

Full Length Research Paper

## Isolation of Aerobic Bacteria from Wounds of Diabetic Patients Undergoing Hyperbaric Oxygen Therapy in Khartoum State

<sup>1\*</sup> Ahmed Ibrahim Hashim <sup>1</sup> Amal Mubarak Hassan Mubarak, <sup>1</sup> Nosiba Ahmed Hassan Hamed, and <sup>2\*</sup> Ahmed Bakheet Abd Alla

<sup>1\*</sup> Department of Microbiology- College of Medical Laboratory Science, Sudan University of Science and Technology.

<sup>2</sup> Department of Parasitology and Medical Entomology -College of Medical Laboratory Science, Sudan University of Science and Technology.

Accepted 07 June, 2022

**Background:** Diabetic wound infection is one of the complications of diabetes mellitus, hyperbaric Oxygen Therapy (HBOT) can be used as an adjacent treatment for diabetic wound infection, it involves inhalation of 100% oxygen under a pressure greater than 1 atmospheric absolute. This was a longitudinal case study, in which the wound specimens were collected from AL-Dirby Centre and further processing was done at the Microbiology Laboratory, Sudan University of Science and Technology (SUST), between January and July 2016. **Objective:** The aim of this study was to evaluate the effect of hyperbaric oxygen therapy (HBOT) on aerobic bacteria isolated from the wounds of diabetic patients attending Al-Dirby Center. **Methodology:** A total of 22 diabetic patients were involved in this study, their ages between 40 and 98 years. The majority of the patients were males 17 (77.3 %) and 5 (22.7%) were females, Insulin dependent diabetic patients were 15 (68.2%) and insulin independent diabetic patients were 7 (31.8%). A Questionnaire was used to collect data from the patients. The data was analyzed by using SPSS version (11.5). The results showed (78.1%) Gram-negative rods and (21.9%) Gram- positive cocci. The most frequent isolated bacteria were *Proteus mirabilis* 8(25%) followed by *Pseudomonas aeruginosa* 6(18.7%), *Staphylococcus aureus* and *Escherichia coli* 5 (15%), *Proteus vulgaris* 4(12.5%), and (*Sterptococcus pyogenes*, *Serratia marcescens*, *Viridans streptococci*, and *Klebsiella pneumoniae*) 1 (3.1%). Gram negative rods were more sensitive to Imipenem while Gram positive cocci were more sensitive to Gentamicin. Complete wound healing and bacterial eradication was observed in 17(77.3%) of the diabetic patients while incomplete wound healing and bacterial persistence was observed in only 5(22.7%). **Conclusion:** This study showed that HBOT could be a useful tool in woundstherapy.

**Keywords:** Diabetic wound, hyperbaric Oxygen Therapy, AL-Dirby, *Proteus mirabilis*.

## INTRODUCTION

The skin is the largest organ of the body, consisting of an external cellular layer, the epidermis, and a deep connective tissue layer called the dermis (Venus et al., 2010). The skin is critical in the body defense against injury and dehydration, as well as against foreign bodies, it is equally important in sensation and the regulation of temperature (Davies & Tovey 2014; Ford, 2010). The skin is colonized by an array of organisms which forms its normal flora (Drake et al., 2008). The relatively arid area of the fore and back arm is colonized with fewer organisms, predominantly Gram-positive bacteria and yeasts in moister areas such as the groin and the armpits, where the organisms are more numerous, more varied and include Gram-negative bacteria. The normal flora of the skin plays an important role in protecting the surface from foreign invaders (Koreck et al., 2003; Goering et al., 2013).

Skin infections may be either primary or secondary, primary skin infections are mainly caused by staphylococci or streptococci, staphylococci infection present as furuncles, carbuncles, superficial folliculitis and scalded skin syndrome (Rhody, 2000). Streptococcal infections present as impetigo, ecthyma, erysipelas and cellulitis. Secondary bacterial infections of pre-existing wounds, burn and dermatitic skin are usually present (Tognetti et al., 2012).

Wound infections occur primarily beside skin and soft infection as a result of a break in the skin surface (Tille, 2014). A great variety of organisms are involved in wound infection, the bacteria most frequently isolated differ according to anatomic site and predisposing factors (Dryden, 2010). Traumatic open wound infections are caused primarily by members of the soil flora such as *Clostridium perfringens* (DiNubile & Lipsky, 2004). Surgical wound infections are usually due to *Staphylococcus aureus* (Levinson, 2008). Swabs or preferably pus obtained directly from the wound or abscess is adequate to find the causative organism (Greenwood et al., 2007).

Normal wound healing occurs rapidly, after sequential phases including; homeostasis, inflammation, remodeling of granulation tissue and re-epithelialization (Rao et al., 2016). Healing process could be delayed or fail due to oxygen deficiency as a result of diabetes mellitus, irradiation, small vessel atherosclerosis or chronic infection (Ekmektzoglou, & Zografos, 2006). Diabetic patients with foot ulcers are prone to infection by multidrug resistant microorganisms due to; inadequate treatment, chronic nature of the wounds or

frequent hospital admissions (Perim et al., 2015). Oxygen is needed in all stages of healing process; proliferation, angiogenesis and remodeling. Oxygen therapy has been applied as a treatment of wounds since 1960s (Rao et al., 2016). Hyperbaric oxygen therapy (HBOT) is the therapeutic administration of 100% oxygen at pressure higher than 1 absolute atmosphere (ATA) (Bhutani, & Vishwanath, 2012). It is administered by placing the patient in a multiplace or mono place chamber where the vessels are pressurized to 1.5-3.0 ATA for a period between 60-120 minutes once or twice a day. In the monoplace chamber the patient breathes the oxygen directly from a chamber but, in the multiplace chamber this is done through a mask (Barata et al., 2011). Hyperbaric oxygen therapy decreases wound tissue hypoxia by enhancing perfusion, reducing edema, down regulating inflammatory cytokines, promoting fibroblast proliferation and collagen synthesis (Benjamin, 2010). HBOT has been used as a potent tool in increasing the oxygen content of blood. HBOT has been advocated for the treatment of various ailments including air embolism, carbon monoxide poisoning, wound healing and ischemic stroke (Yutsis, 2003). Despite being useful in treating many ailments hyperbaric oxygen therapy has several side effects and complications with varying degree of seriousness (Huang et al., 2015). The most frequent side effects include middle ear barotraumas, progressive myopia and pulmonary dyspnea with cough and aspiratory pain (Camporesi, 2014).

## MATERIALS AND METHODS

### Study design

This was a longitudinal case study.

### Study area

This study was conducted in Khartoum State. The specimens were collected from patients admitted to Al-Dirby Centre where initial processing was done.

### Study duration

The study was carried out between January and July 2016

\*Corresponding Author's Email: [ahashima@gmail.com](mailto:ahashima@gmail.com)

## Study population

Twenty-two male and female diabetic patients with wound infections attending Al-Dirby Centre, Khartoum were enrolled in this study. Sampling was repeated weekly during HBOT.

## Ethical consideration

The study was approved by the ethical committee of the College of Medical Laboratory Science- Sudan University of Science and Technology (SUST) and Al-Dirby Centre. Verbal consent was taken from all patients before collection of the specimens.

## Inclusion criteria

Males and females diabetic patients with wound infections

## Exclusion criteria

Males and females diabetic patients without wound infections

## Data collection

A structured questionnaire was used for collection of data from the patients.

## Collection and processing of specimens

Wound swabs were collected from each patient attending *AL-Dirby* Centre before dressings. The swabs were inoculated onto Amies transport slopes for transport to the laboratory of Microbiology Department, College of Medical Laboratory Science, SUST for isolation, identification and sensitivity testing of the clinical isolates. Swabs were inoculated onto basic and selective media, then the isolates were identified through conventional methods (Mandell et al., 2010) and the identified isolates were preserved in nutrient agar slopes.

## Antimicrobial Sensitivity test

This test was done to determine the sensitivity and resistance of the clinical isolates to selected antibiotics following the Kirby-Bauer (Mandell et al., 2010). The following antibiotics were selected for sensitivity testing of wound pathogens including Ampicillin, Co-trimoxazole, Erythromycin, Penicillin, Tetracycline, and

Gentamicin for Gram-positive isolates. Co-trimoxazole, Tetracycline, Gentamicin, Amikacin, Ciprofloxacin, Imipenem, Ceftriaxone, and Amoxicillin were used for Gram-negative isolates. Standard strains of *Escherichia coli* ATCC (25922) and *Staphylococcus aureus* ATCC (29213) were used for quality control.

## Data analysis

All data were analyzed using Statistical Package of Social Science (SPSS) (version 11.5; corp, college station, Tex), using Chi square test, *P*. value <0.05 considered as significant, then data were presented in tables and graphs using Excel.

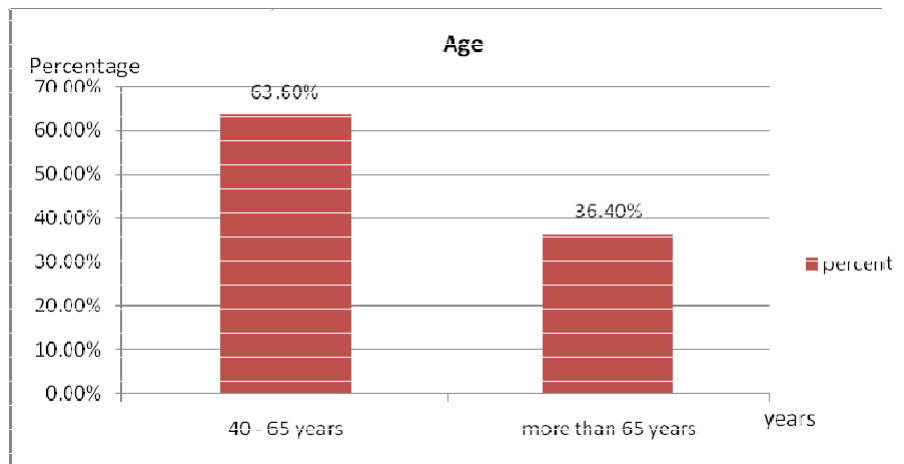
## RESULTS

A total of twenty-two patients were sampled, of whom 17 (77.3%) were males and 5 (22.7%) were females. Insulin dependent diabetic patients were 15 (68.2%), and Insulin independent were 7 (31.8%). Frequencies of gender with IDDM and INDDM were presented in Table (4.1). Patient's age groups presented in Figure (4.1). The study revealed that the wounds of 20 (90.9%) of the patients were in feet, and only 2 (9.1%) wounds were in the hands. A total of 32 bacteria representing 9 different bacteria were isolated as shown in Table (4.3). Patients with single bacterial infection (monomicrobial) during the course of HBOT were 13 (59.1%) while patients infected with more than one bacterium (poly microbial) were 9 (41.9%). The antimicrobial sensitivity tests were done to evaluate the sensitivity and resistance patterns of isolates against selected antibiotics and the results presented in Tables (4 and 5) respectively.

The outcome of Hyperbaric Oxygen Therapy on isolated bacteria in this study was statistically significant with *P*. value <0.05. Bacterial eradication and wound healing was successful in 17 (77.3%) patients, while bacterial persistence was observed in 5 (22.7%) patients, 4 of them had amputation Table (4.6).

## DISCUSSION

Frequency of gender with Insulin dependent diabetes mellitus and Insulin independent diabetes mellitus in this study is similar to that reported by Al-Saimary, (2016) in Iraq. The category which was frequently affected with



**Figure (4.1):** Distribution of enrolled patients according to age groups

**Table (4.1):** Frequency of IDDM and NIDDM among male and female patients

		IDDM	NIDDM	Total
Sex	Male	12 (54.5%)	5 (22.7%)	17 (77.3%)
	Female	3 (13.6%)	2 (9.1%)	5 (22.7 )
	Total	15 (68.2%)	7 (31.8%)	22 (100%)

**Table(4.2):DistributionofbacterialisolatesaccordingtotheirGramreaction**

Type of Bacteria	Frequency
Gram positive cocci	7 (21.9%)
Gram negative rods	25 (78.1%)
Total	32 (100%)

**Table No (4.3): Distribution of bacteria isolated from infectedwounds**

Bacterial isolates	Frequency (%)
<i>Proteus mirabilis</i>	8 (25.0%)
<i>Proteus vulgaris</i>	4 (12.5%)
<i>Staphylococci aureus</i>	5 (15.6%)
<i>Pseudomonas aeruginosa</i>	6 (18.7%)
<i>Escherichia coli</i>	5 (15.6%)
<i>Klebsiella pneumoniae</i>	1 (3.1%)
<i>Streptococcus pyogenes</i>	1 (3.1%)
<i>Serratia. Marcescens</i>	1 (3.1%)
Viridans streptococci	1 (3.1%)
Total	32 (100%)

**Table (4.4): The results of the sensitivity tests of Gram negative rods**

Bacteria frequenciesand percentages	Antibiotic discs															
	Ciprofloxacin		Gentamicin		Amoxycillin		Co-Trimoxazole		Amikacin		Ceftriaxone		Imipenem		Tetracycline	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
<i>P. mirabilis</i>	3 37.5%	5 62.5%	6 75%	2 25%	3 37.5%	5 62.5%	2 25%	6 75%	8 100%	0 0%	2 25%	6 75%	8 100%	0 0%	0 0%	8 100%
<i>Ps. aeruginosa</i>	5 83.3%	1 16.7%	5 83.3%	1 16.7%	Not tested		Not tested		6 100%	0 0%	2 33.3%	4 66.6%	Not tested		Not tested	
<i>E.coli</i>	3 60%	2 40%	4 80%	1 20%	Not tested		Not tested		4 80%	1 20%	4 80%	1 20%	Not tested		Not tested	
<i>P. vulgaris</i>	1 25%	3 75%	3 75%	1 25%	Not tested		Not tested		4 100%	0 0%	0 0%	4 100%	Not tested		Not tested	
<i>S. marcescens</i>	0 0%	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%	1 100%	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%	0 0%	1 100%
<i>K. pneumoniae</i>	1 100%	0 0%	1 100%	0 0%	0 0%	1 100%	0 0%	1 100%	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%	0 0%	1 100%

**Key: S: Sensitive, R: Resistant**

**Table (4.5): The results of sensitivity tests of Gram positive cocci**

Bacteria	Antibiotic discs									
	Gentamicin		Erythromycin		Penicillin		Tetracycline		Cefixime	
	sensitive	Resistant	sensitive	resistant	sensitive	resistant	sensitive	resistant	sensitive	resistant
<i>S. aureus</i>	5 100%	0 0%	5 100%	0 0%	0 0%	5 100%	1 20%	4 80%	Not tested	
<i>St. pyogenes</i>	Not tested		1 100%	0 0%	1 100%	0 0%	Not tested		1 100%	0 0%
Viridans streptococci	1 100%	0 0%	0 0%	1 100%	0 0%	1 100%	Not tested		0 0%	1 100%

**Table (4.6): Distribution of bacterial species before and after Hyperbaric Oxygen Therapy (HBOT)**

Bacterial isolates	Total No. before therapy	Total No. after therapy
<i>Proteus mirabilis</i>	8	2
<i>Proteus vulgaris</i>	4	0
<i>Staphylococcus aureus</i>	5	2
<i>Pseudomonas aeruginosa</i>	6	1
<i>Escherichia coli</i>	5	1
<i>Klebsiella pneumoniae</i>	1	0
<i>Streptococcus pyogenes</i>	1	0
<i>Serratia marcescens</i>	1	0
Viridans streptococci	1	1
Total number	32	7

P .value ≤0.05

**Table (4.7): Outcome of HBOT in relation to age group**

Age / year	Eradication of bacteria	Persistence of bacteria	Total
40-65	12 (85.7%)	2 (14.3%)	14 (100%)
More than 65	5 (62.5%)	3 (37.5%)	8 (100%)
Total	17 (77.3%)	5 (22.7%)	22 (100%)

**Table (4.8): Hyperbaric oxygen therapy (HBOT) in diabetic patients in relation to the duration of Diabetes Mellitus**

Duration of DM/years	Eradication of bacteria	Persistence of bacteria	Total
Less than 10	8 (88.9%)	1 (11.1%)	9 (100%)
11-15	4 (66.7%)	2 (33.3%)	6 (100%)
16-20	4 (100%)	0 (0%)	4 (100%)
21-25	0 (0%)	1 (100%)	1 (100%)
26-30	1 (50%)	1 (50%)	2 (100%)
Total	17 (77.3%)	5 (22.7%)	22 (100%)

**Table (4.9): Treatment outcome of diabetic wounds after Hyperbaric Oxygen Therapy (HBOT)**

Description	Frequency (%)
Complete Healing	17 (72.7%)
Incomplete Healing	5 (27.3%)
Total	22 (100%)

wound infections was those of age group 40- 65 years old, this result is similar to those reported by Ahmed, (2003) in Sudan. The commonest site of infection in this study was the feet 90.9% infection, which is similar to that reported by Mahgoub and Omer, (2015) in Sudan which was 98.7%. There was bacterial growth from all the swabs; probably this because diabetic patients are more prone to infections. Moreover, the increased incidence of infections could be related to impairment of the immune response in the diabetic patients (Ahmed, 2003).

Monomicrobial infection was observed in (59.1%) of the cases, while polymicrobial infection was observed in (41.9%) of the cases, these results disagree with those reported by Mahgoub and Omer, (2015) in Sudan, in which monomicrobial and polymicrobial infection were (77.3%) and (22.7%) respectively. Monomicrobial infection was close to that reported by Sugandhi and Prasanth, (2014) in India which was (56%) unlike polymicrobial infection which was (44%). Moreover, it was less than the rate of monomicrobial (83.5%) and of polymicrobial (16.4%) reported by Turhan *et al.*, (2013) in Turkey. In contrast to monomicrobial infections polymicrobial infection is reported in serious infections that fail to respond to previous antibiotic therapy. Gram negative isolates were more than Gram positive isolates as they were (68.1%) and (21.9%) respectively. These results disagree with those reported by Ali, (2005) in Sudan, where Gram negative isolates were (57.8%) and Gram positive isolates were (42.8%). However, these results are similar to those reported by Mahgoub and Omer in Sudan, where Gram negative isolates were (77.3%) and Gram positive isolates were (22.7%).

The most common bacteria isolated during this study was *Proteus mirabilis* (25%), which is more than those reported by Abbass, (2009) in Sudan which was (19%). In another study in Iran by Akhi *et al.*, *S. aureus* was the predominant isolate (28%) (2015). The variation between the most frequent isolate in different studies and percentages of Gram positive and Gram negative bacteria could be attributed to the source of infection, severity of infection and even the immune status of diabetic patients. Moreover, the high incidence of Gram negative isolates compared to Gram positive isolates among diabetic wound patients could be justified by the complexity of Gram negative cell walls which makes them more resistant to antimicrobial therapy. In this study *P. mirabilis* was more frequent than *P. vulgaris*, and *S. marcescens* which was also found in diabetic wound infection, this is similar to that reported by Ali, (2005) in Sudan. Moreover, *Proteus species* were resistant to many antibiotics and this resistance pattern was also reported by Mordi and Momoh, (2008) in Nigeria. This observation could be explained by the wide spread of plasmid resistance genes among *Proteus species* (Mordi & Momoh, 2009).

In this study, Gram negative bacteria were sensitive to Imipenem, this is similar to those reported by Sugandhi and Prasanth, (2014), Mohanasoundaram, (2012) in India, and Turhan *et al.*, (2013) in Turkey. In this study all Gram negative isolates were resistant to Tetracycline; this is similar to that reported by Abbas, (2009) in Sudan, and Mordi and Momoh, (2008) in Nigeria. The most effective antibiotic against *Ps.aeruginosa* was Amikacin,



this is similar to that reported by Abdel Wahab *et al.*,(2013) in Sudan.All *S. aureus* in this study were sensitive to Erythromycin, which was similar to that reported by Mohanasoundaram, (2012) in India. Furthermore, all *S. aureus* were resistant to penicillin which is similar to that reported by Ahmed, (2003) in Sudan. The routine use of antibiotics in both medical and veterinary medicine has led to wide spread of antibiotic resistance and development of antibiotic resistance genes particularly within the Gram negative organisms (Mordi& Momoh,2008).

The results of pre-and post-procedural wound cultures were statistically significant at *P*.value<0.05, which is similar to those reported by Doctor, (1992) in India. The study revealed that, the frequency of cases that showed healing were (77.3%), which is close to those reported by Ong, (2008) in Singapore, in which the success rate after HBOT was(71%).

## CONCLUSIONS

In conclusion, diabetic wound infections continue to cause problems particularly to the feet where they affect more than 90% of the patients in this study. The wound infections are caused by Gram positive and Gram negative bacteria where they were 7 (21.9%) and 25 (78.1%) respectively. The most common pathogen that caused diabetic wound infections in this study was *P. mirabilis* (25) %. All Gram negative rod isolates were sensitive to Imipenem, and all of them were resistant to Tetracycline. All Gram positive cocci isolates were sensitive to Gentamicin. Complete healing and bacterial eradication was observed in 17(72.7%), while persistence and incomplete healing was observed only in 5 (27.3%)after HBOT.HBOT is an effective enhancement tool of antimicrobial therapy against aerobic bacteria that cause diabetic wound infections.

## REFERENCES

- Abbass EI (2009). Frequency and Sensitivity Pattern of Aerobic Bacteria isolated from Wounds of Diabetic Patients. M.Sc thesis. Sudan University of Science and Technology.
- Abdel Wahab WF, Bakhiet MA, Mahadi SI, Mahmoud SM, Widataal AH, Ahmed ME (2013). Diabetic Foot Infections with Pseudomonas: Jabir Abueliz Diabetic Center Khartoum Experience. *Clinical Research on Foot & Ankle*. 83(S3):1-3.
- Ahmed EO (2003). Bacteriology of Diabetic Foot Infections in Sudan. Clinical MD thesis. University of Khartoum.
- Akhi MT, Ghotaslou R, Asgharzadeh M, Varshochi M, Pirzadeh T, Memar MY (2015). Bacterial etiology and antibiotic susceptibility pattern of diabetic foot infections in Tabriz, Iran. *GMS Hygieneand Infection Control*, 10:1-6.
- Ali AAS (2005). Isolation and identification of bacteria associated with diabetic foot infections. M.Sc thesis. University of Khartoum.
- Al-Saimary IA (2010). Bacterial wound infections in diabetic patients and their therapeutic implications, *Medical Practice and Review*. 1 (2): 12-15.
- Barata P, Cervaens M, Resende R, Camacho O, Marques F (2011). Hyperbaric oxygen effects on sports injuries. *Therapeutic Advances in Musculoskeletal Disease* 3 (2): 111-121.
- Benjamin LA (2010). Hyperbaric Oxygen Therapy for Diabetic Foot Wound. *Diabetic Care*, 33(5):1143-1145.20-
- Bhutani S, Vishwanath G (2012). Hyperbaric oxygen and wound healing. *Indian Journal of Plastic Surgery: Official Publication of the Association of Plastic Surgeons of India*, 45(2), 316.
- Camporesi EM (2014).Side effects of hyperbaric oxygen therapy. *The Journal of Undersea and Hyperbaric Medical Society*, 41
- Davies S, Tovey M (2014). Investigation of wound, tissue, and genital samples. *Medical Microbiology*, 366(11), 1028-1037.
- DiNubile MJ, Lipsky BA (2004). Complicated infections of skin and skin structures: when the infection is more than skin deep. *Journal of Antimicrobial Chemotherapy*, 53(suppl\_2), ii37-ii50.
- Doctor A (1992). Hyperbaric oxygen therapy in diabetic foot. *Journal of Postgraduate Medicine*, 38(3),112-114.
- Drake DR, Brogden KA, Dawson DV, Wertz PW (2008). Thematic review series: skin lipids. Antimicrobial lipids at the skin surface. *Journal of lipid research*, 49(1), 4-11.
- Dryden MS (2010). Complicated skin and soft tissue infection. *Journal of Antimicrobial chemotherapy*, 65(suppl\_3), iii35-iii44.
- Ekmektzoglou KA, Zografos GC (2006). A concomitant review of the effects of diabetes mellitus and hypothyroidism in wound healing. *World Journal of Gastroenterology: WJG*, 12(17), 2721.
- El-Tahawy AT (2000). Bacteriology of diabetic foot Infections. *Saudi Medical Journal*, 21 (4):344-347.
- Ford M (2010). Medical Microbiology, 1st edition, Oxford University Press, New York, p 143.
- Goering RV, Dockrell HM, Zuckerman M, Roitt IM, Chiodini PL (2013). Mims' Medical Microbiology, fifth edition, Elsevier saunders, China, p335.
- Greenwood D, Slack R, Peutherer J, Barer M (2007). Medical Microbiology, seventeenth edition, Churchill livingstoneelsevier, China, p 662.
- Huang ET, Mansouri J, Murad MH, Joseph WS, Strauss MB, Tettelbach W, Worth ER (2015). A clinical practice guideline for the use of hyperbaric oxygen therapy in the treatment of diabetic foot ulcers. *Undersea Hyperb Med*, 42(3),205-247.
- Koreck A, Pivarcsi A, Dobozy A, Kemeny L (2003). The role of innate immunity in the pathogenesis of acne. *Dermatology*, 206(2), 96-105.
- Levinson, W. (2008). Review of Medical Microbiology and Immunology, tenth edition, Mc-Graw- Hill Companies, Sanfrancisco, p 66.
- Mahgoub EM, Omer MEA (2015). Aerobic bacteria isolated from diabetic septic wounds. *American Journal of Research Communication*. 3 (10): 91-99.
- Mandell GL, Bennett JE, Dolin R (2010). Mandell, Douglass and Bennett's Principles and Practice of Infectious Diseases, Seventh Edition, Mandell, Douglas, and Bennetts Principles and Practice of Infectious Diseases, Churchill Livingstone, Elsevier, Philadelphia, USA, pp 278-279
- Mohanasoundaram KM (2012). The Microbiological Profile of Diabetic Foot Infections. *Journal of Clinical and Diagnostic Research*. 6 (3): 409-411.
- Mordi RM, Momoh MI (2009). Incidence of Proteus species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. *African Journal of Biotechnology*.8(5):725-730.
- Ong M (2008). Hyperbaric oxygen therapy in the management of diabetic lower limb wounds. *Singapore medical journal*, 49(2),105.

- Perim MC, Borges JC, Celeste SRC, Orsolin EF, Mendes RR, Mendes GO (2015). Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections. *Revistada Sociedade de MedicinaTropica*, 48 (5):546-554.
- Rao C, Xiao L, Liu H, Li S, Lu J, Li J, Gu S (2016). Effects of topical oxygen therapy on ischemic wound healing. *The Journal of Physical Therapy Science*, 28 (1):118-123.
- Rhody C (2000). Bacterial infections of the skin. *Primary Care: Clinics in Office Practice*, 27(2),459-473.
- Sugandhi P, Prasanth DA (2007). Bacteriological Profile of Diabetic Foot Infections. *International Journal of Innovative Research in Science, Engineering and Technology*. 3 (7): 14688-14692.
- Tille PM (2014). Diagnostic Microbiology, thirty edition, chapter 76, Elsevie, China, p961,
- Tognetti L, Martinelli C, Berti S, Hercogova J, Lotti T, Leoncini F, Moretti S (2012). Bacterial skin and soft tissue infections: review of the epidemiology, microbiology, aetiopathogenesis and treatment. *Journal of the European Academy of Dermatology and Venereology*, 26(8),931-941.
- Turhan V, Mutluoglu M, Acar A, Hatipoğlu M, Önem Y, Uzun G (2013). Increasing incidence of Gram-negative organisms in bacterial agents isolated from diabetic foot ulcers. *The Journal of infection in Developing Countries*. 7 (10):707-712.
- Venus M, Waterman J, McNab I (2010). Basic physiology of the skin. *Surgery-Oxford International Edition*, 28(10), 469-472.
- Yutsis PI (2003). *Oxygen to the Rescue: Oxygen Therapies, and How They Help Overcome Disease, Promote Repair, and Improve Overall Function*. Basic Health Publications,Inc..