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Isolation and Identification of Pathogenic Bacteria from Patients with Dental Plaque in Sana'a City, Yemen

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Abstract

Oral flora is important for maintaining dental health, but some situations may disturb the oral microbiota, which can support the growth of Microbial Pathogens. 28 pathogenic bacteria:17 G+ve and 11 G-ve) were isolated from dental plaques of 192 dental patients and identified by the VITEK 2 Compact System after culturing and studying their morphological features using Gram stain. About 13 (9 G+vecocci and 4 G-ve bacilli) and 24 (13 G+ve cocci, 1 G+ve bacilli, and 10 G-ve bacilli) pathogenic bacteria were isolated from the dental plaques of 46 males and 146 Female patients, respectively. Moreover, *Streptococcus mitis* exhibited the highest prevalence among the 28 isolated pathogenic bacteria. Furthermore, *Serratia marcescens*, and *Streptococcus parasanguinis* showed the highest mean number of colonies among the pathogenic bacteria isolated from male patients with dental plaques. Similarly, *Serratia odorifera* exhibited the highest mean number of colonies among the pathogenic bacteria isolated from female Patients with dental plaques.

Article Info

Keywords:
Dental Plaque, Pathogenic Bacteria, Yemeni Dental Patients, VITEK 2 Compact System, Gram-Positive Bacteria , Gram-Negative Bacteria.

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1. INTRODUCTION

Oral health plays a vital role in maintaining physiological homeostasis; one of the natural elements that play a significant function in protecting the oral cavity from the colonization of exogenous pathogenic bacteria is oral flora [1]. The oral flora, which is also known as the normal flora, represents an essential part of the human microbiota and includes several hundred diverse species, such as *Staphylococcus* spp. and *Streptococcus* spp. including *S. sanguinis*, *S. mitis*, *S. parasanguinis*, and *S. salivarius*, which helps in maintaining oral hygiene by preventing pathogenic kinds from attaching to the mucosal surface [2]. There is a homeostatic balance exists between the resident microbiota in the oral cavity and the host, but under particular conditions, a perturbation of the oral microbiota can be caused, which can contribute to the growth of non-oral pathogens (are commonly found in other parts of the human body) that are hard to be eliminated because of their higher resistance to antimicrobials [2, 3]. Moreover, Hoceini *et al.* [4], Derafshi *et al.* [5], Bogacz *et al.* [6], and Zaatout [7] reported that many non-oral bacteria species, such as *Acinetobacter baumannii*, *Aeromonas hydrophila*, *Burkholderia cepacia*, *B. pseudomallei*, *Enterobacter cloacae*, *E. aerogenes*, *E. gergoviae*, *E. sakazaki*, *E. agglomerans*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *K. oxytoca*, *Morganella morganii* , *Proteus mirabilisand*, *Pseudomonas aeruginosa*, *P. luteola*, *P. putida*, *P. stutzeri*, *Raoultella ornithinolytica*, *Serratia odorferia*, *S. ficaria*, *S. liquefaciens*, *Staphylococcus aureus*, *S. epidermidis*, and *Stenotrophomonas maltophilia* were isolated from different parts of the oral cavity including; Tongue dorsum, Gingiva, Saliva, Root canal, Periodontal pocket and

plaque (comprising the sub and supragingival plaque). The VITEK 2 Compact System is an automatic procedure for identifying the majority of microorganisms that contaminate production areas and assessing their antimicrobial susceptibility [8]. Samples are examined in the VITEK 2 Compact System by using a kinetic analysis of fluorescence, turbidity, and colorimetric signals. Results for identification are available within 3 hours, whereas susceptibility testing takes 2.5-18 hours. Several studies investigated the accuracy of the VITEK 2 Compact System in identifying G+ve and G-ve bacteria; the outcomes of these investigations indicate that VITEK 2 cards are appropriate for fast identification and susceptibility testing of G+ve and G-ve bacteria [8–10]. On the other hand, few studies have been conducted to identify the oral and non-oral flora in Yemeni patients who attend dental clinics using the standard technique of identification. In 2005, Al-Hebshi and Skaug [11] reported that 14 types of Periodontal bacteria (*Actinobacillus actinomycetemcomitans*, *Actinomyces israelii*, *Campylobacter rectus*, *Capnocytophaga gingivalis*, *Eikenella corrodens*, *Eubacterium nodatum*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Streptococcus constellatus*, *S. intermedius*, *Tannerella forsythia*, and *Veillonella parvula*) in sub- and Supragingival plaque have been isolated from young khat chewing Yemeni males. Moreover, Al-Qadasi [12] isolated and identified five oral bacteria (*Streptococcus mutans*, *S. sobrinus*, *Lactobacillus casei*, *L. fermentum*, and *L. acidophilus*) from the oral cavity of 249 Yemeni patients suffering from dental caries. Furthermore, in 2018, Al-Shamahy *et al.* [13] studied the prevalence of bacterial and fungal oral infections among patients receiving dental care at Al-Gumhouri Teaching Hospital in Sana'a City, and they cited that G+ve bacteria represented 73.3 % while G-ve bacteria represented 26.6% of the total collected pathogenic bacteria. Among them, *S. aureus* and *Bacteroides* ssp. were isolated from Dental abscess, Periodontal abscess, Gingivitis, periodontitis, and Pulmonary samples. However, *S. pyrogens* were isolated from Periodontal abscess and Periodontitis samples, while *S. epidermidis* was isolated from Dental abscess and Periodontal abscess, whereas *S. mutans* was isolated from Dental caries samples only. According to the previous studies on the oral and non-oral flora in Yemen, it appears that the standard method (direct microscope examination, cultural standard techniques, and antibiotic susceptibility testing) for identifying bacteria was the only technique used to determine the oral and non-oral flora in Yemeni patients; however, this technique requires time and a selective culture media, which may not be available. Therefore, this study aims to identify oral and non-oral floral bacteria isolated from sub- and supragingival plaque of dental patients using the VITEK 2 Compact system and to determine the quantified bacterial colonization in dental plaque.

2. MATERIALS AND METHODS

Type of Study:

The study was designed as a cross-section study [14].

Dental Plaque Sampling:

Samples of dental plaque were obtained from 200 (48 male and 152 female) patients receiving dental care at 3 major hospitals in Sana'a City (Althawrah Hospital, Al-Gumhouri Teaching Hospital, and Alkuwait Hospital) by using a simple random study design. All patients differ in their dietary habits, age (ranging from 20 to 35 years), and how often they brush their teeth. Plaque samples from each patient were collected individually by using sterilized cotton swabs and placed in a plain tube containing 4 ml of sterilized Peptone Water Buffer (PWB) transport medium [15, 16].

Colony Counting:

Serial 10-fold dilutions of the transport medium (PWB) that contains plaque samples from each patient were prepared (0.1 ml of the transport medium that contained the Plaque sample was added to 0.9 ml distilled water, then mixed, 0.1 ml of the mixture (10^{-1}) is added to a tube containing 0.9 ml distilled water and mixed (10^{-2}), this process is repeated until the required dilution is obtained) to the dilution 10^{-4} [15, 17]. A Sample (0.1 ml) from each dilution 10^{-4} was inoculated on blood agar plates separately; cultured plates were incubated for 48 hours at 37^0C,then the created colonies were counted under a stereomicroscope [15].

Bacterial Morphological Diagnosis:

For Morphological Diagnosis, a thin smear from each colony was prepared and evaluated under a microscope after staining with Gram stain [18].

Bacterial Identification:

Isolates from each colony were tested by the VITEK 2 Compact System for microbial identification and antimicrobial susceptibility at the National Central of Public Health Laboratory (NCPHL) – Sana'a, Yemen.

3. RESULTS

Pathogenic bacteria (isolated from dental plaque samples, which were collected randomly from 200 dental patients receiving dental care at three main hospitals in Sana'a City) were identified by employing the VITEK 2 Compact System after culturing and studying their morphological features using gram stain. Table 1 and Figure 1 show that Pathogenic Bacteria were isolated from the dental plaques of 192 patients (representing

Table 1: Dental Patients under investigation.

Individuals	Patients	Male	Female
Dental patients receiving dental care	200	48	152
No. of dental plaque Patients with Pathogenic Bacteria	192	46	146
No. of Patients without any Pathogenic Bacteria	8	2	6

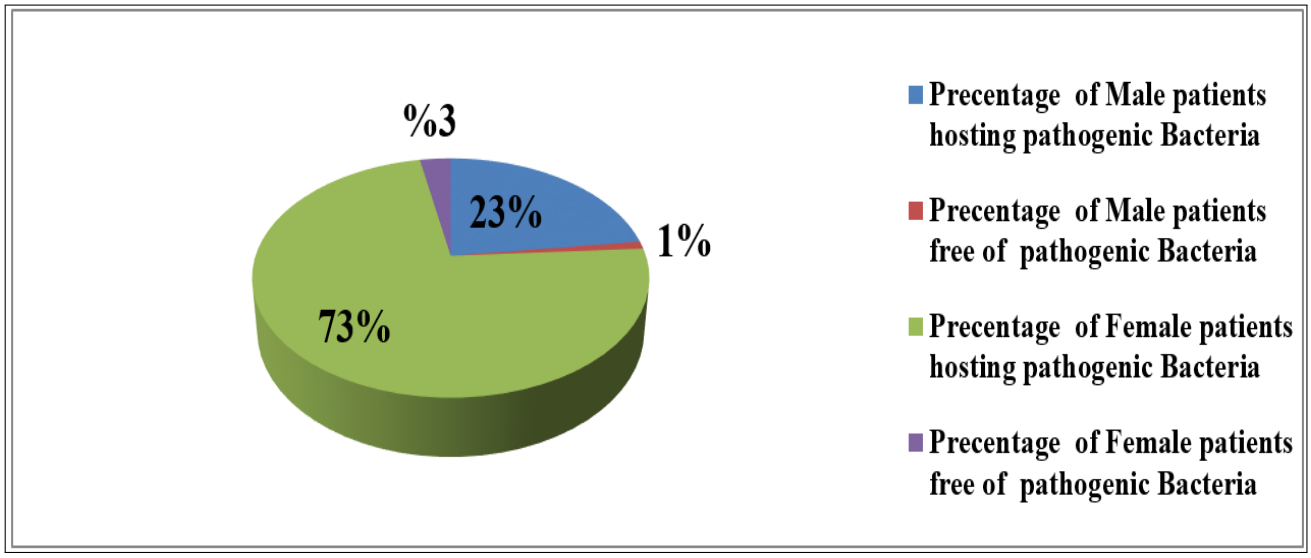

Figure 1. Percentage of Dental Patients.

96% out of all examined dental patients) out of 200 patients receiving dental care. Among them, samples were collected from 46 male patients (illustrating 23% out of all examined dental patients) and samples collected from 146 (exhibiting 73% out of all examined patients) female patients, while the samples were collected from the remaining 8 patients (accounting 4% (2 (1%) male and 6 (3%) female) out of all examined dental patient) did not contain any pathogenic bacteria. According to Tables 2 & 3 and Figures 2-4, about 28 Pathogenic Bacteria were isolated from the dental plaques of 192 (46 male and 146 female) dental patients. Among them, 17 G+ve pathogenic Bacteria were isolated from 128 (39 male and 89 female) dental patients, and 11 G-ve pathogenic bacteria were isolated from 64 (7 male and 57 female) dental patients.

Moreover, the Positive gram pathogenic bacterium comprises 16 cocci-shaped bacteria (*Streptococcus parasanguinis*, *Staphylococcus aureus*, *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus alactolyticus*, *Kocuria rosea*, *Aerococcus viridans*, *Streptococcus pseudoporcinus*, *Streptococcus pluranimalium, Streptococcus sanguinis*, *Enterococcus faecalis*, *Gemella morbillorum*. *Kocuria kristinae*, and three unidentified Bacteria; Unidentified1, Unidentified2, and Unidentified3) isolated from dental plaque collected from 126 dental patients (39 male and 87 female) and one bacilli-shaped bacteria

Table 2: Number of isolated G+ve and G-ve Pathogenic Bacteria.

Total No. of Isolated Bacteria Pathogen	Gram stain		
	(+) ve		(-) ve
	Cocci	Bacilli	Bacilli
28	16	1	11

(*Rothia dentocariosa*) isolated from dental plaque samples collected from 2 female dental patients (Tables 2-4 and Figures 2-5).

However, the negative gram pathogenic bacterium (Tables 2, 3& 5 and Figures 2-4 & 6) includes 11 bacilli-shaped bacteria (*Serratia marcescens*, *Serratia odorifera*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *K. pneumonia*, *Enterobacter cloacae*, *Sphingomonas paucimobilis*, *Raoultella ornithinolytica*, *Escherichia coli*, *Aeromonas hydrophila* and *Acinetobacter haemolyticus*) isolated from dental plaque samples collected from 64 dental patients (7 male and 57 female). Moreover, 13 Pathogenic bacteria species (accounting for 46.4 % out of total pathogenic bacteria that have been isolated from dental plaque samples collected from 192 dental patients): *S. parasanguinis*, *S. marcescens*, *S. mitis*, *S. pseudoporcinus, S.aureus*, *K. rosea*, Unidentified3, *S. salivarius*, *G. morbillorum*, *A. hydrophila*, *K. pneumonia*, *S. paucimobilis*, and Unidentified2 were isolated from the dental plaques of 46 male patients (Tables 4 & 5 and

Table 3: Number of Patients Hosting G+veand G-ve Pathogenic Bacteria.

Patients	Gram Stain		
	(+) ve		(-) ve
	Cocci	Bacilli	Bacilli
Total Number of Patients	126	2	64
No. Male Patient	39	0	7
No. Female Patient	87	2	57

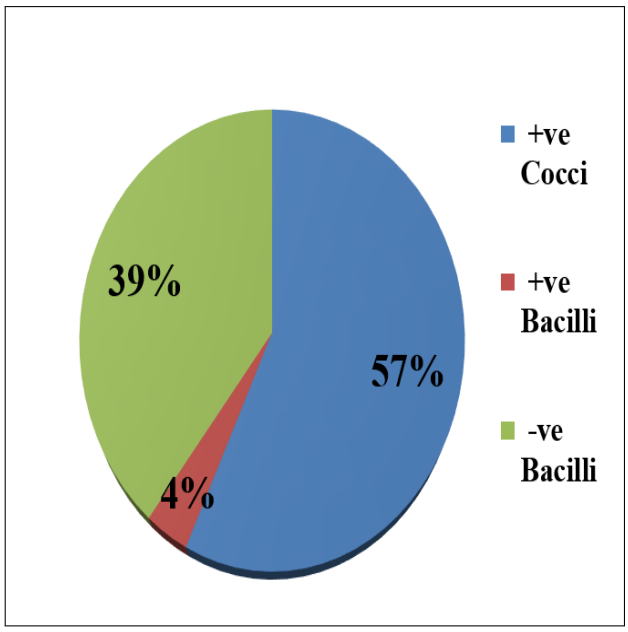

Figure 2. Isolated Pathogenic Bacterial.

Figures 5 & 6).

Furthermore, the 13 Pathogenic bacteria species isolated from dental plaques taken from 46 male patients were divided into two types according to gram stain: G+ve and G-ve pathogenic bacteria (Table 4 &5 and Figures 5 & 6). The first type includes nine G+ve cocci pathogenic bacteria (Tables 4 & 5 and Figures 5 & 6): *S. parasanguinis*, *S. mitis*, *S. pseudoporcinus*, *S. aureus*, *K.rosea*, *S. salivarius*, *G. morbillorum*, Unidentified2 and Unidentified3 (exhibiting 32.1% out of total pathogenic bacteria that have been isolated from dental plaque samples which were collected from 192 dental patients and 69.2% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from male patients). However, the second type comprises 4 G-ve bacilli pathogenic bacteria (Tables 4 &5 and Figures 5 & 6): *S. marcescens*, *A. hydrophila*, *K. pneumonia*, and *S. paucimobilis* (exhibiting 14.3% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from 192 dental patients and 30.8% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from male patients).
In addition, 24 Pathogenic bacteria species (accounting for 85.7% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from 192 dental patients) were isolated from the dental plaques of 146 dental Female patients; *S. odorifera*, *P. aeruginosa*, *K. oxytoca*, *E. cloacae*, *S. alactolyticus*, *R. ornithinolytica*, *S.aureus*, *E. coli*, *K. pneumonia*, *S. marcescens*, *S. paucimobilis*, *S. mitis*, *A. viridans*, *S. pluranimalium*, *S. parasanguinis*, *S. salivarius*, *S. sanguinis*, *E. faecalis*, *K.rosea*, *K. kristinae*, *S. pseudoporcinus*, *R.dentocariosa*, *A. haemolyticus*, and Unidentified1(Tables 4 &5 and Figures 5 & 6). Moreover, the 24 Pathogenic bacteria species isolated from dental plaque samples collected from 146 female patients were divided into two types according to gram stain: G+ve and G-ve pathogenic bacteria (Tables 4 & 5 and Figures 5 & 6). The first type includes 13 G+ve cocci pathogenic bacteria (Tables 4 & 5 and Figures 5 & 6): *S. alactolyticus*, *S. aureus*, *S. mitis*, *A. viridans*, *S. pluranimalium*, *S. parasanguinis*, *S. salivarius*, *S. sanguinis*, *E. faecalis*, *K. rosea*, *K. kristinae*, *S. pseudoporcinus*, and Unidentified1(exhibiting 46.4% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from 192 dental patients and 54.2% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from female patients) and one G+ve bacilli pathogenic bacteria (Tables 4 & 5 and Figures 5 & 6) *Rothia dentocariosa* (Illustrates 3.6% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from 192 dental patients and 4.1% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from female patients).

However, the second type comprises 10 G-ve bacilli pathogenic bacteria (Tables 4 & 5 and Figures 5 & 6): *S. odorifera P. aeruginosa*, *K. oxytoca*, *E. cloacae*, *R. ornithinolytica*, *E. coli*, *K. pneumonia*, *S. marcescens*, *S. paucimobilis* and *A. haemolyticus* (exhibiting 35.7% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from 192 dental patients and 41.7% out of total pathogenic bacteria that have been isolated from dental plaque samples collected from female patients). Moreover, Tables 4 & 5 and Figure 4 illustrate that among the 28 isolated pathogenic bacteria, *S. mitis* exhibited the highest prevalence as it was isolated from dental plaques of 69 dental patients (accounting for 35.9% out of total dental patients carrying pathogenic bacteria in their dental plaque samples); 18 male and 51 female, while 5 pathogenic bacteria demonstrated the lowest prevalence; 3+ve gram (*E. faecalis, S. alactolyticus,* and *S. pluranimalium*) and 2-ve gram (*A. hemolyticus* and *S. odorifera*) where each pathogen was isolated individually from a single dental plaque (Tables 4 & 5 and Figure 4) of a female patient (each

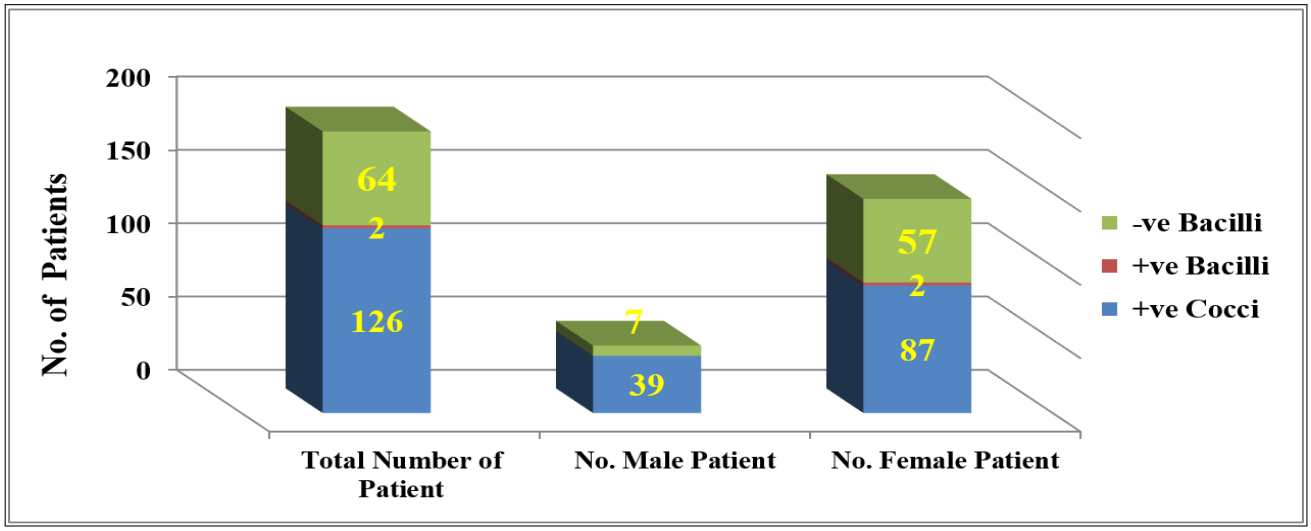

Figure 3. Number of Dental Plaque with with G+ve and G-ve Pathogenic Bacteria.

Table 4: G+ve Pathogenic Bacteria Isolated from Dental Plaque and their Frequency.

Characters		Bacteria	Male Patients		Female Patients		Total	
			No. of Patients	Mean number of colonies	No. of Patients	Mean number of colonies	No. of Patient	Total No. of Colonies (mean)
+ve Gram	Cocci	*A. viridans*	0	0	2	142	2	142
		E. faecalis	0	0	1	108	1	108
		G. morbillorum	3	79	0	0	3	79
		K. kristinae	0	0	4	79	4	79
		K.rosea	2	116	3	82	5	198
		S.aureus	2	123	2	177	4	300
		S. alactolyticus	0	0	1	210	1	210
		S. mitis	18	139	51	145	69	284
		S. parasanguinis	2	300	2	137	4	437
		S. pluranimalium	0	0	1	140	1	140
		S. pseudoporcinus	4	128	2	14	6	142
		S. salivarius	4	106	7	133	11	239
		S. sanguinis	0	0	9	121	9	121
		Unidentified1	0	0	2	149	2	149
		Unidentified2	2	1	0	0	2	1
		Unidentified3	2	109	0	0	2	109
	Bacilli	*R. dentocariosa*	0	0	2	14	2	14
Total			39	1101	89	1651	128	2752

Table 5: G-ve Pathogenic Bacteria Isolated from Dental Patients and their Frequency.

Characters		Bacteria	Male Patients		Female Patients		Total	
			No. of Patients	Mean number of colonies	No. of Patients	Mean number of colonies	No. of Patient	Total No. of Colonies (mean)
-ve Gram	Bacilli	*A.haemolyticus*	0	0	1	7	1	7
		A. hydrophila	2	76	0	0	2	76
		E. cloacae	0	0	20	232	20	232
		E. coli	0	0	2	177	2	177
		K. oxytoca	0	0	6	258	6	258
		K. pneumonia	1	59	8	177	9	236
		P. aeruginosa	0	0	2	300	2	300
		R. ornithinolytica	0	0	6	200	6	200
		S. marcescens	2	300	3	172	5	472
		S. odorifera	0	0	1	382	1	382
		S. paucimobilis	2	58	8	147	10	205
Total			7	493	57	2052	64	2545

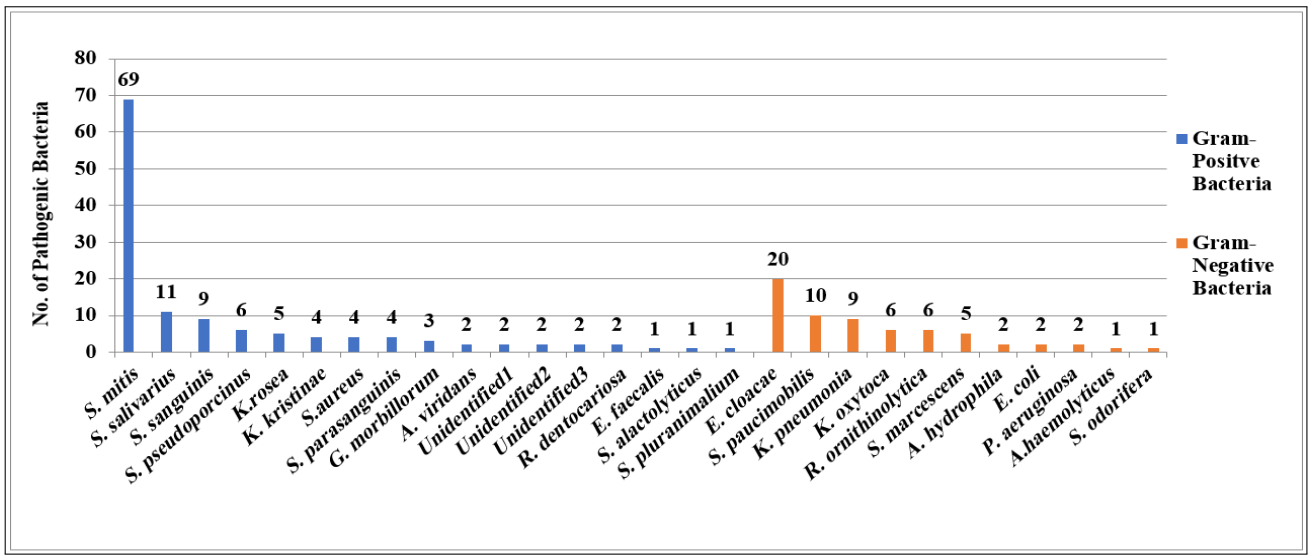

Figure 4. Prevalence of Pathogenic Bacteria Isolated from Patient with Dental Plaque.

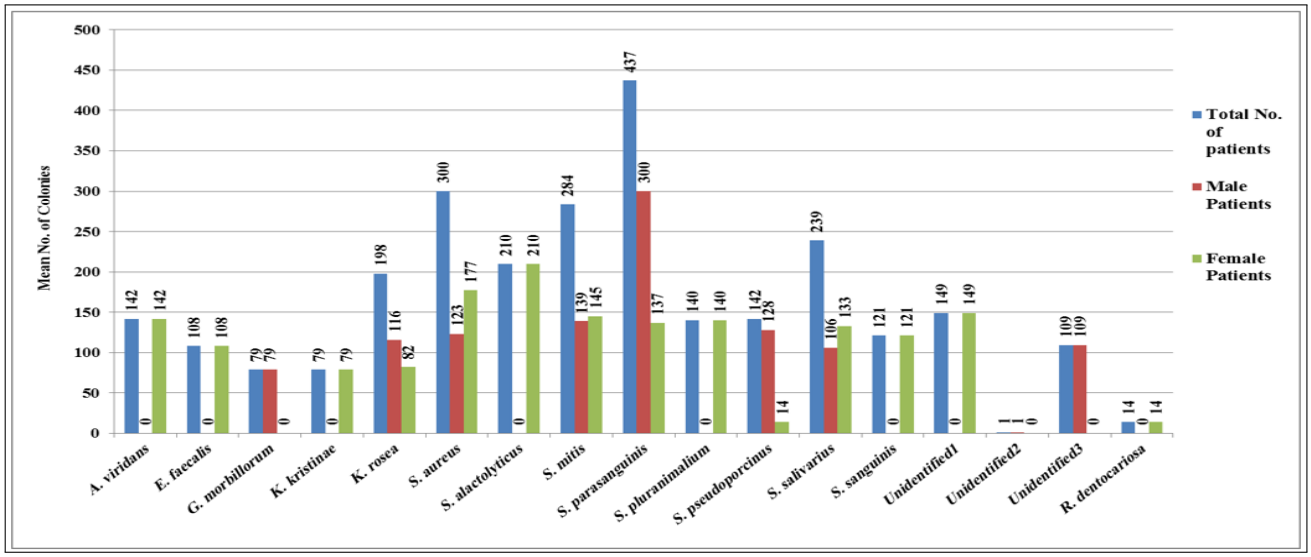

Figure 5. G+ve Pathogenic Bacteria Isolated from Patient with Dental Plaque and their Frequency.

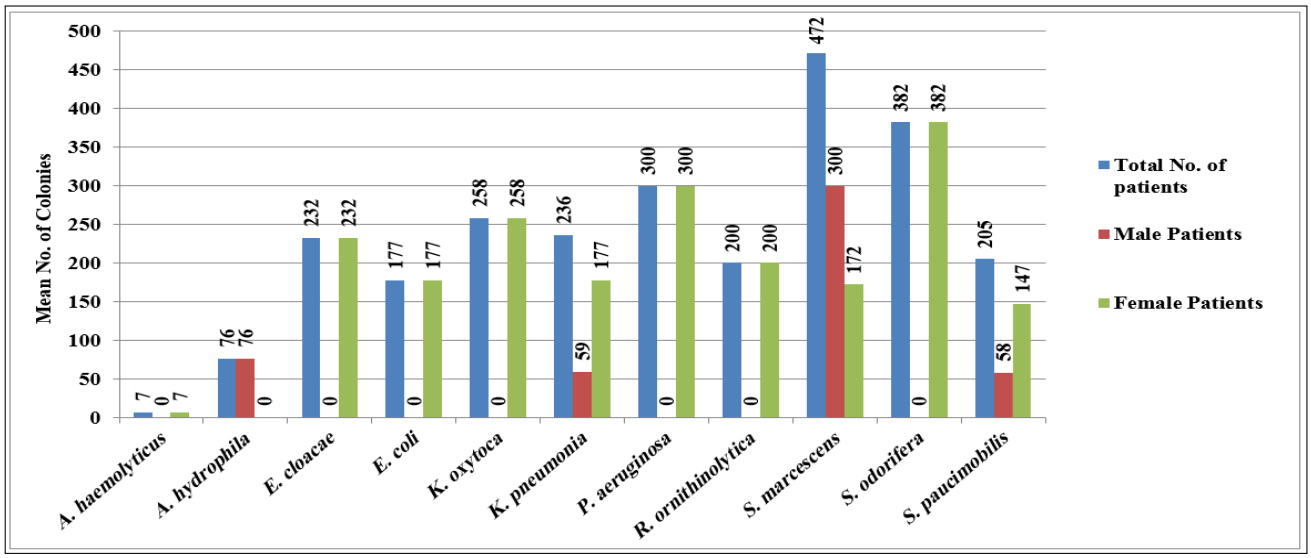

Figure 6. G-ve Pathogenic Bacteria Isolated from Patient with Dental Plaqueand their Frequency.

female patient represents 0.5% of all dental patients that carries pathogenic bacteria in their dental plaque samples). In addition, Tables 4 & 5 and Figures 5 & 6 reveal that *S. marcescens* and *S. parasanguinis*, which were isolated from the dental plaques of 2 male patients separately, showed the highest mean number of colonies (300 colonies in dilution 10^{-4}) among the 13 Pathogenic bacteria species (9 +ve gram and 4-ve gram) isolated from dental plaque samples collected from 46 male patients followed by *S. mitis* (139 colonies), *S. pseudoporcinus* (128 colonies), *S. aureus* (123 colonies), *K. rosea* (116 colonies), Unidentified3 (109 colonies), *S. salivarius* (106 colonies), *G. morbillorum* (79 colonies), *A. hydrophila* (76 colonies), *K. pneumonia* (59 colonies), *S. paucimobilis* (58 colonies) and Unidentified2 (1 colony) which were isolated from the dental plaques of 18, 4, 2, 2, 2, 4, 3, 2, 1, 2 and 2 male patient respectively. On the other hand; Table 4 & 5 and Figure 5 & 6 illustrates that *S. odorifera* which was isolated from dental plaque samples collected from 1 female patients exhibited the highest mean number of colonies (382 colonies in dilution 10^{-4}) among the 24 Pathogenic bacteria species (14+ve gram and 10-ve gram) isolated from dental plaque samples collected from 146 female patients followed by *P. aeruginosa*, *K. oxytoca*, *E. cloacae*, *S. alactolyticus*, *R. ornithinolytica, K. pneumoni*, *E. coli*, *S. aureus*, *S. marcescens*, Unidentified1, *S. paucimobilis*, *S. mitis*, *A. viridans*, *S. pluranimalium*, *S. parasanguinis*, *S. salivarius*, *S. sanguinis*, *E. faecalis*, *K. rosea*, *K. kristinae*, *R. dentocariosa*, *S. pseudoporcinus* and *A. haemolyticus* with mean number of colonies; 300, 258, 232, 210, 200, 177, 177, 177, 172, 149, 147, 145, 142, 140, 137, 133, 121, 108, 82, 79, 14, 14 and 7 correspondingly, isolated from dental plaque samples collected from 2, 6, 20, 1, 6, 8, 2, 2, 3, 2, 8, 51, 2, 1, 2, 7, 9, 1, 3, 2, 2 and 1 female patients sequentially.

4. DISCUSSION

The results in Table 1 and Figure 1 indicate that female patients attend dental clinics for dental care, especially orthodontic treatment, more than male patients; this agrees with Jones [19], Tang & Wei [20], Alkawari [21], Albarakati [22] and Harris and Glassell [23] where they concluded that girls/women exhibit more interest in and seek out for dental treatment especially orthodontic treatment than boys do because they are more often (and strongly) unsatisfied with their teeth look. Moreover, Table 1 and Figure 1 exhibit that females are more likely than males to be hosts for oral pathogens, including dental plaque pathogenic bacteria; this may be due to the presence of caries risk factors in females, which include different salivary composition and flow rate, hormonal fluctuations, dietary habits, genetic variations, and particular social roles in their family. In addition, many systemic diseases that are associated with caries have also been found to have an association with the female gender [24].

Furthermore, the results presented in Tables 2-5 and Figure 2-5 Illustrate that G+ve (particularly G+ve cocci) bacterial pathogens are predominant in dental plaque isolates either in their taxa (about 17 species of G+ve bacterial pathogen verusus11 species of G-ve bacterial pathogens) or their frequency, as the total mean number of G+ve pathogenic bacteria colonies is higher (2752 colonies) than the total mean number of G-ve pathogenic bacteria colonies (2545 colonies). Moreover, the number of patients (128 total; 39 male and 89 female) with a dental plaque containing G+ve pathogenic bacteria is greater (Table 3 & Figure 3) when compared to patients with a dental plaque containing G-ve pathogenic bacteria (64 total; 7 male and 57 female). These outcomes are compatible with the findings of Duggal & Curson [25] and Paula *et al.* [26], where they cited that G+ve bacterial pathogens are predominant in dental plaque isolates, especially in the first stages of dental plaque and G-ve bacterial pathogens replace G+ve during the structural development of dental plaque. This replacement depends on the physiologic changes in the microenvironment. On the other hand, several previous studies support the results in Tables 4 & 5 and Figures 4 & 5, which indicate that *A. haemolyticus* [27], *A. viridans* [4], *A. hydrophila* [4], *E. cloacae* [7], *E. faecalis* [7], *E. coli* [7], *G. morbillorum* [28], *K.oxytoca* [29], *K. pneumonia [7], K. kristinae* [30], *K. rosea* [31], *P. aeruginosa* [7], *R. ornithinolytica* [5], *R. dentocariosa* [31], *S. marcescens* [32], *S. odorifera* [6], *S. paucimobilis* [33]; *S. aureus* [13]; *S. mitis* [7], *S. parasanguinis* [7], *S. pluranimalium* [31], *S. pseudoporcinus* [34], *S. salivarius* [31] *and S. sanguinis* [7] were presented in different parts of dental patients oral cavity, including dental plaque. Moreover, Mylonas *et al.* [35] concluded that the oral cavity should be considered a potential source of *S. alactolyticus*. Furthermore, the previous results confirm with Peterson *et al.* [36], where they recorded that *S. mitis* is one of the most prevalent species found in the dental plaque samples (presented by 25%) collected from dental patients.

5. CONCLUSION

According to the previous results, the pathogenic bacteria isolated from the dental plaques were G+ve pathogenic bacteria than those G-ve ones. Moreover, the most pathogenic bacteria species were isolated from female patients with dental plaque than male patients. Finally, *Serratia marcescens*, and *Streptococcus parasanguinis* showed the highest mean number of colonies among male patients with dental plaques and *Serratia odorifera* exhibited the highest mean number of colonies among female patients with dental plaques.

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