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THE ROLE OF MYCOPLASMAS IN ORO-DENTAL DISEASES

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Abstract

This study aimed to isolate *mycoplasmas* from oral cavity of patients with oro-dental diseases, to evaluate their role in pathogenesis of these diseases, and to evaluate the effects of some antibiotics on them.

The population enrolled in this study was individuals attending the dental out-patient clinics in the dental specialty center in Basrah, from private dental clinic, and from otolaryngology out–patient clinic. It consisted of 154 patients with periodontal diseases (gingival diseases & periodontal abscesses) and oral ulcers of different types, in addition to that, 50 healthy subjects were included as control group. It included 112 males and 92 females, their age ranged from 6 to 68 years. This study extended from the period between August 2010 to December 2011. Samples from gingival sulcus or periodontal spaces were obtained by using paper points and swabs from ulcer surface, all specimens were cultured within one hours of sampling. For the isolation of *mycoplasma*, each specimen was directly inoculated into the liquid phase of MDCS.

Mycoplasma spp. were isolated from 120 individuals out of 204 enrolled in this study, males affected more than females, the commonly affected age group was 20-29 years. Smoking play a clear role in causation of dental diseases and *mycoplasma* found more in smoker patients, (62 were smokers, 36 were non-smokers).Gingival diseases were commonly presented (62 patients), followed by inflammatory oral ulcers (45 patients).

Mycoplasma salivarium was the frequently isolated species (70.8%), followed by *mycoplasma* orale (isolated from 16.6%). Single *mycoplasma* infection was found in 4 cases, 3 in gingival diseases which were 2 *mycoplasma* salivarium and one M.orale. The last spp. was M. pneumoniae, it was isolated from periodontal abscess. Tertracycline and erythromycin showed good inhibitory effects against *mycoplasmas*.

In conclusion, *Mycoplasma* isolated from males with oro dental diseases more than females. The mostly affected age group was 20-29 years of age. *Mycoplasma* salivarium was the commonest isolated species, followed by *mycoplasma* orale. It is rare to find single *mycoplasma* infection in the oral cavity, just 4 cases were documented from total 98 patients. Tetracycline and erythromycin can significantly inhibit the growth of *mycoplasma*.

Introduction

Mycoplasma refers to a genus of Gram-negative bacteria that lack a cell wall[1]. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other beta-lactam antibiotics that target cell wall synthesis. They can be parasitic or saprotrophic. Several species are pathogenic in humans, including M.pneumoniae, which is an important cause of atypical pneumonia and other respiratory disorders, and M.genitalium, which is believed to be involved in pelvic inflammatory diseases. Microorganisms from the oral cavity have been shown to cause a number of oral infectious diseases, including caries (tooth decay), periodontitis (gum disease), endodontic (root canal) infections, alveolar osteitis (dry socket), and tonsillitis. Mouth ulcer is a very common oral lesion. Epidemiological studies show an average prevalence between 15% and 30%. Trauma

to the mouth is a common cause of ulcers[2]. Infection whether viral, fungal and bacterial play a major role in causation of oral ulcers. Many researchers view the causes of aphthous ulcers as a common end product of many different disease processes, each of which is mediated by the immune system[3].

Materials and methods

The population enrolled in this study was individuals attending the dental out-patient clinics in the dental specialty center in Basrah, from private dental clinic, and from otolaryngology outpatient clinic. The sample obtained consisted of 154 patients with periodontal diseases (gingival diseases and periodontal abscesses) and oral ulcers of different types, in addition to that, 50 healthy subjects included as control group. It included 112 males and 92 females, their age ranged from 6 to 68 years. This study extended from the period between August 2010 to December 2011. A special questionnaire forma was prepared and filled by same researcher. Samples from gingival sulcus or periodontal spaces were obtained by using paper points. Dentist selects localizations of interest and remove supra-gingival plaque from dental surface with a cotton. Then, insert a sterile size 45 paper point into the gingival sulcus or periodontal pocket, also swabs taken from ulcers surface, regarding control group the swabs taken from their saliva. All specimens were cultured within one hours of sampling. For the isolation of *mycoplasma*, each specimen was directly inoculated into the liquid phase of Monophasic diphasic culture setup (MDCS), Mixed up well and tilted for a while, once or twice, to cover the upper slanted portion in (MDCS) prior to inoculation[4]. For the isolation of bacteria other than *mycoplasmas*, another 2 swabs from same lesions were obtained from same patients and control, then, each specimen after being transported to the laboratory was directly cultured into MacConkey and blood agar by the streaking method then incubation done.

Results

Table I shows 120 positive *mycoplasma* cultures in both patients and control group (comprises 58.8%), this divided into 98 positive cultures in patient group and 22 in control group, while 84 individuals in this study, had negative *mycoplasma* culture from which 56 were real patients and 28 subjects from the control group, these results are not statistically significant, ($P > 0.05$, $X^2 = 3.322$).

Table I :Mycoplasma culture results in patient and control groups.

Culture results	Patients	Controls	Total
Mycoplasma +ve cultures	98 (63.6%)	22 (44%)	120(58.8%)
Mycoplasma –ve cultures	56 (36.4%)	28 (56%)	84 (41.2%)
Total	154	50	204

P>0.05

Table II, shows that males were 67 while females were 31, just 5 patients with *mycoplasma* were below 9 years of age (5.1%), and only one patient above the age of 60 (1.2%), 26 patients belonged to age group of (20-29) years, 24 patients in age group (30-39) and 22 patients belonged to (10-19) years group comprised, 26.5%, 24.4%, and 22.4% respectively, these results are statistically significant ($P<0.01$, $X^2=8.36$).

Table II: Age and sex distribution of patients with *mycoplasma*

Age sex	Males	Females	Total
≤ 9	4	1	5(5.1%)
10 – 19	16	6	22(22.4%)
20 – 29	17	9	26(26.5%)
30 – 39	16	8	24(24.4%)
40 – 49	7	5	12(12.2%)
50 – 59	6	2	8(8.1%)
≥ 60	1	-	1(1.2%)
Total	67	31	98

P < 0.01

Figure 1, shows the relation between patients with *mycoplasma* and smoking habit, it is found that 62 patients were cigarette smokers, divided into 58 males and 4 females, while 36 patients were non-smokers, divided into 9 males and 27 females the differences are statistically significant (P <0.01 , X^2 = 4.122).

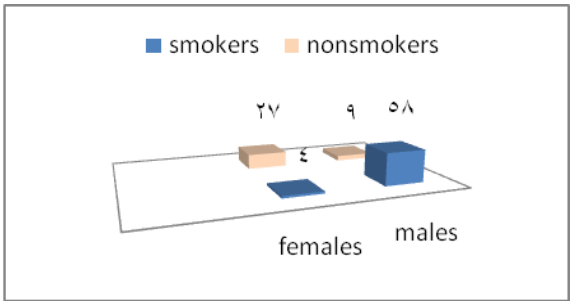

Figure 1: Smoking among patients with *mycoplasma*.

Table III, shows different species of isolated *mycoplasma*, the commonest species was *mycoplasma* salivarium 85(70.8%), followed by *mycoplasma* orale (20,16.6%), *mycoplasma* hominis (10,8.3%), and *mycoplasma* pneumoniae (5,4.1%). These results are statistically significant.

Table III: Frequency of different species of *mycoplasma* in patients and control groups

Groups m .species	Patients	Controls	Total
M.salivarium	72 (73.4%)	13(59%)	85(70.8%)
M. orale	11 (11.2%)	9(41%)	20 (16.6%)
M. hominis	10 (10.2%)	-	10 (8.33%)
M. pneumoniae	5 (5.1%)	-	5 (4.1%)
Total	98	22	120

P <0.01

It is found that just 4 patients out of 98 how had *mycoplasma* positive culture, had single *mycoplasma* infection (4.2%) i e single species of *mycoplasma*, 3 of them had gingival diseases and one patient with periodontal abscess, no single *mycoplasma* infection was detected in any types of oral ulcers this clearly shown in Table IV, the results are statistically significant (P<0.01, $\chi 2$=7.333). The majority of *mycoplasma*

infections in the present study were mixed (94 out 98 patients = 95.9%).

Table IV: Relation of oro-dental diseases with single *mycoplasma* infection and mixed infection.

	Oro-dental diseases	Single M. infection	Mixed infection	Total
Periodontal diseases	Gingival diseases	3	43	46
	Periodontal abscesses	1	17	18
Oral ulcers	Inflammatory	0	27	27
	Traumatic	0	5	5
	Neoplastic	0	2	2
Total		4	94	98

P< 0.01.

Figure 2, shows that tetracycline forms inhibitory zones of 20 mm around M. orale, 18 mm around M. pneumoniae, 17 mm around M.salivarium and 12 mm around M. hominis. Regarding erythromycin, its zones of inhibition were 20 mm form. salivarium, 18 mm form. orale, 12 form. pneumonia and 10 mm form. hominis. Neomycine and streptomycine showed the least inhibitory zones for all the isolated *mycoplasmas*.

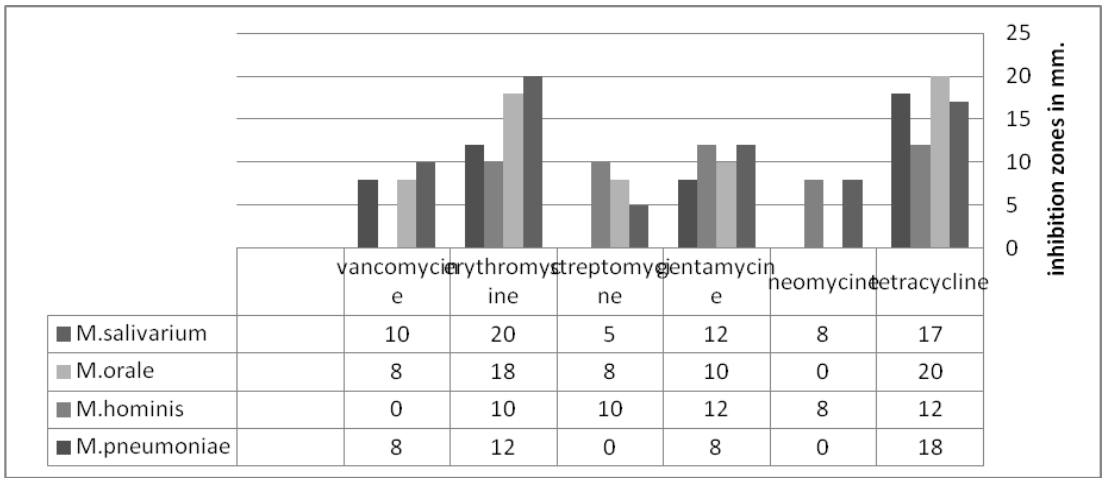

Figure 2: Sensitivity of Mycoplasma spp. toward some antibiotics

Discussion

According to reviewing of some libraries and internet literatures, this is the first study performed on *mycoplasma* of oral cavity in Iraq and middle east, also this is the first study which isolates pathogenic *mycoplasma* (*mycoplasma* hominis and *mycoplasma* pneumonia) from oral cavity in Iraq.

The method of monophasic-diphasic culture setup (MDCS)[4], was used in this study, for the rapid isolation and identification of oral *mycoplasma*.

It is found that the frequently presented age of oro-dental diseases was between 20-29, while affection of ages below 9 years and above 60 years is uncommon. Regarding the patients with oro-dental diseases and *mycoplasma* positive culture, the age group

usually affected was 20-29 years, while the presence of *mycoplasma* in these patients was very low in those below 9 years and above 60 years, this probably due to small sample of investigations at these age groups, this results are relatively similar to Tsai et al[5] study, they found that the common age group affected was those between 20–39 years.

In the present study, it is found that 67 patients with oro–dental diseases and had *mycoplasmas* of different species were males and the rest were females (31 patients), in other word the males to females ratio of oro-dental patients with Mycoplasma was 2.16:1, while out of 22 persons (control) with *mycoplasma* of different species 15 were males and 7 were females, the ratio was 2.14:1, these results are comparable with Engil et al study[6], but the reverse is true regarding Watanabe et al[7] study.

The results of the current study showed comparable results regarding the gender to those affected by chest infections due to *mycoplasma*, Grayston et al[8] and Alexender et al[9], found males affected more than females especially at young age groups.

The present study revealed that smoking habit in patients with *mycoplasma* was significantly different from non-smokers, Martin et al published a research on murine model called interaction between cigarette smoke and *mycoplasma* infection: a murine model, they got a fact that there was a significant connections between smoking and *mycoplasma* infections[10].

In 1983, Ismail et al[11] analyzed smoking and periodontal disease and found that after adjusting for potential confounding variables such as age, oral hygiene, gender and socioeconomic status, smoking remained a major risk indicator for periodontal diseases.

The majority of the studied patients with oro-dental diseases presented with gingival diseases in form of gingivitis and gingival abscesses (62 patients out of 154, represents 40.2%), 46 of them (74.2%) had positive Mycoplasma culture.

Periodontal abscesses found in 25 patients, 18 of them had *mycoplasma* (72%), both groups of diseases belonging to the main group of disease called peri-odontal disease, it was stated that *mycoplasmas* play an etiological role in periodontal diseases by facilitating infiltration, accumulation, or retention of inflammatory cells in gingival connective tissue[12].

Mycoplasma was isolated from the periodontal pockets of periodontally diseased subjects at a significantly higher rate than from the gingival sulci of healthy subjects (87 versus 32%)[6], and is also the predominant *Mycoplasma* species in dental plaque[13]. On the basis of this significant correlation between the presence of M. salivarium and periodontal disease, it is speculated that the organism might participate in periodontal disease[6].

Mycoplasma also isolated from 27 out of 45 patients with inflammatory oral ulcers, mainly aphthus ulcers, Gordon et al[14] found that *mycoplasmas* were isolated from the saliva of 15 of the 33 patients with recurrent ulcers, and from the saliva of eight out of 39 healthy controls. In both groups, M. orale was the species Most commonly recovered.

It is found that *mycoplasma* salivarium isolated from 72 patients out of total 98, comprising 73.4%, followed by *mycoplasma* orale, *mycoplasma* hominis and *mycoplasma* pneumoniae, the shape of the colonies of *mycoplasma* spp.

It was stated that most common *mycoplasma* species of the oral cavity are M. salivarium and M. orale[6]. M. salivarium is usually found in 60 to 80% of throat specimens from adults and is also frequently found in inflamed tonsils[15].

Some types of antibiotics were tried, to detect the sensitivity of different isolated species of *mycoplasma* to them, it is found that, all the isolated *mycoplasma* species were susceptible to tetracycline apart from *mycoplasma* hominis, for erythromycin, *mycoplasma* salivarium and *mycoplasma* orale were susceptible but *mycoplasma* hominis and *mycoplasma* pneumoniae were

resistant, all the isolated species of *mycoplasmas* showed to resist gentamycine and streptomycine. Cassell et al[16] stated that, erythromycin, was the first introduced macrolide antibiotic, which still being used in the treatment of *mycolasma* infections. The isolated *mycoplasma* spp. In the present study showed results comparable with David and Christiane[17] study.

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