CLINICAL FEATURES, AGE AND SEX DISTRIBUTIONS, RISK FACTORS AND THE TYPE OF BACTERIA ISOLATED IN PERIODONTITIS PATIENTS IN SANA'A, YEMEN

ABSTRACT

Background: Periodontitis is plaque induced inflammation of surrounding tooth structures. It is a major factor in adult tooth loss. There is paucity of information on associated clinical features , risk factors and microbial etiology of periodontitis in Sana'a, Yemen. **Aim:** The study focused on associated clinical features , risk factors and the isolation and identification of bacteria in periodontitis and associated risk factors among patients attending dental clinics is Sana'a city. **Materials and methods:** This was a cross sectional study that was conducted from October 2017 to September 2018. A total of 296 samples were collected from patients suffering from dental diseases comprising of 49 patients with periodontitis. Sterile paper points were used for the sample collection. Standard culture and biochemical techniques were used for the isolation and identification. Structured questionnaires were used to record clinical features, demographic variables and other risk factors of periodontitis. Analysis was done with Epi info Version 6. Categorical variables were reported using descriptive statistics. **Results:** A total of 130 microorganisms were isolated from 49 patients with periodontitis. Male patients accounted for 14.4% and females 18.9% from the total dental patients attending to our clinics. There was no significant association between gender and periodontitis occurrence while there was significant association the younger age groups (45.4% in <26 years of age). The most common signs and symptoms were swollen or puffy gums (91.9%), bleed easily gums (96%), halitosis (96%), painful chewing (87.8%), pus between teeth and gums (71.4%), loose teeth or loss of teeth (44.9%), gingival recession (83.7%), spitting out blood when brushing or flossing teeth (79.6%), and tender gums (93.9%). There was a significant correlation between a frequent history of gingivitis (OR = 3.2), qat chewing (OR = 5.3), obesity (OR = 2.7), using some drugs that cause dry mouth or gingival changes (OR = 11.8), conditions that cause decreased immunity (OR = 4.3) and certain diseases, such as diabetes, rheumatoid arthritis, and Crohn's disease (OR = 6.1). Multi-infections occurred in 89.8% of the periodentitis patients and the most common bacteria isolated were *Actinobacillus actinomycetemcomitans* (79.6%), followed by *Streptococcus pyogens* (73.5%) and *Staphylococcus aureus* (53.1%). **Conclusion:** This study is new in Sana'a city. The clinical features of preiodentitis in Yemen and the risk factors are similar to those reported in the literature elsewhere, but the isolated bacteria differ in frequency from those reported elsewhere, as some upper respiratory tract pathogens such as *Streptococcus pyogens* are commonly isolated in this study. Knowledge of the clinical features, bacterial causes of gum disease, and risk factors is the key to successful periodontal therapy.

Keywords: **bacteria agents, clinical features, periodontitis, risk factors, Sana'a, Yemen**

INTRODUCTION

There is a significant spread of periodontal disease globally, and identifying the etiology is the key to controlling it. Periodontal disease is a chronic bacterial infection characterized by persistent inflammation, breakdown of connective tissue and destruction of the alveolar bone [1]. Periodontal disease is generally classified into gingivitis and periodontitis. Gingivitis is inflammation of the gums initiated by the proliferation of dental plaque and is reversible. Gingivitis possibly common in children up to 5 years old [2]. It is the result of inappropriate oral hygiene practices[3]. Periodontitis is a chronic inflammatory disease that begins with a buildup of dental biofilm plaque and continues through a disorganized immune response and is commonly started by gingivitis leading to irreversible demolition of the supportive tissues adjoining the tooth, together with the alveolar bone [1,2,4]. . Periodontal disease is a multi-microbial, multifactorial disease, with numerous host factors involved in influential an individual's susceptibility to disease[5]. Several reports have been reported that the onset and progression of the disease is not only similar to the presence of pathogenic bacterial strains in the gums but also due to the absence or minimum levels of beneficial equivalents commensals in the susceptible host[5-7]. A limited number of periodontal pathogens have been reported in the complex biofilms to initiate periodontal disease. Data definite that some bacterial strains in the gingival environment can cause gingivitis and bone destruction. These bacterial strains are known as periodontal pathogens[8,9].

It is known that these periodontal disease pathogens possess, when present in even very small quantities, the ability to damage the gingival structure [5]. It is known that most periodontal pathogens are anaerobes, but biofilm can also accommodate facultative aerobes, capnophilles, and microaerophiles whose number depends on the

environment in the developing biofilms and gingival pocket[5]. It became known that of 800–1000 species that colonized the oral cavity, 50 species have been identified with strong links to periodontal disease [10]. These complexes were classified Socratic and Hafidian into 5 complexes, which are the yellow or early colonized complex, the green or secondary colonized complex, then the orange, purple and red complexes. The red compound is the secondary colony that is the main pathogens associated with bleeding upon investigation [11]. There is strong bacterial progression in oral cavity infection that may be influenced by age, diet, or site of infection[12].

Nevertheless, in addition to periodontal disease pathogens, genetic and environmental factors predispose to disease progression. The risk of gum disease is determined by several factors including any health condition that leads to bacterial defense mechanisms defect such as human immunodeficiency virus (HIV), diabetes, and neutropenia. Obesity, tobacco smoking, poor diet, and a inactive lifestyle are associated with an increased risk of periodontitis[13]. In the early stages, periodontitis has few symptoms, and the disease has progressed significantly in many individuals before they seek treatment. Symptoms may include redness or bleeding of the gums while brushing the teeth, the use of dental floss or gnawing on solid food, frequent swelling of the gums, Halitosis, Gingival recession, Deep pockets between the teeth and the gums, Loose teeth, and Drifting of incisors [1, 3,14].

Periodontitis is the leading cause of tooth loss in adults globally, and these people are at risk of multiple tooth loss, edentulism, and masticatory impairment as a result of which a negative impact on nutrition, quality of life and self-esteem and thus a significant societal imposition - the economic impact and the cost of health care [3, 14,15]. Most of the information about the causes of periodontitis emerged from studies conducted in Europe and the United States of America, and some third world countries. Although there are some studies on oral and dental problems in Yemen [16-25], no study has been conducted in Yemen on periodontitis [26]. So this study focused on associated clinical features , risk factors and the isolation and identification of bacteria in periodontitis and associated risk factors among patients attending some dental clinics is Sana'a city.

SUBJECTS AND METHODS

Patients

This study included 49 patients suffering from periodontitis, who were admitted to the dental clinic at the Republican University Hospital and private dental clinics in Sana'a, during a period of about one year, which started in December 2019 and ended in November 2020, of whom 22 were males and 27 were females.

Data collection and processing

A questionnaire was filled out for each patient with the patient's personal, clinical data and risk factors. This included age, gender, occupation and relevant clinical information regarding bacterial oral infections. Also risk factors of contracting periodontitis. Cultures were obtained from the collected pocket by probes in order to isolate the various bacterial causative agents. First, the supragingival plaque was removed (without disturbing the subgingival plaque) and a bacterial sample was collected from the deepest periodontal pockets with a sterile probe. The samples were then placed in a vial containing 2 ml of liquid thioglycolate enriched medium, sealed immediately and transported to the laboratory within 30 minutes. Bacteriological procedures were performed within one hour of sample collection. For germ cultures, the following media and conditions were used: TSA blood (5%) and MacConkey agar plates - incubated at 35 ° C under 5% CO_2 and examined at 24 and 48 hours; Brucella agar enriched with Vitamin K1 and CDC + amikycin blood agar - incubated at 35 ° C anaerobically in a Gaspak jar (Oxoid Ltd). Cultures were examined for the presence of bacteria at 48 and 96 h. Plates showing bacterial growth were retained until final processing and organism identification by standard techniques[27].

Data analysis

Clinical, personal, and risk factors data as well as sample culture results entered into the questionnaire were analyzed by Epi Info, Version 6. All subjects with pockets less than 2 mm were considered to have periodentitis. To correlate the clinical features and potential risk factors for periodentitis, the data were examined in the form of case-control studies. For people with periodentitis, people with other dental diseases have been matched. Differences in categorical variables were assessed using Fisher's exact tests as appropriate. Ninety-five percent confidence intervals for odds ratios were calculated according to the Cornfield limits and 95% confidence intervals were calculated for simple ratios by an exact binomial method. The significance of the difference in the ratio and the odds ratio was analyzed, and a chi-square ($\chi2$) greater than 3.84 and a probability value (p) less than 0.05 were considered statistically significant.

ETHICAL APPROVAL

Ethical approval was obtained from the Medical Research & Ethics Committee of the Faculty of Medicine & Health Sciences at Sana'a University. All data, including patient identification were kept confidential.

RESULTS

Table 1 shows the age and gender distribution of patients with periodontitis. Male patients accounted for 14.4% of the total periodontitis patients, and the female percentage was 18.9% from the total dental patients attending

to our clinics. There was no significant association between gender and periodontitis occurrence. When age was considered a dependent factor for periodontitis, the rate of periodontitis was highest in the younger age groups (45.4% in <26 years of age), while the rate in> 45 years was 13.3%. Table 2 shows the importance periodontitis signs and symptoms among dental patients in Sana'a, Yemen. When compared with other oral diseases, we find that the swollen or puffy gums occurred in 91.9% of periodontitis patients and occurred more than 30.2 times (CI = 10.4-87, p <0.001) of other oral diseases.Bleed easily gums occurred in 96% of periodontitis patients and occurred more than 67 times (CI = 15-284, p <0.001) of other oral diseases. Halitosis occurred in 96% of periodontitis patients and occurred more than 88 times (CI = 20-374, p <0.001) of other oral diseases. Painful chewing occurred in 87.8% of periodontitis patients and occurred more than 13.4 times (CI = 5.4-32, p <0.001) of other oral diseases. Pus between teeth and gums occurred in 71.4% of periodontitis patients and occurred more than 15.3 times (CI = 7.6-32, p <0.001) of other oral diseases. Loose teeth or loss of teeth occurred in 44.9% of periodontitis patients and occurred more than 5.2 times (CI = 2.6-10.3, p <0.001) of other oral diseases. Other symptoms and signs occurred 83.7% for gingival recession, 79.6% for spitting out blood when brushing or flossing teeth, 93.9% for tender gums with significant occurrence for all these signs as compared with other oral diseases. Table 3 shows the risk factors associated with periodontitis among dental patients in Sana'a, Yemen. There was a significant correlation between a frequent history of gingivitis (OR = 3.2, CI = 1.7-6.1, p <0.001), qat chewing (OR = 5.3, CI = 2.8-10.2, p <0.001), obesity (OR = 2.7, CI). = 1.3-5.4, p = 0.004), some drugs that cause dry mouth or gingival changes (OR = 11.8, CI = 3.4-41, p <0.001), conditions that cause decreased immunity (OR = 4.3, CI = 1.1- 16.6, p = 0.02), and some diseases, such as diabetes, rheumatoid arthritis, and Crohn's disease (OR = 6.1, CI = 2.8-13.2, p <0.001); and the occurrence of periodontitis. Table 4 shows the number and percentage of the cultivated microorganisms from the 49 patients suffering from periodentitis . Multi-infections occurred in 89.8% of the periodentitis patients and the most common bacteria isolated were *Actinobacillus actinomycetemcomitans* (79.6%), followed by *Streptococcus pyogenes* (73.5%) and *Staphylococcus aureus* (53.1%). While the *Bacteriodes* species (20.4%), *Streptococcus mutans* (16.3%) and *Anaerobic lactobacillus* (4%) were less isolated from our periodentitis patients. *Candida albicans* was isolated in 4 cases (10.2%).

DISCUSSION

Periodontal disease is a growing health problem in Yemen. Currently, no work has been done to determine the clinical features, etiological and risk factors for periodontitis in Sana'a City, but little works have been done, they dealt with the spread of oral and dental diseases; and some dental and oral disorders[16-25]. In the current study, there was no significant association between sex and the incidence of periodontitis, and many other researchers appear to favor the female preference[28, 29]. Also, our work contrasts with that of Ababneh and others, who reported a predisposition to males but our result is similar to that reported by Susin and Albander, which reported an equal distribution[30, 31]. Age was a dependent factor for periodontitis in the current study, with periodontitis rate being higher in the younger age groups (45.4% in <26 years), while it was lower at> 45 years (13.3%) (Table 1). Several authors believe that age is not a determining factor but a lifetime accumulation of disease [30-32]. For people over the age of 31, the probability of developing gingivitis increased by 5.17 times, and the likelihood of developing periodontitis increased by 2.28 times[30-32].

In the current study, when comparing the clinical features of periodontitis patients with other oral diseases, the clinical signs and symptoms in Table 2 such as swollen or puffy gums, bleed easily gums, halitosis , painful chewing, pus between teeth and gums , loose teeth or loss of teeth, gingival recession, spitting out blood when brushing or flossing teeth, and tender gums occur more frequently in periodontitis patients than in patients with other oral diseases. This finding is similar to that reported in the literature in which previous signs and symptoms appeared in periodontitis more than other dental diseases [[33- 35]]. In the current study, bleeding gums occurred in 96% of periodontitis patients and occurred 67 times more (CI = 15-284, p <0.001) than other oral diseases. In a previous study by Maduakor *et al.* (2019) [36] patients with bleeding gums showed an odds increase of 38.41 times the incidence of gingivitis and 2.58 times the incidence of periodontitis compared to patients without bleeding gums. It has also been reported that bleeding gums is one of the early signs of developing gum disease. This confirms the effect of maintaining good oral health and hygiene associated with preventing inflammation [37, 38].

In the current study higher prevalence of periodontitis among subjects with low education (27.3%) with a significant correlation between a low education level and periodontitis, in which subjects with low school education were 2.9 times more likely to have periodontitis than subjects with a higher level of education (OR = 2.9 times, CI = 1.5- 5.4, p <0.001) (Table 3). A high prevalence of periodontitis has been reported among low-education patients in Nigeria, Jordan and Thailand [30,36,39]. This confirmed that in the United States of America, people with a low school education were three times more likely to have periodontitis than people with a higher education level[40] and it has been reported in many studies that there is a correlation between gum disease and educational level. In the current study, patients with a previous history of recurrent gingivitis were approximately 3.8 times more likely to have periodontitis than those without a previous history of gingivitis

(OR = 3.2, CI = 1.7-6.1, p <0.001) (Table 3). A previous frequent positive history of gingivitis has been reported by several researchers as a risk factor for developing gingivitis [30,36,39]. In the current study, poor oral health habits were not risk factors for periodontitis (odds ratio = 0.4). This finding differs from most of the reported studies in that poor oral health habits (oral hygiene) lead to a risk factor for gingivitis and periodontitis [36-38]. This finding may be because in our study we compared patients with other oral disorders and not with healthy individuals.

Khat or qat (*Catha edulis*) is a flowering plant native to Ethiopia. Khat contains the alkaline cathinone, which is a stimulant that causes excitement, loss of appetite and euphoria. Chewing khat has a history as a social habit going back thousands of years similar to the use of coca leaves in South America and betel nuts in Asia[41]. It is estimated that up to 90% of adult males chew qat three to four hours per day in Yemen. There was a significant association between Khat chewing and periodontitis (OR = 5.3, CI = 2.8-10.2, P <0.001). These results are similar to previous studies in Yemen where Khat chewing is a risk factor for oral diseases [24], and better research on Khat chewing and its potential association with oral and dental disorders should be conducted on a large scale.

Aside from the physiological causes of xerostomia, the iatrogenic effects of medications are the most common cause[42]. A drug known to cause dry mouth can be called xerogenic[43]. More than 400 drugs are associated with dry mouth. Although dry mouth caused by medications is usually reversible, the conditions for which these medications are prescribed are often chronic [44]. The likelihood of developing a xerostomia increases compared to the total number of drugs taken, whether or not individual drugs are dehydrating[45]. The sensation of dehydration usually begins shortly after starting the offending drug or after increasing the dose [42]. There was a significant association between the use of drugs that cause xerostomia or gingival changes and the development of periodontitis (OR = 11.8, CI = 3.4-41, P <0.001). These results are similar to previous studies that reported several medications associated with dry mouth, which is a risk factor for periodontitis [36].

There was a significant correlation between obesity and develop periodontitis, (OR = 2.7, CI). = 1.3-5.4, p = 0.004). This result is similar to previous studies in which obesity is predisposing factors for dental and gums disorders [36]. There was a significant association between conditions causing decreased immunity and the development of periodontitis (OR = 4.3, CI = 1.1- 16.6, p = 0.02); and some diseases such as diabetes, rheumatoid arthritis, and Crohn's disease (OR = 6.1, CI = 2.8-13.2, p <0.001); with the occurrence of periodontitis. These disorders can be described in several different ways: by the component (s) of the affected immune system, whether the immune system is overactive or inactive, and whether the condition is congenital or acquired [46]. These conditions usually make people more susceptible to dental and other local or systemic infections [46].

The polymicrobial pattern that is a feature of periodontal disease was evident in this study, 89.8% in periodontitis, and this is in agreement with the work of many other researchers [12, 47-49]. The presence of polymicrobial infection has important implications for management as it modifies the clinical course of disease, influences the choice of antimicrobial therapy and the expected response to treatment especially when it comes to pathogens that commonly exhibit antimicrobial resistance such as *Staphylococcus aureus* [50].

Aggregatibacter actinomycetecomitans was the most prevalent facultatative anaerobe in periodontitis, 79.6% . Of all the microorganisms in biofilm, three are said to be important in the initiation and progression of periodontal disease: *A. actinomycetecomitans* is named key pathogens or "red complex" bacterium [2,51]. *A. actinomycetecomitans* in aggressive periodontitis patients and in chronic periodontitis patients [52]. They are small short, straight or curved rods with rounded ends that is non motile and Gram negative and it is reported to be strongly associated with destructive periodontal lesions [5]. It possesses many virulence factors including leukotoxin, collagenase, protease, endotoxin, fibroblast inhibition factor inducing bone resorption [5]. *Staphylococcus aureus* was isolated from 53.1% of our periodontitis patients, as did several researchers [52,53]. These microbes are known to easily become resistant to antibiotics, and may reach climax with super-infection. The ability to form biofilm has enabled *Staphylococcus aureus* to survive in this environment also [49,54]. *Streptococcus pyogenes* was isolated from 73.5% and *Streptococcus mutans* in 16.3% of our patients, and this result is similar to that previously reported in which *Streptococcus* species was detected in large numbers by several researchers [12, 52]. Some *streptococci* are beneficial to the host as colonization of the pocket in large numbers can delay the periodontal disease process [55].

In our study, *Enterobacteriaceae* was isolated in 8% of patients with periodontitis (Table 4). *Enterobacteriaceae* is unusual in patients with periodontitis [56]. Several studies have linked enteric bacilli to periodontal disease[52]. According to Botero and colleagues, their role in periodontitis is not clear but is thought to indicate super-infection [57]. They are thought to be opportunists that thrive after periodontal treatment. The drug of choice for the treatment of periodontal disease includes doxycycline, tetracycline, amoxicillin, and metronidazole. The gut bacteria show resistance to these drugs and may therefore persist after taking them. Further studies are needed to explain its presence in the plaque biofilm and explain its role in periodontal infection[57].

Candida albicans was isolated in our study in 8% of patients with periodontitis, and several researchers including Daniluk *et al.* have reported that *Candida albicans* could have a role in the ultrastructure of gingival microbial plaques and in their adherence to periodontal tissue[25,58,59] .

CONCLUSION

This study is new in Sana'a city. The clinical features of periodontitis in Yemen and the risk factors are similar to those reported in the literature elsewhere, but the isolated bacteria differ in frequency from those reported elsewhere, as some upper respiratory tract pathogens such as *Streptococcus pyogens* are commonly isolated in this study. Knowledge of the clinical features, bacterial causes of gum disease, and risk factors is the key to successful periodontal therapy.

AUTHOR CONTRIBUTION

All authors participated in overseeing clinical and laboratory work, data analysis, and manuscript writing and review.

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CONFLICT OF INTEREST

"No conflict of interest associated with this work".

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Table 1:The age and sex distribution of patients suffering from periodontitis

Sex	Periodontitis n=49		OR	95% CI	χ^2	p
	No.	%				
Male n=153	22	14.4	0.7	0.3-1.3	1.1	0.29
Female n=143	27	18.9	1.3	0.7-2.5	1.08	0.29
Age in years						
< 16 n=36	8	22.2	1.5	0.6-3.5	0.9	0.32
16-25 n=56	13	23.2	1.7	0.8-3.5	2.2	0.13
26-35 n=64	10	15.6	0.91	0.4-1.9	0.05	0.82
36-45 n=50	6	12	0.68	0.3-1.5	0.9	0.34
> 45 n=90	12	13.3	0.7	0.34-1.4	0.9	0.32
Total n=296	49	16.6				

Table 2: The significance of signs and symptoms of periodontitis among dental disease patients in Sana'a Yemen

Signs and symptoms	Periodontitis n= 49		OR	95% CI	χ^2	p
	No.	%				
Swollen or puffy gums n=112	45	91.9	30.2	10.4-87	72	<0.001
Bright red, dusky red or purplish gums n=145	44	89.8	12.7	4.8-32	39	<0.001
Tender gums n=195	46	93.9	10	3.1-331	20	<0.001
Bleed easily gums n=111	47	96	67	15-284	85	<0.001
Bleeding during toothbrush or after brushing n=143	41	83.7	7.2	3.2-16	29	<0.001
Spitting out blood when brushing or flossing teeth n=89	39	79.6	15.3	7.1-32	68	<0.001
Halitosis n=99	47	96	88	20-374	102	<0.001
Pus between teeth and gums n=69	35	71.4	15.6	7.6-32	76	<0.001
Loose teeth or loss of teeth n=55	22	44.9	5.2	2.6-10.3	26	<0.001
Painful chewing n=129	43	87.8	13.4	5.4-32	46	<0.001
Gingival recession n=188	41	83.7	3.4	1.5-7.7	10.2	<0.001
Total n=296	49	16.6				

OR= odds ratio, CI= confidence limit 95%, χ^2 = Chi-square ≥ 3.84 (significant), p= probability value < 0.05 (significant)

Table 3: the associated risk factors of periodontitis among dental disease patients in Sana'a Yemen

Risk factors	Periodontitis n=49		OR	95% CI	χ^2	p
	No.	%				
Low Education level n=95	26	27.3	2.9	1.5 -5.4	11.8	<0.001
Repeated History of Gingivitis n=95	29	59.2	3.2	1.7-6.1	14.4	<0.001
Poor oral health habits n=151	16	32.6	0.4	0.21-0.77	7.9	0.004
Smoking or chewing tobacco n=45	12	24.4	0.74	0.2-1.8	0.39	0.52
Chewing Qat n=91	31	63.2	5.3	2.8-10.2	29	<0.001
*Hormonal changes n= 45	6	12.2	0.74	0.2-1.8	0.39	0.52
*Recreational drug use n=6	1	2	1.01	0.11-8.8	0.001	0.99
Obesity n=57	17	34.7	2.7	1.3-5.4	8.9	0.002
Inadequate nutrition, including vitamin C deficiency n=71	10	20.4	1.2	0.5-2.5	0.23	0.6
*Certain medications that cause dry mouth or gum changes n=12	8	16.3	11.8	3.4-41	22	<0.001
*Conditions that cause decreased immunity n=9	4	8.2	4.3	1.1-16.6	5.2	0.02
Certain diseases n= 34	16	32.7	6.1	2.8-13.2	25.8	<0.001
Total n=296	49	16.6				

Hormonal changes= such as those related to pregnancy or menopause.

Recreational drug use = such as smoking marijuana or vaping.

Certain medications that cause dry mouth (xerostomia): Antihistamines, Antidepressants, Antipsychotics, Lung inhalers.

Conditions that cause decreased immunity= such as leukemia, HIV/AIDS and cancer treatment.

Certain diseases, such as diabetes, rheumatoid arthritis and Crohn's disease.

OR= odds ratio, CI= confidence limit 95%, χ^2 = Chi-square ≥ 3.84 (significant), p= probability value < 0.05 (significant)

Table 4: The number and percentage of the cultivated microorganisms from the 49 patients suffering from periodontitis

Bacterial isolates	Total n=49		Male n=22		Female n=27	
	No.	%	No.	%	No.	%
Streptococcus pyogens	36	73.5	17	77.3	19	70.3
Staphylococcus aureus	26	53.1	14	63.6	12	44.4
Bacteroides species	10	20.4	7	31.8	3	11.1
Actinobacillus actinomycetemcomitans	39	79.6	19	86.4	20	74
Streptococcus mutans	8	16.3	3	13.6	5	18.5
Anaerobic lactobacillus	2	4	2	9	0	0
Enterobacteriaceae	4	8	2	9	2	7.4
Candida albicans	4	8	2	9	2	7.4
Multi-infections (more than 2 isolates)	44	89.8	20	90.9	24	88.9
No growth of potential pathogens	5	10.2	2	9.1	3	11.1
Total significant growth	130					