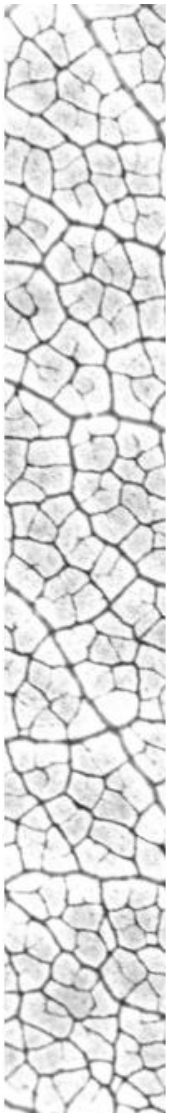

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 4217-4223 (2020)

Study the Adhesion Capacity on abiotic surfaces by Acinetobacter baumannii isolated from drinking water

Sanaa R. Oleiwi [1*], Entissar F. Ahmed [1], Shaymaa F. Rasheed [1]

[1] Department of biology, College of science, University of Baghdad, IRAQ
*Corresponding author: sanaeleiwi@yahoo.com

Abstract

Acinetobacter baumannii ability to form biofilm makes it to be opportunistic pathogen causing of nosocomial infections and to be good survivor in adverse environmental conditions including medical devices and hospital environments**.**

Six isolates of *A. baumannii* were isolated from drinking water and tested to investigate biofilm formation capacity on three different type of abiotic surface, also several factors were examined such as hydrophobicity, PH and temperature.

All *A. baumannii* isolates displayed a positive biofilm on congored aga test CRA (pigmented colonies with black color) and Christensen's test (adhesive layer of stained material to the inside surface of the tube).The obtained data of microbial adhesion to hydrocarbons assay (MATH) assay revealed that the percentage of all isolates ranged between (45-75%).

Results of recent study revealed that optical density OD values were consistently higher on catheter than on that of the polystyrene and glass at any of the PH and temperature Temperature 37◦C and PH 4 have greatest positive effect on biofilm formation process than other values, Current study may help in additional understanding of *A. baumannii* ability to form biofilm on abiotic surface which may be is used in medical devices' manufacturer and role of this in spreading of this pathogen in hospital environment.

Keywords: biofilm, hydrophobicity, Christensen test, congored aga test, catheter

Oleiwi SR, Ahmed EF, Rasheed SF (2020) Study the Adhesion Capacity on abiotic surfaces by Acinetobacter baumannii isolated from drinking water. Eurasia J Biosci 14: 4217-4223.

INTRODUCTION

A. baumannii is a Gram-negative, aerobic, non-fermenting bacterium which is frequently found and isolated from different environments, including surface water or soil (Zhang et al, 2013; Bartram, 2015), also named as "Iraqi bacter" because of its emergence in US military treatment facilities during the Iraqi War in Iraq, (Taitt et al, 2014). *A. baumannii* are commonly isolated from unpolluted sites, like, surface, tap or ground water (WHO,2017), these environments consider great sources of this species with potential to be significant health care-related bacteria, especially immune-compromised persons, such as, via room humidifiers and water baths (WHO, 2017), in this matter, the biofilm formation capacity of *A. baumannii* is recorded to be great virulence factor (Bhargava et al, 2010; Simoes et al, 2010) and this bacteria was found to contaminate healthcare workers' hand and colonize different objects, involving medical tools,equipment, hospital furniture, gloves and gowns of healthcare workers (Morgan et al, 2010; La Fauci et al, 2019). Its ability to formation of biofilm makes it to be opportunistic pathogen causing of nosocomial infections and to be good survivor in adverse environmental conditions including medical devices and hospital environments (Pakharukova et al, 2018).The most frequent infections that are resulted by *A. baumannii* in hospitals involve urinary tract infection, pneumonia, peritonitis, bacteraemia, meningitis, and surgical wound infection (Fournier et al, 2006). The aim of this work was to investigate adhesive property of *A. baumannii* and study the effect of PH and temperature on biofilm formation on abiotic surfaces (catheter, glass and polystyrene) that may have potential application in prevent biofilm formation by this species in hospital environment.

MATERIALS AND METHODS

Isolation and identification

Drinking water were collected and subjected to microbiology tests according to (Standing Committee of Analysts, 2017), all samples were cultured on some bacteriological media. All bacterial isolates were examined morphologically by Gram's stain and

Received: October 2019
Accepted: March 2020
Printed: October 2020

subjected to some biochemical tests and further confirmation tests (API 20E and ID 32 mini API systems) were used.

Detection of slime layer production

Congo red agar method

This medium was prepared as previously described, a positive result indicated by black colonies and the non-slime producers usually remained pink (Freeman et al, 1989).

Christensen's method

This test was done as previously described, a positive result was revealed by the founding of an adherent layer of stained material to the inside surface of tube (Christensen et al, 1982). Microbial adhesion to hydrocarbons assay (MATH); Hydrophobicity was determined by using affinity test to xylene was previously described (Rosenberg et al, 1980; Lather et al, 2016).The hydrophobicity index (HI) was calculated by using the following equation: HI = (A660 control-A660 test)/A660 control. The isolates were considered as strongly hydrophobic when the hydrophobicity index was above (70%), and hydrophilic when the hydrophobicity index was below (30%) (Rosenberg et al, 1980; Lather et al, 2016).

Selection of abiotic surfaces

Polystyrene, glass, catheter were selected for the adhesion tests, were cut into uniform size of 1cm2 coupons. Prior to adhesion tests, polystyrene and catheter were swabbed by absolute ethanol then washed and rinsed thoroughly eight times with deionized water, glass coupons were directly autoclaved at 121◦C for 15min (Mafu et al, 2011).

Study effect of pH and temperature on A. baumannii biofilm formation on abiotic surfaces.

This study investigate the biofilm formation by A. baumannii isolate on three different surfaces: Polystyrene, catheter and glass surfaces under different temperature and pH values by crystal violet binding assay,the most efficient biofilm producers isolate was selected.

A single, isolated colony was grown in 50 ml of BHI-broth that incubated at 37◦C for 18 h. Working culture was containing 2 ml of the bacterial suspension in BHI-broth and 18 ml of low nutrient medium BHI-broth (Brain Heart Infusion Broth was prepared according to the instructions of Manufacturer Company, and then diluted ten times (Krolasik et al, 2010).

The effect of pH was examined by production of biofilm in low nutrient broth BHI-broth modified at pH values (4,7 and 9) with NaOH or HCl, while the temperature effect was examined by incubation under different temperature 5 ◦C, 37 ◦C and 40 ◦C at pH 7. The inoculation step was achieved by adding 2 ml of bacterial suspension of A. baumannii on different types of coupons.

Negative controls were achieved by adding 2 ml of low nutrient medium instead of 2 ml of A. baumannii suspension then incubation for (24, 48, 72) hr.

Quantification of biofilm

Crystal violet method was used to measure the formation of biofilm which measures the total biofilm biomass, including bacterial cells and extracellular matrix. This assay was adapted from (Adetunji and Isola, 2011; Pui et al, 2011; Tang et al, 2012). At the end of each incubation periods, a set of coupons were aseptically removed, these coupons were washed 3 time by using 1 ml distilled water in order to remove the loosely attached bacterial cells, then coupons were air dried and adherent bacteria were stained with 2 ml of crystal violet stain 0.1% (w/v) for each coupon at 37°C for twenty minute. The exceed stain fluid were discard from the coupons, washed with one milliliter distilled water three times and coupons were kept for drying at room temperature. After drying, the adherent stain was dissolved with 2 milliliter of 99.9% ethanol for twenty minutes, finally the crystal violet concentration was detected by measurement optical density of de-staining solution at 620 nm (OD620 value- C value). This experiment conducted twice with duplicate in each time.

RESULTS AND DISCUSSION

Isolation and identification

A. baumannii has emerged worldwide as an serious hospital acquired bacteria that lead to outbreaks and infection of critically ill, hospitalized patients (de Breij et al, 2009). Six isolates of *A. baumannii* (8.6 %) were obtained from drinking water samples. Isolates were identified according to their morphology and biochemical characteristics, also API 20E and ID 32 mini API test systems were used to confirmation bacteria as *A. baumannii*.

Recent isolation rate is similarly to (Samie et al, 2012) who found that recovery rate of Acinetobacter from Household Drinking-water is (7.5 %) and less than Bifulco et al, (1989) was 16%.

Some former studies proposed Some degree of pathogenic potential for isolates obtained from drinking water and consider as opportunistic bacteria (Bifulco et al, 1989; Leclerc and Moreau, 2002; WHO 2008; Eliwi et al, 2013;Narciso-da-Rocha et al, 2013; Umezawa et al, 2015).

Detection of slime layer production by Congored agar and Christensen's tests

All *A. baumannii* isolates (100 %) displayed a positive biofilm on congored aga test CRA (pigmented colonies with black color) and Christensen test (adhesive layer of stained material to the inside surface of the tube), recent results greater than findings of (Babapour et al, 2016), who used congo red to qualitative investigation of biofilm production, Bifulco et al,(1989) found that isolates obtained from drinking

water is producer of slim material and was not significantly differ from clinical isolates.

Microbial adhesion to hydrocarbons assay (MATH)

The results of (MATH) assay revealed that the percentage of all isolates ranged between (45-75%), that means, all isolates have hydrophobic cell surface, this results in accordance with M'hamedi et al, (2014).

Hydrophobicity of the bacterial cell surface is one of the most essential factors which control the mechanism of bacterial attachment to animate and inanimate surfaces and biofilm formation (Donlan 2002) i.e., rising of bacterial hydrophobocity causing of rising of attachment capacity (Akiyama et al, 1998). Most fimbriae that have been tested consist of a large percentage amino acid residues with hydrophobic property (Rosenberg and Kjelleberg,1986). Also fimbriae have an effect on cell surface hydrophobicity and adherence, perhaps by reducing the introductory electrostatic repulsion barrier that occur between substratum and the cell (Corpe, 1980).

Study effect of pH and temperature on A. baumannii biofilm formation on abiotic surfaces

In general, the results of recent study revealed that *A. baumannii* attached and formed biofilm on catheter coupons, greater than other surfaces (P<0.05) at all examined factors followed by polystyrene, finally glass coupons, showed the least intensity of bacterial adhesion on investigated surfaces, these results came in agreement with Musleh and Jebur, (2014) who found similar pattern of adhesion on catheter and glass (Pour et al, 2011 Musleh and Jebur, 2014).

Recent results of adhesion pattern on examined surface can be explained by physiochemical properties of these surfaces, such as catheter is made of hydrophobic material like polymers while glass was less biofouled, the most hydrophobic surfaces have a higher capability to attract bacterial cell and form biofilm, in other hand, less attraction of bacterial cell and biofilm formation were observed in the most hydrophilic surfaces (Donlan, 2002; Houdt and Michiels, 2010; De-la-Pinta et al, 2019).

Several previous studies revealed that roughness is the most factor for bacterial attachment and biofilm formation (Pier-Francesco et al, 2006; Al-Ahmad et al, 2010; Zaugg et al, 2017) and other suggested that hydrophobicity is the main affecting factor (Koseki et al, 2014; Jindal et al, 2016). However, most researchers regard both factors, hydrophobicity and roughness, essential for attachment and biofilm development (Gyo et al, 2008; Zhao *et al*, 2014) depending their influence on the specific micro-organism (Wassmann et al, 2017).

According to recent data, maximum optical density OD values (which represented biofilm density) was reach at 24 hr incubation period compared to other periods (48 and 72).The first period correlates to

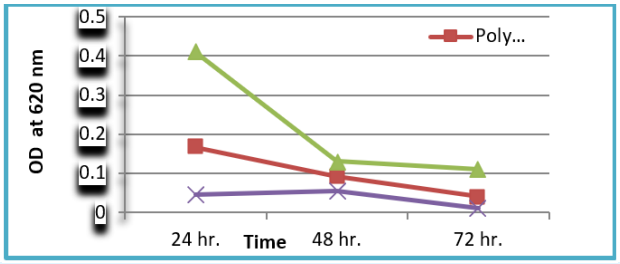

Fig. 1. Mean OD value of biofilm formation by *A. baumannii* on three surfaces at PH 4

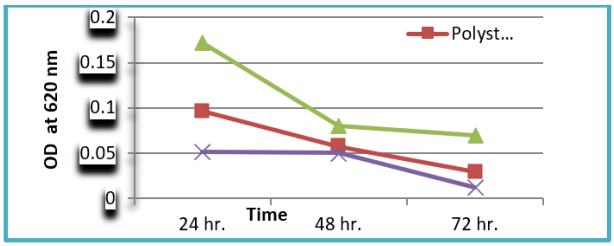

Fig. 2. Mean OD value of biofilm formation by *A. baumannii* on three surfaces on at PH 7

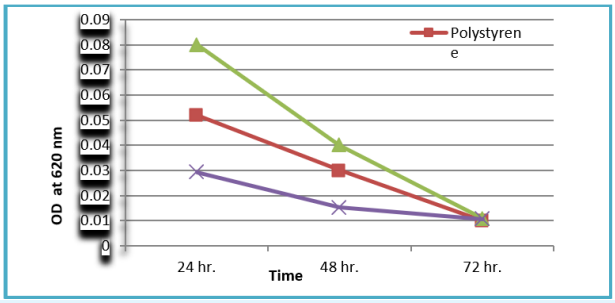

Fig. 3. Mean OD value of biofilm formation by *A. baumannii* on three surfaces at PH 9

Table 1. Relationship between three surface and mean value of biofilm formation by *A. baumannii* (OD 620) at pH 4

Surface	days			LSD value
	24 hr.	48 hr.	72 hr.	
Polystyrene	0.167	0.092	0.04	0.094 *
Catheter	0.410	0.130	0.111	0.116 *
Glass	0.045	0.055	0.01	0.042 NS
LSD value	0.l07 *	0.063 *	0.075 *	----

* (P<0.05).

significant biofilm production and the second to biofilm dispersal, with a dispensation of bacterial cells into the culture medium (Djeribi et al, 2012).

pH

In general, If the pH differs from the optimal range may slow growth of bacteria, causing in death of bacteria then stopping of biofilm formation. However, this is only the case in the first stage of biofilm formation. (Jones et al, 2015).

In recent study the impact of cultivation pH was examined at pH values (4, 7 and 9).The results were summarized in **Figs. 1**, **2**, and **3**, **Tables 1**, **2** and **3** which showed the ability of *A. baumannii* to adherence to all

Table 2. Relationship between three surface and mean value of by *A. baumannii* biofilm formation (OD 620) at pH 7

Surface	Days			LSD value
	24	48	72	
Polystyrene	0.096	0.057	0.03	0.063 *
Catheter	0.172	0.08	0.07	0.070 *
Glass	0.0515	0.0502	0.012	0.043 NS
LSD value	0.066 *	0.029 NS	0.064 NS	----

* (P<0.05).

Table 3. Relationship between three surface and mean value of Biofilm formation by *A. baumannii* (OD 620) at pH 9

Surface	days			LSD value
	24 hr.	48 hr.	72 hr.	
Polystyrene	0.052	0.0301	0.0101	0.042 NS
Catheter	0.08	0.04	0.011	0.071 *
Glass	0.0293	0.0152	0.0107	0.018 NS
LSD value	0.0496 *	0.033 NS	0.0094 NS	----

* (P<0.05).

Table 4. Relationship between three surface and mean value of biofilm formation by *A. baumannii* (OD 620) at 4 C

Surface	days			LSD value
	24 hr.	48 hr.	72 hr.	
Polystyrene	0.06	0.034	0.017	0.044 *
Catheter	0.197	0.063	0.043	0.085 *
Glass	0.087	0.0603	0.0101	0.059 *
LSD value	0.074 *	0.034 NS	0.032 NS	----

* (P<0.05).

Table 5. Relationship between three surface and mean value of biofilm formation by *A. baumannii* (OD 620) at 37 C

Surface	days			LSD value
	24 hr.	48 hr.	72 hr.	
Polystyrene	0.101	0.08	0.05	0.053 NS
Catheter	0.222	0.091	0.04	0.088 *
Glass	0.06	0.042	0.02	0.046 NS
LSD value	0.079 *	0.055 NS	0.032 NS	----

* (P<0.05).

Table 6. Relationship between three surface and mean value of biofilm formation by *A. baumannii* (OD 620) at 40 C

Surface	days			LSD value
	24 hr.	48 hr.	72 hr.	
Polystyrene	0.082	0.041	0.041	0.046 NS
Catheter	0.152	0.085	0.083	0.0618 *
Glass	0.08	0.051	0.022	0.056 *
LSD value	0.079 *	0.055 NS	0.032 NS	----

* (P<0.05).

three coupons surfaces under all selected pH values, but in different degrees,in which increased in pH (pH4) lead to a higher biofilm production in catheter coupons surfaces (P<0.05), followed with neutral pH (pH7), while at the alkaline environment (pH9) a less biofilm production was observed, that refer to the alkalinity have inhibiter role on biofilm formation process.

Bacteria turn into more resistant against acidity after the initial attachment phase, because the protection of the Exopolysaccharides matrix, while planktonic cell are more sensitive to acidity (Liu et al, 2015), the resistance of *A. baumannii* at different levels of this factor could clarify the noticed persistence at the inanimate surfaces in hospital. (Martí et al, 2011).

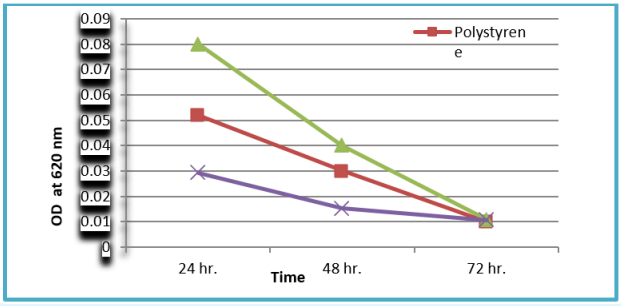

Fig. 3. Mean OD value of biofilm formation by *A. baumannii* on three surfaces at PH 9

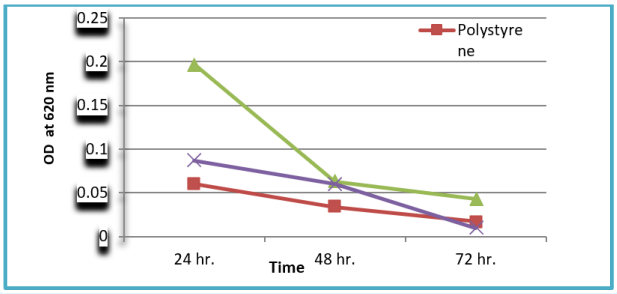

Fig. 4. Mean OD value of biofilm formation by *A. baumannii* on three surfaces at 4 ◦C

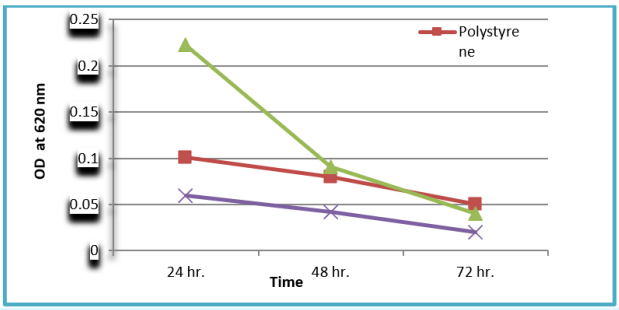

Fig. 5. Mean OD value of biofilm formation by *A. baumannii* on three surfaces at 37 ◦C

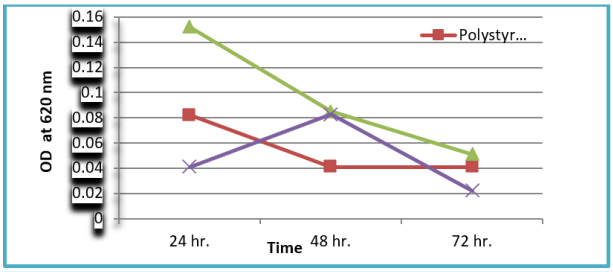

Fig. 6. Mean OD value of biofilm formation by *A. baumannii* on three surfaces at 40 ◦C

According to recent data, pH 9 is considered as inhibitor factor of biofilm formation process that in agreement with (Qin et al, 2015; Nostro et al, 2012), alkalinity could be hopeful towards the reducing of bacterial colonization and therefore the decreasing of the biofilm-associated infections, by treatment surfaces like catheters or indwelling medical devices with alkaline solutions (Qin et al, 2015 ; Nostro et al, 2012)

Current results were somewhat different from the values reported by (Pour et al, 2011) who found that biofilm production by *A. baumannii* was maximal at 30 ◦C, pH 7.0, this may be due to the difference in source of isolates.

Temperature

Temperature is another important factor that affect the first phases of biofilm formation (Moldoveanu, 2012). According to the obtained data from recent study, temperature 37◦C has greatest positive effect on biofilm formation process than other temperature values followed by 4 ◦C finally 40◦C, previous researchers (M'hamedi et al, 2014) reported that this process is found to be more significant at 30 than at 37°C.

Bacterial metabolism is directly related to enzyme s' presence, which means that the biofilm production relies upon the reaction rates of the enzymes. Important factor that affects the rate of enzyme s' reaction is the temperature, as it is related to the production of cells forming a biofilm (Garrett et al, 2008). In addition, studies have shown that the bacteria possess an increased surface area when compared to higher temperatures (35 ◦C) at low temperatures (10 ◦C) (Nisbet et al,1984; Herald and Zottola, 1988 ; Rampadarath et al., 2017; Vásquez-Ponce et al, 2017; Govaert et al, 2018). Also biofilm formation is related to appendages such as flagella, the number of flagella from bacteria is dependent on the temperature. (Townsley and Yildiz, 2015).

CONCLUSION

Recent study showed the capacity of drinking water *A. baumannii* isolates to form biofilm on three type of abiotic surfaces also the effect of several factors on biofilm ability such as PH and temperature.

Optical density OD values were consistently higher on catherter than on that of the polystyrene and glass at any of the PH and temperature.

The interesting feature of biofilm formation by *A. baumannii* in recent study is the increasing of optical density OD value in PH 4.

REFERENCES

Adetunji VO and Isola T O (2011). Crystal violet binding assay for assessment of biofilm formation by Listeria monocytogenes and Listeria spp on wood, steel and glass surfaces. Global Veterinaria, 6(1), pp.6-10.

Akiyama H, Yamasaki O, Kanzaki H, Tada J, and Arata J (1998). Adherence characteristics of Staphylococcus aureus and coagulase-negative staphylococci isolated from various skin lesions. J. Dermatol. Sci., 18(2), pp.132-136.

Al-Ahmad A W, Al-Ahmad M, Faust J, Bächle M, Follo M, Wolkewitz M, Hannig C, Hellwig E, Carvalho C and Kohal R, (2010). Biofilm formation and composition on different implant materials in vivo. J Biomed Materi Res Part B: Appl Biomater. 2010;95B:101–9.

Babapour E, Haddadi A, Mirnejad R, Angaji S A and Amirmozafari N (2016). Biofilm formation in clinical isolates of nosocomial Acinetobacter baumannii and its relationship with multidrug resistance. Asian Pac. J. Trop. Biomed., 6(6), pp.528-533.

Bartram J ed (2015). Routledge handbook of water and health. Routledge

Bhargava N, Sharma P, and Capalash N, (2010). Quorum sensing in Acinetobacter: an emerging pathogen. Crit. Rev. Microbiol.36 (4), pp.349-360.

Bifulco J M, Shirey J J, and Bissonnette G K (1989). Detection of Acinetobacter spp. in rural drinking water supplies. Appl. Environ. Microbiol., 55(9), pp.2214-2219.

Christensen G D, Simpson W A, Bisno A L, and Beachey E H, (1982). Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect. Immun 37(1), pp.318-326.

Corpe W A (1980). Microbial surface components involved in adsorption of microorganisms onto surfaces. Adsorption of microorganisms to surfaces, New York: John Wiley & Sons;. (1980). 105-44

de Breij A, Gaddy J, van der Meer J, Koning R, Koster A, van den Broek P, Actis L, Nibbering P and Dijkshoorn L (2009). CsuA/BABCDE-dependent pili are not involved in the adherence of Acinetobacter baumannii ATCC19606T to human airway epithelial cells and their inflammatory response. Res. Microbiol. 160:213-218

De-la-Pinta I, Cobos M, Ibarretxe J, Montoya E, Eraso E, Guraya T, and Quindós G (2019). Effect of biomaterials hydrophobicity and roughness on biofilm development. J Mater. Sci. Mater. Med., 30(7), p.77.

Djeribi R, Bouchloukh W, Jouenne T, and Menaa B, (2012). Characterization of bacterial biofilms formed on urinary catheters. Am. J. Infect. Control, 40(9), pp.854-859.

Donlan R M (2002). Biofilms: microbial life on surfaces. Emerg. Infect. Dis.8(9): (2002). 881-890.

Eliwi S, Musleh R M, Sabah M A and Ibraheem S (2013). Study on Aeromonas spp. Isolated from raw and drinking water in Baghdad city. Iraqi J Sci., 54(5), pp.1068-1077.

Fournier P E, Richet H, and Weinstein R A (2006). The epidemiology and control of Acinetobacter baumannii in health care facilities. Clin. Infect. Dis., 42(5), pp.692-699.

Freeman D J, Falkiner F R and Keane C T (1989). New method for detecting slime production by coagulase negative staphylococci. J. Clin. Pathol., 42(8), pp.872-874.

Garrett T R, Bhakoo M, and Zhang Z, (2008). Bacterial adhesion and biofilms on surfaces. Prog. Nat. Sci. 1049-1056.

Govaert M, Smet C, Baka M, Ećimović B, Walsh J L, and Van Impe J (2018). Resistance of L. monocytogenes and S. Typhimurium towards cold atmospheric plasma as function of biofilm age. Appl. Sci., 8(12), p.2702.

Gyo M, Nikaido T, Okada K, Yamauchi J, Tagami J, and Matin K (2008). Surface response of fluorine polymer-incorporated resin composites to cariogenic biofilm adherence. Appl Environ Microbiol. 74:1428–35.

Herald P J, and Zottola E A (1988). Attachment of Listeria monocytogenes to stainless steel surfaces at various temperatures and pH values. J. Food Sci., 53(5), 1549-1562.

Houdt R V, and Michiels C (2010). Review Article: Biofilm formation and the food industry, J. Appl. Microbiol., 109, pp.1117-1131.

Jindal S, Anand S, Huang K, Goddard J, Metzger L and Amamcharla J (2016). Evaluation of modified stainless steel surfaces targeted to reduce biofilm formation by common milk sporeformers. J Dairy Sci. ;99:9502–13.

Jones E M, Cochrane C A and Percival S L (2015). The effect of pH on the extracellular matrix and biofilms. Adv. wound care, 4(7).431-439.

Koseki H, Yonekura A, Shida T, Yoda I, Horiuchi H, Morinaga Y, Yanagihara K, Sakoda H, Osaki M, and Tomita M (2014). Early staphylococcal biofilm formation on solid orthopaedic implant materials: in vitro study. PloS one, 9(10),.e107588

Krolasik J, Zakowska Z, Krepska M and Klimek L (2010). Resistance of bacterial biofilms formed on stainless steel surface to disinfecting agent. Pol J Microbiol, 59(4), pp.281-287.

La Fauci V, Costa G B, Genovese C, Palamara MAR, Alessi V, and Squeri R (2019). Drug-resistant bacteria on hands of healthcare workers and in the patient area: an environmental survey in Southern Italy's hospital. Revista Española de Quimioterapia, 32(4), p.303.

Lather P, Mohanty A K, Jha P and Garsa A K (2016). Contribution of cell surface hydrophobicity in the resistance of Staphylococcus aureus against antimicrobial agents. Biochem. Rese. Intern. pp1-5.

Leclerc H, and Moreau A (2002). Microbiological safety of natural mineral water. FEMS Microbiol Rev 26:207–222

Liu Y, Tang H, Lin Z and Xu P (2015). Mechanisms of acid tolerance in bacteria and prospects in biotechnology and bioremediation. Biotechnol. Adv., 33(7), pp.1484-1492.

Mafu A A, Plumety C, Deschênes L, and Goulet J (2011). Adhesion of pathogenic bacteria to food contact surfaces: influence of pH of culture. Inter. Jour. of Microbiol. Article ID 972494, 10.

Martí S, Rodríguez-Baño J, Catel-Ferreira M, Jouenne T, Vila J, Seifert H and Dé E (2011). Biofilm formation at the solid-liquid and air-liquid interfaces by Acinetobacter species. BMC research notes, 4(1), pp.1-4.

M'hamedi I, Hassaine H, Bellifa S, Lachachi M, Terki I K, and Djeribi R (2014). Biofilm formation by Acinetobacter baumannii isolated from medical devices at the intensive care unit of the University Hospital of Tlemcen (Algeria). Afr. J. Microbiol. Res., 8(3), pp.270-276.

Moldoveanu A M (2012). Environmental factors influences on bacterial biofilms formation. Ann. Romanian Soc. Cell Biol. 17(1)):118-126.

Morgan D J, Liang S Y, Smith C L, Johnson J K, Harris A D,Furuno J P, Thom K A, Snyder G M, Day H R, and Perencevich, E.N., 2010. Frequent multidrug-resistant Acinetobacter baumannii contamination of gloves, gowns, and hands of healthcare workers. Infect Control Hosp Epidemiol: the official journal of the Society of Hospital Epidemiologists of America, 31(7), p.716.

Musleh R M, and Jebur A Q (2014). Crystal Violet Binding Assay for Assessment of Biofilm Formation by Klebsiella pneumoniae on Catheter, Glass and Stainless-steel Surfaces. Iraqi J Sci., 55(3B), pp.1208-1212.

Narciso-da-Rocha C, Vaz-Moreira I, Svensson-StadlerL, Moore E R, and Manaia C M, (2013). Diversity and antibiotic resistance of Acinetobacter spp. in water from the source to the tap. Appl. Microbiol. Biotechnol., 97(1), pp.329-340.

Nisbet B A, Sutherland I W, Bradshaw I J, Kerr M, Morris E, and Shepperson W A (1984). XM-6: a new gel-forming bacterial polysaccharide. Carbohydr. Polym., 4(5), pp.377-394.

Nostro A, Cellini L, Di Giulio M, D'Arrigo M, Marino A, Blanco A R, Favaloro A, Cutroneo G, and Bisignano G (2012). Effect of alkaline pH on staphylococcal biofilm formation. Apmis, 120(9), pp.733-742

Pakharukova N, Tuittila M, Paavilainen S, Malmi H, Parilova O, Teneberg S, Knight S D and Zavialov A V (2018). Structural basis for Acinetobacter baumannii biofilm formation. Proc. Natl Acad. Sci 115(21), pp.5558-5563.

Pier-Francesco A, Adams R J, Waters MG, and Williams D W (2006). Titanium surface modification and its effect on the adherence of Porphyromonas gingivalis: an in vitro study. Clin. oral implants res., 17(6), pp.633-637.

Pour NK, Dusane D H, Dhakephalkar P K, Zamin F R, Zinjarde S S and Chopade B A (2011). Biofilm formation by Acinetobacter baumannii strains isolated from urinary tract infection and urinary catheters. FEMS Immunol. Med. Mic., 62(3), pp.328-338

Pui C F, Wong W C, Chai L C, Lee H Y, Tang JY, Noorlis A, Farinazleen M G, Cheah Y K, and Son R (2011). Biofilm formation by Salmonella Typhi and Salmonella Typhimurium on plastic cutting board and its transfer to dragon fruit. Inter.Food Research Jour., 18(1)

Qin H, Zhao Y, Cheng M, Wang Q, Wang Q, Wang J, Jiang Y, An Z, and Zhang X (2015). Anti-biofilm properties of magnesium metal via alkaline pH. RSC advances, 5(28), pp.21434-21444.

Rampadarath S, Bandhoa K, Puchooa D, Jeewon R, and Bal S (2017). Early bacterial biofilm colonizers in the coastal waters of Mauritius. Electron. J. Biotechnol, 29.13-21.

Rosenberg M, Gutnick D, and Rosenberg E (1980). Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS microbiology letters, 9(1), pp.29-33.

Rosenberg M, and Kjelleberg S (1986). Hydrophobic interactions: role in bacterial adhesion. Advances Microb. Ecol.9: (1986). 353-93.

Samie A, Mashao M B, Bessong P O, NKgau T E, Momba M NB. and Obi C L (2012). Diversity and antibiograms of bacterial organisms isolated from samples of household drinking-water consumed by HIV-positive individuals in rural settings, South Africa. J Health. Popul. Nutr, 30(3), p.241

Simoes L C, Simoes M and Vieira M J (2010) Influence of the diversity of bacterial isolates from drinking water on resistance of biofilms to disinfection. Appl Environ Microbiol, 76(19), pp.6673-6679

Standing Committee of Analysts.The Microbiology of Water and Associated Materials (2017) Practices and Procedures for Laboratories Methods for the Examination of Waters and Associated Materia

Taitt C R, Leski T A, Stockelman M G, Craft D W, Zurawski D V, Kirkup B C and Vora G J (2014). Antimicrobial resistance determinants in Acinetobacter baumannii isolates taken from military treatment facilities. Antimicrob. Agents Chemother., 58(2), pp.767-781.

Tang P L, Pui C F, Wong W C, Noorlis A and Son R (2012). Biofilm forming ability and time course study of growth of Salmonella Typhi on fresh produce surfaces. Inte. Food Res. J. 19(1): 71-76.

Townsley L, and Yildiz F H (2015). Temperature affects c-di-GMP signalling and biofilm formation in Vibrio cholerae. Environ. Microbiol., 17(11), pp.4290-4305.

Umezawa K, Asai S, Ohshima T, Iwashita H, Ohashi M, Sasaki M, Kaneko A, Inokuchi S, and Miyachi H (2015). Outbreak of drug-resistant Acinetobacter baumannii ST219 caused by oral care using tap water from contaminated hand hygiene sinks as a reservoir. Am. J. Infect. Control 43(11), pp.1249-1251.

Vásquez-Ponce F, Higuera-Llantén S, Pavlov M S, Ramírez-Orellana R, Marshall S H, and Olivares-Pacheco J (2017). Alginate overproduction and biofilm formation by psychrotolerant Pseudomonas mandelii depend on temperature in Antarctic marine sediments. Electron. J. Biotechnol., 28, pp.27-34.

Wassmann T, Kreis S, Behr M and Buergers R (2017). The influence of surface texture and wettability on initial bacterial adhesion on titanium and zirconium oxide dental implants. Int. J Implant Dent, 3(1), pp.1-11.

World Health Organisation. (2008) Guidelines for drinking water. World Health Organization, Geneva

World Health Organisation (2017) Guidelines for drinking water. World Health Organization, Geneva

Zaugg L K, Astasov-Frauenhoffer M, Braissant O, Hauser-Gerspach I, Waltimo T, and Zitzmann N U, (2017). Determinants of biofilm formation and cleanability of titanium surfaces. Clin Oral Implants Res. 2016;27:918–25

Zhang H Z, Zhang J S, and Qiao L (2013). The Acinetobacter baumannii group: a systemic review. World J. Emerg. Med., 4(3), p.169.

Zhao B, Van Der Mei H C, Subbiahdoss G, de Vries J, Rustema-Abbing M, Kuijer R, Busscher H J,and Ren Y (2014). Soft tissue integration versus early biofilm formation on different dental implant materials. Dental Mater.;30:716–27.30(7), pp.716-727.