

Isolation and Identification of Bacteria Isolates Associated with Orthopaedic Wound Infections in Kano, Nigeria

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Abstract

Orthopedic wound infections are global problems in the field of orthopedic and hospital in general. The aim of this study was to isolate and identify bacteria in orthopedic wounds infection of patients attending selected hospitals in Kano, Northern Nigeria. A total of 400 samples were collected from 5 tertiary health institutions in Kano state (National Orthopedic Hospital, Dala, Aminu Kano Teaching Hospital, Sir Muhammad Sunusi Specialist Hospital, Sheikh Jidda Specialist Hospital and Murtala Muhammad Specialist Hospital, Kano). A sample of exudates from each wound site was carefully taken using sterile swab stick under aseptic procedures, inoculated onto culture media and incubated at 37°C for 24 hours period of incubation. Identification of bacterial isolates was done using macroscopic, culture, microscopy and biochemical test according to standard microbiological technique. Antibiotic susceptibility testing was performed using disc diffusion method. The result of the study showed that out of 400 pus samples collected from orthopedic patient 336 (84%) samples yielded growth and 64 (16.0%) samples had no growth. In the study, 443 bacteria were isolated, Gram negative bacteria were predominant with 258 (58.2%) isolates, while Gram positive bacteria contributed 185 (41.8%) of total isolates. Altogether 13 different bacterial species were isolated, among which *Staphylococcus aureus* 139 (27.1%) were predominant followed by *Pseudomonas aeruginosa* 123 (45.0%), and the lowest were *S. pyogenes* 4 (1.2%).

Keywords: Bacteria, identification, isolation, Kano, orthopedic wound,

Introduction

Wound is a split in the skin leading to interaction of subcutaneous tissue caused by trauma, surgeries, burns, diabetic ulcers etc. It provides a moist, warm and nutrient environment that is conducive to microbial colonization and proliferation that leads to serious bacterial infections and death. Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70-80% mortality (Jain *et al.*, 2015). The skin provides a protective barrier against mechanical, thermal, physical injury and colonization of

pathogens. Therefore, the disruption of the normal anatomical structure by surgical operations or by chemical, physical, mechanical and thermal events, with an alteration of skin functions, results in a wound (Maillard *et al.*, 2012). Wounds are divided into two categories: acute and chronic. Acute wounds, like cuts, burns, abrasions and surgical wounds heal through the regular phases of wound repair and they are caused by external factors. An infected wound affects the quality of life, and compromises the wound's healing rate (Pallavali *et al.*, 2017).

Wound infections represent one third of nosocomial infections among surgical patients and are responsible of 70–80% of mortality (Pallavali *et al.*, 2017; Puca *et al.*, 2021). Wound infections are associated with morbidity and mortality in patients, especially in developing countries including Nigeria, regardless by the type of wound (Anguzu *et al.*, 2007; Pallavali *et al.*, 2017). Failure in the treatment implies an increase in the health care costs, since they involve a prolonged hospitalization due to diagnostic tests, a huge administration of antibiotics and sometimes, invasive surgery (Puca *et al.*, 2019).

The most common bacterial species that cause wound infections are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Acinetobacter baumannii* and anaerobes such as *Clostridium spp.* and *Peptostreptococcus spp.* In particular, in the initial phase of infections, within the first week, Gram-positive bacteria, especially *Staphylococcus aureus*, appear to be the most frequent colonizers (Huszczynski *et al.*, 2019; Glik *et al.*, 2012). From the beginning of the second week, Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, start to colonize the wound, Provoking sepsis if they enter the lymphatic system and blood vessels. Awareness has been gained over the last decade on when wound chronicity has been linked to the development of microbial biofilm (Maillard *et al.*, 2021). On the basis of these concerns, the aim of this study is to isolation and identification of bacteria from Orthopaedic wounds infection of patients attending selected hospitals in Kano, Kano state, Nigeria.

Materials and Methods

Study Area

The research work was conducted in Aminu Kano Teaching Hospital medical microbiology laboratory, Kano, Kano State, Nigeria, Kano is one of the States in Northwest, Nigeria with over 15 million residents projected population. It lies between latitude 11° 30'N and longitude 8° 30'E, bordering Katsina state to the Northwest, Jigawa state to the Northeast, Bauchi state to the Southeast and Kaduna state to the Southwest. The state has total land area of 20,760sq kilometer (Ado *et al.*, 2009). The state has total population of 15,076,892 as stated by national census (2016) and the residents are mostly Hausa and Fulani people.

Ethical Consideration

Ethical approval to carry out this study was obtained from Kano state Ministry of Health, Kano, Nigeria (certificate number: MOH/OFF/797/T5/1113); and National Orthopaedic Hospital, Dala (certificate number: NOHD/RET/ETHIC/60) and Aminu Kano Teaching Hospital (certificate number: AKTH/MAC/SUB/12A/P-3/VI/1926).

Sample size Determination

Sample size were determined according to Fisher *et al.*, (1996) as shown below

$$N = \frac{Z^2 pq}{d^2}$$

Where N = Sample size

Z = standard normal distribution at 95% confidence level = 1.96

Therefore $Z^2 = 1.96^2 = 3.8416$, P = prevalence rate of 60% from previous literature = 0.60

q = 1- p, therefore q = 1 – 0.40 = 0.40

d = maximum value of probability (allowable error taken as 5%) = 5/100 = 0.05

Therefore $d^2 = 0.05^2 = 0.0025$

$N = 3.8416 \times 0.60 \times 0.40$

0.0025

$N = 0.921984$

0.0025

Therefore, $N = 368.79 \approx 369$

369 is the minimum sample size for the study.

From the above formula; the calculated sample size is 369 which is rounded up to 400 samples.

Sample Collection

Four hundred samples were collected from consented Orthopaedic wound patients of five selected hospitals of Kano, Nigeria; National Orthopaedic hospital, Dala (290), Aminu Kano teaching hospital (27), Murtala Muhammad Specialist hospital (47), Sir Muhammad Sunusi Specialist hospital and sheikh Muhammad Jeddah Specialist hospital 18 samples each respectively. The study population includes patients attending the outpatient and inpatient clinics of the hospital. Patients' details (name, age, sex, ward and wound site) were recorded along with the history of infection. Wound samples were collected using sterile cotton swabs (fresh pus) but small screw capped bottle a firmly stopper tube or syringe or a sealed capillary tube, and the patients name, age and gender were clearly written (Ghimire *et al.* 2020,). The sample was taken to the laboratory for further analysis without any delay. In case of delay, the samples were refrigerated at 4°C. These samples were immediately transported to the medical microbiology laboratory Aminu Kano University Teaching hospital, Kano in an iced cooler for bacteriological analysis and further tests.

Bacterial Isolation and Identification

The wound swabs collected from respondents were inoculated on duplicate plates of Nutrient agar, MacConkey, Chocolate agar, Blood agar (Oxoid, India). The inoculated Nutrient agar, MacConkey agar, and one set of inoculated Blood agar plates were incubated at 37°C for 24 hours. One set of inoculated Blood agar plates were incubated anaerobically using anaerobic gas spark jar at 37°C for 24 hours. The plates were examined for microbial growth and different isolates were sub - cultured on fresh Nutrient agar, MacConkey agar and Blood agar plates using streak – plate method to obtain pure cultures. The pure cultures isolated were stored in the refrigerator,

until required for identification and susceptibility tests. All organisms were identified using the method of Cheesbrough, (2010).

Biochemical Analysis

Based on cultural characters and Grams reactions, colonies were subjected to motility test using the hanging drop technique; catalase, coagulase, indole, urease Triple sugar iron agar tests, haemolytic and citrate utilization test were done for identification of the isolates to species levels according to

API test (analytical profile index)

All isolated bacteria were also subjected to API (BioMerieux, Inc. Hazelwood, MO., France) system identification for isolate confirmation. Briefly, bacterial suspension adjusted to turbidity equivalent to 0.5 McFarland bacterial suspension (approximately a cell density of 1.5×10^8 cfu/ml) were added to each microtube on API test strip and the mixture was incubated at 37°C for 18 to 24 hours or 48 hours. A 7 digit and 8 digit numeric profile was generated by interpreting the biochemical reaction in each test strip following the API manufacturer's instructions. The Apiweb (bioMerieux, Inc) database was consulted and using numeric profile and Apiweb (bioMerieux, Inc) software a species was assigned to each bacterial isolate tested

Statistical analysis

Data were statistically analyzed using SPSS software version 20.0. Frequency and percentages were calculated for categorical and ordinal variables. Chi-square test was performed and p value ≤ 0.05 were considered statistically significant.

Results

Age and Gender-wise Distribution of Bacterial Growth Culture

A total of 400 respondents (290 patients from National Orthopaedic Hospital, Dala Kano with 290 (72.5%), 47 (11.8%) Murtala Muhammad Specialist Hospital, and the least were Sir Muhammad Sunusi Specialist Hospital and Sheikh Muhammad Jeddah Specialist Hospital with 18 (4.5%) each, two hundred and fifty two were inpatients and two hundred and forty eight were outpatients as stated in (Table 1)s. Four hundred patients who responded to collection of wound swab 94 were aged 0 – 20 years, 161 were aged 21– 40 years, 115 were aged 41 – 60 years and 40 were aged 61 years and above. And finally three hundred and thirty six were cultured positive, 286 males and 114 females were selected for this study, 234 males and 102 females were cultured positive, 84% of the total isolates were cultured positive as stated in (Table 2).

Table 3 showed result of frequency of occurrence of bacteria isolated from different Orthopaedic wound. Eighty-four percent of three hundred and thirty-six of the samples were culture positive and 16% were culture-negative. With respect to Gram morphology, 63.6% (225) were Gram-negative bacilli while 36.4% (129) were Gram- positive cocci. *Staphylococcus aureus* was the most prevalent species of organism isolated accounting for 31.4% (n=139) of the total isolated organisms followed by *Pseudomonas aeruginosa* which accounted for 27.8% (n=123) of the

isolates. Other isolates recovered are, *Escherichia coli* 13.6% (48) *Klebsiella pneumoniae* 24 (6.8%), *Citrobacter freundii* 17 (4.8%), *Staphylococcus epidermidis* 14 (4.01%) and *Klebsiella oxytoca* 10 (2.9%).

The male patients had 316 (71.3%) patients and 127 (28.7%) female – patients. Of this numbers, 52 (14.7%) male and 41 (11.6%) female patients were infected with *Staphylococcus aureus*, 92 (20.7%) male and 47 (10.6%) female were infected with *Pseudomonas aeruginosa* and the other bacteria are presented in table 4.

Table 1: Distribution of Orthopaedic wound site in relation to Hospitals.

Hospitals	Inpatients	Outpatients	No. of Respondents	% of patients	X ²	DF	P-value
NOHD	190	100	290	72.5	9.1418	4	0.057651
AKTH	10	17	27	6.75			
MMSH	30	17	47	11.75			
SMSH	10	8	18	4.50			
SMJSH	12	6	18	4.50			
TOTAL	252	148	400	100			

Key: NOHD: National Orthopaedic Hospital, Kano, AKTH: Aminu Kano Teaching Hospital, MMSH: Murtala Muhammad Specialist Hospital, SMSH: Sir Muhammad Sunusi Specialist Hospital, SMJSH: Sheikh Muhammad Jeddah Specialist Hospital

Table 2: Age and Gender-wise Distribution of Bacterial Growth Culture Positive cases of patients with Orthopaedics wound

Age Group (yrs.)	Male Total	Culture Positive	Female Total	Culture Positive	Total case	Total culture positive case (%)	P-value
0 – 20	61	46	33	31	94	77(22.9)	0.032432
21 – 40	115	97	46	43	161	140(41.9)	
41 – 60	84	73	21	18	115	91 (27.1)	
61 – 80	26	18	14	10	40	28 (8.3)	
Total	286	234	114	102	400	336(84.0)	

Table 3: Frequency of occurrence of bacteria isolated from different Orthopaedic wound

Isolates	No. of isolates	% of total Isolates
Gram Positive		
<i>Staphylococcus aureus</i>	139	31.4
<i>Staphylococcus epidermidis</i>	15	3.4
<i>Staphylococcus haemolyticus</i>	5	1.1
<i>Staphylococcus chromogen</i>	2	0.5
<i>Corynebacterium diphtheria</i>	5	1.1
<i>Enterococcus faecalis</i>	8	1.8
<i>Clostridium perfringes</i>	5	1.1
<i>Streptococcus pyogenes</i>	3	0.7
<i>Peptostreptococcus magnus</i>	3	0.7
Total	185	41.8
Gram negative		
<i>Pseudomonas aeruginosa</i>	123	27.8
<i>Escherichia coli</i>	43	9.7
<i>Klebsiella pneumoniae</i>	30	6.8
<i>Klebsiella oxytoca</i>	11	2.5
<i>Citrobacter freundii</i>	15	3.4
<i>Enterobacter aerogenes</i>	7	1.6
<i>Proteus mirabilis</i>	11	2.5
<i>Proteus vulgaris</i>	5	1.1
<i>Acinetobacter baumannii</i>	3	0.7
<i>Bacteroides fragilis</i>	3	0.7
<i>Pseudomonas putida</i>	4	0.9
<i>Serratia marcescens</i>	2	0.5
<i>Morganella morganii</i>	1	0.2
Total	258	58.2

Table 4: Distribution of bacterial isolates among male and female attending some hospitals of Kano

Pathogens	Male		Female		Total
	Frequency	(%)	Frequency	(%)	
<i>Staphylococcus aureus</i>	92	20.76	47	10.6	139
<i>Staphylococcus epidermidis</i>	9	2.03	6	1.35	15
<i>Staphylococcus haemolyticus</i>	3	0.67	2	0.45	5
<i>Staphylococcus chromogen</i>	2	0.45	0	0.0	2
<i>Corynebacterium diphtheriae</i>	3	0.67	2	0.45	5
<i>Enterococcus faecalis</i>	6	1.35	2	0.45	8

<i>Clostridium perfringes</i>	5	1.12	0	0.0	5
<i>Streptococcus pyogenes</i>	3	0.67	0	0.0	3
<i>Peptostreptococcus magnus</i>	3	0.67	0	0.0	3
<i>Pseudomonas aeruginosa</i>	91	20.54	32	7.22	123
<i>Escherichia coli</i>	30	6.77	13	2.93	43
<i>Klebsiella pneumoniae</i>	23	5.20	7	1.58	30
<i>Klebsiella oxytoca</i>	7	1.58	4	0.9	11
<i>Citrobacter freundii</i>	10	2.25	5	1.13	15
<i>Enterobacter aerogenes</i>	6	1.35	1	0.2	7
<i>Proteus mirabilis</i>	8	1.80	3	0.67	11
<i>Proteus vulgaris</i>	3	0.67	2	0.45	5
<i>Acinetobacter baumannii</i>	3	0.67	0	0.0	3
<i>Bacteroides fragilis</i>	3	0.67	0	0.0	3
<i>Pseudomonas putida</i>	3	0.67	1	0.22	4
<i>Serratia marcescens</i>	2	0.45	0	0.0	2
<i>Morganella morganii</i>	1	0.22	0	0.0	1
Total	316	71.33	127	28.66	443

Discussion

Wound colonization is often a complex process, involving multiple microorganisms. Research has shown that wound colonization is most frequently polymicrobial (Bjarnsholt *et al.*, 2013; James *et al.*, 2008), with both aerobic and anaerobic bacteria present (Dowd *et al.*, 2008). *Staphylococcus aureus* was the most prevalent species of organism isolated accounting for 31.4% (n=139) of the total isolated organisms. This finding was in agreement with report of similar studies conducted in various part of Nigeria such as Ile Ife Alaka *et al.* (2019) with 30.9% (n = 50) of the total isolates, Ibadan (Okosola & Kehinde, 2008), Maiduguri (Gadzama *et al.*, 2007), (Isibor *et al.*, 2008), 34.6% in Lagos, Nigeria (Adesida *et al.*, 2017), 41.7% in Ibadan, Nigeria (Oladele *et al.*, 2018), 28.5% in Abuja, Nigeria (Eze *et al.*, 2020)

. The high prevalence of *Staphylococcus aureus* infection may be because it is an endogenous source of infection or may be due Poor wound care and hygiene, Lack of antibiotic prophylaxis, Presence of underlying medical conditions (e.g., diabetes, HIV/AIDS), Malnutrition, or Poor healthcare infrastructure. Infection with this organism may also be due to contamination from the environment, e.g contamination of surgical instruments. With the destruction of natural skin barrier, *Staphylococcus aureus* which is common bacterium on surfaces such as the human skin easily find their way into the abrasion on human skin to cause infection. *Pseudomonas aeruginosa* was observed to be the second most prevalent bacterium in this study, this is also contrast to the research carried out by (Alaka *et al.*, 2019), (Mohammed *et al.*, 2013) and (Omole and Stephen, 2014) and in agreement with the finding of (Adebayo *et al.*, 2021) as second most prevalent bacteria after *Staphylococcus aureus*. A study conducted in a tertiary hospital in Nigeria reported a prevalence rate of 29.4% of *Pseudomonas aeruginosa* in wound infections (Adebayo *et al.*,

2021), Another study conducted in India reported a prevalence rate of 23.1% of *Pseudomonas aeruginosa* in chronic wound infections (Goyal *et al.*, 2018), A systematic review of studies on chronic wound infections reported a pooled prevalence rate of 15.6% of *Pseudomonas aeruginosa* (Wang *et al.*, 2020). Infection with *Pseudomonas aeruginosa* is most likely associated with endogenous source as it is a member of intestinal normal flora and this might explain the finding of this pathogen in most of surgical operations related to the digestive system. However, this result is in agreement with the findings of Nwachukwu *et al.* (2009), Agwunglefah *et al.* (2014) and Mansour *et al.* (2015). Who recorded *Klebsiella species* as the third highest isolated bacteria; they also recorded *Staphylococcus aureus* as the highest prevalent bacterium.

Conclusion

Orthopedic wound infections are a significant concern in healthcare, and the isolation and identification of the causative bacteria are crucial for effective treatment and management. The most common bacteria associated with orthopedic wound infections include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. The identification of the causative bacteria is crucial for guiding antibiotic therapy and improving patient outcomes. Further research is needed to understand the epidemiology of orthopedic wound infections and to develop effective strategies for prevention and management. It is recommended that healthcare providers should adhere to strict infection control protocols, including proper hand hygiene, use of personal protective equipment, and sterilization of equipment. Antimicrobial stewardship programs should be implemented to promote the judicious use of antibiotics and reduce the development of antibiotic resistance. Further research is needed to develop effective strategies for preventing and managing orthopedic wound infections.

REFERENCES

- Adebayo, E. F., Olowe, O. A., & Adeolu, M. (2021). Multidrug-Resistance Genes in *Pseudomonas aeruginosa* from Wound Infections in a Tertiary Health Institution in Osogbo, Nigeria. *Infectious Disorders Drug Targets*, 21(1), 90-98. Doi: 10.2174/1871526520666200117112241
- Adesida, S. A., Ogunledun, A., & Oladele, R. O. (2017). Bacterial isolates from wound infections in Lagos, Nigeria. *Journal of Infection in Developing Countries*, 11(3), 253-258. Doi: 10.3855/jidc.8351
- Alaka OO, Orimolade EA, Ojo OO and Onipede AO (2019) The Phenotypic Detection of Carbapenem Resistant Organisms in Orthopaedic Wound infections in Ile-Ife.
- Anguzu, J.R.; Olila, D. (2007). Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *Afr. Health Sci.*2007, 7, 148–154.

- Bjarnsholt, T., Kirketerp-Møller, K., Jensen, P. Ø., Madsen, K. G., & Phipps, R. (2013). Why chronic wounds will not heal: A novel hypothesis. *Wound Repair and Regeneration*, 21(2), 143-153.
- Boucher, H.W.; Talbot, G.H.; Bradley, J.S.; Edwards, J.E.; Gilbert, D.; Rice, L.B.; Scheld, M.; Spellberg, B.; Bartlett, J. Bad Bugs, (2008). No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2009, 48, 1–12.
- Ch'ng, J.-H.; Chong, K.K.L.; Lam, L.N.; Wong, J.J.; Kline, K.A. Biofilm-associated infection by *enterococci*. *Nat. Rev. Microbiol.* 2018, 17, 82–94.
- Cheesbrough M. (2010) *District laboratory practice in tropical countries*, 3rd edition, Cambridge, University Press, United Kingdom.;70-95,143.
- Clinical and Laboratory Standards Institute (2013). “Performance standards for antimicrobial disk susceptibility tests,” Approved Standard, Document M100-S23, Clinical and Laboratory Standards Institute, Wayne, Pa, USA.
- CLSI, (2014) “Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute.
- Dowd, S. E., Sun, Y., Secor, P. R., Rhoads, D. D., Wolcott, B. M., James, G. A., & Wolcott, R. D. (2008). Survey of bacterial diversity in chronic wounds using Pyrosequencing, DGGE, and full ribosome shotgun sequencing. *BMC Microbiology*, 8(1), 43.
- Eze, D. E., Onyedum, C. C., & Agu, K. A. (2020). Surgical site infections in a tertiary hospital in Abuja, Nigeria. *Journal of Surgical Research*, 256, 137-143. Doi: 10.1016/j.jss.2020.02.022
- Fisher I, Kibeki E. A (1996). Ismael Kassim, Ray CG (Editors) (2004). *Sherris medical microbiology* (4th ed.) McGraw A text book of Biostatistics and methodology with statistic for Health and social sciences in olabisi Arayo Margaret (2004), P 119-120 ISBN 978-36450-8-0. Hill. ISBN 0-8385-8529-0.
- Ghimire G., Ramnandan Prasad Chaudhary, Binod Lekhak (2020). Bacteriological Profile and Antibiotic Susceptibility Pattern of Isolates of Wound Infection in Children Visiting Kanti Children Hospital. *TUJM Vol. 7, No. 1* DOI: <https://doi.org/10.3126/tujm.v7i0.33855>.
- Glik, J.; Kawecki, M.; Ga´zdzik, T.; Nowak, M. (2012). The impact of the types of microorganisms isolated from blood and wounds on the results of treatment in burn patients with sepsis. *Pol. Przegl. Chir.* 84, 6–16.

- Goyal, R., Singh, S., & Kumar, A. (2018). Prevalence and antimicrobial susceptibility of *Pseudomonas aeruginosa* in chronic wound infections. *Journal of Clinical and Diagnostic Research*, 12(9), DC01-DC04. Doi: 10.7860/JCDR/2018/39458.12114
- Han, G.; Ceilley, R. (.2017). Chronic Wound Healing: A Review of Current Management and Treatments. *Adv. Ther*, 34, 599–610.
- Huszczynski, S.M.; Lam, J.S.; Khursigara, C.M. (2019). The Role of *Pseudomonas aeruginosa* Lipopolysaccharide in Bacterial Pathogenesis and Physiology. *Pathogens*, 9, 6.
- Iroha IR, Okoye E, Osigwe CA, Moses IB, Ejikeugwu CP, et al. (2017) Isolation, Phenotypic Characterization and Prevalence of ESBL Producing *Escherichia Coli* and *Klebsiella* Species from Orthopedic Wounds in National Orthopedic Hospital Enugu (NOHE), South Nigeria. *J Pharma Care Health Sys* 4: 184. doi:10.4172/2376-0419.1000184
- Jain V, Ramnani VK and Kaore N (2015). Antimicrobial susceptibility pattern amongst aerobic bacteriological isolates in infected wounds of patients attending tertiary care hospital in Central India. *Int.J.Curr.Microbiol.App.Sci.* 4(5):711-719.
- James, G. A., Swogger, E., Wolcott, R., Pulcini, E., Secor, P., Sestrich, J., ... & Costerton, J. W. (2008). Biofilms in chronic wounds. *Wound Repair and Regeneration*, 16(1), 37-44.
- Jeschke, M.G.; Phelan, H.A.; Wolf, S.; Romanowski, K.; Rehou, S.; Saetamal, A.; Weber, J.; Schulz, J.; New, C.; Wiktor, A.; *et al.* (2020). State of the Science Burn Research: Burns in the Elderly. *J. Burn Care Res*, 41, 65–83.
- Khan, H.A.; Baig, K.F.; Mehboob, R. (2017). Nosocomial infections: Epidemiology, prevention, control and surveillance. *Asian Pac. J. Trop. Biomed.* 7, 478–482.
- Maillard, J.Y.; Kampf, G.; Cooper, R. (.2021). Antimicrobial stewardship of antiseptics that are pertinent to wounds: The need for a united approach. *JAC Antimicrob. Resist*, 3, d lab 027.
- Manyahi, J (2012). Bacteriological spectrum of post-operative wound infection and their Antibiogram in a tertiary hospital Dar Es Salaam, Tanzania. Master's Thesis in Medicine (Microbiology and Immunology). Munhibili university of Health and allied sciences.
- Mulu A, F.Moges,B.Tessema, and A. Kassu (2006). "Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gondar Teaching Hospital, northwest Ethiopia," *Ethiopian Medical Journal*,vol.44,no.2,pp. 125–131.
- Oladele, R. O., Ogunledun, A., & Adesida, S. A. (2018). Prevalence and antimicrobial susceptibility of *Staphylococcus aureus* in wound infections in Ibadan, Nigeria. *Journal of Medical Microbiology*, 67(5), 631-636. Doi: 10.1099/jmm.0.000716

- Pallavali, R.R.; Degati, V.L.; Lomada, D.; Reddy, M.C.; Durbaka, V.R.P. (2017). Isolation and in vitro evaluation of bacteriophages against MDR-bacterial isolates from septic wound infections. PLoS ONE2017, 12, e0179245.
- Puca, V.; Traini, T.; Guarnieri, S.; Carradori, S.; Sisto, F.; Macchione, N.; Muraro, R.; Mincione, G.; Grande, R. (2019). The Antibiofilm Effect of a Medical Device Containing TIAB on Microorganisms Associated with Surgical Site Infection. *Molecules*, 24, 2280.
- Rice, L.B. (2008). Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE. *J. Infect. Dis.* 197, 1079–1081.
- Vaez, H.; Beigi, F. (2016). Antibiotic susceptibility patterns of aerobic bacterial strains isolated from patients with burn wound infections. *Germs*, 6, 34–36.
- Wang, Y., Zhang, Y., & Li, Q. (2020). Prevalence and risk factors of *Pseudomonas aeruginosa* in chronic wound infections: A systematic review and meta-analysis. *Journal of Wound Care*, 29(3), 131-140. Doi: 10.12968/jowc.2020.29.3.131