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Full Length Research Paper

Oral *Candida* colonization in patients with fixed orthodontic appliances: The importance of some nutritional and salivary factors

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We investigated the relationship between fixed orthodontic appliances and oral *Candida* colonization. The influence of some important nutritional and salivary factors was also investigated. These factors included: hemoglobin, vitamin B12, ferritin and folate levels, salivary flow rate, salivary pH, tobacco smoking and dietary habits. Patients enrolled in this study were adolescent patients aged 12-18 years who attended the Orthodontics Department/University of Jordan Hospital for the provision of fixed metallic orthodontic appliances. Salivary samples were collected on the first visit prior to bonding, one month and four months later. All patients were investigated for serum vitamin B12, serum ferritin, red-cell folate, complete blood count, salivary pH, and salivary flow rate. Data were obtained from the patients regarding tobacco smoking and dietary habits. We found that *Candida* colonization did not increase after bonding of fixed orthodontic appliances. None of the local oral factors investigated was correlated with *Candida* colonization. Only two systemic factors (serum vitamin B12 and red-cell folate) were significantly (P<0.05) associated with *Candida* colonization during the study period. It was obvious that metallic fixed orthodontic appliances did not encourage oral *Candida* colonization during the four months study period. On the other hand, it seems that nutritional factors like serum vitamin B12 and red-cell folate can influence oral *Candida* colonization more than tobacco smoking, dietary and salivary factors.

Key words: Oral, Candida, Orthodontic appliances, nutritional deficiency, smoking.

INTRODUCTION

Candida species is a commensal yeast which colonizes the oropharyngeal region of up to 60% of all healthy immune-competent individuals (Brawner and Cutler, 1989; Fotos et al., 1992). Healthy individuals with yeast colonization have, on average, 300 to 500 colony forming units of *Candida* per milliliter of saliva (Muzyka, 2005).

The ability of *Candida* to become a pathogenic micro-organism capable of causing infections is attributed to a number of factors. An important factor is nutritional deficiency of vitamin B12, folate and iron. Deficiency of these factors is known to contribute to *Candida* opportunistic infection (Samaranayake, 1986), particularly *Candida*-associated lesions like angular cheilitis (Muzyka, 2005).

Local oral factors may also influence oral *Candida* carriage and these mainly include wearing removable dentures, fixed and removable orthodontic appliances, dry mouth, high-sugar diet, poor oral hygiene (Vazques and Sobel, 2002), and possibly tobacco smoking (Abu-Elteen and Abu-Alteen, 1998).

Studies investigating the effect of fixed orthodontic appliances on oral *Candida* colonization reported differing results (Arslan et al., 2008; Brusca et al., 2007; Hagg et

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al., 2004). A recent literature review confirmed that there is lack of studies investigating oral *Candida* colonization in orthodontic patients (Hibino et al., 2009). Furthermore, significantly little attention has been paid to the nutritional factors which may be involved in the pathogenesis of oral fungal diseases (Paillaud et al., 2004). In recent years, a substantial percentage of Jordanian adolescents (49%) demanded orthodontic treatment (Abu Alhaija et al., 2004). As a developing country, Jordan has a high prevalence of anemia (Kilbride et al., 2000), as well as vitamin B_{12} deficiency (Barghouti et al., 2009).

This study was carried out to address the nutritional and local factors that may influence oral *Candida* colonization in the susceptible group of apparently healthy orthodontic patients.

The aim of this study was to investigate the relationship between fixed orthodontic appliances and oral colonization by *Candida* in association with some important nutritional and blood parameters namely, hemoglobin, vitamin B12, ferritin and red-cell folate levels. The effect of local factors such as salivary flow rate, salivary pH, dietary habits and tobacco smoking on *Candida* colonization is also investigated.

MATERIALS AND METHODS

Patients and questionnaire

Patients included in this study were adolescent male and female patients aged 12-18 years who attended the orthodontics department for the bonding of fixed orthodontic appliances. All subjects reported no history of diseases or intake of medications. All included patients were referred to a periodontist and a restorative dentist to obtain the required level of oral hygiene and to achieve the necessary periodontal and dental health. Patients who failed to maintain plaque-free tooth surfaces were excluded from the study.

The nature of the research was explained to the patients and their parents. Participants signed a consent form which was specifically prepared when the ethical approval was obtained through the University of Jordan Research council. Patients filled a questionnaire that asked specific questions about the patient's oral hygiene practices including the use of tooth brush, dental floss and mouth wash. It also had questions pertaining to dietary habits namely daily consumption of carbonated drinks, sugar-containing sweets and fresh produce. There were also questions about history of antibiotic use in the last 3 months, and current tobacco use including smoking cigarettes and water-pipe. To ensure validity and reliability of the questionnaire, it was first given to 10 patients who were asked to read it loudly and point to any ambiguity. Unclear and vague questions were discussed and modified until no unclear or vague questions were reported any more. The questionnaire was then tested on groups of 10 patients and retested after two weeks on the same subjects. The questions were modified when necessary until the final group of patients gave the same responses.

Collection of salivary and blood samples

To perform salivary studies and to obtain a saliva sample for *Candida* culture, saliva was collected in a sterile container by asking the patient to expectorate over a period of 5 min. Patients did not eat or drink for at least one hour prior to salivary collection. Venous blood samples were collected in plain tubes to estimate the levels of serum vitamin B_{12}, and serum ferritin. More blood samples were collected in EDTA tubes to estimate red cell folate and complete blood count.

Signing the consent form, filling-up the questionnaire and collection of salivary and blood samples were all performed in the first visit. On the same visit, the patient was provided with a fixed orthodontic appliance. Fixed appliances delivered to patients were all made of nickel-titanium wires and stainless steel brackets and bands. One month later, on the second visit, another salivary sample was collected using the same technique. Three months later a third salivary sample was collected. All salivary samples collected over the three visits were cultured for *Candida*.

Salivary studies and microbiological investigations

Salivary pH was determined and salivary flow rate was estimated for all Saliva specimens. To evaluate oral *Candida* colonization, salivary samples were cultured by inoculating 0.1 ml saliva on Sabouraud dextrose agar (Mast ID, UK) supplemented with chloramphenicol (0.05 g/l). The plates were incubated at 37ºC and inspected after 24 and 48 h. All growth of yeast-like colonies was subsequently identified by the germ-tube production test and by subculture of two or three representative colonies on CHROMagar Candida medium (Mast ID) and incubation at 37°C for /48 h to detect the common *Candida* species .. The *Candida* isolates were identified by detection of various color characteristics on CHROMagar *Candida* plates and confirmed by using biochemical Remel system (RapID™ YEAST PLUS, USA). Yeast growth was counted as follows: few (1-10 colonies), moderate (11-50 colonies) and heavy (>50 colonies). Reference standard strains of *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC, 2001), and *C. parapsilosis* (ATCC 22019) were subcultured on the same medium as control strains (Odds and Bernaerts, 1994).

Statistical analysis

Statistical Package for Social Sciences (SPSS) for Windows version 11.5 (SPSS Inc., Chicago Ill, USA) was used to calculate values of significant differences between the groups and to carry out regression analysis. Two sample t-test was used when the examined values were parametric (laboratory tests). Mann-Whitney, and Kruskal Wallis tests were used when the examined values were non-parametric (gender, smoking). Chi-square test was used when data were cross-tabulated. Differences between the groups was indicated by the p value that was considered significant when ≤ 0.05.

RESULTS

With the exception of three patients who declined to participate in this study, a total of 96 patients were first included. However, 15 patients failed to attend in subsequent visits, so they were excluded from the study and their data were removed. Hence, the final number of patients was 81 (24 males and 57 females).

Baseline microbiological, salivary and hematological results

Prior to bonding of their fixed orthodontic appliances, 42

Table 1. Mean values of hematological and salivary parameters of all patients. Hgb: hemoglobin, B12: vitamin B12, RCf: red-cell folate, SFR: salivary flow rate, SpH: salivary pH.

Status of *Candida* carriage	Ferritin (µg/l)	RCf (ng/ml)	B12 (pg/ml)	Hgb (g/dl)	S.pH	S.F.R (ml/5 min)
Carriers	27.2	260.9	291.2	13.6	6.5	1.9
Non-carriers	30.7	257.8	338.9	13.6	6.7	2.4

Table 2. Characteristics of *Candida* colonization during the study period.

	Mild colonization (n=34)	Moderate colonization (n=20)	Heavy colonization (n=1)
First visit (n=39)	17: C. *albicans* 3: C. *glabrata* 2: C. *albicnas+glabrata* 1: C. *krusei* 1: Others	12: C. *albicans* 2: C. *albicnas+glabrata*	1: C. *glabrata*
Second visit (n=6)	4: C. *albicans*	2: C. *albicans*	
Third visit (n=10)	4: C. *albicans* 2: C. *glabrata*	4: C. *albicans*	

patients (52%) did not show evidence of *Candida* colonization while 39 (48%) patients did. *Candida* colonies count, was mild (1-10 colonies) in 25 patients, moderate (11-50 colonies) in 13 patients and was heavy (>50 colonies) in one patient. Twenty eight patients showed colonization only in the first visit, and they had no colonization in subsequent visits.

The mean values of hematological and salivary parameters of patients with *Candida* colonization (n=39) and patients who did not have colonization are shown in Table 1. There was a significant difference between the two patient groups in the values of vitamin B12 only (P=0.04). Furthermore, regression analysis showed that the values of vitamin B12 correlate and are directly related to the occurrence of colonization prior to bonding (r =0.228, P=0.041).

Salivary flow rate in all subjects in this study ranged from 0.25 - 9.5 ml/5 min, and pH ranged from 5 – 8.

Nutritional habits in terms of quantities of sweets, fresh fruits and vegetables and fizzy drinks that were consumed daily, did not have any influence on *Candida* colonization prior to bonding (P value was: 0.3, 0.9 and 0.2 respectively) or after bonding (P value was: 0.06, 0.6 and 0.6 respectively).

Microbiological data of the second and third visits

On the second visit, saliva cultures showed *Candida* colonization in only 6 patients with the colonization being mild in 4 patients and moderate in 2 patients. When cross-tabulated, there were significantly more patients with *Candida* colonization on the first visit compared to the second visit (P<0.000).

Statistical analysis showed that those patients did not have significantly different values for vitamin B12, ferritin, red cell folate, haemoglobin, salivary flow rate or salivary pH when compared to patients who showed no colonization at the second or third visits, (P values >0.05 for all). However, the mean values of vitamin B_{12} and red cell folate were higher in patients who did not have colonization (mean values: 337.9 pg/ml, 267.5 ng/ml for B_{12} and red cell folate respectively) compared to patients who had colonization (mean values: 293.3 pg/ml and 237.7 ng/ml for B_{12} and red cell folate respectively).

Only one female patient showed evidence of *Candida* colonization in the three visits. This smoker patient had a reduced level of vitamin B12 (145 pg/ml) and ferritin, (3.6 ng/ml) and normal values for the other laboratory tests. *Candida glabrata* was the isolated species in this subject.

On the third visit, *Candida* colonization was detected in 10 patients, 6 of them had mild and 4 had moderate colonization. Although 5 of these patients were colonized on the first visit as well, 4 patients became colonized only in the third visit. The number of patients with colonization at the third visit was less than that at the first visit, and when these data were cross-tabulated, the difference was statistically significant (P<0.000).

Moreover, regression analysis showed that the status of colonization at the first visit itself is significantly related to the status of colonization at the second visit (r = 0.294, P= 0.008), but not to that at the third visit (r = 0.161, P=0.15).

Tobacco smoking was not found to be associated with candida colonization at all visits (P> 0.05) Isolated *Candida* spp during the study period were as follows: *C. albicans, C. glabrata, C. tropicalis, C. Krusei* and one unidentified isolate. Number of total positive *Candida* cultures in the three visits was 55 (Table 2). *C. albicans* was identified in 85.5% of these.

DISCUSSION

It was the aim of this study to investigate oral *Candida* colonization in patients provided with fixed orthodontic appliances in association with important related factors namely: nutritional, dietary and other local oral factors (salivary flow rate, salivary pH and smoking). Oral hygiene was not a variable in this study since all patients practiced optimum oral hygiene habits that were a requirement for orthodontic appliance provision. Furthermore, the assumption that dental plaque is correlated with oral *Candida* colonization is probably overestimated; a recent study found no correlation between oral *Candida* carriage rate and levels of dental plaque (Darwazeh and Al-Jamaei, 2010).

Forty eight percent of patients had *Candida* colonization prior to orthodontic treatment which was in accordance with the reported prevalence of oral *Candida* colonization of up to 60% (Brawner and Cutler, 1989; Fotos et al., 1992). Ninety eight % of patients with *Candida* colonization had low or moderate *Candida* colonization. In contrast to a number of studies (Addy et al., 1982; Arslan et al., 2008; Hagg et al., 2004), prevalence of *Candida* colonization did not increase after one month or even 4 months of orthodontic treatment. In fact, 28 patients or 71.8% of the patients that were carriers prior to therapy, became non-carriers after provision of the appliances. On the other hand 4 patients only became carriers after 4 months of therapy. This result may be attributed to the metallic nature of the fixed orthodontic appliance. Brusca et al. (2007) attributed the adherence of *Candida albicans* to orthodontic brackets to the type of these brackets (Brusca et al., 2007). They found that composite brackets favored adherence, whereas metallic ones did not. All our patients received 18/8 stainless-steel brackets and nickel/titanium wires and showed a decrease in *Candida* colonization with time. Several studies have shown that titanium (Berry et al., 1992; Muranyi and Wunderlich, 2010), and nickel (Yasuyuki et al., 2010) are associated with an antibacterial activity. The findings of this study suggest that these metals may have an antifungal property, highlighting the need for more studies to investigate this possibility.

Sampling technique for *Candida* culture was different from other studies investigating the same issue. In this study, resting saliva samples were collected and cultured, as opposed to another study which used more than one technique (Hagg et al., 2004). In that study the overall *Candida* prevalence rates obtained using the oral rinse technique did not show an increase in *Candida* density after fixed orthodontic appliance insertion, in contrary to the imprint technique which showed a significant increase (Hagg et al., 2004). On the other hand, the 4-month period of the study is relatively short when considering the length of time that fixed orthodontic appliances may remain intraorally with durations that may exceed one or two years. The longer the orthodontic patients are followed and investigated, the more accurate the results will be.

Certain systemic factors like serum ferritin and hemoglobin had no association with *Candida* colonization. However, two other systemic factors were associated with oral *Candida* colonization after orthodontic treatment: vitamin B_{12} and red-cell folate. In this study it was decided to investigate red cell folate instead of serum folate because of the limitations of the assay for serum folate to measure tissue folate stores (Snow, 1999).Only low Vitamin B_{12} values were significantly associated with *Candida* colonization prior to orthodontic treatment.

The role of folate and Vitamin B_{12} in oral *Candida* colonization was confirmed by a number of studies (Challacombe, 1986; Fletcher et al., 1975). On the other hand, some studies found no correlation between depletion of iron, folate and B_{12} and the development of *Candida* disease (Jenkins et al., 1977; Samaranayake and MacFarlane, 1981). In a previous study carried out to investigate oral *Candida* colonization of removable denture wearers, it was found that patients with heavy *Candida* colonization had low levels of Vitamin B_{12} (Dar-Odeh and Shehabi, 2003). In this category of susceptible patients, it seems that lower Vitamin B_{12} values may facilitate epithelial invasion by hyphae of *Candida* and contribute to heavy colonization (Dar-Odeh and Shehabi, 2003). It has also been reported that Vitamin B_{12} gives tissues more resistance to *Candida* infection and that deficiency of Vitamin B_{12} and folic acid may subsequently lead to mucosal atrophy (Bottero et al., 1997). However, one must not overlook the fact that the nature of acrylic appliances like surface roughness and porosity, may encourage *Candida* adhesion and growth more than the metallic orthodontic appliances with their smooth polished surfaces.

None of the investigated local factors had an association with *Candida* colonization. Four patients had reduced and 8 patients had low salivary flow rate, however, no association was detected with *Candida* colonization. According to some researchers tobacco smoking is not related to oral candidiasis (Campisi et al., 2008). On the other hand, a number of studies showed a correlation between hyposalivation and *Candida* colonization (Campisi et al., 2008; Leung et al., 2008; Shimizu et al., 2008). It is noticed that in the last three cited studies, the study group was of older age and not as young as the patients in our study. This lack of association found in our patients is perhaps attributed to the fact that immune responses in this group of young healthy individuals are well-developed, and that other host factors may compensate for the reduced salivary flow rates. Also, another study could not detect this correlation, although the study groups were institutionalized elderly (Yamanaka et al., 2005). The exact effect of salivary pH on *Candida* colonization is yet to be determined, while a study in pregnant women could not detect a correlation (Scheffer et al., 1981), acidic pH in HIV positive patients

was correlated with oral candidosis (Sanchez-Vargas et al., 2002). Most of our patients (no=54) had acidic pH, however, no association with *Candida* colonization was found. It is believed that salivary pH is not a determinant for *Candida* growth; however, it could affect the adherence and invasiveness of the yeast (Sanchez-Vargas et al., 2002).

Within the limitations of this study, it can be concluded, that the provision of fixed orthodontic appliances reduces *Candida* colonization. Moreover, the effect of local factors on Candida colonization becomes diminished probably because of this effect of the orthodontic appliances. This finding can be utilized clinically by using metallic brackets for patients at a risk of *Candida* infection, such as patients having immunocompromising conditions. In this group of patients, a sensible clinical practice should be followed by avoiding the use of removable acrylic orthodontic appliances or non-metallic fixed appliances. Patients who have low levels of vitamin B12 and red cell folate may be considered at risk of *Candida* colonization after the insertion of fixed orthodontic appliances.

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REFERENCES

Abu-Elteen KH, Abu-Alteen RM (1998). The prevalence of Candida albicans populations in the mouths of complete denture wearers. New Microbiol., 21:41-48.

Abu Alhaija ES, Al-Nimri KS, Al-Khateeb SN (2004). Orthodontic treatment need and demand in 12-14-year-old north Jordanian school children. Eur. J. Orthod., 26:261-263.

Addy M, Shaw WC, Hansford P, Hopkins M (1982). The effect of orthodontic appliances on the distribution of Candida and plaque in adolescents. Br. J. Orthod., 9:158-163.

Arslan SG, Akpolat N, Kama JD, Ozer T, Hamamci O (2008). One-year follow-up of the effect of fixed orthodontic treatment on colonization by oral Candida. J. Oral Pathol. Med., 37:26-29.

Barghouti FF YN, Halaseh LJ, Said TT, Ghraiz SM (2009). High frequency of low serum levels of vitamin 12 among patients attending Jordan University Hospital. East Mediterr. Health J., 15:853-860.

Berry CW MT, Safar JA, Henry CA, Wagner MJ (1992). Antibacterial activity of dental implant metals. Implant Dent., 1:59-65.

Bottero A, Lauritano D, Spadari F, Zambellini Artini M, Salvato A (1997). [Atrophy of the oro-pharyngeal mucosa caused by vitamin B12 and folic acid deficiency. Etiopathologic aspects and clinico-therapeutic problems]. Minerva Stomatol., 46:359-374.

Brawner DL, Cutler JE (1989). Oral Candida albicans isolates from nonhospitalized normal carriers, immunocompetent hospitalized patients, and immunocompromised patients with or without acquired immunodeficiency syndrome. J. Clin. Microbiol., 27:1335-1341.

Brusca MI, Chara O, Sterin-Borda L, Rosa AC (2007). Influence of different orthodontic brackets on adherence of microorganisms *in vitro*. Angle Orthod., 77:331-336.

Campisi G, Panzarella V, Matranga D, Calvino F, Pizzo G, Lo Muzio L, Porter S (2008). Risk factors of oral candidosis: a twofold approach of study by fuzzy logic and traditional statistic. Arch. Oral Biol., 53:388-397.

Challacombe SJ (1986). Haematological abnormalities in oral lichen planus, candidiasis, leukoplakia and non-specific stomatitis. Int. J. Oral Maxillofac. Surg., 15:72-80.

Dar-Odeh NS, Shehabi AA (2003). Oral candidosis in patients with removable dentures. Mycoses, 46:187-191.

Darwazeh AM, Al-Jamaei AA (2010). The relationship between oral hygiene and oral colonization with Candida species in healthy adult subjects. Int. J. Dent. Hyg., 8:128-133.

Fletcher J, Mather J, Lewis MJ, Whiting G (1975). Mouth lesions in iron-deficient anemia: relationship to *Candida albicans* in saliva and to impairment of lymphocyte transformation. J. Infect. Dis., 131:44-50.

Fotos PG, Vincent SD, Hellstein JW (1992). Oral candidosis. Clinical, historical, and therapeutic features of 100 cases. Oral Surg. Oral Med. Oral Pathol., 74:41-49.

Hagg U, Kaveewatcharanont P, Samaranayake YH, Samaranayake LP (2004). The effect of fixed orthodontic appliances on the oral carriage of *Candida* species and Enterobacteriaceae. Eur. J. Orthod., 26:623-629.

Hibino K, Wong RW, Hagg U, Samaranayake LP (2009). The effects of orthodontic appliances on *Candida* in the human mouth. Int. J. Paediatr. Dent., 19:301-308.

Jenkins WM, Macfarlane TW, Ferguson MM, Mason DK (1977). Nutritional deficiency in oral candidosis. Int. J. Oral. Surg., 6:204-210.

Kilbride J BT, Parapia LA, Khoury SA (2000). Incidence of iron-deficiency anaemia in infants in a prospective study in Jordan. Eur. J. Haematol., 64:231-236.

Leung KC, McMillan AS, Cheung BP, Leung WK (2008). Sjogren's syndrome sufferers have increased oral yeast levels despite regular dental care. Oral Dis., 14:163-173.

Muranyi P SC, Wunderlich J (2010). Antimicrobial efficiency of titanium dioxide-coated surfaces. J. Appl. Microbiol., 108:1966-1973.

Muzyka BC (2005). Oral fungal infections. Dent. Clin. North Am., 49:49-65, viii.

Odds FC, Bernaerts R (1994). CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol., 32:1923-1929.

Paillaud E, Merlier I, Dupeyron C, Scherman E, Poupon J, Bories PN (2004). Oral candidiasis and nutritional deficiencies in elderly hospitalised patients. Br. J. Nutr., 92:861-867.

Samaranayake LP (1986). Nutritional factors and oral candidosis. J. Oral Pathol., 15:61-65.

Samaranayake LP, MacFarlane TW (1981). A retrospective study of patients with recurrent chronic atrophic candidosis. Oral Surg. Oral Med. Oral Pathol., 52:150-153.

Sanchez-Vargas LO, Perez-Rios P, Romo-Garcia J, Corona-Izquierdo FP, Hidalgo-Loperena H, Franco-Martinez F (2002). [Salivary pH and culture determinations in HIV infected and non-HIV infected patients with oral candidosis]. Rev. Iberoam. Micol., 19:155-160.

Scheffer P, Mautaint C, Dublanchet A (1981). Salivary pH during pregnancy. Relationship with the presence or absence of *Candida albicans*. A study of 200 cases (author's transl)]. Rev. Stomatol. Chir. Maxillofac., 82:140-142.

Shimizu C, Kuriyama T, Williams DW, Karasawa T, Inoue K, Nakagawa K, Yamamoto E (2008). Association of oral yeast carriage with specific host factors and altered mouth sensation. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 105:445-451.

Snow CF (1999). Laboratory diagnosis of vitamin B12 and folate deficiency: a guide for the primary care physician. Arch. Int. Med., 159:1289-1298.

Vazquez JA, Sobel JD (2002). Mucosal candidiasis. Infect. Dis. Clin. North. Am., 16:793-820, v.

Yamanaka K, Nakagaki H, Morita I, Maeda N, Ohara H, Tomatsu S, Nakashima T, Watanabe Y, Ohta N, Shibata K (2005). Relationship between oral *Candida* species and risk factors with reference to drugs with xerostomic side effects among institutionalised elderly in Aichi Pref., Japan. Commun. Dent. Health, 22:19-24.

Yasuyuki MKK, Kurissery S, Kanavillil N, Sato Y, Kikuchi Y (2010). Antibacterial properties of nine pure metals: a laboratory study using *Staphylococcus aureus* and *Escherichia coli*. Biofouling, 26:851-858.