# Prevalence of Bacterial Hemolytic Strains and Their Antimicrobial Resistance Pattern in Relation to Antibiotic Misuse by Misurata Dental Patients

Asma Abass*, Hend Salama, Howida Elsheekh

*Department of Industrial Pharmacy and Medical Microbiology, Faculty of Pharmacy, Misurata University, Libya.*
**Corresponding Author Email: cavity95@yahoo.com*

## Abstract

Fifty gum infected patients were swabbed for oral cavity to report the prevalence of heamolytic and non-heamolytic bacterial strains and their drug resistance condition against commonly used antibiotics. Obtained results revealed the predominance of heamolytic strains than non-heamolytic ones. In addition, both types showed high antimicrobial resistance patterns against most tested antibiotics. Positive correlations were observed between incidence of drug resistances and many unhealthy attributes like smoking, infrequent teeth brushings and mainly; uncontrolled use of antibiotics. Noteworthy was also that antimicrobial resistance was noticed mainly against amoxicillin/clavulonic acid, cefoxitin and trimethoprim/sulphamethoxazole. This study highlighted the hazard of misuse of antibiotics and the necessity of both controlling and awareness programs among both physicians and community.

**Keywords**: Antibiotic misuse; Antimicrobial resistance; Dental problems.

## 1. Introduction

A healthy gum is one that is free of pain and infection, with pink gum tissue, do not bleed on brushing and the mouth should be moist, with no evidence of tooth decay [1]. Gum disease is typically progressed slowly and painlessly, so it can easily reach an advanced state before patient is aware of its presence. Protection from gum diseases could be done through learning more about gum disease and taking action to ensure better overall health [2].

Gum disease or "clinically termed periodontal disease" involves destruction of tooth and supporting tissue (gum and bone),usually occur in a cyclic manner, with bursts of destruction and periods of inactivity [3]. The gum diseases or gum infections are responsible for 70 percent of all tooth diseases and are the principal cause of tooth extraction [4-7]. Periodontal disease can be defined as multifactorial infectious disease, being characterized by symptoms and clinical signs that may include inflammation (visible or invisible). Varying degrees of spontaneous gingival bleeding or

bleeding on probing, pocket formation related to loss of attachment of alveolar bone, and tooth mobility which may lead to tooth loss are associated with gum diseases [8].

Bacteria can produce toxins, which irritate the gum and cause infections [9]. In the early stage of gum disease, the gum can become red, swollen, and bleed easily, as it is not normal for gum to bleed [2]. Earlier intervention can minimize the damage to gum and teeth and positively impact overall health [10].

Many bacteria, fungi and viruses are associated with gum infections, although the major cause is bacteria, which infect gum, ligaments and supportive bones. Oral bacteria usually live in a film called plaque [11]. Plaque is a thick and sticky film of bacteria that's build up on the teeth, which could be hardened to become calculus, known also as tartar [12].

Plaque is spreading its growth on the teeth and down into the crevice on the gum and tooth, when the underlying bone is lost.This crevice is called the pockets. In very large amounts, plaque can be seen as a fuzzy unclean coating on the teeth.Bacteria of plaque may secrete toxin that damage the gums and underlying bone [13].

In dentistry, the routine use of antibiotics before or after extractions or endodontics has not been shown to be effective [14,15]. Therefore, routine prescribing for every extraction or endodontic procedure must be discouraged in order to fight the problem of antimicrobial resistance.

This study was conducted aiming many objectives. Initially, to detect microbial resistance among bacterial strains isolated from gum infection patients at Misurata's dental clinics. Secondly, to conclude the effect of different unhygienic and unhealthy habits on incidence of gum infections.

## 2. Materials and Methods

### 2.1. Patient Survey

A standardized survey designed to report many parameters. This survey provides a standardized methodology that allows objective and meaningful comparisons.The survey is composed of 9 questions. Question were designed to provide answer on different related topics to antibiotic resistance of oral and gum microbial infection. In addition, this survey was aimed to explore different habits that may affect gum health.

### 2.2. Collection of samples

Fifty samples of sub-gingival swab were collected during the period from 14 December 2013 to 18 March 2014, from"Misurata dental clinics" with the assistance of dentists and under aseptic conditions.The swab samples were sent to the laboratory as quickly as possible within 2 hours of collection.

### 2.3. Preparation of sample

Each swab sample was inoculated separately into nutrient broth and incubated at 37 ℃ for 24hr to enhance the growth of microorganisms.

### 2.4. Bacteriological examination of samples

#### 2.4.1. Isolation of bacteria from sub-gingival swap sample

A loopful from incubated swab soaking nutrient broth were streaked on blood agar, chocolate agar and MacConkey agar and incubated at 37ºC for 24hr according to the method recommended by Arura and Arora [16].

#### 2.4.2. Cultural characteristics

Cultural characteristics of isolated bacterial colonies after culturing them on different media such as size, shape and color were recorded.

#### 2.4.3. Purification and Identification of isolated bacteria

Different colonies were picked up and streaked on slope agar, then incubated at 37ºC for 24hr for further purification and identification.

#### 2.4.4. Microscopical examination

Gram stained film from bacterial growth was observed under oil immersion lens of a light microscope.The shapes of bacteria cell, stain reaction and arrangement were recorded.

#### 2.4.5. Antimicrobial susceptibility testing

The susceptibility of bacterial isolates to antimicrobial agents was determined by using the disc diffusion method on nutrient agar according to the method recommended by Arura and Arora [16]. The bacterial isolates were evenly streaked on Muller hinton agar plates, then the antimicrobial discs were distributed on the plate and incubated for 18-24hr. About 5-6 discs were equally distributed on each cultured plate. Afterwards, the diameter of halo zone of non-growth around each disc was measured and the results were recorded as sensitive (+), relatively resistant (+/-), and resistant (-) and compared to Cappuccino and Sherman [17] Table (1).

Eleven antimicrobials were chosen to represent the mostly prescribed antimicrobials among dental clinics patients. The antimicrobial agents used in this study included: Tetracycline (30 µg), Amoxicillin/clavulanic acid (20/10µg), Erytromycin (30 µg), Chloramphenicol(30 µg), Nalidixic acid (30 µg), Ciprofloxacin (5µg), Nitrofurantoin (50 µg), Cefoxitin (30µg), Imipenem (10µg), Ceftriaxon (30µg) and Trimethoprim/sulphamethoxazole (1.25/23.75 µg).

**Table (1):** Interpretation chart of zone size in Kirby-Bauer disc diffusion method

| Antimicrobial agent | Diameter of zone of inhibition (mm) | | |
|---|---|---|---|
| | Resistant | Moderately susceptible | Susceptible |
| Erythromycin | ≤13 | 14_17 | ≥18 |
| Tetracycline | ≤14 | 15_20 | ≥21 |
| chloramphenicol | ≤12 | 13_17 | ≥18 |
| Nalidixic acid | ≤13 | 14_18 | ≥19 |
| Nitrofurantoin | ≤14 | 15_17 | ≥18 |
| Trimethoprim \ Sulphamethoxazole | ≤10 | 11_15 | ≥16 |
| Ciprofloxacin | ≤15 | 16_20 | ≥21 |

## 3. Results and Discussion

This study was designed to isolate bacterial strains predominated in patients with periodontitis, to explore the antibiotic susceptibility to different antibiotics commonly prescribed as treatment or prophylaxis, and to study different factors affecting both prevalence of periodontal diseases and microbial resistance among them via a detailed questioner.

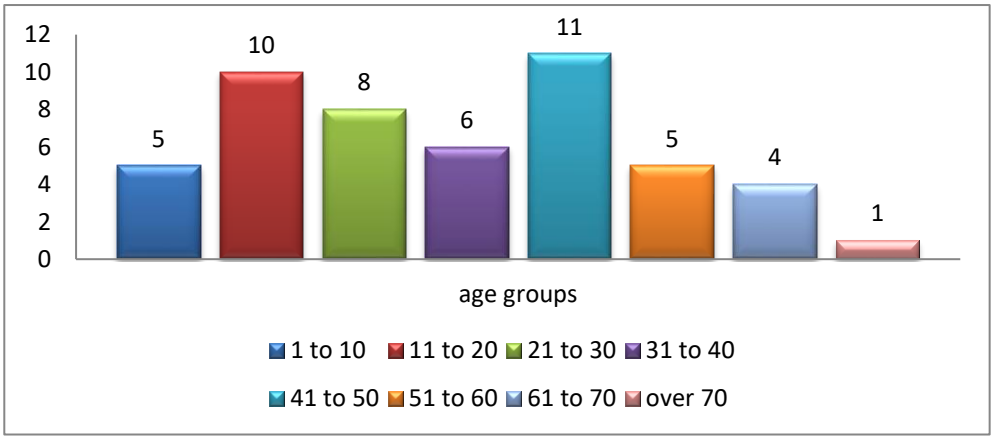


**Figure (1):** Groups of age of patient included in the study.

Regarding age of patients, Figure (1) presented a view for all the age of examined patients, and it indicated that the gum infection was most prevailed at age from 41-50 years. Very small population in the age group of 70 years was found to have a healthy periodontium. Although, periodontitis is also reported in different ages.

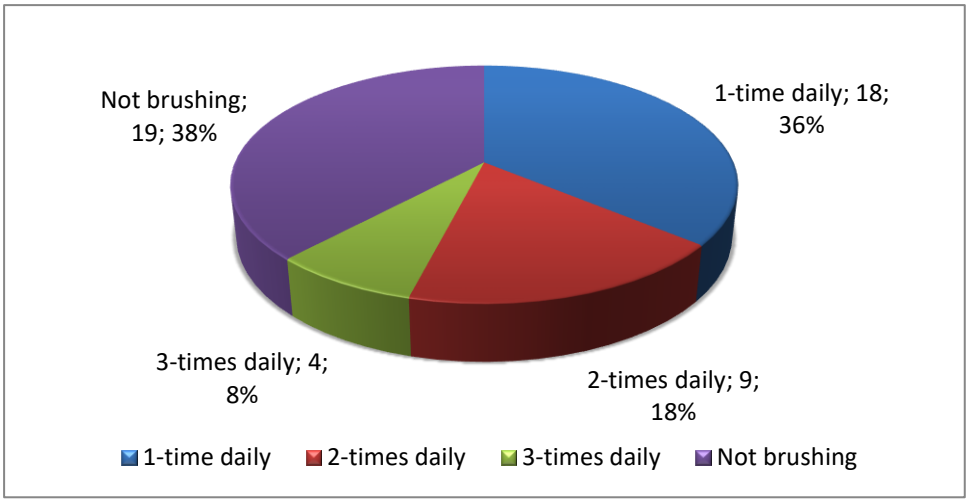


**Figure (2):** Distribution of patients according to frequency of daily teeth brushing.

Important factor affecting the incidence of periodontal diseases is the habit of proper cleaning of oral cavity via teeth brushing. Figure (2) presented that the majority of examined patients (38%) were not practicing brushing with a toothpaste, which represent a risky percentage when compared to previous study conducted on Libyan patients [37]. In addition, only 8 % of examined patients were practicing teeth brushing regularly of 3-times daily.It is very clear that the frequency of tooth brushing was significantly associated with the incidence of gum infections. While regarding the most important factor affecting the microbial resistance of antibiotics, the uncontrolled use of antibiotics was found to be of high impact.

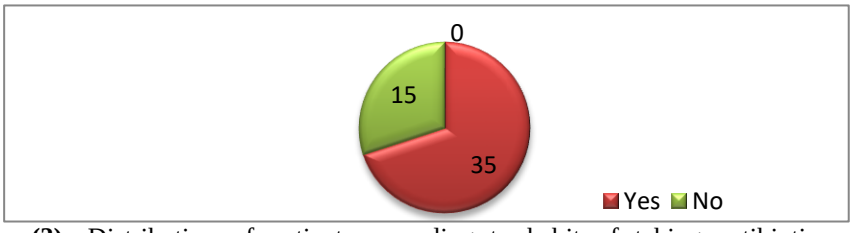


**Figure (3):** Distribution of patients according to habit of taking antibiotic without physician prescription.

Figure (3) showed that the majority of examined patients were taking antibiotics without physician's prescription. In line with previous study Peeran *et al* [18]**,** the severity of infection, Figure (4) showed that 40 % of examined patients suffered from gum bleeding. Different organisms were isolated from gum infections from included Misurata dental clinics' patients. Out of fifty swabs, which were collected from those patients, the number of isolated hemolytic bacterial strains was 29, which indicated the prevalence of hemolytic bacteria than non-hemolytic bacteria from those patients Figure (5).

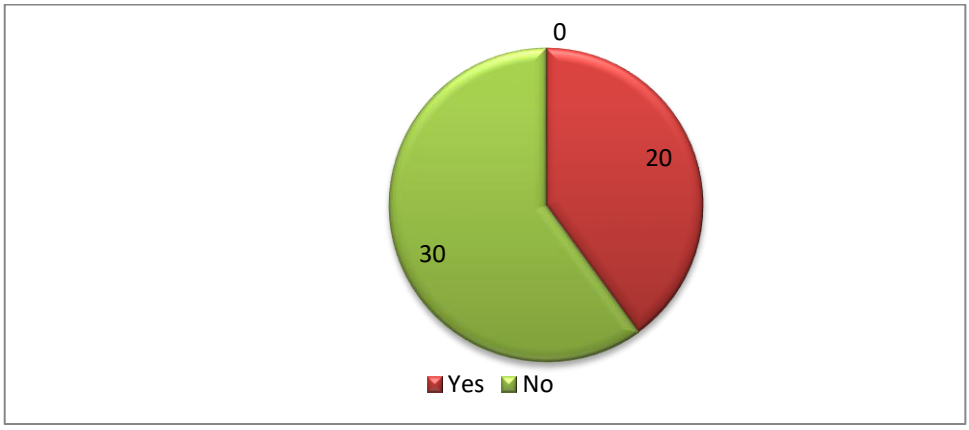


**Figure (4):** Distribution of patients according to history of gum bleeding.

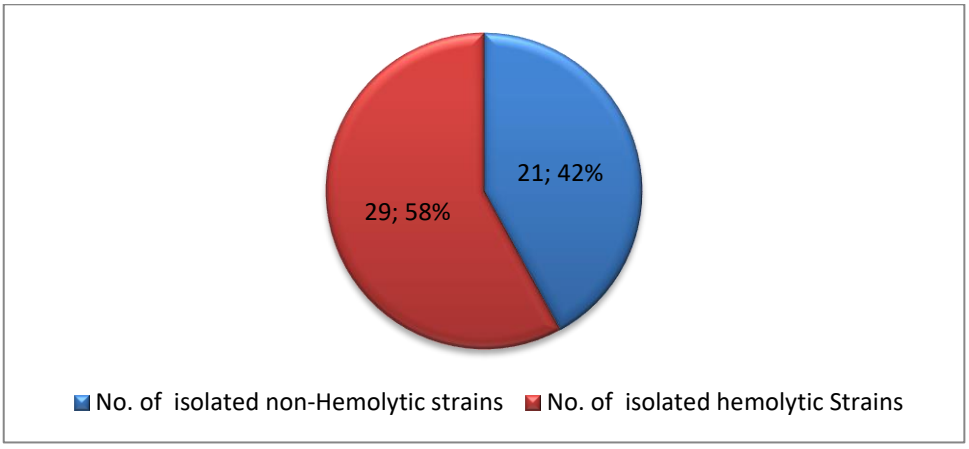


**Figure (5):** Prevalence of hemolytic and non-hemolytic strains isolated from gum infection Patients.

There are many international prevalence studies regarding isolation of microbial causative agents of gum infections, although, as far as our knowledge, only one literature regarding the gum infection status of the

population in Misurata by Khemaleelakul *et al*. [19], in which they reported nearly similar findings to our study except that the likelihood of the prevalence of periodontal disease was much less during adulthood. In order to determine the antimicrobial sensitivity pattern of isolated organisms, eleven antimicrobial agents were chosen in this study to compare the sensitivity of these isolated organisms. The selection of these antimicrobial agents was done based on the most comonly prescribed antimicrobial among Misurata Dental clinics.

Organisms varied in their response to these antimicrobial agents according to their types and/or strains differences. As for the antibiotic susceptibility of hemolytic isolated strains, the highest rate of resistance was found against Amoxicillin/Clavulanic acid and Trimethoprim/ Sulphamethoxazole, in which nearly 48 and 58 % of isolated heamolytic strains were resistant to these antimicrobial agents, respectively (Table 2). Imipenem and ciprofloxacin were found to have the greatest antimicrobial effect against heamolytic strains.

**Table (2):** Antibiotic resistance pattern of hemolytic isolated strains.

| **Antibiotics** | **Percentage of resistant Strains** | **Percentage of intermediate Strains** | **Percentage of susceptible Strains** |
|---|---|---|---|
| Amoxicillin/Clavulanic acid | 48.3 | 34.5 | 17.2 |
| Trimethoprim/ Sulphamethoxazole | 58.6 | 27.6 | 13.8 |
| Cefoxitin | 51.7 | 17.3 | 31.0 |
| Erythromycin | 37.9 | 44.8 | 17.3 |
| Tetracycline | 44.0 | 27.0 | 29.0 |
| Imipenem | 41.3 | 7.0 | 51.7 |
| Ceftriaxone | 44.8 | 20.7 | 34.5 |
| Ciprofloxacin | 38.0 | 10.0 | 52.0 |
| Nalidixic Acid | 69.0 | 20.7 | 10.3 |
| Nitrofurantoin | 34.5 | 34.5 | 31.0 |
| Chloramphenicol | 31.0 | 24.1 | 44.9 |

While regarding the antibiotic susceptibility of non-hemolytic strains, Amoxicillin/Clavulanic acid was found to be the most antimicrobial agent to which isolated organisms have developed a resistance against Table (3).

**Table (3):** Antibiotic resistance pattern of non-hemolytic isolated strains.

| Antibiotics | Percentage of resistant Strains | Percentage of intermediate Strains | Percentage of susceptible Strains |
|---|---|---|---|
| Amoxicillin/Clavulanic acid | 66.7 | 23.8 | 9.5 |
| Trimethoprim/ Sulphamethoxazole | 33.3 | 52.4 | 14.3 |
| Cefoxitin | 42.8 | 28.6 | 28.6 |
| Erythromycin | 52.4 | 33.3 | 14.3 |
| Tetracycline | 30.6 | 43.6 | 25.8 |
| Imipenem | 24.0 | 19.0 | 57.0 |
| Ceftriaxone | 33.0 | 48.8 | 19.0 |
| Ciprofloxacin | 24.0 | 24.0 | 52.0 |
| Nalidixic Acid | 33.3 | 52.4 | 14.3 |
| Nitrofurantoin | 57.2 | 33.3 | 9.5 |
| Chloramphenicol | 19.1 | 47.6 | 33.3 |

Similarly, to heamolytic strains, imipenem and ciprofloxacin were found to have the greatest antimicrobial effect against non-heamolytic strains. While in another comparison between heamolytic and non heamolytic strains Tables (4), (5) and (6) imipenem and ciprofloxacin were found to be the highly effective antimicrobial agents against both types of isolated strains (highly sensitive and less resistant strains). At the contrary, Nalidixic Acid was found to be the most non effective antimicrobial against heamolytic strains, and Amoxicillin/Clavulanic acid against non-heamolytic strains.

**Table (4):** Comparison of antibiotic significance among isolated susceptible hemolytic and non-hemolytic strains.

| Antibiotics | No. of hemolytic Susceptible strains | No. of non-hemolytic Susceptible strains |
|---|---|---|
| Amoxicillin/Clavulanic acid | 5 | 2 |
| Trimethoprim/ Sulphamethoxazole | 4 | 3 |
| Cefoxitin | 9 | 6 |
| Erythromycin | 5 | 3 |
| Tetracycline | 9 | 5 |
| Imipenem | 15 | 12 |
| Ceftriaxone | 10 | 4 |
| Ciprofloxacin | 16 | 10 |
| Nalidixic Acid | 3 | 3 |
| Nitrofurantoin | 9 | 2 |
| Chloramphenicol | 13 | 7 |

**Table (5):** Comparison of antibiotic significance among isolated intermediate hemolytic and non-hemolytic strains.

| Antibiotics | No. of hemolytic intermediate strains | No. of non-hemolytic intermediate strains |
|---|---|---|
| Amoxicillin/Clavulanic acid | 10 | 5 |
| Trimethoprim/ Sulphamethoxazole | 8 | 11 |
| Cefoxitin | 5 | 6 |
| Erythromycin | 13 | 7 |
| Tetracycline | 8 | 9 |
| Imipenem | 2 | 4 |
| Ceftriaxone | 6 | 10 |
| Ciprofloxacin | 3 | 5 |
| Nalidixic Acid | 6 | 11 |
| Nitrofurantoin | 10 | 7 |
| Chloramphenicol | 7 | 10 |

Many different antibiotic regimens have being subscribed at clinics making the correct choice difficult due to development of resistance and increase in opportunistic microorganisms. In addition, no justification of antibiotic prescription in the treatment of chronic, slowly-progressive forms of periodontal diseases are taken, which was also mentioned in previous studies [20, 21]. Recognizing specific types of periodontal infections can significantly influence the choice of antimicrobial treatment. Therapy should be tailored to differences in antibiotic susceptibility between various periodontal pathogens. The choice of antibiotic is empirical and based on the clinical signs.

**Table (6):** Comparison of antibiotic significance among isolated resistant hemolytic and non-hemolytic strains.

| Antibiotics | No. of hemolytic resistant strains | No. of non-hemolytic resistant strains |
|---|---|---|
| Amoxicillin/Clavulanic acid | 14 | 14 |
| Trimethoprim/ Sulphamethoxazole | 17 | 7 |
| Cefoxitin | 15 | 9 |
| Erythromycin | 11 | 11 |
| Tetracycline | 13 | 6 |
| Imipenem | 12 | 5 |
| Ceftriaxone | 13 | 7 |
| Ciprofloxacin | 11 | 5 |
| Nalidixic Acid | 20 | 7 |
| Nitrofurantoin | 10 | 12 |
| Chloramphenicol | 9 | 4 |

The most commonly prescribed antibiotic treatments for periodontitis are systemic antibiotic therapy for periodontal treatment which usually involves

monotherapy based on the β-lactams (amoxicillin with or without clavulanic acid), tetracyclines (tetracycline, doxycycline, and minocycline), clindamycin and ciprofloxacin. The obtained results from this study have concluded that the use of antibiotics in dental practice is characterized by prescription based on clinical and but not microbial susceptibility bacteriological diagnosis. In turn, an increased number of bacterial strains resistant to conventional antibiotics are found in the oral cavity in our study which relatively simulate a previous study by Roda *et al.* [22].

Microbial analysis should be used to determine the specific antimicrobial susceptibility pattern of the suspected pathogens, which can help in choosing the appropriate antibiotics, and may be followed-up with additional testing to verify the elimination or suppression of the putative pathogens. Unfortunately, for some clinicians, microbial analysis may be reserved for cases that are refractory to an initial course of antimicrobial therapy.

## References

[1]. Petersen, P.E. and Yamamoto, T. (2005). Improving the oral health of older people: the approach of the WHO Global Oral health program. Community Dent Oral Epidemiology 33:81-92.

[2]. Grau, D.M., Silvestre, F.J., Miralles, L. and Roig, J. M. (2002). La secreción saliva ldurante el embarazo. Rev Eurodontoestomatol; XIV: 93-8.

[3]. Henry, J. L., and Sinkford, H. C. (1977). The Economic and Social Impact of Periodontal Disease: Position Report and Review of Literature" in International Conference on Research in the Biology of Periodontal Disease (Ann Arbor, Mich.: University of Michigan).

[4]. Ingle, J.I. (1975). The Health, Economic, and Cultural Impact of Periodontal Disease on our Aging Population," presented at the National Institutes of Health Conference on Aging, Louisville, KY.

[5]. U.S. Department of Health, Education, and Welfare (1971). "Edentulous Persons, United States," National Center for Health Statistics, series 10, No. 89.

[6]. Laine, M. A. (2002). Effect of pregnancy on periodontal and dental health. Acta Odontol Scand; 60: 257-64.

[7]. Chilton, N. W., and Miller, M. F. (1977). Epidemiology of Periodontal Disease: Position Report and Review of Literature. in International Conference on Research in the Biology of Periodontal Disease (Ann Arbor, Mich.: University of Michigan).

[8]. Trombelli, L. and Tatakis D N. (2003): Periodontal diseases: current and future indications for local antimicrobial therapy. Oral Diseases 9: 11-5

[9]. Marsh, P. D. (2003). Plaque as a biofilm: pharmacological principles of drug delivery and action in the sub- and supragingival environment. Oral Dis; 9: 16-22.

[10]. Meng HX. Periodontal Abscess. Ann Periodontol;4:79-83.

[11]. Ranta, H., Haapasalo, M., Kontiainen, S., Kerosuo, E. and Valtonen, V. (1988). Bacteriology of odontogenic apical periodontitis and effect of penicillin treatment. Scand J Infect Dis. 20:187-92.

[12]. Brook, I. and Frazier, E. (1995). Clinical features and aerobic and anaerobic microbiological characteristics of cellulitis. Arch Surg 130: 786-92.

[13]. Oguntebi. B., Slee. A. M., Tanzer J. M. and Langeland, K. (1982): Predominant microflora associated with human dental periapical absceses. J Clin Microbiol. 15:964-6.

[14]. Kroes, I., Lepp, P. W. and Relman, D. A. (1999). Bacterial diversity within the human subgingival crevice. Proc Natl Acad Sci USA 96: 14547-52.

[15]. Sakamoto, M., Umeda, M., Ishikawa, I. and Benno Y. (2000) Comparison of the oral bacterial flora in saliva from a healthy subject and two periodontitis patients by sequence analysis of 16S rDNA libraries. Microbiol Immunol 44: 643-52.

[16]. Arura, B and Arora, D. R (2007): Practical Microbiology. CBS publishers, New Delhi, India.

[17]. Cappuccino, M and Sherman, G. (1998): Microbiology, a laboratory manual. 5th edition. Benjamin/Cummings Science Publishing. Menlo Park, California.

[18]. Peeran, S., Singh R., Alagamuthu G., Kumar, N., (2013). Periodontal status and its risk factors among young adults of the Sebha city. Libya Dental Research Journal, 10(4): 102-106.

[19]. Khemaleelakul, S., Craig Baumgartner, J. and SumaleePruksakorn, M. (2002): Identification of bacteria in acute endodontic infections and their antimicrobial susceptibility. Endodontics 94(6): 746-755.

[20]. Dahlen, G., Wikström, M. and Renvert, S. (1996). Treatment of periodontal disease based on microbiological diagnosis. A 5-year follow-up on individual patterns. J Periodontol 67:879-87.

[21]. Baehni, P.C. and Takeuchi, Y. (2003). Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Dis. 9: 23-9.

[22]. Roda R., Bagán J.V., Sanchis-Bielsa J.M. and Carbonell-Pastor, E. (2007). Antibiotic use in dental practice. A review. Med Oral Patol Oral Cir Bucal 12:E186-92.