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EVALUATION OF SOME ANTIBIOTICS'RESISTANCE BY DIFFERENT TYPES OF ORAL BACTERIA IN PATIENTS WITH GINGIVITIS AND PERIODONTITIS

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ABSTRACT

Background

Periodontal diseases are highly prevalent in Iraq. They may become serious conditions if they faced by unsuitable treatment and resulting in particularly cardiovascular systemic disorders. Further, antibiotic resistance in general has been witnessed prevalence, which may lead to failure of periodontal disease treatment. **The aim** of this study is evaluation of susceptibility and resistance to different antibiotics by dental plaque bacterial isolates.

Material and methods

Thirty seven infected patients were included in this study. From their dental plaque, samples were taken aseptically then bacteria has been isolated as well as antibiotic susceptibility tests agar diffusion methods had been applied.

Results

Among three types of bacteria, *Streptococcus mutans* occupied the largest percentage (62%) in dental plaque isolates. Imipenem registered profound susceptibility 78.3% via *Streptococcus mutans* followed by each of erythromycin, ciprofloxacin, and ceftriaxone (60.9%). This pathogen isolates showed resistance to amikacinchioramphenicol, tetracycline and amoxicillin. *Pseudomonas aeruginosa* appeared the highest susceptibility to chloramphenicol and ceftriaxone by 90% while *Staphylococcus aureus* had been existed complete susceptibility to imipenem 100% followed directly by tetracycline and chloramphenicol by about 75% of each. The lowest susceptibility was resulted toward amoxicillin, amikacin, and ciprofloxacin with 25%. Generally speaking, amoxicillin exposed to highest resistance 70.3% in comparison to others, whereas both of imipenem and ceftriaxone witnessed highest susceptibility 67.6% by the same pathogens.

Conclusions

Amoxicillin occupied the first position in bacterial resistance, this may support the assumption that improper administration of amoxicillin will lead to therapy failure.

KEYWORDS: Antibiotics, Gingivitis, Periodontitis, Resistance, Susceptibility

INTRODUCTION

Gingivitis is a superficial inflammation of the soft tissues surrounding the teeth capable of being reversed. It is started only post a few period of insufficient oral hygiene via local plaque often of bacterial deposits close to the highly vascularized tissues of gingiva[1]. Despite of apical migration of the epithelium junction does not occur, these areas of tissues become erythematous. Furthermore, gum bleeding may happen post chewing, tooth brushing as well as even after simple excitation in severe cases[2].

Interestingly, gingivitis affects most people[3,4,5,6]. In general, it is assumptive that this reversible inflammatory situation, if neglected and left without treatment, progression to period ontitis may ultimately occur[7].

Periodontitis, an irreversible periodontal infection, is characterized by loss of alveolar bone, loss of ligament, periodontal pockets formation and eventually followed by loss of tooth[8,9]particularly in susceptible subjects[10].

The major leading cause of gingivitis progression is bacterial plaque that is carrying responsibility for gingival tissues destruction and loss of periodontal attachment[11,12].Subsequently,invasion of the epithelial barrier via oral bacteria itself in addition to their products into the systemic circulation become easy. Previous studies, have been reported that an infected period ontium may be a source of some of systemic disorders[13,14]. Additionally, periodontal pathogens have also been found in both of abdominal aortic aneurysms and in athermanous plaques[15,16].

Therefore, treatment of patients with gingivitis or those suffering from period ontitis is of particular clinical importance, thus it has been resulting in reduction of atherosclerosis parameters and then improvement of endothelial function[17], [18]. In order to eliminate invasive pathogens, mechanical debridement alone is inadequate[19]. Therefore, systemic antibiotics is mandatory where it can enhance the therapeutic response to scaling and root planning[20].

However, without effective antibiotics, it is probable that both of oral and systemic health will be hampered and may result in a considerable rising in morbidity and mortality from infections. Many of antibiotics are now seriously threatened by increasing its resistance by different pathogens which is a leading reason for asking to depression in prescription of a usual antibiotic that are indicated for widely prevalent disease. The great prevalence of gingivitis in Iraq[21,22]may be rever berate of spread of antibacterial resistance. The primary focus of this study is the evaluation of susceptibility and resistance of most common administer edantibiotic for patients with gingivitis or periodontitis.

MATERIALS AND METHODS

Subjects

This prospective randomized study was conducted at the University of Thi-Qar/College of Pharmacy from March 2014 to May 2014. Participants were recruited from staff and student population with gingivitis or period ontitis of the College of Pharmacy. Thirty seven people 17 (46%) males and 20(54%) females, aged 22 to 37 years. Each participant was given verbal information that explained the nature of the study. Eligible subjects included participants equal or older than 19 years of age who were in general in a good health and they were not administer in gantibiotics or immunosuppressant drugs within at least the last one month.

Laboratory Diagnostic Methods

Plaque samples were collected in the morning, approximately twelve hours after evening tooth brushing. No food intake and drink or even oral hygiene was allowed in the morning before sampling. Samples from each patient were collected aseptically then transferred to the Eppendorf tube containing 140 µL buffer (10 mmol·L^{-1}Tris-HCl, 1.0 mmol·L^{-1} ethylene diaminetetraacetic acid (EDTA)).All samples were stored under -70°C.

Growth Media for Isolation of Bacteria

Basal salt medium with yeast extract (BSMY I) were utilized in this study. BSMY I[23], used for the bacterial isolation from samples of dental plaque which contained: 1.0 g yeast extract, 0.14 g MgSO4.7H2O, 0.3 g (NH4)2SO4, 0.1 g NaCl, 0.2 g CaCl2.2H2O, 0.05 g KH2PO4, 0.6 mg H3BO3, 0.05 g K2HPO4, 0.17 mg CoCl2.6H2O, 0.1 mg

MnCl2.4H2O, 0.22 mg ZnCl2, 0.09 mg CuCl2.2H2O, 10 g glucose in one liter of Tris-HCl buffer (pH 8.0). All the chemicals were applied of analytical step.

Isolation of Bacteria from Dental Plaque

Each sample was inoculated separately on 25 ml of BSMY I broth. Glasses that inoculated were incubated at 35℃ ± 1℃ for about 48 hours. Newly grown culture of one milliliter from each dental plaque was regularly diluted with distilled water up to 10-5 ml. Then, 100 µl serially samples (diluted) were diffuse over BSMY I agar plates. The inoculated plates were incubated at 37℃ for about three days under aerobic conditions. After wards, the isolated colonies were hiked and then streaked on slant of BSMY I for preservation of pure culture.

Antibiotic Susceptible Test

The susceptibility tests were achieved on Mueller-Hinton agar using modified Kirby-Bauer disc diffusion method[24]. The susceptibility pattern of each bacterial isolate was translated based on the standard criteria of Clinical and Laboratory Standards Institute. The antimicrobial agents tested were amoxicillin, erythromycin, tetracycline, vancomycin, chloramphenicol, ceftriaxone, ciprofloxacin, imipenem, and amikacin.

Statistical Analysis

Data were analyzed using SPSS version 19 statistical software and Excel. The chi-square test (χ^2) and Student's *t*-test were applied.

RESULTS

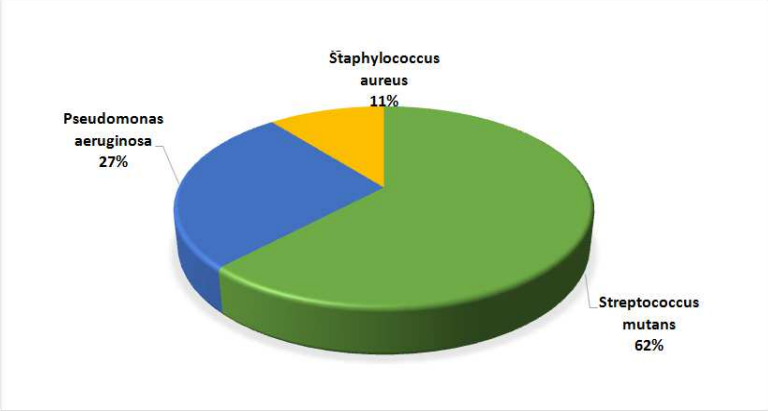

Figure 1: Frequency of Isolation of Causative Bacteria of Gingivitis and Periodontitis

Thirty seven patients with gingivitis or period ontitis had been completed this trial. Figure 1 represents different distribution of bacterial isolates from dental plaque. Interestingly, *Streptococcus mutans* showed the predominance 62% among other isolates. Whereas, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were with a distribution of 27% and 11% respectively

Regarding to figure 2 which represents susceptibility to different antibiotics by three bacterial isolates and it highlights that *Streptococcus mutans* had been existed highest susceptibility to imipenem78.3% followed by each of erythromycin, ciprofloxacin, and ceftriaxone which had equivalent susceptibilities 60.9% by this organism. The next was vancomycin 52.2% while others had been showed very low susceptibilities by the same pathogen ranging from (39.10% to amoxicillin - 26.1% to amikacin).

The second predominance bacteria *Pseudomonas aeruginosa* was with the highest susceptibility to chloramphenicol and ceftriaxone by 90% followed by ciprofloxacin and erythromycin by 70% then to amikacin and tetracycline with 60% whereas its susceptibility to the rest of antibiotics was below the half of isolates.

Notably, the lowest predominance pathogen (*Staphylococcus aureus*) had been existed complete susceptibility to imipenem 100% followed directly by tetracycline and chloramphenicol by about 75% of each. Vancomycin, ceftriaxone, and erythromycin were coming then by 50%. The lowest susceptibility was reflected by amoxicillin, amikacin, and ciprofloxacin with 25%.

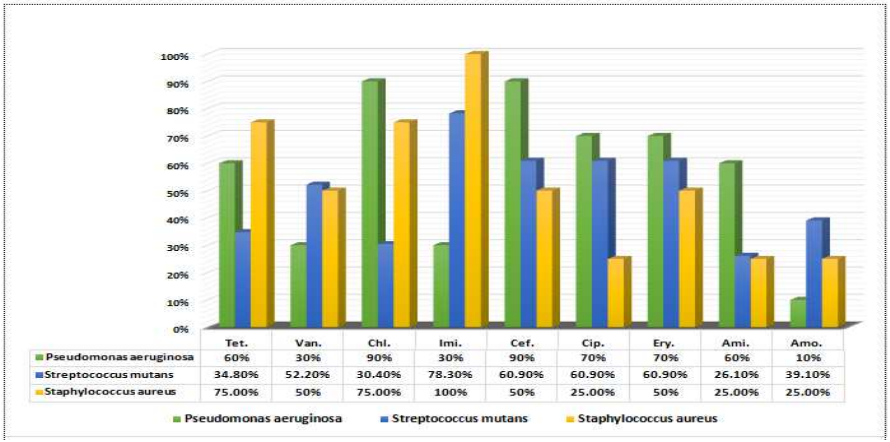

Figure 2: Susceptibility of Different bacterial Isolates to Some Antibiotics

Tet., tetracycline; Van., vancomycin; Chl., chloramphenicol; Cef., ceftriaxone; Cip, ciprofloxacin; Ery., erythromycin, Ami, amikacin; Amo., amoxicillin.

Moving to figure 3 which re presents the resistance of each type of bacterial isolates to the diverse antibacterial medications. This figure illustrates the opposite of the figure 2 where the organism with highest susceptibility to certain antibiotic in figure 2 would be found with the lesser resistance to the same antibiotic and vice versa.

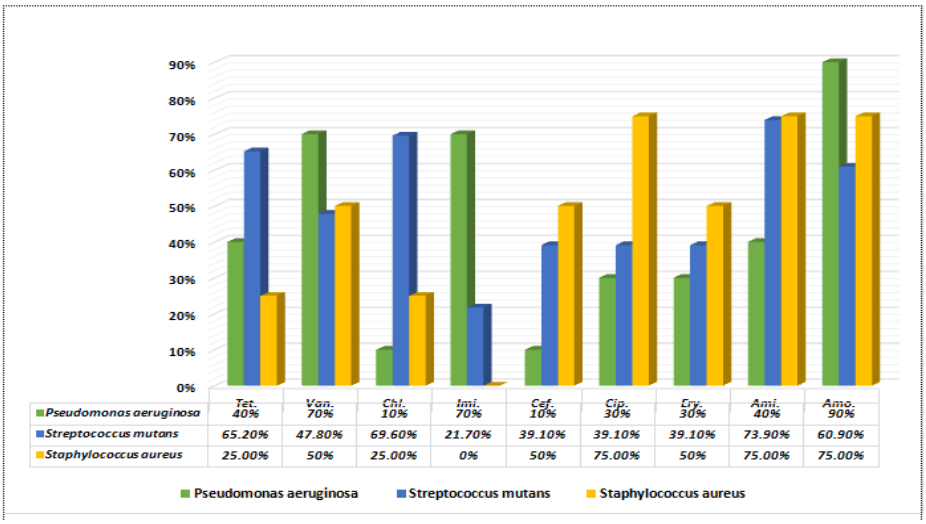

Figure 3: Resistance of Bacterial Isolates to Various Types of Antibiotics

Tet., tetracycline; Van., vancomycin; Chl., chloramphenicol; Cef., ceftriaxone; Cip, ciprofloxacin; Ery., erythromycin, Ami, amikacin; Amo., amoxicillin.

Figure 4 highlights percentages of resistance that exposed to each antibiotic by bacterial isolates from dental plaque in comparison to percentages of susceptibility to the same antibiotics. This figure reveals that amoxicillin generally faced highest resistance 70.3% in comparison to others, whereas both of imipenem and ceftriaxone exposed to highest susceptibility67.6% by the same pathogens.

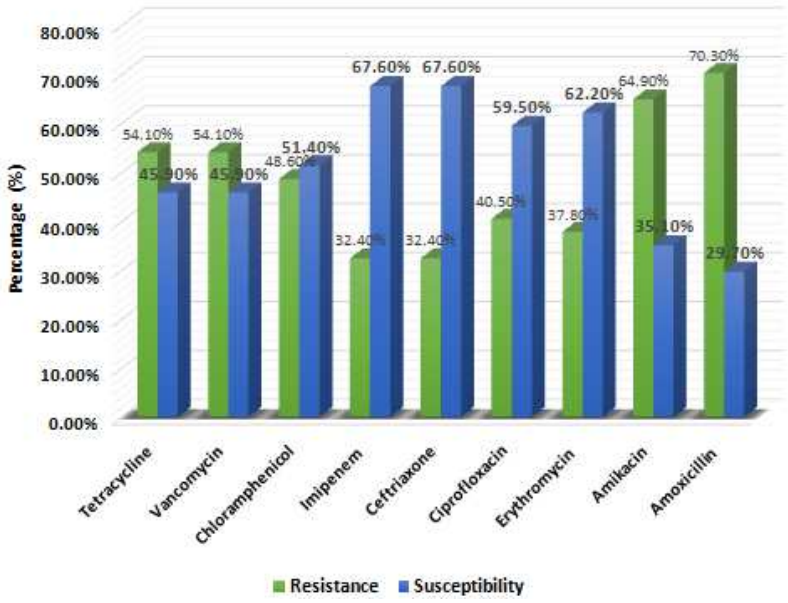

Figure 4: Resistance and Susceptibility to Studied Antibiotics by Bacterial Isolates

DISCUSSIONS

Untreated intraoral diseases as gingivitis and periodontitis can ultimately progress to, in response to bacterial accumulation, serious problems ranging from teeth loosening reaching to even systemic diseases[25,26].Moreover, growing prevalence of these inflammatory conditions[22] from one hand and of microbial resistance to antibiotics[27,28]from the other hand makes widespread of systemic diseases of a common sense.

Our study revealed higher percentage of periodontal diseases was found in females 54% than in males 46%. This result is disagree with Beltrn*et al.* study where men occupied a higher disease prevalence than women [29]. This difference may be analyzed as that the lower number of male students accepted in our Pharmacy Collage (the place of this study) than female which leads to lower cases of male in comparison to female.

In contrast to some studies[10,30,31],this trial reported that the predominant bacterial isolates were of *Streptococcus mutans* with percentage 62.2%, whilst in the Sweden study[32] the results were concordant with that of ours. The divergence can be interpreted by the fact "bacterial spectrum may alter from one area to another". However, the tendency of *Pseudomonas aeruginosa* prevalence in dental plaque gets much resemblance to that of previous published study[33]

When we discuss the most important part of this study "susceptibility and resistance" of three different types of the tested bacteria to several antibiotics, we can clearly understand the variation in response of patients to their antibiotic treatment.

Equivalent resistance to both tetracycline and vancomycinhas been registered in the current study by general

tested microbes (54.1%) which was just exceeding their susceptibility by the same bacteria. This percentage of resistance could be related to the extensive administration of tetracycline and inappropriate option of vancomycin for treatment of dental plaque bacteria. This have seemed to be similar to that previously reported literature[33]which was disseminated in the distribution of the microbial resistance to tetracycline.

Although chloramphenicol use has been rare in field of dentistry, both authors recorded 51.4% susceptibility tochloramphenicol which is slightly exceeded the frequency of bacterial resistance. This may be due to its broad antibacterial spectrum of chloramphenicol. Additionally this tendency of resistance has been highlighted by in *strep to coccivirida*ns and *enterococci*, despite of resistant of some gram-negative rods to this agent. This have been demonstrated in Brazilian study in 2007[34].

Ceftriaxone have been varied greatly in its susceptibility to β-lactamases, where it has been exposed to highest susceptibility 67.6% among other antibiotics (except imipenem) by tested bacteria. *Pseudomonas aeruginosa* reported the towering susceptibility 90% followed by each of *Streptococcus mutans* (60.0%) *and Staphylococcus aureus* (50%). In contrast, the cephalosporins have been more resistant to hydrolysis by β-lactamases of *staphylococci*. A reasonable interpretation of this difference is that in our country, Iraq, ceftriaxone prescription for periodicities or gingivitis is infrequent.

Like ceftriaxone and in agreement with the outcomes of Italian study, imipenem also showed the highest susceptibility 67.6% by general trialed pathogens with complete 100% susceptibility by *Staphylococcus aureus*, 78.3% via *Streptococcus mutans* while *Pseudomonas aeruginosa* isolates were resistant with 70%. However, the resistance to imepenem was observed by Pillar, *et* al and it may restrict by a few isolates including *P. aeruginosa*and*staphylococci* [35]. Due to the relatively lower rates of microbial resistance to imipenem and to its high activity against the dental bacteria that associated with periodontal diseases, it is suggested to consider imipenem as a promising medication for periodontitis.

The susceptibility to ciprofloxacin was 59.5% but, its resistance was mainly restricted to isolates of genera *Staphylococcus*75%, while other two types of the tested microorganisms were highly susceptible. This outcome was consistent with Huang, *et al*[36]who revealed high levels of resistance to this drug in *S. aureus* (100%) and much reduced in *streptococci* to reach to(33%), as well as in. *P. aeruginosa* (9%). However, it contrasted with data that published in previous literatures [37,38]Since this antibiotic has not been either frequently used by Iraqi dentists or as part of patient self-drug.

Going to erythromycin which is first discovered macro lide. Because of the increasing prevalence of bacterial resistant to erythromycin previously from one hand, and due to the knowledge that this agent is considered the antibiotic with the worst undesired gastrointestinal adverse effects from the other hand, this pay physician (both in medicine and in dentistry)to limit its prescription for a patient on the last decade[10,134]. These factors can clarify why our results reflect approximately high levels of susceptibility 62.2%.

Amikacin is one of the amino glycoside antibiotics that have not been usually recommended forod on to genic infections treatment. The susceptibility to it was high among most of the tested microorganisms. Similar findings have been described in many studies [38,34]

In respect to amoxicillin (broad spectrum antibacterial agent) which is most common antibiotic that extremely prescribed by dentists[39,40,41]the investigation of our study exhibited towering in resistance reaching to 70.3% via studied

organisms in comparison to others. This result is agree with studies held in Colombia [42], Philadelphia [43] and Brazil where they showed higher levels of amoxicillin resistance in clinical isolates. This greatly attributed to the above reasons in addition to the self-use of this medication without prescription. Thus, our work highlights the low antimicrobial potential of amoxicillin in vitro, may restrict its clinical use for gingivitis or periodontitis.

CONCLUSIONS

From outcomes of our study we can conclude that failure to treat periodontal disease is largely attributed to the resistance to antibiotic action. Declining in amoxicillin prescription is required due to maximum resistance to its action. Simultaneously, imipenem and ceftriaxone may be recommended as the first options among others for periodontal diseases due to profound positive susceptibilities followed by erythromycin then ciprofloxacin.

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