH using microscopic morphology. In this table it was shown that Gram staining, ZN staining, and 10% KOH mount indicated that 143(36.6%) of the organisms were Gram-negative bacilli, 3(0.97%) acid fast bacilli and 16(4.1%) fungi elements were seen respectively, while 235(60.1%) showed no growth, 388(99.2%) no AFB seen and 375(95.9%) indicated that there was no fungi element seen respectively. This is in agreement with the study conducted in Okolobiri, Niger Delta area of Nigeria where it was also found that Gram-negative bacteria were the most commonly isolated pathogens (Pondei *et al.,* 2013). The present study disagree with the study carried out in India (Shashikala *et al.,* 2016) with predominance of Gram positive organisms (75.6%) while 24.4% were Gram negative organisms and als in disagreement with the study by Awad *et al.* (2017) which showed that Gram-positive organisms were predominant causative organisms of wound infection. The result from a study in Cameroun (Pondie *et al.,* 2013) corroborates the present study and elsewhere having Gram-negative organisms as the major causes of wound infection. The predominance of Gram negative organisms has been noted in several studies (William *et al*., 2011).

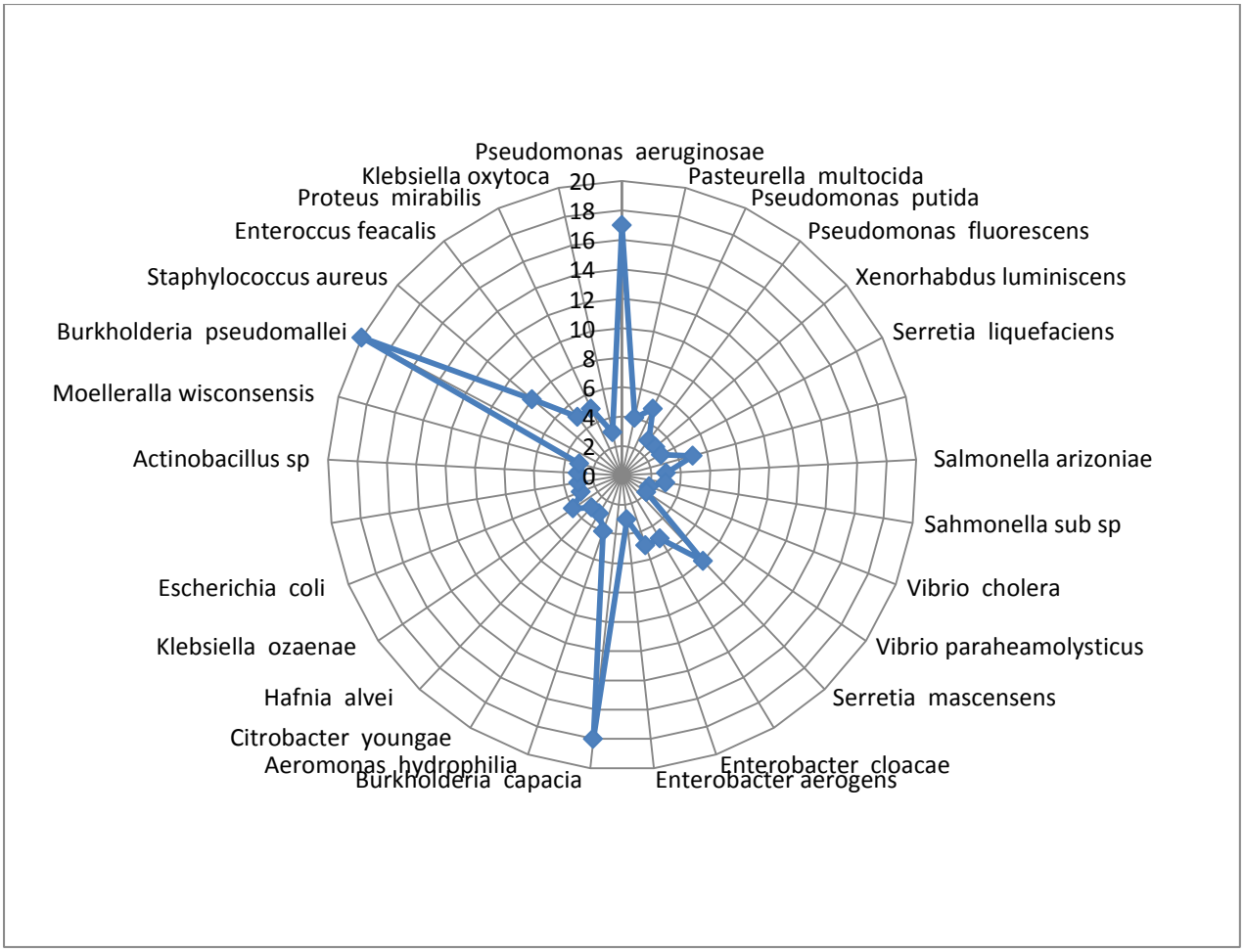

Figure 4: Distribution of bacteria isolated from wound of patients attending OOUTH

The figure 4 showed distribution of bacteria isolated from wound of patients attending OOUTH. This figure revealed that *Burkhoderia pseudommallei* has the highest frequency of 20(13.3%). Followed by *Burkhoderia cepacia* 18(12.0%), *Pseudomonas aeruginosa* 17 (11.3%) respectively while *Vibrio cholerae and Vibrio parahaemolyticus* has the least frequency of 2 (0.7%) respectively and was not in agreement with the study carried out by Pondei *et al.* (2013) where *Staphylococcus aureus* (40.3%) was the most predominant isolate followed by *Pseudomonas aeruginosa* (23.9%), while *Citrobacter* sp. was the least (0.5%) (Pondei *et al.*, 2013) and the study conducted in India Shashikala *et al*. (2016) where *Staphylococcus aureus* and *Escherichia coli* were the most commonly isolated organisms and the findings of a study by Baba *et al*. (2018) in which 76% of the microbes were Gram – negative and 24% were Gram-positive organisms corresponds to the findings in the present study. However, a study conducted in Malaysia reported *Pseudomonas aeruginosa* to be the predominant Gram- negative organism (Onajobi *et al*., 2020). The result of the present study

may be due to the effective diagnostic methods employed in identification of organisms. However, certain studies (Lipsky *et al*., 2009; Onajobi *et al*., 2020) have established a higher proportion of Gram positive organisms to corroborate this study.

4.0 Conclusion

The study shows that distributions of patients with wound infections are more frequent among those attending general outpatient departments (GOPD). Age range 41 to 45 years were mostly affected which was significantly associated with females. Study reveals that varieties of oxidase positive and oxidase negative gram negative bacilli were isolated out of which *Burkhoderia pseudomallei* is predominant. Out of 391 wound swab samples collected, *Burkholdera pseudomalei* was the highest showing 13.3% occurrence rate, followed by *Burkoldera cepacia* (12.0%), *Pseudomonas aeruginosa* (11.3%) and least *Vibro cholera* and *Vibro parahaemolyticus* (2.0%) respectively. The present study also reveals the effective diagnostic methods employed in identification of organisms. Laboratory capacity should be strengthened to accommodate improved diagnostic techniques and facilities for effective wound management. Due to increasing rate of antibiotics resistant bacterial strains in wound infections among patients attending OOUTH, Sagamu, Ogun state, Nigeria, proper personal hygiene and awareness on the use of antibiotics and practical intervention programs are needed to prevent and control infections in OOUTH, Sagamu, Nigeria.

References

Akinjogunla OJ, Adegoke AA, Mboto CI, (2009). Bacteriology of automobile accident wounds infection. *International Journal of Medicine and Medical Sciences;* **1**(2): 023-027.

Anapurba D, Bhart KK, Sapna UK (2003). Sensory and Physico-chemical properties of commercial samples of honey, *Food Research International*; **36**: 18-191.

Awad ME, López-Galindo A, Setti M, El-Rahmany MM, Iborra CV. (2017). Kaolinite in pharmaceutics and biomedicine. Int J Pharm 533:34–48.

Baba J., Ajaegbu1 V.E., Mohammed S.B., Abdullahi M., Olutimayin A.T., ZakariY.(2018): Antibiotic Susceptibility Studies of Pseudomonas aeruginosa Isolated from Wounds of Patients Attending General Hospital Minna, Nigeria , *Nigeria of Journal Pure & Applied. Science* Vol. 31.

Caflisch K.M. et al., (2018). Antibacterial activity of reduced iron clay against pathogenic bacteria associated with wound infections, International Journal of Antimicrobial Agents, https://doi.org/10.1016/j.ijantimicag.07.018

Charkoudian N (2003) Skin blood flow in adult human thermoregulation: How it works, when it does not, and why. *Mayo Clinic Proceedings*; **78**(5): 603-612.

Clinical Laboratory Standard Institute, (2017). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI.

Egbe CA, Omoregie RA, Igbarumah I, Onemu S. (2011). Microbiology of Wound Infections Among patients of Tertiary Hospital in Benin City, Nigeria. *J Res Health Sci*; 11(2):109-113.

Fuchs E and Valerie Horsley (2008). More than one way to skin. *Genes & Development*; **22**: 976 - 985.

Fuchs E. (2008). Skin stem cells: Rising to the surface. *The Journal of Cell Biology*; **180**(2): 273 - 284.

Guyton AC and Hall JE. (2006). Textbook of Medical Physiology, 11th edition; (Chapter 33: Resistance of the body to infection: I. Leukocytes, Granulocytes, the Monocytes-Macrophage system and inflammation); pages 429 - 438. Elsevier Saunders; Philadelphia, Pennyslvania, USA.

Kemebradikumo Pondei, Beleudanyo G. Fente, and Oluwatoyosi Oladapo. (2013): Current Microbial Isolates from Wound Swabs, Their Culture and Sensitivity Pattern at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria.

Lipsky BA. (2008). New developments in diagnosing and treating diabetic foot infections. *Diabetes /Metabolism Research and Reviews*; **24** (Suppl 1):S66-71.

Mofikoya et al, (2011): Predictors of surgical site infections of the abdomen in Lagos Nigeria.

Morrison KD, Williams SN, Williams LB. (2017). The anatomy of an antibacterial clay deposit: A new economic geology. Econ Geol;112:1551– 1570.

Nallapareddy SR, Singh KV, Sillanpää J, (2006) Endocarditis and biofilm-associated pili of *Enterococcus faecalis. Journal of Clinical Investigation*; **116**(10): 2799-2807.

Nwachukwu, N.C,F.A and Okike, U.M. (2009). Antibiotic susceptibility patterns of Bacterial isolates from surgical wounds in Abia state University Teaching Hospital (ABSUTH), Aba-Nigeria, Research Journal of Medical Sciences, 4(2) 575-579.

Oladeinde, BH Omoregie R, Olley M, Anunibe JA,and Onifade A A. (2013) A 5 - year surveillance of wound infections at a rural tertiary hospital in Nigeria.

Olutayo I. Falodun, Yetunde M. Morakinyo and Obasola E. Fagade. (2018). Determination of Water Quality and Detection of Extended Spectrum Beta-Lactamase Producing Gram-Negative Bacteria in Selected Rivers Located in Ibadan, Nigeria. Jordan Journal of Biological Sciences.Volume 11, Number 1.Pages 107-112.

Onajobi I. B., Idowu E. O., Adeyemi J. O., Samson O. J., Ogunyinka P. I., Fagade O. E.. (2020). In vitro antibacterial activities and molecular characterization of bacterial species isolated from farmlands against selected pathogens, Biotechnology Reports, Volume 27, e00513, ISSN 2215-017X, https://doi.org/10.1016/j.btre.2020.e00513.

Pondei Beleudanyo G. Fente, Oluwatoyosi Oladapo (2013): Current Microbial Isolates from Wound TMH Swabs, Their Culture and Sensitivity Pattern at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria Kemebradikumo

Thiobodeau GA and Patton KT (2007) Anatomy and physiology 6th ed. (Chapter 6; Skin and its appendages, pages195-228). Mosby Elsevier. St Louis, Missouri, USA.

THISDAYLIVE. (2019). Restoring Olabisi Onabanjo Varsity Teaching Hospital's Past Glory. Retrieved 2022-04-22.

Vanguard News. (2020). We will return OOUTH to its glorious days ― Coker. Retrieved 2022-04-22.

Williams LB, Metge DW, Eberl DD, Harvey RW, Turner AG, Prapaipong P, et al. (2011). What makes a natural clay antibacterial? Environ Sci Technol 45:3768–73.

Williams LB. (2017). Geomimicry: Harnessing the antibacterial action of clays. Clay Miner 52:1–24.

Zhang JT, Liang X (2014) One-way ANOVA for functional data via globalizing the pointwise FF-test. Scand J Stat 41:51–71