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Antibiofilm Effect of Biopolymer Dextran – Gentamycin- PVP Blend in Catheters

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Abstract : Objectives: The objective of this study was to determine the antibiofilm effect of biopolymer dextran produced by *Leuconostoc mesenteroides* ssp. *Mesenteroides* and their blends with gentamycin and Polyvinylpyrrolidone(PVP)against pathogenic bacteria in catheters.

Methods: Minimum inhibitory concentration (MIC) values of dextran, gentamycin and Polyvinylpyrrolidone(PVP) were determined against bacteria isolated from catheters. Antibiofilm effect of biopolymer dextran and its blenders (gentamycin – PVP) was determined alone and as blends (dextran- gentamycin), (dextran-PVP), (dextran - gentamycin -PVP) using a pre-coated method in micro titer plate and catheters.

Results:The MIC of dextran was found to be 32mg/ml for *E.coli* , *P.aeruginosa*, *S.aureus* isolates , the MIC for *P.mirabilis* isolates was between (16 –32) mg/ml while in *S.epidermidis* was between (32 – 64)mg/ml. The MIC of gentamycin, PVP was found to be 16 µg/ml, 256 mg/ml respectively for all bacterial isolates. Biopolymer blend shad the ability to inhibit biofilm formation in micro titer plate and catheters, the highest biofilm inhibition ratio 80% was recorded of biopolymer dextran - gentamycin –PVP blend against *S.epidermidis*(Se_1) in microtiterplate, while in catheters the same biopolymer blend had antibiofilm effect with biofilm inhibition ratio reached to 90% and 81% against *E.coli*(E_2) and *S. aureus*(Sa_2) respectively after 72h.

Conclusion: The biopolymer dextran- gentamycin – PVP blend had antibiofilm properties against pathogenic bacteria isolated from catheters. Also had a potential to be used as antibiofilm coating for catheters.

Keywords : Dextran,Polyvinylpyyrodine, Gentamycin, Antibiofilm, Catheter.

Introduction

The most common medical device infection is the catheter- acquired urinarytract infection where the period of time of catheters insertion to the body is quite enough for bacterial growth in the catheter[1,2]. Catheters provide a suitable surface for bacterial attachment where 24 hours is enough for bacterial attachment and can easily enter urinary system[3]. The most important cause of bacteriuria along the catheter surface is the ability of these pathogens to form the biofilm where bacteria can attach quickly to the surface of catheter[3,4,5]. Bacteria associated with catheters characterized by their ability to form biofilm which is complex organic material forming of microorganisms growing in colonies within an extra-cellular mucopolysaccharide substance.Biofilm formation begins immediately after catheter insertion when organisms adhere to a consistingfilm of host proteins which forms along the catheter surface, both the interior and exterior catheter surfaces are involved[6] .

Dextran is a bacterial homo-polysaccharide cationic polymer which the main chain consist of several α-glucans linked by α-(1-6) glycosidic bonds with different mount of branched linkages such as α-(1-2) , α-(1-3) and α-(1-4) linked as a single unite or lengthen side chain where the degree of branch depend on bacterial strain use for production[7,8,9] .Certain lactic acid bacteria can produce dextran such as genera belong to *Leuconostoc* , *Lactobacillus* , *Streptococcus, Pediococcus* and *Weissella*[10,11,12] . Dextran can be used as plasma/blood volume expanders, blood plasma substituent and drug delivery vehicle for a variety of drugs for its excellent biocompatibility, high biodegradability, wide availability and relatively low- cost modification through its reactive hydroxyl chemistry[13].

Polyvinylpyrrolidone (PVP) is define as a class of hydrophilic water-soluble polymer which attributing for electronegative groups of the carbonyl in the pyrrolidone structure that are able to form hydrogen bond with water [14], it is regarded as bulky, non-toxic,colorless , non-ionic , temperature-resistant, pH-stable , biocompatibility polymer with molecular formula of $(C_6H_9NO)_n$ and C=O, C-N , CH_2 functional groups[15,16,17] . PVP can be used in medicine field as solubilisers , stabilizers , medical additive, polymeric modifier , for sustaining drug and delivery [18]. Attributing for its hygroscopicity , crosslinkability and low coefficient of friction , PVP can be used in catheter coating[19].

The objective of this study was to determine the antibiofilm effect of biopolymer dextran produced by *Leuconostocmesenteroides* and their blends with gentamycin and PVP against pathogenic bacteria in catheters.

Materials and Methods

Microorganisms

An isolate of *Leuconostoc mesenteroides* ssp. *mesenteroides* was used for dextran production, which isolated from the fish intestine, then identified throughout cultural, microscopically and biochemical test according toGarvie[20] and Vitek 2 system.

Ten isolates of bacteria isolated from catheters (collected from Iraqi males patients)included two isolates for each of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*were used forantibiofilmtest. These isolates were identified throughout cultural, microscopically, biochemical test according to the criteria established by Forbes[21] and Vitek 2 system.

Productionof Dextran from *L. mesenteroides*ssp. *mesenteroides*

Aninoculum of*L. mesenteroides*ssp. *mesenteroides* was prepared for dextran production by inoculating 10 ml of sucrose broth with a loop full of 24 h old culture of the isolate . After incubation for 24 h at 30°C, 1ml of this containing (9×10^8cfu/ml) was added to 100ml of the medium described bySarwat*et al.*[22]which contained (g/l): (sucrose 150 g, peptone 5.0 g , K_2HPO_4 15.0 , $MnCl_2.4H_2O$ 0.01, yeast extract 5.0, NaCl 0.01 , $CaCl_2$ 0.05). Incubation was done at 30°C for 24 h[23].

Precipitation and Purification of Dextran:

For dextran precipitation the equal volume of chilled ethanol was added to culture medium, shaken using the vortex, centrifuged at 8000 rpm for 20 min and the supernatant was decanted. This step was repeated twice[23].The precipitated dextran was dried in oven approximately at 40°C for 45 minutes.

For dextran purification, the precipitated dextran was dissolved in distilled water then the dextran slurry that gained was precipitated with equal volume of chilled ethanol. This procedure of re-dissolving, precipitation and washing were repeated three times to eliminate cells debris[22].Purified dextran was dried by using the electrical oven at 40°C for 45 minutes, and then dried dextran was calculated on dry weight basis.

Minimum Inhibitory Concentration (MIC) of Dextran, Gentamycin,PVP.

Minimum inhibitory concentration (MIC) values were determined against bacteria isolated from the catheters using broth dilution method as described byMorello *et al*[24]. Briefly, a stock solution of dextran from *L.mesenteroides*ssp. *mesenteroides*, gentamycin(Bioanalyse/Turkey) , PVP (Sino reagent /China) separately in

sterilized distilled water was diluted to concentrations ranging from (2-512)mg/ml , (2-512)µg/ml and (2-512)mg/ml respectively.

Antibiofilm Effect of Biopolymer Dextran - Gentamycin – PVP Blend in Micro titer Plate

The antibiofilm activity of biopolymerdextran produced by *L. mesenteroides*sssp. *mesenteroides* and blenders (gentamycin and PVP) against bacteria isolated from catheters were quantified using a plastic Microtiter plate as a primary method to detect the biopolymer and blenders abilities to coated surfaces and inhibition of biofilm formation, according to the procedure described by Ali [25] with modification , control wells full with 200µl of distilled water ,while other wells full with (200µl) subMIC of biopolymer, gentamycin , PVP alone and blends separately , the covered microtiter plate was sealed with parafilm during incubation at 37^{o}C for 24 h , wells contents were decanted and washed with distilled water and dried for 15 min at room temperature , after drying (200µl) of bacterial suspension was added to the wells then sealed microtiter plate with parafilm and incubated at 37^{o}C for 24 h . After incubation, wells contents were decanted then washed with distilled water. After drying at room temperature for 15 min 200µl of crystal violet (0.1%) was added tothe wells for 20 min . The stained wells were rinsed three times with distilled water, allowed to dry at room temperature for 15min then extracted with 200µl of 95% ethanol and the absorbance of each well was measured at 630nm using ELISA Reader. The inhibition of biofilm formation percentage of biopolymer, gentamycin, PVP and their blends was calculated as equation described by Namasivayam*et al*[3] .

$$\%\text{Inhibition of biofilm formation} = \frac{O.D\ control - O.D\ treatment}{O.D\ control} \times 100$$

Antibiofilm Effect of Biopolymer Dextran –Gentamycin – PVP Blend in Catheters

The effect of biopolymerdextran-gentamycin-PVP blend on biofilm formation of bacteria in catheters was examined according to method of Namasivayam*et al*[3] with modification. Briefly, catheter was cut into pieces of 1.5cm and autoclaved, then the 1.5cm pieces were immersed separately in subMIC of biopolymer dextran, gentamycin, PVP , biopolymerdextran-gentamycin blend (1:1) , biopolymer dextran-PVP blend (1:1) and biopolymer dextran -gentamycin-PVP blend (1:1:1). A control catheter piece was without any coating treatment and then incubated at 37^{o}C for 24 h in order to coating catheter pieces. After coating, the pieces were put on filter paper to removed solution and drying at 40^{o}C. The dried pieces were immersedin 10 ml of nutrient broth that inoculated with *E.coli* and *S.aureus* separately, and then they incubated at 37^{o}C for 24 h. After incubation, the broth was decanted then all coated and not coated catheter pieces were stained with (0.1) crystal violet for 30 min at room temperature. After staining, catheter pieces were washed with distilled water to remove excess stain and washed three times with 95% ethanol, then ethanol was collected for measuring the absorbance of each piece at 630nm using spectrophotometer and inhibition of biofilm formation percentage was calculated as equation described by Namasivayam *et al*[3] .

Results and Discussion:

Minimum Inhibitory Concentration (MIC) of Dextran, Gentamycin, PVP.

The antibacterial activity of biopolymer dextran was determined on the basis of minimum inhibitory concentration (MIC) values. Results showed that the MIC of Biopolymer dextran was found to be 32mg/ml for *E.coli* , *P.aeruginosa* , *S.aureus* isolates , the MIC for *P.mirabilis* isolates was between (16 –32) mg/ml while in *S.epidermidis* was between (32 – 64)mg/ml.

The MIC of gentamycin was found to be 16 µg/ml for all bacterialisolates. The bactericidal activity of aminoglycosides such as gentamycin is dependent on the concentration. According to CLSI [26] , the MIC of gentamycin for most of Enterobacteriaceae such as *E.coli* , *P.mirabilis* and *P.aeruginosa* was ≥16 µg\ml as well as *S.aureus* and *S.epidermidis* .

The MIC of PVP was found to be 256 mg/ml for all bacterial isolates. Abdel-Aziz and Aeron [27] showed that the polymer was capable of inhibiting ≥ 99% of *S.epidermidis* , *E.coli,* and *S.aureus* . Oyanagi*et al*[28]

showed that 50% of bacteria died after treated with PVP. The presence of higher concentration of PVP improved membrane performances in the antibacterial activity[29].

Antibiofilm Effect of Biopolymer Dextran - Gentamycin – PVP Blend in Micro titer Plate

Antibiofilm effect of biopolymer dextran and blenders (gentamycin and PVP) against bacteria isolated from catheters was studied using micro titer plate. Results showed the ability of biopolymer dextran and its blenders to inhibit biofilm formation. Biopolymer dextran was recorded inhibition of biofilm formation for pathogenic bacteria isolated from the catheters with inhibition ratio ranged between (15-71) % and the maximum inhibition was against *P.aeruginosa* (Ps_2), while gentamycin recorded biofilm inhibition between (3-79)% and the maximum inhibition was against *S.epidermidis* (Se_2). In PVP it was between (39-70)% with maximum inhibition against *S.epidermidis*(Se_2), while biopolymer dextran - gentamycin blend had biofilm inhibition ranged between (22-70)% and the maximum inhibition was against *P.mirabilis* (Pr_2) , for dextran - PVP blend biofilm inhibition (23-65)% was recorded with maximum inhibition against *P.mirabilis* (Pr_2) and in dextran-gentamycin-PVP blend , it was between (58-80)% with maximum inhibition against *S.epidermidis* (Se_1) (Table 1).

From the results above, biopolymer dextran produced from *L.mesenteroides* ssp. *mesenteroides*and its blenders were able to inhibit biofilm formation of gram positive and gram negative bacteria isolated fromcatheters.

Table(1):Antibiofilm effect of biopolymer dextran-gentamycin-PVP blend (Microtiter plate).

Bacterial isolates	Biofilm inhibition %					
	Dextran	GM	PVP	Dex+GM	Dex+PVP	Dex+GM+PVP
E.coli(E_1)	55	58	51	39	41	58
E.coli(E_2)	50	57	59	50	34	60
P.aeruginosa(Ps_1)	15	3	39	28	49	63
P.aeruginosa(Ps_2)	71	64	45	63	39	70
P.mirabilis(Pr_1)	55	42	54	51	42	55
P.mirabilis(Pr_2)	50	64	49	70	65	73
S.aureus(Sa_1)	49	13	45	22	23	51
S.aureus(Sa_2)	31	57	59	49	46	65
S.epidermidis(Se_1)	38	50	46	49	39	80
S.epidermidis(Se_2)	28	79	70	67	61	63

Dex: dextran, GM : gentamycin , PVP : polyvinylpyrrolidone

Antibiofilm Effect of Biopolymer Dextran –Gentamycin – PVP Blend in Catheters

The effect of biopolymer dextran produced by *L. mesenteroides* ssp. *mesenteroides* and blenders (gentamycin and PVP) were studied to inhibit biofilm formation in the catheters. Results showed that biopolymer dextran was able to inhibit biofilm formation of pathogenic bacteria in catheters with biofilm inhibition 65% for *E.coli*(E_2) after incubated for 24 h and reached to 78% after incubated for 72 h , while against *S.aureus*(Sa_2) it was 57% after 24 h and reached to 58% after 72 h , while results showed that PVP have limited ability to inhibit biofilm formation of pathogenic bacteria on catheters , with 2% biofilm inhibition against *E.coli*(E_2) after 24 h and reached to 5% after 72 h , while against *S.aureus*(Sa_2) , was 15% after 24 h and reached to 20% after 72 h .

On other hand results showed that gentamycin was able to inhibit biofilm formation of pathogenic bacteria on catheters , where biofilm inhibition against *E.coli*(E_2) was 62% after 24 h but it was 46.9% after 72 h , while against *S.aureus*(Sa_2) was 59% after 24 h but it was 55% after incubated for 72 h .Our results also showed that biopolymer dextran – Gentamycin blend was able to inhibit biofilm formation of pathogenic bacteria on catheters ,where against *E.coli* (E_2) was 69% after 24 h but it was 85% after 72 h while against *S.aureus*(Sa_2) was 52% after 24 h but it was 75% after incubated for 72 h .Also results showed that biopolymer dextran – PVP blend was able to inhibit biofilm formation of pathogenic bacteria on catheters ,where against *E.coli* (E_2) was 68% after 24 h but it was 43% after 72 h while against *S.aureus*(Sa_2) was 59%

after 24 h but it was 50% after incubated for 72 h .In dextran - gentamycin - PVP blenders, results showed ability of blend to inhibit biofilm formation of pathogenic bacteria on catheters, where biofilm inhibition against *E.coli* (Sa_2) was 71% after 24 h but it was 90 % after 72 h , while against *S.aureus*(Sa_2) was 63% after 24 h but it was 81% after incubated for 72 h (Table2), (Figure1) .

Biopolymer dextran as an anti-adhesive polymer that worked as antibiofilm[30]where dextran with Iron oxide nanoparticle can act as antibiofilm against *E.faecalis* and *P.aeruginosa*[31] . Exopolysaccharide hadantibiofilm activity against pathogenic bacteria such as EPS that produced by *Lactobacillus fermentum*[32] . In another study, Wu *et al*[33] observed antibiofilm activity of exopolysaccharide extracted from marine bacteria against *P.aeruginosa* while Sayem*et al*[34] reported novel exopolysaccharide isolate from *Bacillus licheniformis* with the antibiofilm activity.

Moreover,Kanmani *et al*[35] used exopolysaccharide extracted from *E.faecium* as antibiofilm against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* . Antibiofilm effect of polysaccharide (as sugar polymers) are explicated to their ability to alter the physical characteristics of bacterial cells or abiotic surfaces , act as signaling molecules that affect the gene expression of bacteria or by inhibiting carbohydrate- protein interactions , thereby interfering with adhesion[27] .

In PVP , studies on antibiofilm activity of PVP - Fe_3O_2 combination on the surface of a medical device like the catheter showed that PVP - Fe_3O_2 had the ability to inhibited biofilm formation against *S.aureus* and *P.aeruginosa*[36] . Dispersion forces between the poly chain and the bacterial cells prevent bacteria from binding to the surface and initiating biofilm growth. Modification of the surface change of polymers has also proven to be an effective means of biofilm prevention[27] .

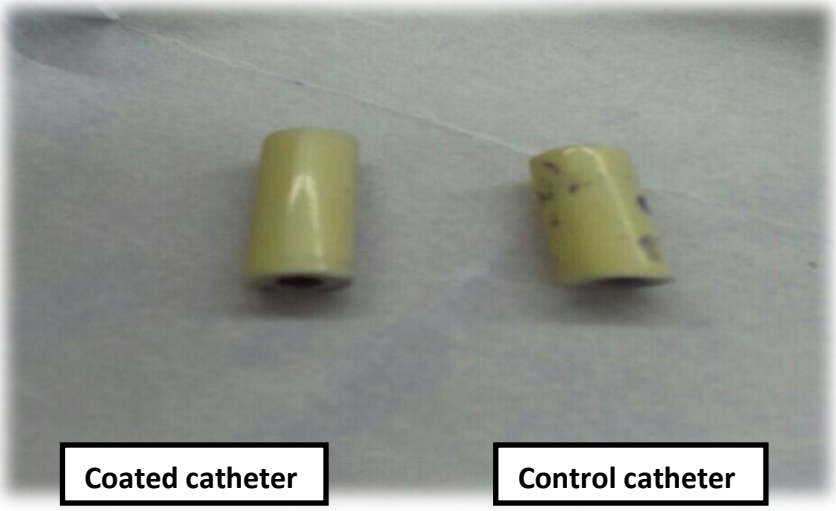
Other studies used gentamycin with a biodegradable polymer to prevent biofilm formation by *S.aureus* on metallic surgical implants[37] while Lin *et al*[38] observed the ability of gentamycin loaded titania nanotube to inhibited biofilm formation . Such studies, Machado *et al*[39] revealed the ability of *P.aeruginosa* to form biofilm under pressure of antibiotic like ciprofloxacin and gentamycin . However, none of the antibiotics could eradicate biofilm completely. Henry-stanley*et al*[40] demonstrated the ability of aminoglycoside group (gentamycin) to inhibited of *S.aureus* biofilm formation while Mu *et al*[41] used chitosan with the gentamycin as antibiofilm to improved antibiotic penetration .

The essential challenge for restore functions of catheters and prevent trauma, disease or ageing is by producing a coating with an antibacterial activity which resist for a long period of time in the device , not eliminated by contact with urine and have a board spectrum activity without spread bacterial resistance . A biomaterial coating is the most biocompatibility agent can decrease biofilm formation and more comfortable for the patient when catheter inserted in the body or in urethra[42] . Fisher *et al*[43] suggested biomaterial modification of urinary catheters with antimicrobials activity where polymer of three antibiotics combination of rifampicin, sparfloxacin and triclosan were used to prevent colonization by common uropathogenic *P.mirabilis, S. aureus* and *E. coli* to give long-term broad spectrum antibiofilm activity while Islas *et al*[44] used poly (vinyl chloride) (PVC) urinary catheters grafted with polymer to preventing biofilm formation in catheters and avoid bacteria adhesion. Also, ciprofloxacin-loaded catheters inhibited the growth of *E. coli* and *S. aureus* surroundings the catheter and prevented bacteria adhesion .

Table(2):Antibiofilm effect of biopolymer dextran-gentamycin-PVP blend in catheters.

Bacterial isolates	Incubation time (h)	Biofilm inhibition (%)					
		Dextran	GM	PVP	DEX.GM	DEX. PVP	DEX.GM . PVP
Escherichia coli (E_2)	24	65	62	2	69	68	71
	48	71	57	3	76	53	78
	72	78	47	5	85	43	90
Staphylococcus aureus(Sa_2)	24	57	59	15	52	59	63
	48	57	58	17	63	55	74
	72	58	55	20	75	50	81

Dex: dextran, GM : gentamycin , PVP : polyvinylpyrrolidone



Figure(1):Antibiofilm effect of biopolymer dextran – gentamycin – PVP blend against *E.coli* in catheter after 72 h .

Conclusion

In conclusion, the biopolymer dextran- gentamycin – PVP blends had antibiofilm properties against pathogenic bacteria isolated from catheters. Also had the potential to be used as antibiofilmcoating for catheters.

Reference

1. Newman, D.K.(2010). Prevention and Management of Catheter-Associated UTIs. In Infectious Disease Special Edition .Macmohan publishing . pp. 13-20.
2. Nicolle, L.E. (2012).Urinary catheter-associated infections. Infect Dis ClinNorth .(26):13–28.
3. Namasivayam, S.K.R. ;Preethi, M. ; Bharani, A.R.S. ; Robin, G. and Latha. B.(2012). Biofilm inhibitory effect of silver nanoparticles coated catheter against *Staphylococcus aureus* and evaluation of its synergistic effects with antibiotics. Int J Biol Pharm Res .,3(2): 259-265.
4. Stickler, D.J. (2008).Bacterial biofilms in patients with indwelling urinary catheters. Nat ClinPract Urol., (5):598–608.
5. Nicolle, L.E. (2014). Urinary catheter-associated infections. Infec. Dis. Clinic. North Am., 26: 13–27.
6. Jordan, R.P. and Nicolle, L.E. (2014). Preventing Infection Associated with Urethral Catheter Biofilms. Biofilms in Infection Prevention and Control: A Healthcare Handbook.,pp. 287-295.
7. Samal, S. K.; Dash, M.; Van Vlierberghe, S.; Kaplan, D. L.; Chiellini, E.; Van Blitterswijk, C. and Dubruel, P. (2012). Cationic polymers and their therapeutic potential. Chemical Society Reviews., 41(21): 7147-7194.
8. Juvonen, R.; Honkapää, K.; Maina, N.H.; Shi, Q.; Viljanen, K.; Maaheimo, H. and Virkki, L. (2015). The impact of fermentation with exopolysaccharide producing lactic acid bacteria on rheological, chemical and sensory properties of pureed carrots (Daucuscarota L.). International journal of food microbiology., 207:109–18.
9. Karsma, A. (2015). Bioprocessing with enzymes and lactic acid bacteria for production of new functional faba bean ingredients.(Master Thesis). Aalto University. Finland. Helsinki.
10. Bounaix, M-S.; Gabriel, V.; Morel, S.; Robert, H.; Rabier, P.; Remaud- Siméon, M.; Gabriel, B. and Fontagné-Faucher, C.(2009). Biodiversity of exopolysaccharides produced from sucrose by sourdough lactic acid bacteria. J Agric Food Chem., 57:10889–10897.
11. Galle, S.; Schwab, C.; Arendt, E. and Gänzle, M. (2010). Exopolysaccharide-forming Weissella strains as starter cultures for sorghum and wheat sourdoughs. Journal of agricultural and food chemistry., 58(9): 5834-5841.

12. Malunga, L.N.; Zinal, E.; Shoubi, I.; Barel_dadon, S.; Berkovich, Z. and Reifen, R. (2012). Effect of combined germination, dehulling and boiling on mineral, sucrose, stachyose, fibrulose, and phytic acid content of different chickpea cultivars. African Journal of Food, Agriculture, Nutrition and Development., 12:6853–6868.
13. Almeida, J. F.; Ferreira, P.; Alves, P.; Lopes, A. and Gil, M. H. (2013). Synthesis of a dextran based thermo-sensitive drug delivery system by gamma irradiation. International journal of biological macromolecules., 61:150-155.
14. Kibbe, A.H. (2004). Handbook of Pharmaceutical Excipients. 4thed. Povidone , In: Rowe RC, Sheskey PJ, Weller , editors. London : Royal Pharmaceutical Society of Great Britain., pp. 508–13.
15. Liu, X.; Lin, T.; Gao, Y.; Xu, Z.; Huang, C.; Yao, G. and Wang, X. (2012). Antimicrobial electrospunnanofibers of cellulose acetate and polyester urethane composite for wound dressing.Journal of Biomedical Materials Research Part B: Applied Biomaterials., 100(6): 1556-1565.
16. Jadhav, S.V.; Nikam, D.S.; Khot, V.M.; Thorat, N.D.; Phadatare, M.R.; Ningthoujam, R. S. and Pawar, S. H. (2013). Studies on colloidal stability of PVP-coated LSMO nanoparticles for magnetic fluid hyperthermia. New Journal of Chemistry., 37(10): 3121-3130.
17. Ziaei-Azad, H. and Semagina, N. (2014). Bimetallic catalysts: Requirements for stabilizing PVP removal depend on the surface composition.Applied Catalysis A: General., 482: 327-335.
18. Bahadur, I.; Momin, M. I.; Koorbanally, N. A.; Sattari, M.; Ebenso, E. E.; Katata-Seru, L. M. and Ramjugernath, D. (2016). Interactions of polyvinylpyrrolidone with imidazolium based ionic liquids: Spectroscopic and Density Functional Theory studies. Journal of Molecular Liquids., 213:13-16.
19. Petersen, S.; Minrath, I.; Kaule, S.; Köcher, J.; Schmitz, K. P. and Sternberg, K. (2013). Development and in vitro characterization of photochemically crosslinked polyvinylpyrrolidone coatings for drug-coated balloons. Coatings., 3(4): 253-267.
20. Garvie, E.I.(1986).Gram -positive cocci. Genus *Leuconostoc* , in Bergeys Manual of Systematic Bacteriology Vol 2.Eds.P.H.Sneath, N.S.Mair and M.E.Sharpe, Williams-Wilkins,Baltimore.pp.1071.
21. Forbes, B.A. ;Sahm, D.F. and Weissfeld, A.S. (2007). Bailey and Scotts Diagnostic Microbiology . 12th ed. USA, Philadelphia . Mosby Elsevier Company., PP: 216-245 and 856-870.
22. Sarwat, F.; Qader, S. A. U.; Aman, A. and Ahmed, N. (2008). Production and characterization of a unique dextran from an indigenous Leuconostoc mesenteroides CMG713. Int. J. Biol. Sci., 4(6): 379-386.
23. Salman, J.A.S.andSalim, M.Z. (2016). Production and characterization of dextran from *Leuconostoc mesenteroides* ssp.*mesenteroides* isolated from Iraqifish intestine. European Journal Of Biomedical And Pharmaceutical Sciences., 3(8): 62-69.
24. Morello , J.A. ; Granato , P.A. and Mizer , H.E. (2003). Laboratory Manual and Workbook in Microbiology : Applications to patient care . 17th ed . The Mc Grow – Hill Companies .pp. 97 – 99.
25. Ali , O.A. (2012) . Prevention of *Proteus mirabilis* biofilm surfactant solution .Egypt .Acad .J. Bioloy .Sci ., 4(1) :1-8 .
26. CLSI . (2011). Performance standard for antimicrobial susceptibility testing ; Twenty – First informational supplement . M100 – S21. Vol 31 . No.(1) .
27. Abdel-Aziz, S. M. and Aeron, A. (2014). Bacterial biofilm: dispersal and inhibition strategies. SAJ Biotechnology., 1(1): 1.
28. Oyanagi, T.; Tagami, J. and Matin, K. (2012). Potentials of mouthwashes in disinfecting cariogenic bacteria and biofilms leading to inhibition of caries. The open dentistry journal., (6):23-30.
29. Basri, H.; Ismail, A.F. and Aziz, M. (2011). Assessing the Effect of PVP of Various Molecular Weight (MW) in PES-Ag Membranes: Antimicrobial Study Using E. Coli. Journal of Science and Technology., 3(2):59-66.
30. Junter, G.A.; Thébault, P. and Lebrun, L. (2016). Polysaccharide-based antibiofilm surfaces. Actabiomaterialia., 30:13-25.
31. Prodan, A. M.; Andronescu, E.; Trusca, R.; Beuran, M.; Iconaru, S. L.; Barna, E. Ş. and Marutescu, L.(2014). Antibiofilm activity of dextran coated iron oxide nanoparticles.U.P.B. Sci. Bull., 76(4):82-88.
32. Salman, J.A.S. Khalaf, KH.J. andJasim, I.I.(2014).Extraction and partial purification of polysaccharide produced from *Lactobacillus acidophilus* and *Lactobacillus fermentum* and their antibiofilm activity against some pathogenic bacteria .Al-Mustansiriyah Journal of Sciences.,25(4):17-24.
33. Wu, S.; Liu, G.; Jin, W.; Xiu, P. and Sun, C .(2016) .Antibiofilm and Anti-Infection of a Marine Bacterial Exopolysaccharide Against Pseudomonas aeruginosa. Front. Microbiol., 7:102.

34. Sayem, S. M.; Manzo, E.; Ciavatta, L.; Tramice, A.; Cordone, A.; Zanfardino, A. and Varcamonti, M. (2011). Anti-biofilm activity of an exopolysaccharide from a sponge-associated strain of *Bacillus licheniformis*. Microbial cell factories., 10(1): 1.
35. Kanmani, P.; Suganya, K.; Yuvaraj, N.; Pattukumar, V.; Paari, K.A. and Arul, V. (2013).Synthesis and functional characterization of antibiofilm exopolysaccharide produced by Enterococcus faecium MC13 isolated from the gut of fish. Applied biochemistry and biotechnology., 169(3): 1001-1015.
36. Limban, C.; Missir, A.V.; Grumezescu, A.M.; Oprea, A.E.; Grumezescu, V.; Vasile, B.Ş. andGălăţeanu, B. (2014). Bioevaluation of Novel Anti-Biofilm Coatings Based on PVP/Fe3O4 Nanostructures and 2-((4-Ethylphenoxy) methyl)-N-(arylcarbamothioyl) benzamides. Molecules., 19(8): 12011-12030.
37. McMillan, D.J.; Lutton, C.; Rosenzweig, N.; Sriprakash, K.S.; Goss, B.; Stemberger, M. and Steck, R. (2011). Prevention of *Staphylococcus aureus* biofilm formation on metallic surgical implants via controlled release of gentamicin. Journal of Biomedical Science and Engineering., 4(8): 512-535.
38. Lin, W. T.; Tan, H. L.; Duan, Z. L.; Yue, B.; Ma, R.; He, G. and Tang, T. T. (2014). Inhibited bacterial biofilm formation and improved osteogenic activity on gentamicin-loaded titania nanotubes with various diameters. International journal of nanomedicine., 90: 12-15.
39. Machado, I.; Graça, J.; Lopes, H.; Lopes, S. and Pereira, M. O. (2012). Antimicrobial Pressure of ciprofloxacin and Gentamicin on biofilm development by an endoscope-Isolated Pseudomonas aeruginosa. ISRN biotechnology., 2013(2013): 2-10.
40. Henry-Stanley, M.J.; Hess, D.J. and Wells, C.L. (2014). Aminoglycoside inhibition of Staphylococcus aureus biofilm formation is nutrient dependent. Journal of medical microbiology., 63(6): 861-869.
41. Mu, H.; Guo, F.; Niu, H.; Liu, Q.; Wang, S. and Duan, J. (2014). Chitosan improves anti-biofilm efficacy of gentamicin through facilitating antibiotic penetration. International journal of molecular sciences., 15(12): 22296-22308.
42. Carberry, B.J. ; Farrell, J. and Kennedy, J. E. (2015). Evaluation and characterization of urinary catheter coating utilizing Hansen solubility parameters and FEA analysis. Surface and Coatings Technology., 276:456-463.
43. Fisher, L. E.; Hook, A. L.; Ashraf, W.; Yousef, A.; Barrett, D.A.; Scurr, D. J. and Parkinson, R. (2015). Biomaterial modification of urinary catheters with antimicrobials to give long-term broadspectrum antibiofilm activity. Journal of Controlled Release., 202: 57-64.
44. Islas, L.; Alvarez-Lorenzo, C.; Magariños, B.; Concheiro, A.; del Castillo, L.F. and Burillo, G. (2015). Singly and binary grafted poly (vinyl chloride) urinary catheters that elute ciprofloxacin and prevent bacteria adhesion. International journal of pharmaceutics., 488(1): 20-28.

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