Original Research Article

Pyogenic Anaerobes of Wound Infection and the Associated Risk Factors Among Patients in Uyo, Southern Nigeria.

ABSTRACT

Aims: Anaerobic causes of pyogenic wound infection are not usually investigated due to difficulties in cultural techniques, lack of equipment and technical man-power. Therefore, this study aimed to investigate the anaerobic bacterial agents of pyogenic infection and the associated risk factors among patients in a tertiary hospital.

Study design: This was a descriptive cross-sectional study conducted at the University of Uyo Teaching Hospital, Uyo and carried out on 136 wound samples from patients. These samples were collected from all consented patients with pyogenic wound infection that met the inclusion criteria.

Place and duration of study: The study was conducted at Uyo, the capital city of Akwa Ibom State, Nigeria between April and October, 2018.

Methodology: Deep wound swabs or aspirated pus samples were collected and inoculated into fresh 25% Sheep Blood Agar plate and incubated in an anaerobic jar containing anaerobic indicator and Gas-pak at 37^{o}C for 48 – 72 hours Identification of isolates was performed following standard procedures. Data were obtained through a well- structured questionnaire and analyzed using SPSS software.

Results: Of the 136 samples collected, 127 yielded microbial growth with a total of 202 isolates which included 50 and 2 pure growths of aerobes and anaerobes respectively and 75 combined growths of aerobes and anaerobes. Overall, more aerobes (125) were isolated when compared to the anaerobes (77). Nevertheless, the predominant anaerobe was *B. fragilis* 26(33.8%). There was a statistical significant relationship between the age of the patient and infection by gram- positive anaerobes ($p = 0.002$).

Conclusion: *Bacteriodes fragilis* is mainly involved in anaerobic pyogenic wound infections in Uyo, however, only the age of the patient was found to be a factor in the prevalence of infection by gram-positive anaerobes.

Keywords: Pyogenic, anaerobes, wound infections, Southern Nigeria.

1. INTRODUCTION

Infections of the human skin and soft tissues are generally associated with pus formation and the bacteria involved are said to be pyogenic or pus producing. Pyogenic infections are characterized by local and systemic inflammation usually with pus formation.[1] These may originate endogenously through auto-infections caused by resident microflora, or from exogenous sources. A break in the skin can provide a portal of entry to the surface bacteria which start multiplying locally. The body's defense mechanism responds by bringing immune cells to the site to fight against bacteria. The accumulation of these cells produces pus which is a thick whitish fluid composed of dead white blood cells (WBCs), cellular debris, and necrotic tissues. [2,3,4,5] A wide variety of aerobic and anaerobic bacteria have been implicated in wound infections either singly or in combination. The most predominant aerobic pyogenic bacteria are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pneumonococcus, Coliform bacilli* such as *Escherichia coli* and environmentally acquired bacteria such as *Proteus species* and *Pseudomonas aeruginosa*. Anaerobic organisms are mainly *Clostridium perfringens* and other *Clostridia*, *Bacteroides* species and anaerobic cocci.[6]

Anaerobic bacterial infections which often present as abscesses are a common cause of wound infections, some of which can be serious and life-threatening.[7] Anaerobic bacteria are the main members of the indigenous, normal human flora, including the skin and the oral, gastrointestinal, and vaginal mucosa.[8] Because of their fastidious nature, anaerobes are hard to culture and isolate and are often not recovered from wound infected sites. The isolation of

anaerobic bacteria requires adequate methods for collection, transportation and cultivation of clinical specimens.[9]

The often notable conditions predisposing to anaerobic infections include: exposure of a sterile body location to a high inoculum of indigenous bacteria of mucous membrane flora origin, inadequate blood supply and tissue necrosis which lower the oxidation and reduction potential thereby supporting the growth of anaerobes. Conditions that can lower the blood supply and can predispose to anaerobic infection are: trauma, foreign body, malignancy, surgery, edema, shock, colitis and vascular disease. Other predisposing conditions include splenectomy, neutropenia, immunosuppression, hypogammaglobinemia, leukemia, collagen vascular disease and cytotoxic drugs and diabetes mellitus.[7, 9]

2. METHODOLOGY

2.1 STUDY DESIGN

This study was a descriptive cross-sectional hospital-based study of patients with pyogenic infections at different wards, units and clinics. It was carried out at the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, South-South region of Nigeria. Uyo lies between latitude 5.5^{o}N and 6.0^{o}N, and longitude 6.0^{o}E and 6.5^{o}E of the Greenwich Meridian.

2.2 SAMPLE SIZE

A total of 136 wound samples were used for this study which was calculated using a prevalence rate of 8.8% as obtained from a related study at the University of Uyo Teaching Hospital, Uyo.[10] All wound samples that meet up with the inclusion criteria were selected.

All pus samples from various wound sites of adult male and female patients on admission in the different hospital wards and outpatient departments were included in the study.

While children and adult patients with wound infection involving the eyes and upper respiratory tracts or on catheter were excluded.

Data on demographics and other information bordering on predisposing factors were collected from patients through self-administered well structured-questionnaire.

2.3 SAMPLE COLLECTION

Sample Collection included cleaning the surface of a wound and/or abscess with normal saline and 70% alcohol respectively before collecting samples with swabs for deep wounds or aspirates for abscesses. Aspirates were transported in anaerobic transport tubes while swabs were placed in sterile tubes containing carbon dioxide in free oxygen for immediate processing in the microbiology laboratory.[11]

2.4 SAMPLE PROCESSING

For anaerobic Culture; the specimen was inoculated onto fresh 25% Sheep Blood Agar plate and incubated in an anaerobic jar containing anaerobic indicator and Gas-pak at 37^{o}C for 48 – 72 hours.[12] Isolates were inoculated onto Thioglycollate broth (for storage) and Blood Agar plates and incubated aerobically for confirmation of obligate anaerobes which do not grow when cultured aerobically.[13]

Biochemical Identification of Anaerobes was carried out with the following tests:

(a) The 20% Bile-inhibition test (Bacteroides Bile Esculin Test): This test was used for the preliminary identifications of *Bacteroides fragilis.* Anaerobic isolates were inoculated onto 20% Bile Esculin agar prepared according to manufacturers' manual and incubated anaerobically. This was observed after 18-48 hours. Esculin hydrolysis is indicated by browning or blackening in the medium surrounding a colony.

(b) Egg Yolk Base Agar Test: Egg Yolk Agar is used for the isolation and differentiation of *Clostridium* species and other relevant anaerobic organisms based on lecithinase and lipase activity. The medium was allowed to reach room temperature and an appropriate organism for anaerobic culture was inoculated and Incubated anaerobically at 33-37^{o}C for 48-72 hours. This was examined for lecithinase and lipase activity. An opaque precipitate in the medium surrounding the colonies showed positive Lecithinase production, while negative test was identified by the absence of opaque precipitate. Lipase production was indicated by an iridescent sheen of oil on water appearance on the surface of growth and the surrounding medium while negative test showed no iridescent sheen. *Clostridium perfringens* was lecithinase negative and lipase positive, while *Bacteroides fragilis* was lecithinase and lipase negative.

(c) Antibiotic Agar Presumptive Disk Identification system for Anaerobes: This system was used to further identify anaerobic organisms based on susceptibility reaction to various antibiotics. Antibiotic susceptibility testing was done for the presumptive identifications of anaerobes using Modified Kirby Bauer's Disc Diffusion Method on Mueller Hinton Agar containing 5% sheep blood at 0.5 MacFarland Turbidity Standard and interpreted as per the CLSI guidelines.[14]

The following standard antimicrobial agents, Kanamycin (1000 µg), Penicillin (2 µg), Erythromycin (60 µg), Clindamycin (30 µg) and Vancomycin (5 µg) were used (Oxoid, UK). Presumptive identification criteria for anaerobes are; *Bacteroides fragilis* was resistant to Penicillin, Vancomycin and sensitive to Erythromycin. Other *Bacteroides* were sensitive to Erythromycin and Penicillin and resistant to Kanamycin. *Prevotella* species were sensitive to Erythromycin and Penicillin but Resistant to Kanamycin. *Peptostreptococci* and *peptococci* species were sensitive to Penicillin and Vancomycin and resistant to Colistin. *Clostridium perfrigens* was sensitive to Penicillin and Vancomycin.

Bacteroides fragilis (ATCC 25285) and *P. aeruginosa* (ATCC 27853) for isolation of anaerobes were used as quality control strains.

2.5 STATISTICAL ANALYSIS

Samples were collected from all consented patients with pyogenic wound infection that met the inclusion criteria. The completed questionnaires and the results of the processed wound samples were handled using computer applications and software. Data analysis was carried out using SPSS (Statistical Package for Social Sciences) Version 21 and Mini TaB Version 17. Proportions were compared using Chi-square test with Confidence Interval 95% value put at $p < 0.05$ deemed statistically significant.

2.6 CONSENT AND ETHICAL CONSIDERATION

Consent was sought and obtained from all participants prior to inclusion in this study. Ethical clearance and approval (reference no.: UUTH/AD/3/96/VOL.XXI/J4, dated April 1, 2017) was gotten from the Ethical Review Board of the University of Uyo Teaching Hospital prior to commencement of this study.

3. RESULTS

Out of 136 included patients, 76 (55.9%) were males and 60 (44.1%) were females. Patients between ages 30-39 were highest in number just as those with secondary school education 45 (33.1%), were more. The majority of the patients were involved in business as their occupation 39 (28.7%) and reside mainly in the urban area 88 (64.7%). Although most of the patients have no underlying disease, a number of them have diabetes mellitus 14(10.3%) and the majority of the patients on admission 91 (66.9%), were in the Orthopaedic ward (Table 1). There was mixed growth of bacteria comprising of aerobes and anaerobes were dominant 75 (55.1%) in the analysed samples with few pure isolates 2 (1.5%) of anaerobes. Overall, the gram-negative anaerobes were predominant with *Bacteroides fragilis* 26 (33.8%), having the highest prevalence while *Peptococci* spp 8 (10.4%), has the highest prevalence among gram-positive anaerobes (Tables 2, 3 and 4). Among the assessed risk factors in patients, only age was found to have a statistically significant relationship with the prevalence of gram-positive anaerobes ($p = 0.02$), Table 5.

Table 1. Risk factors in patients associated with pyogenic infection

Variables	Categories	Frequency	Percent
Underlying Diseases	None	96	70.6
	Cancer	8	5.9
	Diabetes	14	10.3
	foot ulcer	3	2.2
	HIV	4	2.9
	Renal Impairment	2	1.5
	Sickle Cell	2	1.5
	Others	7	5.1
	Total	**136**	**100**
Duration	≥1 week	31	22.8
	≥2 week	32	23.5
	≥3 week	73	53.7
	Total	**136**	**100**
Patient Settings	Inpatient	91	66.9
	Outpatient	45	33.1
	Total	**136**	**100**
Department	Outpatient	45	33.1
	A/E	7	5.1
	Amenity	4	2.9
	Burn	11	8.1
	Family Medicine	12	8.8
	Gynaenacology	2	1.5
	Orthopaedics	36	26.5
	Surgery	19	14.0
	Total	**136**	**100**

Table 2. Pyogenic Anaerobic isolates according to their species

Class	No. Isolated (%) n=136	Species	Frequency (%) n=77
Pure growth of Aerobes	50 (36.8)	-	50
Pure growth of Anaerobes	2 (1.5)	-	2
Mixed growth of Aerobes and anaerobes	75 (55.1)	-	150
Total	**127 (93.4)**	-	**202**
Gram -Positive Anaerobes	17 (12.5)	*Clostridium perfrigens*	3 (3.9)
		Peptococci spp.	8 (10.4)
		Peptostreptpcocci spp.	6 (7.8)
Gram-Negative Anaerobes	60 (44.1)	*Bacteroides fragilis*	26 (33.8)
		Fusobacterium spp.	23 (29.8)
		Prevotella melaninogenica	11 (14.3)
Total	**77 (56.6)**		**77 (100)**

Table 3. Distribution of gram-positive anaerobes by the presumptive antibiotic disc identification method

Gram-positive anaerobes	Kanamycin			Penicillin			Erythromycin			Vancomycin			Clindamycin			Colistin		
	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S
C. perfringens N=3			3		0	3		0	3			3			3			3
% =			100		0	100		0	100			100			100			100
Peptostrepcocci spp n = 8			8			8			8			8						8
% =			100		100	100		100	100			100			100			100
Peptococci spp n = 6					0	6			6		0	6						6
% =						100			100			100						100

Keys: I- Intermediate, R- Resistance, S- Sensitivity

Table 4. Distribution of Gram-negative anaerobes by the presumptive antibiotic disc identification method

Gram Negative Anaerobes	Kanamycin			Penicillin			Erythromycin			Vancomycin			Clindamycin			Colistin		
	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S
B. fragilis n = 26		26	0		26	0			26		26						26	
% =		100	0		100	0			100		100						100	
Fusobacterium spp. n = 23			23		0	23			23		23							
% =			100		0	100			100		100				100			
*p. melaninogoc*us n=11		11				11			11		11				11			
% =		100				100			100		100				100			

Table 5. The relationship between patients' sociodemographic characteristics and prevalence of anaerobes in pyogenic infections

Variables	Category	Gram-positive anerobes			Total	χ^2	p-value
		C. Perfrigens	*Peptococcocci* spp.	*Peptostrepto-cocci* spp.			
Age	20-29	2(66.7)	1(33.3)	0	3(15.0)	21.7	0.02
	30-39	0	4(50.0)	4(50.0)	8(40.0)		
	40-49	1(100.0)	0	0	1(5.0)		
	50-59	1(50.0)	0	1(50.0)	2(10.0)		
	60-69	0	3(100.0)	0	3(15.0)		
	70 above	1(33.3)	0	2(66.7)	3(15.0)		
	Total	5(25.0)	8(40.0)	7(35.0)	20(100.0)		
Gender	Male	4(33.3)	5(41.7)	3(25.0)	12(60.0)	1.77	0.42
	Female	1(12.5)	3(37.5)	4(50.0)	8(40.0)		
	Total	5(25.0)	8(40.0)	7(35.0)	20(100.)		
Education	No Edu	1(100.0)	0	0	1(5.0)	7.52	0.27
	Primary	0	0	2(100.0)	2(10.0)		
	Secondary	2(20.0)	5(50.0)	3(30.0)	10(50.0)		
	Tertiary	2(28.6)	3(42.9)	2(28.6)	7(35.0)		
	Total	5(25.0)	8(40.0)	7(35.0)	20(100.0)		
Residence	Urban	3(23.1)	6(46.2)	4(30.0)	13(65.0)	0.61	0.73
	Rural	2(28.6)	2(28.6)	3(42.9)	7(35.0)		
	Total	5(25.0)	8(40.0)	7(35.0)	20(100.0)		
Occupation	Unemployed	4(25.0)	6(37.5)	6(37.5)	16(80.0)	8.59	0.2
	Business	0	0	0	0		
	Employ	0	2(10.0)	0	2(10.0)		
	Retired	1(5.0)	0	0	1(5.0)		
	Student	0	0	1(5.0)	1(5.0)		
	Total	5(25.0)	8(40.0)	7(35.0)	20(100.0)		
Underlying Diseases	None	4(30.)	4(30.8)	5(38.5)	13(65.0)	6.49	0.59
	Cancer	0	1(5.0)	0	1(5.0)		
	Diabetes	1(5.0)	2(10.0)	1(5.0)	4(20.0)		
	foot ulcer	0	1(5.0)	0	1(5.0)		
	HIV	0	0	0	0		
	Renal Impairment	0	0	1(5.0)	1(5.0)		
	Sickle Cell	0	0	0	0		
	Others	0	0	0	0		
	Total	5(25.0)	8(40.0)	7(35.0)	20(100.0)		

4. DISCUSSIONS

Wound infections have become the most important cause of morbidity and mortality.[15] According to studies, colonized wounds contain one-third of anaerobic bacteria while infected wounds contain 50% of anaerobic bacteria.[16] Hence, the pyogenic infection involves not only one type of potential pathogen but numerous types of aerobic and anaerobic pathogens in polymicrobial wounds.[17, 16] Despite this and due partly to lack of equipment and trained personnel, anaerobes are rarely assessed for in wound infection involving pyogenic bacteria in routine laboratory practice.

In this study, there were more wound samples from males 76 (55.9%), than from females 60 (44.1%). This was also observed in similar studies by Duggal *et al.* and Siddiqi *et al.* which reported male preponderances 57.66% and 72% respectively.[18, 19] The reason may likely be that males are more involved with outdoor activities that can lead to trauma and eventually pyogenic infection than females. Also, the majority of the patients were of the age group 30-39 years. This same age group had the highest occurrence of pathogenic bacteria which conforms with findings from various other studies.[20, 21, 22] These findings could be because the age group 30-39 years is taken to be more engaged in lots of risky and/or occupational activities such as farming, forestry and transport business to provide for the family; which exposes them to hazards leading to pyogenic infections.[23, 24]

The isolation rate of pyogenic bacteria in wounds was 93.4%. This is similar to a report of another study done in a neighbouring Rivers State in Nigeria, with an isolation rate of 94%.[25] However, studies done in Gondar, Addis-Ababa and Dessie, reported lower isolation rates of 83.9%, 42% and 70.5% respectively.[26, 27, 28] The observed differences in the isolation rates may

not be unconnected with the differences in wounds managements and bacteriological techniques used.

Among the 136 samples collected, 50 (36.7%) of the samples had pure growth of aerobic bacterial isolates only, 2 (1.5%) had pure growth of anaerobic bacteria only, while 75 (55.1%) had growths of aerobic and anaerobic bacteria isolates from same patient's sample similar to what has been reported elsewhere. However, an overall 202 bacterial isolates were gotten, out of which 125 (61.9%) were aerobes, and 77 (38.1%) were anaerobes which conforms with some studies [29, 30] but which also differs with some others, that reported higher isolation of anaerobes.[31]

Gram-negative anaerobes were predominant among the anaerobic isolates with *Bacteriodes fragilis* 26 (33.8%), having the highest number as against the reported predominance of *Peptostreptococcus* [32, 33] and *Clostridium* species [34, 29] which are Gram-positive anaerobes by some studies. No special reason for these differences can immediately be deduced although there were observable low cases of gas gangrene in our study area. This may have also contributed to the more *Peptococccci* and *Peptostreptococci* spp and few of *Clostridia* spp among the Gram-positive anaerobes revealed by this study.

As regards the associated risk factors of anaerobic pyogenic wound infections among patients, age was found to have a statistically significant relationship with the prevalence of Gram-positive anaerobes only. Age of patient, was also reported to be a risk factor for developing Gram-positive anaerobic pyogenic infection by studies elsewhere.[35]

5. CONCLUSION

In conclusion, there was a preponderance of wound infection in males than in females admitted to a tertiary hospital in Uyo. The majority of the patients were of the age group 30-39 years whose wound infection was majorly (93.5%) due to pyogenic bacteria. Of note is the 38.1% isolation rate of both anaerobic and aerobic from samples belonging to the same patients. Only very few samples from patients with wound infection (1.5%) yielded the pure growth of anaerobic bacteria alone. There is the preponderance of *Bacteriodes fragilis* a Gram-negative anaerobe, in wounds of patients in this study. Interestingly, even with the lower prevalence of Gram-positive anaerobic cases of pyogenic wound infection, the age of patients was found to be a statistically significant relationship with its rate of occurrence.

CONSENT

Consent was sought and obtained from all participants prior to inclusion in this study.

ETHICAL APPROVAL

Ethical clearance and approval (reference no.: UUTH/AD/3/96/VOL.XXI/J4, dated April 1, 2017) was gotten from the Ethical Review Board of the University of Uyo Teaching Hospital prior to commencement of this study.

REFERENCES

1. Koneman WK, Allen SD, Janda WM, Schreckenberger PC, Propcop GW, Woods GL. Philadelphia Color Atlas and Textbook of Diagnostic Microbiology, 6th Edition: Lippincott publishers; 2005.
2. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology and epidemiology of bacterial resistance. Microbiology Mol Biol Rev. 2001; 65(2): 232-260. 2001.

3. Cogen AL, Nizet V, Gallo RL. Skin microbiota a source of disease or defence? British J Dermatol 2008; 158(3): 442–455.

4. Dryden MS. Complicated skin and soft tissue infection. J Antimicrob Chemo 2010; 65(3): 35–44.

5. Scalise A, Bianchi A, Tartaglione C. Microenvironment and microbiology of skin wounds: the role of bacterial biofilms and related factors. Seminars Vas Surg 2015; 28(34): 151–159.

6. Collee, J. G., Mackie, T. J., McCartney, J. E. (2014). Mackie and McCartney Practical Medical Microbiology, 14th edition. New York, Churchill Livingstone, 70p

7. Stotts NA. Wound infection: diagnosis and management. In: Bryant RA, Nix DP, Editors. Acute and chronic wounds: Current management concepts. 4th Edition Saint Louis, MO: Elsevier-Mosby; 2012. pp 270-278.

8. Hentges DJ. The anaerobic microflora of the human body. Clinical j Infect Dis 1993; 164: 175–180.

9. Brook, I. (2007). Anaerobic Infections Diagnosis and Management. A Textbook. Informa Healthcare USA, Inc. New York. 27-35.

10. Etok, C. A., Edem, E. M., Ochang, E. (2012). Aetiology and antimicrobial studies of surgical wound infections in University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria. *Open Access Scientific Reports,* 1: 340-341.

11. CLSI (Clinical and Laboratory Standards Institute) (2014). *Methods For Antimicrobial Susceptibility Testing Of Anaerobic Bacteria.* 27-33.

12. Collee, J. G., Mackie, T. J., McCartney, J. E. (2014). Mackie and McCartney Practical Medical Microbiology, 14th edition. New York, Churchill Livingstone, 70p

13. Cheesebrough, M. (2010). *District Laboratory Practice in Tropical Countries* pp80-85. 2nd Edition, Part2, Cambridge University Press. Examination of Pus Ulcer Material and Skin Specimens, 137-150.

14. CLSI (Clinical and Laboratory Standard Institute) (2014). Performance Standards for Antimicrobial Susceptibility Testing. 20th Informational Supplement. *Clinical and Laboratory Standards Institute*, 20p.

15. Ezekiel, O., Abdul-Rashid, A., Adebayo, L. (2014). Pattern of pathogens from surgical wound infections in a Nigerian Hospital and their antimicrobial susceptibility profiles. *African Health Sciences,* 14(4): 802-809.

16. Brook, I., Frazier, E. H. (1998). Aerobic and anaerobic microbiology of chronic venous ulcers. *International Journal of Dermatology*, 37: 426-428.

17. Mousa, H. A. (1997). Aerobic, anaerobic and fungal burn wound infections. *Journal of Hospital Infection,* 37: 317-323.

18. Duggal, S., Khatri, P. K., Parihar, R. S., Arora, R. (2015). Antibiogram of various bacterial isolates from pus samples in a tertiary care centre in Rajasthan. *International Journal of Science and Research.* 4(5): 1580-1584.

19. Siddiqi, F., Madahihah, E., Masood, N. U. S., Abdus S., Mazhar, Q. and Ali, A. Q. (2002). Antibiogram sensitivity pattern of Methicilline resistant *Staphylococus aureus* isolates from Pus Sample. *Pakistan Journal of Biological Science,* 5(4): 491-493.

20. Sukumar, N., Rajesh, S. (2017). Aerobic bacterial isolates and their antibiotic susceptibility pattern from pus samples in a Tertiary Care Government Hospital in Tamilnadu, India. *International Journal of Current Microbiology and Applied Sciences,* 6(6): 423-442.

21. Bhat, V. G., Vasaikar, S. D. (2010). Bacteriological profile and antibiogram of aerobic Burn wound isolates in Mthatha, Eastern Cape, South Africa. *South Africa journal of Epidemiological Infection,* 25(4): 16-19.

22. Rajeshwar Rao, S., Jaya Lakshmi, L., Pavani, S., Kawle, V., Jaya Prakash, S. (2014). Bacteriological profile, antibiogram of burn wound isolates and detection of MRSA and ESBL production at Tertiary Care Hospital, Hyderabad. *World Journal of Pharmacy and Pharmaceuticals Sciences,* 3: 1691-1698.

23. Ajao, T., Yakubu, S. E. (2015). Identification, characterization and plasmid profiling of multidrug resistant nocomial pathogens isolated from selected hospitals in Ilorin metropolis. *British Microbiology Research Journal,* 1: 33-43.

24. Akindele, P. O., Afolayan, C. O. (2017). Plasmid profile of multidrug resistant bacteria isolated from wound swabs from hospital patients in Akure, Nigeria. *Asian Journal of Medicine and Health,* 2(3): 1-13.

25. Wariso, B. A., Nwachukwu, C. O. (2003). A survey of common pathogens in wound in patients at University of Port Harcourt Teaching Hospital, Port Harcourt. *West African Journal of Medicine*, 22(1): 50–54.

26. Aynalem, M., Mengistu, E. S., Teklay, G., Moges, T., Feleke, M. (2017). Bacterial isolates and their antimicrobial susceptibility patterns of wound infections among inpatients and outpatients attending the University of Gondar Referral Hospital, Northwest Ethiopia. *International Journal of Microbiology,* 3: 1-10.

27. Abraham, Y., Wamisho, B. L. (2009). Microbial susceptibility of bacteria isolated from open fracture wounds presenting to the err of black lion Hospital, Addis Ababa University, Ethiopia. *African Journal of Microbiology Research*, 3: 939-951.

28. Kibret, M., Abera, B. (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *African Health Science*, 11(51): 540 - 545.

29. Sushma, N., Pednekar, S., Pol, S., Sheetal, S., Kamble, S., Smita, Deshpande, K. et al (2015). Drug resistant anaerobic infections: are they complicating diabetic footulcer. *International Journal of Healthcare and Biomedical Research,* 3(3): 142-148.

30. Brook, I., Frazier, E. H. (1999). Aerobic and anaerobic microbiology of surgical-site infection following spinal fusion. *Journal of Clinical Microbiology*, 37(3): 841-843.

31. Brooks, G. F., Brutel, S., Morse, S. A. (2004). *Jawetz, Melnick, and Adelberg's Medical Microbiology,* 23rd Edition./-McGraw-Hill, New York, USA. 3-4.

32. Eslami, G., Fallah, F., Goudarzi, H., Navidinia, M. (2005). The prevalence of antibiotic resistance in anaerobic bacteria isolated from patients with skin infections. *Gene Thermal Molecular Biology*, 9: 263-268.

33. Ritu, G., Kaistha, N., Vars, H., Gupta, J., Chander, J. (2014). Isolation identification and antimicrobial susceptibility of anaerobic bacteria. *Journal of Clinical and Diagnostic Research*, 8(11): 1-2

34. Raymundo, M. and Mendoza, M. T. (2002). The microbiologic features and clinical outcome of diabetic foot infections among patients admitted at International Journal of Herbs and Pharmacological Research. *Philippine Journal of Microbiology and Infectious Disease,* 31: 54-63.

35. Akoachere, J., Francis, T. K., Palle, J. N., Mbianda, S. E., Nkwelang, G., Roland, N. N. (2014). Risk factors for wound infection in health care facilities in buea, cameroon: aerobic bacterial pathogens and antibiogram of isolates. *Pan African Medical Journal,* 5: 18-26.