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Biofilm Production of *Staphylococcus aureus* (MRSA) and its interaction with each *Candida albicans* and *Pseudomonas aeruginosa*

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ABSTRACT

The pathology resulting from *Staphylococcus aureus* MRSA infections is of great importance as one of the community associated bacterial infections and its ability to produce biofilms. Studies have focused on monospecies biofilm production, therefore, this study was designed to detect multispecies biofilm production . Biofilm production was evaluated by Microtiter plate method using crystal violet stain. Different clinical isolates including *Staphylococcus.aureus* (MRSA), *Candida albicans* and *Pseudomonas aeruginosa.* Dual species included *S.aureus* MRSA with *C.albicans* and *S.aureus* MRSA with *P.aeruginosa*. Strong biofilm production of *S.aureus* MRSA showed the highest percentage 70% , while in dual species with *C.albicans* it showed 25% and with *P.aeruginosa* 50%. These results show the different behavior in biofilm production of bacterial species with other bacteria or yeasts in combinations, which may affect the mixed infections and the ability of such microorganisms to resist antibiotics.

Keywords: Biofilm; Interactions; Pathogenic bacteria; Yeasts.

1. INTRODUCTION

Biofilm is defined as an extracellular polymer matrix that surround the microbial population, the microbial cells are embedded in this matrix substance that they have produced in the first place [1]. It has been a very important subject for very long time because of its important relationship with most hospital infections [2]. The relevance of this is explained by their free living forms which are phenotypically different from the biofilm it produce and this increase its resistance to antimicrobial agents and host defenses [3] as well as recognizing it as an important virulence factor [4], these characteristics helps the producing microorganism to resist hostile niches within the host, causing persistent or chronic infections and as a result biofilm producing microorganisms will survive treatment with antibiotics that inhibit only the free form of the microbial population [5].

Polymicrobial biofilm populations may develop according to different interactions that occur between microorganisms, such as synergistic or antagonistic interactions [6]. *Candida albicans, Staphylococcus aureus* methicillin-resistant (MRSA), and *Pseudomonas aeruginosa* are well known microorganisms that are responsible for a wide range of infections separately, on the other hand there are some studies that showed an association between such microorganisms as a co-infector [7]. Infections caused by a single species of a microbe is related to 23% mortality rate compared to a significantly higher mortality rate 70% caused by infections due to polymicrobial biofilm producers [8].

Candida albicans is common eukaryotic yeast which usually colonize the mucus membranes of the oral cavity and vagina [9]. It is known to cause candidiasis and was found to be isolated with *S.aureus* in polymicrobial blood cultures [10]. *Staphylococcus*

aureus (MRSA) is a Gram-positive pathogen that has been known to be one of the most virulent pathogens and cause a wide range of infections due to its ability to produce a well-recognized biofilm which lead to develop resistance against several drugs [11,12]. *Pseudomonas aeruginosa* is a Gram-negative pathogen that cause complicated and life threatening infections such as meningitis, endocarditis, bacteremia, pneumonia and cystic fibrosis [13,14].

Literature showed important evidence of polymicrobial infections, therefore scientists focused on specific interactions between microbial pathogens of different origins (eukaryotic and prokaryotic) [15, 16]. In Infections caused by polymicrobial pathogens, the effect of some specific virulence factors such as specific degradative or hydrolytic enzymes that may effect and increase the pathogenicity of these biofilm forming microorganisms because such enzymes are involved in colonization and other growth processes [17]. Other virulence factors produced by such pathogens may form a large amount of radicals and eventually induce an oxidative damage to each other, as well as the host [18]. Studies on the biofilm production interaction between microorganisms is not totally covered and limited. Thus, the aim of the present study was to examine the biofilm production interaction between *S. aureus* (MRSA) and *C. albicans, S. aureus* (MRSA) and *P. aeruginosa*.

2. MATERIALS AND METHODS

2.1 Strains and Growth conditions:

Candida albicans (10 human clinical vaginal isolates), were collected and maintained into Yeast Pepton medium . The isolates were then sub-cultured onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (0.05g/L) and incubated at 37C° for 24 hr. *Staphylococcus aureus* (MRSA) (20 human clinical isolates from wounds), were collected and maintained into Tryptic soy broth (TSB) medium then isolates were sub-cultured onto Mannitol Salt Agar (MSA) plates and incubated at 37C° for 24 hr. *Pseudomonas aeruginosa* (10 human clinical isolates from burns) were collected and maintained into Tryptic soy broth (TSB) medium then isolates were sub-cultured onto Cetramide agar and incubated at 37C° for 24 hr. (all isolates were previously identified in laboratories of biology department /college of science/Baghdad university).

2.2 Detection of biofilm production:

To prepare the yeast and bacteria inoculum, a loop full of the agar stock cultures was transferred to 5 ml of TSB and supplemented with 100 mM glucose and incubated at 37C° for 18 hr. Each of *Candida albicans, Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* suspensions were spectrophotometrically standardized on OD 540 nm of 1.0 which corresponds to a final concentration of 10^7/ml cells [19].

The ability of isolates to produce biofilm was evaluated by microtiter plat test using crystal violet stain according to [20], with modification. For each isolate 200 *µl* aliquots of previously prepared suspension were inoculated into the wells of polystyrene 96-well tissue culture plates, the plates were incubated at 37C° for 24 hr. (Dual species biofilm formation was performed by adding 75 *µl* of prepared suspension of each microorganism used as associated below : *S.aureus* (MRSA)+ *Candida albicans* and *S.aureus* (MRSA)+ *Pseudomonas aeruginosa*) The well contents were removed by pipetting and were washed three times with 200 *µl* of sterile phosphate buffer saline (pH: 7.2). The plates were then dried in inverted position, the attached biofilm producing cells were fixed for 15 min. with heating at 60C°. Afterward, the plates were stained with 200*µl* (0.1% wt/vol.) aqueous solution of crystal violet for 15 min. at room temperature. Excess crystal violet was rinsed with distilled water and air dried overnight. Bounded crystal violet was released by adding 200 *µl* of 96% ethanol. Absorbance was measured by using the spectrophotometer at 490 *nm* (A490) and was proportional to biofilm biomass.

3. RESULTS AND DISCUSSION

All isolates, *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Candida albicans* were identified according to morphological and biochemical tests performed in the laboratories of Baghdad University/College of Science – Biology Department.

Results of the biofilm production revealed that from 20 *Staphylococcus aureus* (MRSA) isolates there was 14 isolate that gave strong biofilm production as shown in Table (1). Whereas, the number of isolates that gave strong biofilm production out of 10 isolates was 3 for *Pseudomonas aeruginosa* and 2 for *Candida albicans*, Table (2).

Dual species biofilm formation was performed on the 2 highest biofilm producers of *Pseudomonas aeruginosa* and *Candida albicans* with 6 highest biofilm producers of *Staphylococcus aureus* (MRSA), the results showed that in the dual species for *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (12 dual) strong biofilm production was shown in 6 dual species, compared to only 3 dual species for dual species of *Staphylococcus aureus* (MRSA) and *Candida albicans*, Table (2).

Table 1: Number of *Staphylococcus aureus* (MRSA) isolates distributed according to biofilm production.

No. of strains	No. of isolates showing production of		
	Weak biofilm 0.1<OD≤0.2	Moderate biofilm 0.2<OD≤0.4	Strong biofilm 0.4<OD
20	3 (15%)	3 (15%)	14 (70%)

Table 2: Number of *Candida albicans* and *Pseudomonas aeruginosa* isolates as well as dual species with *Staphylococcus aureus* (MRSA) distributed according to biofilm production

Strains	No. of strains	No. of isolates showing production of		
		Weak biofilm 0.08<OD≤0.16	Moderate biofilm 0.16<OD≤0.32	Strong biofilm 0.32<OD
Candida albicans	10	5 (50%)	2 (20%)	3 (30%)
Pseudomonas aeruginosa	10	7 (70%)	1 (10%)	2 (20%)
	Dual species			
Candida albicans 2 + Stapylococcus aureus (MRSA) *6*	12	6 (50%)	3 (25%)	3 (25%)
Pseudomonas aeruginosa 2 + *Stapylococcus aureus* (MRSA) *6*	12	2 (16.1%)	4 (33.3%)	6 (50%)

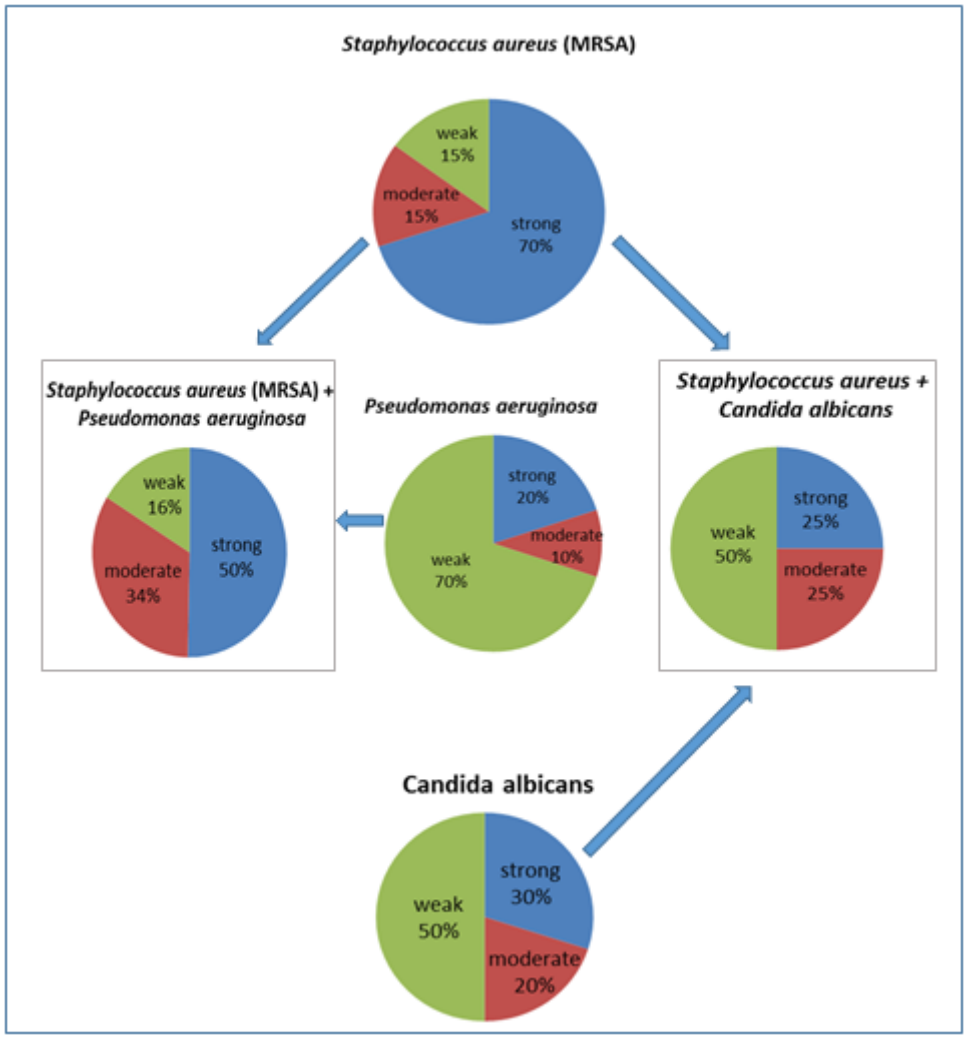

Figure 1: Percentage of biofilm producing microorganisms according to strong, moderate and weak production in single and dual.

The percentage of strong biofilm producing dual species of *Staphylococcus aureus* (MRSA) and *Candida albicans* (25%) was less than both species tested apart for biofilm production , (70%, 30%) respectively, Fig (1). This was accompanied with the mean of OD values of the strong producing dual strains (0.54) which was less than the mean of both strains tested apart (1.4 , 0.84) respectively, Fig (2).

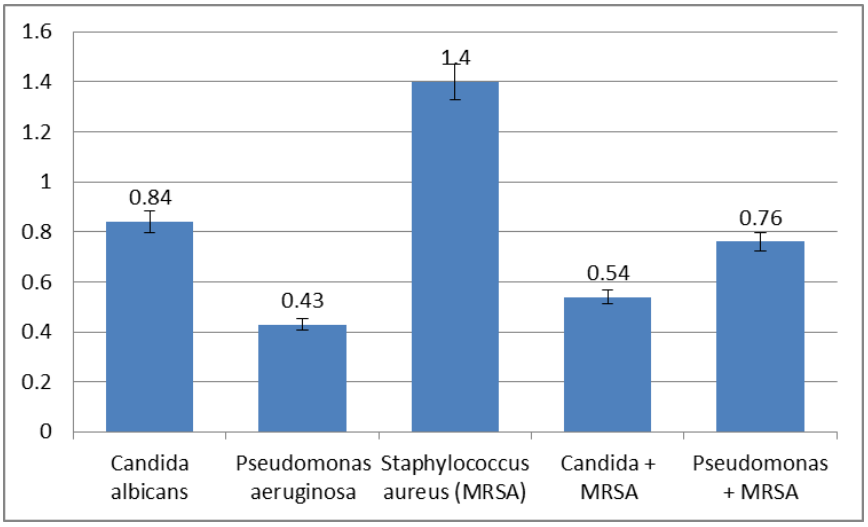

Figure 2: Mean OD absorbance values of CV in all study groups (single and dual) grown in Tryptic soy broth.

This can be explained by the ability of such microorganisms to form amounts of radicals against each other and finally induce an oxidative damage which inhibit each other biofilm production [21], or the fact that *Staphylococcus aureus* does not form biofilm on abiotic surfaces at once but rather require a precoating to the surface as well as a number of nutrient supplements [22]. Other studies showed that *Staphylococcus aureus* increased in dual biofilm production after a period of 48 hour due to the higher quantity of glucose which represent an important component of the matrix produced by *Candida albicans* [23], in which *Staphylococcus aureus* can use this glucose as a carbon source and as a result increase its biofilm production [24]. It was also shown that the interaction of *Staphylococcus aureus* cells with the hyphal form of *Candida albicans* and not the round yeast form which was due to the binding of *Staphylococcus aureus* to the agglutinin-like sequence 3 of *Candida albicans* which has the major role in the adherence of the *Staphylococcus aureus* bacterium to the hyphae [25], thus the interactions between eukaryotic organism like *Candida albicans* and a prokaryotic bacterium such as *Staphylococcus aureus* represent a wide field for further studies.

In comparison with the percentage of strong biofilm producers in the dual species of *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* 50% that was higher than the percentage of *Pseudomonas aeruginosa* biofilm production alone (20%) but less than *Staphylococcus aureus* (MRSA) biofilm production alone (70%), Fig (1). So the mean of the OD values of the strong biofilm producing dual strains (0.76) which was higher than the mean of biofilm production for *Pseudomonas aeruginosa* alone and less than the mean of biofilm production *Staphylococcus aureus* (MRSA) (1.4), Fig (2). These results can be explained by a number of reasons including waste products, such as molecules that bacteria produce which are of no use and not utilized by it known as end-products are released into the environment in which the biofilm is presented, these metabolites may be ammonia, carbon dioxide and many others that have important effect on the surrounding microorganisms in the same biofilm environment [26] .

It has been shown that metabolites produced by *Pseudomonas* may protect *Staphylococcus aureus* from aminoglycosides [27], also low levels of *Pseudomonas aeruginosa* mixed with *Staphylococcus aureus* increased infection rates in a rat model [28].

Studies demonstrated MRSA *Staphylococcus aureus* and *Pseudomonas aeruginosa* are among the most common organisms isolated from both acute and chronic infections especially from infected wounds [29], despite the fact of a number of mechanisms that *Pseudomonas* use as virulent factors and exoproducts which have an inhibitory effect on Staphylococcal growth in vitro [30]. The survival of *Staphylococcus aureus* in such environments is yet an unknown mechanism , but it could be possibly due to the formation of small colony varients of *Staphylococcus aureus* [31]. Investigators have noticed that *Pseudomonas aeruginosa* quickly become the dominant species in mixed cultures [32], which may be explained by the ability of this bacteria to produce bactericidal factors such as Las A protease (staphylolysin), which affect the *Staphylococcus aureus* cells by cleaving the pentaglycine cross-links in its peptidoglycan [33].

Bacteria using a biofilm strategy possess a number of molecular mechanisms for advantaging from other bacteria [34]. In conclusion, biofilm producing microorganisms in different environments tend to be polymicrobial [35], in a single community containing bacterial and /or fungal species, biofilms show a number of advantages to its own species including quorum sensing [36], by product influence [37], passive resistance [38] as well as other benefits, on the other hand these biofilm microorganisms may associate with each other to combat human life and cause serious problems and hardly treated diseases.

5. REFERENCES

1. Donland, RM. And Costerton, JW. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. Clin.Microbiol.Rev. 15(2):167-193.
2. Costerton, JW.; Stewart, PS. and Greenberg, EP. (1999). Bacterial biofilms: a common cause of persistence infections. Science. 284:1318-1322.
3. Lewis, K. (2001). Riddle of biofilm resistance. Antimicrob.Agents Chemother. 45(4):999-1007.
4. Oliveira, M; Bexiga, R.; Nunes, SF.; Carneiro, C.; Covaco, LM.; Bernardo, F.; etal (2006). Biofilm forming ability profiling of *Staphylococcus aureus* and *Staphylococcus epidermidis* mastitis isolates. Vet.Microbiol. 118:133-140.
5. Dubravaka, M.; Lazic, S.; Branka, V.; Jelena, P.; Bugorski, D. and Zorica, S. (2010). Slim production and Biofilm forming ability by *Staphylococcus aureus* bovine mastitis isolates. Acta.Veterinaria. 60(2-3):217-226.
6. Kuramtsu, HK.; Lux, R.; Anderson, MH. and Shi, W. (2007). Interspecies interactions within oral microbial communities. Microbiol.Mol.Biol.Rev. 71:653-670.
7. Harriott, MM. and Noverr,MC. (2010). Ability of *Candida albicans* mutants to induce *Staphulococcus aureus* vancomycin resistance during polymicrobial biofilm formation. Antimicrob.Agents Chemother. 54:3746-3755.
8. Faix, RG. and Kovarik SM. (1989). Polymicrobial sepsis among intensive care nursery infants. J.Perinatol. 9:131-136.
9. Sanita, PV.; Pavarina, AC.; Giampaolo, ET.; Silva, MM.; Mima, EG.; Ribeiro, DG.; etal (2011). *Candida spp.* Prevalence in well controlled type 2 diabetic patients with denture stomatitis. Oral.Surg.Med.Pathol.Radiol.Endo. 111:726-733.
10. Klotz, SA.; Chasin, BS.; Powell, B.; Gaur, NK. and Lipke, PN. (2007). Polymicrobial blood stream infections involving *Candida* species: analysis of patients and review of the literature. Diag.Microbiol. infec.Dis. 59:401-406.
11. Gordon, RJ. and Lowy, FD. (2008). Pathogenesis of Methicillin-Resistant *Staphylococcus aureus* infection. Clin.Infec.Dis. 46:359.
12. Palavecino, E. (2004). Community acquired methicillin resistant *Staphylococcus aureus* infections. Clin.Lab.Med. 24:403-418.
13. Van Delden, C. (2007). *Pseudomonas aeruginosa* blood stream infections: how should we treat them? . Int.J.Antimicrob.Agents. 30 Suppl 1: S71-S75.
14. Ratjen, F.; Munck, A.; Kho, P. and Angyalosi, G. (2010). Treatment of early *Pseudomonas aeruginosa* infection in patients with cystic fibrosis, Thorax. 65(4):286-291.
15. Harriott, MM. and Noverr, MC. (2009). *Candida albicans* and *Staphylococcus aureus* from polymicrobial biofilms : effect on antimicrobial resistance. Antimicrob.Agents.Chemother. 53:3914-3922.
16. Nguyen, AT. and Oglesby-shcrrouse, AG. (2016). Interaction between *Pseudomonas aeruginosa* and *Staphylococcus aureus* during co-infection and polymicrobial infections. Appl.Microbiol.Biotechnol. 100(14):614-618.
17. Sanita,PV.; Zago, CE.; Pavarina, AC.; Jorge, JH.; Machado, AL. and Vergani, CE. (2014). Enzymatic activity profile of a Brazilian culture collection of *Candida albicans* isolated from diabetics and non-diabetics with oral candidiasis. Mycoses. 57:351-357.
18. Lindsay, AK. and Hogan, DA. (2014). Candida albicans: molecular interactions with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Fungal.Bio.Rev. 28:85-96.
19. Peters, BN.; Jabra-Rizk, MA.; Scheper, MA.; Lied, JG.; Costerton, JW and Shirtliff, ME. (2010). Microbial interactions and different protein expression in *Staphylococcus aureus* – *Candida albicans* dual-species EMS Immunol.Med.Microbiol. 59:493-503.
20. Tang, J.; Kang, M.; Chen, H.; Shi, X.; Zhou, R.; Chen, J. and Du, Y. (2011). The Staphylococcal nuclease prevents biofilm formation in *Staphylococcus aureus* and other biofilm forming bacteria. Sci.China.life. 54(9):863-869.
21. Carlson, E.C. and Johnson, R.(1985). Protection by *Candida albicans* of *Staphylococcus aureus* in the establishment of dual infection in mice. Infec.Immun. 50:655-659.
22. Cassat, J.E.; Lee, C.Y. and Smeltzer, M.S. (2007). Investigation of biofilm formation in clinical isolates of *Staphylococcus aureus*. Methods Mol.Biol. 391:127-144.
23. Al-Fattani, M.A. and Douglas, L.J. (2006). Biofilm matrix of *Candida albicans* and *Candida tropicalis* : chemical composition and role in drug resistance. J.Med.Microbiol. 55:999-1008.
24. Costerton, J.W.; Stewart, P.S. and Geenberg, E.P. (1999). Bacterial biofilms: a common cause of persistent infections. Science, 284:1318-1322.
25. Peters,B.M.; Ovchinnikova, E.S.; Krom, B.P.; Schlecht,L.M.; etal (2012). *Staphylococcus aureus* adherence to *Candida albicans* hyphae is mediated by the hyphal adhesion Als3p. Microbiology, 158: 2975-2986.
26. Wolcott,R; Costerton,J.W.; Raoult,D. and Culter,S.J. (2013). The polymicrobial nature of biofilm infection. Clin.Microbiol.Infec. 19:107-112.
27. Hoffman,L.R.; Deziel,E.; D"Argenio,D.A.; etal. (2006). Selection for *Staphylococcus aureus* small-colony variant due to growth in the presence of Pseudomonas aeruginosa . Proc.Natl-Acad.Sci.USA. 103:19890-19895.
28. Hendricks,K.J,; Burd,T.A.; Anglen,J.O.; etal. (2001). Synergy between *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a rat model of complex orthopaedic wounds. J.Bone Joint Surg.Am.83-A: 855-861.
29. Co,E.M.; Keen,E.F. and Aldous,W.K. (2011). Prevalance of Methecillin-resistant *Staphylococcus aureus* in a combat support hospital in Iraq. Mil.Med.176:89-93.
30. Yang,L.; Liu,Y.; Markussen,T.; etal. (2011). Pattern differentiation in co-culture biofilms formed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* . FEMS.Immunol.Med.Microbiol.62:339-347.
31. Biswas,L.; Biswas,R.; Schlag,M.; etal (2009). Small colony variant selection as a survival strategy for *Staphylococcus aureus* in the presence of *Pseudomonas aeruginosa.* Appl.Environ.Microbil.75:6910-6912.
32. Malie,S.; Hill,E.; Hayes,A.; etal (2009). Detection and identification of specific bacteria in wound biofilms using peptide nucleic acid fluorescent in situ hybridization. Microbiol.155:2603-2611
33. Kessler,E.; Safrin,M.; Oslon,J.C.; and OhmanD.E. (1993). Secreted Las A of *Pseudomonas aeruginosa* is a staphylolytic protease. J.Biol.Chem.268:7503-7508.
34. Kurmitso,H.K.; He,X.; Lux,R.; etal (2007). Interspecies interactions within oral microbial communities. Microbiol.Mol.Biol. 71:653-670.
35. Del Pozo,J.L.; and Patel,R. (2007). The chalange of treating biofilm-associated bacterial infections. Clin.Pharmacol.Ther. 82:204-209.
36. Elia,S.and Banin,E. (2012). Multi-species biofilms living with friendly neighbors. FEMS Microbiol.Rev.
37. Carlsson,J.(1997). Bacterial metabolism in dental biofilms. Adv.Dent.Res. 11: 75-80.
38. Weimex,K.E.; Juneau,R.A.; Murrah,K.A.; etal (2011). Divergrnt mechanisms for passive pneumococcal resistance to beta-lactam antibiotics in the presence of haemophilus influenzae. J.Infec.Dis. 203:549-555.

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