International Journal of Microbiological Research 11 (1): 37-47, 2020
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2020.37.47

Antibiotic Resistance among Biofilm Forming Microorganisms and Treatment Methods for Biofilm Degradation

Sonu Bansal and G. Sibi

Department of Biotechnology,
Indian Academy Degree College-Autonomous, Bengaluru, India

Abstract: Bacterial biofilms are multicellular aggregates enclosed in a self-created biopolymer matrix. Biofilm-producing bacteria have become a great public health problem worldwide because biofilms enable these microorganisms to evade several clearance mechanisms produced by host and synthetic sources like antibiotics. Majority of these biofilm forming micro-organisms have developed resistance against most of the antibiotics and hence cause difficulty in treating them with the present antibiotics. There are many other ways to kill the biofilm formed by the bacteria of which few are discussed in this paper. All the methods have proved effective against the biofilm and has helped in the treatment of the disease. But the complete knowledge on the specific method is not known and the way of their action is not completely ascertained. But there is still a need for the development of newer techniques to overcome the problems caused by them. The micro-organisms are developing day by day and are getting resistant to multiple drugs due to the over exploitation of antibiotics, hence there has become an utter need for the development of newer ways to fight against them. The research for new techniques is still in the infancy stage and researches need to work out more to solve the issues of the biofilm forming organisms.

Key words: Biofilm · Quorum Sensing · Degradation of Biofilm · Antibiotics

INTRODUCTION

Due to the emerging and increasing antibiotic resistance, treatment of infectious diseases has become a major concern [1, 2]. Decreasing speed of discovery of new antimicrobials into the market has led to emerging and rapid spreading of the drug resistant isolates. Hence, spreading of these drug resistant isolates is a serious threat to public health [1-4]. Previous studies have shown various associations between antibiotic resistance and biofilm formation. Biofilms have major medical significance as they decrease susceptibility to antimicrobial agents. The decreased susceptibility to microbial agents within a biofilm arises from multiple factors, including physical impairment of diffusion of antimicrobial agents, reduced bacterial growth rates and local alterations of the microenvironment that may impair activity of the antimicrobial agent. Furthermore, the proximity of cells within a biofilm can facilitate plasmid exchange and hence enhance the spread of antimicrobial resistance [5]. Bacteria within the biofilm behave differently from their planktonic counterparts especially in terms of antibiotics, which causes limitation in conventional antibiotic therapies [6].

On the basis of their growth characteristics, microorganisms have primarily characterized as planktonic [7]. It is now widely accepted that about 99% of all microorganisms attached to a surface and grow as a biofilm is a universal microbial strategy for their survival [8]. Biofilms have high cell densities ranging from 10^8 to 10^{11}cells per gram of wet weight. They are persistently attached to both biotic and abiotic surfaces ranging from the human tooth or lung and the intestine of a cow to a rock submerged in a fast-moving stream. Medical devices that can be colonized by biofilms include intrauterine contraceptive devices, implants, prosthetic medical devices, catheters, dental materials, cardiac valves and contact lenses [9].

Corresponding Author: G. Sibi, Department of Biotechnology, Indian Academy Degree College-Autonomous, Bengaluru, India.

It has been found that bacteria inside the biofilm are 10-1000 times more resistant to antimicrobial agents than the planktonic ones [10]. The bacteria within a biofilm are in a distinct metabolic state that differs from the free-floating planktonic state. They express additional virulence factors. Bacteria in a biofilm communicate with each other by sending signals which regulate the gene expression of neighbouring bacteria in a phenomenon known as "quorum sensing" [11]. Once a biofilm has matured, it is difficult to kill the bacteria within it. The main aim of this review article is to highlight the threats caused by the biofilm formation due to its resistance against the available antimicrobial agents. This article also gives a glimpse about the other methods available for the treatment of the biofilm, which has proven effective against it.

Biofilm Formation: A biofilm can be defined as a "matrix-enclosed accretion of single or multiple strains of bacteria that adhere to biological or non-biological surfaces" [12, 13]. Bacteria living in a biofilm are able to resist shear forces in fluids and other external environmental factors than free-floating bacteria. The biofilm matrix which binds the bacteria together also enhances their communication with one another which consequently increases their pathogenicity [14]. There are five stages in biofilm formation: adhesion; irreversible adhesion; colonization; mature biofilm and dispersion [15]. The initial adhesion of bacteria to a surface is weak and microorganisms are able to bind and then detach readily. In the following stage, the binding is irreversible and the bacteria produce extracellular polymeric substances (EPS) which surround and retain them on the surface. At this point, the shear force from fluid flowing past will not readily cause the biofilm to detach. As the colonization stage progresses, microorganisms start to form more complex towers and other three-dimensional (3D) structures. As the biofilm grows in complexity, bacteria secrete chemical signals and communicate with each other [16]. As the biofilm matures further, the towers develop complex shapes due to the shear forces exerted by the movement of fluids. Within the biofilm, bacteria show heterogeneity and play different roles, according to their location. As the biofilm matures, it becomes better at surviving despite unfavourable environmental conditions, such as limited nutrition or low oxygen levels. In the final stage, as competition for nutrients and oxygen becomes greater, bacteria begin to detach from the biofilm and then disperse, to initiate biofilm formation in new locations [15].

Biofilm Forming Bacteria and Their Resistance Against Antibiotics

Correlation Between Biofilm Formation and Carbapenem Resistance: *Klebsiella pneumoniae* is one of the most common human pathogens which is recovered from a wide range of community and hospital acquired infections such as pneumoniae, bacteremia, urinary tract and respiratory tract infections [17]. The major virulence factors of *K. pneumoniae* are capsules, fimbriae, lipopolysaccharides and biofilm formation [4]. The bacterial populations can survive in harsh conditions within host, by the formation of biofilm [18].

Rahdar *et al.* [19] collected a total of 160 K. pneumoniae isolates from various infections of hospitalized patients at Army hospitals and identification of bacterial isolates were performed using phenotypic and biochemical tests according to standard methods [20]. They tested these isolates against carbapenem, which is a highly effective antibiotic agent which is used for the treatment of severe or high-risk bacterial infections. The Carba NP test and molecular methods were used by them for detection of carbapenem resistance isolates of *K. pneumonia* and also studied the ability of biofilm production on all isolates. They observed a significant correlation between biofilm formation ability and carbapenem resistant isolates. Among carbapenem sensitive cases they found a varied biofilm production that is strong, middle and weak.

The increase of carbapenem resistance in biofilm producing isolates of *K. pneumoniae* is to be considered as a serious alert and the basic measures to combat this phenomenon has to be imperative. In conclusion, the specific antibacterial resistance can compromise or enhance biofilm formation among the bacterial population. Therefore, the increasing prevalence towards the resistance of drug and antibiotic is alarming condition in the case *K. pneumoniae* in hospitals and it even indicates the high level of threat from this pathogen.

Biofilm Formation and Antimicrobial Resistance of *E. coli* in Diabetic and Nondiabetic Patients: Diabetes is a chronic, metabolic disease characterized by increased levels of blood glucose, which on overtime leads to serious damage to heart, kidney blood vessels, nerves and eyes. The number of people with diabetes has increased from 108million (1980) to 422million (2014) worldwide, causing 1.6million deaths in 2015 [21, 22]. Diabetic patients are more susceptible to infection compared to nondiabetic counterparts, urinary tract infection being the most common bacterial infection

encountered in these patients. Susceptibility of the infection in these patients increases with longer duration and greater severity of diabetes. The main causative agent for the UTI is *E. coli*. UTI is considered as the most common infectious disease affecting both men and women and the most affected one are women [23]. The main problem associated with UTI is its recurrence and persistence, which is mainly due to the presence of biofilm associated pathogen [24].

Raya *et al.* [25] had collected the urine samples from over 1, 099 patients and they processed the urine samples by standard microbiological techniques; of which 182 samples were from the diabetic group and 917 were from the non-diabetic. They subjected all the isolates to antibiotic susceptibility test by using modified Kirby-Bauer disc diffusion method and the invitro biofilm forming capacity of the isolates were detected by Microtiter plate method. They observed that UTI was significantly high in diabetic people, *E. coli* being the most common causative agent of UTI in both diabetic and non-diabetic. The prevalence of biofilm producing uropathogens was higher in both diabetic and nondiabetic groups. *E. coli* showed higher percentage of resistance to amoxicillin, followed by ciprofloxacin in both diabetic and nondiabetic patients. *E. coli* isolated from diabetic patients were found to be the most resistant to many of the antibiotics compared to nondiabetic patients. Biofilm producing E.coli showed comparatively high resistance rate to tested antimicrobial agents than non-biofilm producing E. coli counterparts. The resistance rate of quinolones, third generation cephalosporin and sulphonamide was statistically higher among biofilm producing *E. coli*. Thus, they concluded that the biofilm forming *E. coli* in diabetics were multi drug resistant. The higher the biofilm production the greater the resistance to in-use antimicrobial agents, this study renders its inefficacy for empirical treatment and point out the importance of biofilm screening to ensure the effective management of infection.

Antibiotic Susceptibility Patterns and Biofilm Formation in Clinical Isolates of *Enterococcus* spp.: *Enterococcus* is a commensal in the intestine and is now emerging as a drug-resistant pathogen. It produces different virulence factors. *Enterococcus* surface protein (ESP) is a virulence factor that helps in adhesion, but its role in biofilm formation is still not exactly known. *Enterococci* are normal flora of oral, gut and female genital tract of humans and are known to cause nosocomial infections [26-29]. E. faecalis is responsible for 80-90 percent and E. faecium 5-10 percent of the human enterococcal infections [30, 31]. Most frequent infections caused by *Enterococcus* spp. are urinary tract infections followed by intra-abdominal abscesses and bloodstream infections [32]. Biofilm protects *Enterococci* from host immune response and antibiotics. Biofilm-producing *Enterococci* cause recurrent, chronic and antibiotic-resistant infections [33-35]. Apart from biofilm-forming ability, *Enterococcus* spp. are known to produce various virulence factors [36]. Moreover, clinical isolates have been reported to harbor gene coding for esp virulence factor rather than the commensal strains [37].

Sridevi and Biranthabail [38] had conducted the experiment to study the prevalence of drug resistance in clinical isolates of *Enterococcus* spp. and to find the association of drug resistance with biofilm formation and esp genes. They tested the antibiotic susceptibility test by Kirby-Bauer disc diffusion and biofilm production was done by microtiter plate method. PCR was performed for detection of esp gene. They isolated the *Enterococci* from clinical samples like pus, sputum, vaginal swab and aspirates. A total of 150 isolates of *Enterococcus* were included in the study. They found out that 82% were *E. faecalis*, 63% were *E. faecium*. They performed a PCR on the isolates for the detection of esp genes and they found that 40 isolates were positive for esp genes. They found out that the relation between esp gene and biofilm formation was significant, but not in all the cases. In this study they found that, 21.9% of *Enterococcal* isolates produced biofilm, which included 27.5% E. faecium and 17.7% E. faecalis. Two *E. faecium* strains resistant to vancomycin and high-level aminoglycoside (HLAR) were non-biofilm-producers and did not harbor *esp* gene. However, other biofilm-producing *E. faecium* harbored *esp* gene. They observed that there was no significant association between biofilm formation and presence of *esp* gene in *E. faecalis.* Moreover, a significant correlation was not found between drug resistance and biofilm production in both *Enterococcus* species. Thus, biofilm formation is not always associated with the presence or absence of *esp* gene and or drug resistance in *Enterococcus* spp. They concluded that *E. faecium* is more resistant than *E. faecalis*. Hence, speciation and antibiotic susceptibility testing are necessary to detect the emergence and changing pattern of drug resistance. Vancomycin-resistant Enterococcus is a significant cause of concern as this might share its resistance gene with other bacterial strains, causing crossover of gene rendering others resistant to vancomycin.

Treatment Methods Against Biofilm Forming Bacteria Hypochlorous Acid Generating Electrochemical Scaffold for Treatment of Wound Biofilms: Biofilm formation causes prolonged wound infections due to dense biofilm structure, differential gene regulation to combat stress and production of extra cellular polymeric substances. Staphylococcus aureus, Pseudomonas aeruginosa and Acinetobacter baumannii are commonly associated with biofilm formation in wound infections [39-42]. Biofilms of *S. aureus* lead to a chronic infectious state in wounds by stimulating the production of pro-inflammatory cytokines and prolonging the inflammatory phase of the wound [43, 44]. Public and healthcare concerns have arisen because of the increasing prevalence of bacteria resistant to antibiotics and antiseptics [45, 46]. Thus, it is crucial to develop new treatment approaches to combat biofilm-infected wounds that do not involve antibiotics.

An experiment was performed by Kiamco *et al*. [47] using H_2O_2-producing electrochemical scaffold (e-scaffold) that eliminated large microbial communities in biofilms. HOCl eradicates bacteria by inhibiting bacterial growth, cell division and protein synthesis; oxidizing sulfhydryl enzymes and amino acids; decreasing adenosine triphosphate production; breaking DNA; and depressing DNA synthesis [48-50]. HOCl is already used as a wound cleansing agent [51] and published work demonstrates that HOCl acts as an antimicrobial, anti-biofilm agent that also promotes wound healing [52]. They hypothesised that HOCl generated electrochemically by the e-scaffold can eliminate biofilms without damaging host tissue. The e-scaffold is a device that oxidizes chloride ions from phosphate-buffered saline (PBS) or physiological chloride ions in blood and wound fluid to chlorine [53], the chlorine then reacts with water to produce HOCl. Low concentration of HOCl can be antimicrobial without causing cytotoxicity to host tissue. To verify HOCl generation, they used custom-made microelectrodes to measure HOCl concentrations near the surface of the e-scaffold. *S. aureus*, *A. baumannii* and *P. aeruginosa* biofilms were subjected to HOCl e-scaffold treatment for various lengths of time. The addition of exogenous HOCl for biofilm treatment was also compared to e-scaffold-generated HOCl. The e-scaffold produces a continuous supply of HOCl at a low concentration, a process that is very different from simply applying HOCl directly to a wound. They also concluded that their e-scaffold did not change the pH in the wound bed and thus did not cause any side effects. They were successful in eradicating the *A. baumannii*, *P. aeruginosa* and *S. aureus* from the wounds.

Non-Antibiotic Antimicrobial Agents: Generally, antibiotics are used to stop the growth of bacteria, but emerging resistance has limited their effectiveness. This is because of the excessive use of antibiotics having led to drug resistance, both within health care settings and wider community [54]. Bacteria in biofilms are less susceptible to antibiotics compared with their free-floating state, as biofilms impair antibiotic penetration. To overcome this challenge, non-antibiotic antimicrobial agents are needed. Here it has been described about two classes of these agents, namely antimicrobial nanoparticles and antimicrobial peptides.

In light of these issues, there is growing interest in the search for non-antibiotic antimicrobial agents. Unlike antibiotics, which target a single metabolic pathway, these agents usually attack multiple sites on bacteria. Non-antibiotic antimicrobial agents, including silver nanoparticles or antimicrobial proteins, may bind to and oxidize thiol groups, block DNA replication, alter bacterial gene expression, denature enzymes, induce reactive oxygen species (ROS), or damage bacterial membranes [55, 56]. By attacking multiple molecular targets, the likelihood of developing drug resistance should be much lower [55]. There is particular interest in developing antimicrobial agents that can work against bacteria with multi-drug resistance (MDR). The main purpose of the usage of these agents is to cause a fewer side effect on the host and to lower the chance of inducing resistance and also to completely wipe out the disease-causing organism from the body of the host.

Antimicrobial Nanoparticles: Inorganic antimicrobial agents include metal ions and photocatalysts, the main particles used in the treatment are silver, zinc oxide and titanium dioxide when used in the form of nanoparticles.

Silver Nanoparticles (Ag-NPs): Ag NPs have a diameter between 1 nm and 100 nm [57]. They have been used widely in many areas, including medical devices (such as wound dressings), pharmaceutical products, food packaging and kitchen appliances [58-61]. Ag NPs can inhibit the growth of many bacteria and fungi because of their unique physiochemical characteristics [62, 63]. Ag NPs can directly influence bacterial cell membranes and can impair bacterial respiration, metabolism and proliferation [64]. Ag NPs are found effective against *Pseudomonas putida* [65] AgNPs have also proven to be effective against biofilms of *S. aureus* [66].

Zinc Oxide Nanoparticles (ZnO-NPs): ZnO NPs can exert antimicrobial effects against many species of bacteria and fungi [67, 68]. They disturb the stability of bacterial cell membrane, causing leakage, which leads to bacterial death [69]. Within the oral cavity, biofilms can form on teeth and on other hard and soft surfaces. Oral biofilms are complex and contain many species of bacteria and fungi. These multi-species biofilms show greater resistance to antibiotics than a single species biofilm [70-72]. ZnO NPs interfere with oral biofilms and kills them. ZnO NPs can inhibit biofilm formation of *Streptococcus pneumoniae*, a common cause of pneumonia [73]. ZnO NPs have also been shown to reduce biofilm formation by uropathogenic *E. coli* strains [74].

Titanium Dioxide Nanoparticles (TiO_2-NPs): TiO2 is a photocatalyst that helps to generate strong oxidizing agents when irradiated with UV light [75]. Matsunaga *et al.* [76] demonstrated that platinum-loaded TiO_2 can kill *E. coli* bacteria dispersed in water. Following this initial report, TiO2 NPs have been examined for use as a disinfectant [77]. They can disrupt biofilms produced by *Listeria monocytogenes*, a common food-borne bacterium that causes health issues in food processing [78]. Likewise, TiO2 NPs can inhibit the growth and reduce biofilm formation by the common oral bacterial species *Streptococcus mitis* [79] and also by methicillin-resistant *S. aureus* (MRSA) [80]. In the same manner, TiO_2 NPs can exert anti-biofilm effects against *Streptococcus* mutants [81]. Because of the effects of TiO_2 NPs against *S. aureus,* it has been suggested that a film of TiO_2 could be used on the surface of medical implants to reduce the levels of contamination by pathogens [82].

Antimicrobial Peptides and Proteins: Antimicrobial peptides which are also known as host defence peptides, play an important role in normal host immune responses to pathogenic microorganisms [83]. The antimicrobial activity of AMPs is not as strong as conventional antibiotics, but is sufficient to kill pathogens. AMP create pores in the cell membrane. In Gram-negative bacteria, AMP can disaggregate lipopolysaccharides (LPS) in the outer layer of the bacterial membrane [84]. LPS are a potent trigger for host inflammatory responses elicited by macrophages and other immune cells [85]. In Gram-positive bacteria, AMP such as lantibiotics can inhibit the replication of bacteria because of their effects on peptidoglycans in the cell wall [86, 87]. Both natural and synthetic AMP can exert antimicrobial effects [88]. Zapotoczna *et al.* [89] showed that synthetic AMP (Bac8c, HB43, P18, Omiganan, WMR, Ranalexin and Polyphemusin) could successfully destroy a *S. aureus* biofilm in a catheter.

Antimicrobial Enzymes: These enzymes are found in many organisms as a part of their innate defence mechanisms against bacterial invasion. Not only these enzymes attack bacteria directly, but they also inhibit the formation of biofilms. The major types of Journal Pre-proof 12 antimicrobial enzymes are proteolytic enzymes and polysaccharide-degrading enzymes [90]. Amongst the proteolytic enzymes, subtilisins, which belong to the serine proteases, are used in industry to control biofilm growth [91]. These enzymes are produced by *Bacillus spp*. Lysostaphin is a proteolytic enzyme from *S. simulans*, this can rupture the cell walls of staphylococci and so is a promising candidate in the search for agents that can inhibit MRSA [92].

All these non-antibiotic antimicrobial agents have an impact on the biofilm producing organisms but their action is not completely understood yet, more research needs to be done on these Nano particles and antimicrobial peptides and proteins as they have a promising effect on the biofilm producing microbes. All these can be combined with other chemicals and some traditional techniques also, the Nano particle's cytotoxicity has also to be kept in calculation before a high dose is administered.

Use of Phages: Methicillin-resistant *S. aureus* (MRSA) biofilm producers represent an important etiological agent of many chronic human infections. Antibiotics and host immune responses are largely ineffective against bacteria within biofilms. In this context, Dakheel *et al*. [93] have demonstrated the usage of phages to destroy MRSA biofilms as an innovative alternative mechanism.

In the light of emerging antibiotic resistance among biofilm producing bacteria and antibiotic-induced biofilm production, the development of selective antibacterial agents with less toxicity towards the treatment of biofilm-induced infections has become imperative [94, 95]. Therefore, the phage-based anti-biofilm strategy would be an attractive solution towards addressing the biofilm menace. Bacteriophages, like all viruses, are obligate intracellular parasites that need a host to multiply. They require actively growing host cells to multiply and reproduce. They are abundant in the water environment [96-98]. Lytic bacteriophages isolated and

characterized from several MSRA strains play crucial roles in the investigation of the potential use of phages and their products as therapeutic agents against infections caused by biofilm-producing MRSA. Bacteriophages have been tested as anti-infectives in human and animals [99, 100]. Phage encoded lytic proteins have also been used to inhibit pathogenic bacteria [101-103].

Dakheel *et al.* [93] had isolated twenty-five MRSA biofilm producers and were used as substrates to isolate MRSA-specific phages. They had isolated two phages (UPMK_1 and UPMK_2). They found both phages varied in their ability to produce halos around their plaques, host infectivity, one-step growth curves and electron microscopy features. They found that the UPMK_1 isolate was not effective against all the 25 MRSA biofilm producers, where the UPMK_2 phage isolate was effective against all the 25 MRSA biofilm producers. The phages basically degraded the biofilm and killed the *S. aureus* strains. They concluded from their experiment that both the phages possessed lytic enzymes that were associated with a high ability to degrade biofilms. Based on the results, phages can be used as a new bio-weapon against these biofilm forming microorganisms.

CONCLUSIONS

Emergence of severe biofilm infections and its resistance to antimicrobial treatment, has posed a great challenge in the medical field. This review provides the information of the mechanism of biofilm formation and antibiotic resistance among biofilm forming bacteria. Details regarding the treatment strategies provide better understanding about the mode of action of antibiotics and non-antibiotic methods can be further used to combat the biofilm producing microorganisms with the previously known target of action.

REFERENCES

1 Ribeiro, S.M., M.R. Felcio, E.V. Boas, S. Goncalves, F.F. Costa, R.P. Samy, N.C. Santos and O.L. Franco, 2016. New frontiers for anti-biofilm drug development. Pharmacology and Therapeutics, 160: 133-144.

2. Fernandez, L. and R.E. Hancock, 2012. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. Clinical Microbiology Reviews, 25: 661-681.

3. Kazemian, H., H. Heidari, R. Ghanavati, S. Ghafourian, F. Yazdani, N. Sadeghifard, H. Valadbeigi, A. Maleki and I. Pakzad, 2019. Phenotypic and genotypic characterization of ESBL-, AmpC- and Carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates. Medical Principles and Practice, 28: 547-551.

4. Aghaei, S.S., M. Keikha, M.K. Zarandi, H.A. Rahdar, A. Javadi, E. Takei and R. Nazarei, 2019. Evaluation and Identification of Carbapenem Resistant *Klebsiella pneumoniae* Isolated from Hospitalized Patients in Qom City, (Iran). Qom University of Medical Sciences Journal, 13: 39-47.

5. Watnick, P. and R. Kotler, 2000. Biofilm, city of microbes. Journal of Bacteriology, 182: 2675-2679.

6. Romling, U. and C. Balsalobre, 2012. Biofilm infections, their resilience to therapy and innovative treatment strategies. Journal of Internal Medicine, 272: 541-561.

7. Donlan, R.M. and J.W. Costerton, 2002. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews, 15: 167-193.

8. McCarty, S., E. Woods and S.L. Percival, 2014. Biofilms: from Concept to Reality. Biofilms in Infection Prevention and Control, pp: 143-163.

9. Jolivet-Gougeon, A. and M. Bonnaure-Mallet, 2014. Biofilms as a mechanism of bacterial resistance. Drug Discovery Today. Technologies, 11: 49-56.

10. Wu, H., C. Moser, H.Z. Wang, N. Hoiby and Z.J. Song, 2015. Strategies for combating bacterial biofilm infections. International Journal of Oral Science, 7: 1-7.

11. Li, Y.H. and X. Tian, 2012. Quorum sensing and bacterial social interactions in biofilms. Sensors (Basel), 12: 2519-2538.

12. Hall-Stoodley, L., J.W. Costerton and P. Stoodley, 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nature Reviews Microbiology, 2: 95-108.

13. Vestergaard, M. and H. Ingmer, 2019. Antibacterial and antifungal properties of resveratrol. International Journal of Antimicrobial Agents, 53: 716-723.

14. Wessel, A.K., T.A. Arshad, M. Fitzpatrick, J.L. Connell, R.T. Bonnecaze, J.B. Shear and M. Whiteley, 2014. Oxygen limitation within a bacterial aggregate. MBio, 5: e00992-14.

15. Gordon, V.D., M.D. Fields, K. Kovach and C.A. Rodesney, 2017. Biofilms and mechanics: a review of experimental techniques and findings. Journal of Physics D: Applied Physics, 50: 223002.
16. Frederick, M.R., C. Kuttler, B.A. Hense and H.J. Eberl, 2011. A mathematical model of quorum sensing regulated EPS production in biofilm communities. Theoretical Biology and Medical Modelling, 8: 8.
17. Podschun, R., S. Pietsch, C. Holler and U. Ullmann, 2001. Incidence of Klebsiella species in surface waters and their expression of virulence factors. Applied Environmental Microbiology, 67: 3325-3327.
18. Turkel, I., T. Yildirim, B. Yazgan, M. Bilgin and E. Basbulut, 2018. Relationship between antibiotic resistance, efflux pumps and biofilm formation in extended-spectrum β-lactamase producing *Klebsiella pneumoniae*. Journal of Chemotherapy, 30: 354-363.
19. Rahdar, H.A., E.S. Malekabad, A.R. Dadashi, E. Takei, M. Keikha, H. Kazemian and M.K. Zarandi, 2019. Correlation between biofilm formation and carbapenem resistance among clinical isolates of *Klebsiella pneumoniae*. Ethiopian Journal Health Sciences, 29: 745-750.
20. Mahmoudi, S., M. Mahzari, M. Banar, B. Pourakbari, M.T. Ashtiani, M. Mohammadi, S. Keshavarz Valian and S. Mamishi, 2017. Antimicrobial resistance patterns of Gram negative bacteria isolated from bloodstream infections in an Iranian referral paediatric hospital: A 5.5 year study. Journal of Global Resistance, 11: 17-22.
21. Gyawali, B., R. Sharma, D. Neupane, S.R. Mishra, E. Van Teijlingen and P. Kallestrup, 2015. Prevalence of type 2 diabetes in Nepal: a systematic review and meta-analysis from 2000 to 2014. Global Health Action., 8: 29088.
22. WHO (World Health Organization), 2019. Diabetes. World Health Organisation.
23. Shahidul, M.K., A. Asthma, F. Farahnaaz and A. Sunjukta, 2013. Determination of antibiotic resistance pattern of biofilm producing pathogenic bacteria associated with UTI. International Journal of Drug Development and Research, 5: 312-319.
24. Ejrnæs, K., M. Stegger, A. Reisner, S. Ferry, T. Monsen, S.E. Holm, B. Lundgren and N. Frimodt-Møller, 2011. Characteristics of *Escherichia coli* causing persistence or relapse of urinary tract infections: phylogenetic groups, virulence factors and biofilm formation. Virulence, 2: 528-537.
25. Raya, S., A. Belbase, L. Dhakal, K.G. Prajapati, R. Baidya and N.K. Bimali, 2019. *In vitro* Biofilm Formation and Antimicrobial Resistance of *Escherichia coli* in Diabetic and Nondiabetic Patients. Biomed Research International, 1474578.
26. Winn, W.C., Jr., S.D. Allen, W.H. Jande and P.C. Koneman, 2006. Konman's Color Atlas and Textbook of Diagnostic Microbiology, pp: 1294-1298.
27. Murray, B.E. and G.M. Weinstock, 1999. Enterococci: New Aspects of An Old Organism. Blackwell Science Inc., 111(4): 328-34.
28. Richards, M.J., J.R. Edwards, D.H. Culver and R.P. Gaynes, 2000. Nosocomial infections in combined medical-surgical intensive care units in the United States. Infection Control and Hospital Epidemiology, 21: 510-515.
29. Mukherjee, K., D. Bhattacharjee, G. Chakraborti and S.S. Chatterjee, 2016. Prevalence and antibiotic susceptibility pattern of *Enterococcus* species from various clinical samples in a tertiary care hospital in Kolkata. International Journal of Contemporary Medical Research, 3: 1565-1567.
30. Moellering, R.C., 1992. Emergence of *Enterococcus* as a significant pathogen. Clinical Infectious Diseases, 14: 1173-1178.
31. Sreeja, S., P.R. Sreenivasa Babu and A.G. Prathab, 2012. The prevalence and the characterization of the enterococcus species from various clinical samples in a tertiary care hospital. Journal of Clinical and Diagnostic Research, 6: 1486-1488.
32. Low, D., N. Keller, A. Barth and R. Jones, 2001. Clinical prevalence, antimicrobial susceptibility and geographic resistance patterns of Enterococci: results from the Sentry antimicrobial surveillance program, 1997-1999. Clinical Infectious Diseases, 32: S133-S145.
33. Costerton, J., 2001. Cystic fibrosis pathogenesis and the role of biofilms in persistent infection. Trends in Microbiology, 9: 50-52.
34. Moniri, R., A. Ghasemi, S.G. Moosavi, K. Dastehgoli and M. Rezaei, 2013. Virulence gene's relationship with biofilm formation and detection of aac (6')/aph (2") in *Enterococcus faecalis* isolated from patients with urinary tract infection. Jundishapur Journal of Microbiology, 6: e6244.
35. Watnick, P. and R. Kolter, 2000. Biofilm, city of microbes. Journal of Bacteriology, 182: 2675-2679.

36. Toledo-Arana, A., J. Valle, C. Solano, M.J. Arrizubieta, C. Cucarella, M. Lamata, B. Amorena, J. Leiva, J.R. Penadés and I. Lasa, 2001. The enterococcal surface protein, esp, is involved in enterococcus faecalis biofilm formation. Applied and Environmental Microbiology, 67: 4538-4545.
37. Upadhyaya, G.P.M., K.L. Ravikumar and B.L. Umapathy, 2009. Review of virulence factors of *Enterococcus:* an emerging nosocomial pathogen. Indian Journal of Medical Microbiology, 27: 301-305.
38. Shridhar, S. and B. Dhanashree, 2019. Antibiotic susceptibility pattern and biofilm formation in clinical isolates of *Enterococcus* spp. Interdisciplinary Perspectives on Infectious Diseases, 2019: 7854968.
39. De la Fuente-Nunez, C., F. Reffuveille, L. Fernandez and R.E.W. Hancock, 2013. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Current Opinion in Microbiology, 16: 580-589.
40. Otto, M., 2008. Staphylococcal biofilms. Current Topics in Microbiology and Immunology, 322: 207-228.
41. Costerton, J.W., P.S. Stewart and E.P. Greenberg, 1999. Bacterial biofilms: A common cause of persistent infections. Science, 284: 1318-1322.
42. Surucuoglu, S., H. Gazi, S. Kurutepe, N. Ozkutuk and B. Ozbakkaloglu, 2005. Bacteriology of surgical wound infections in a tertiary care hospital in Turkey. East African medical Journal, 82: 331-336.
43. Chronic Wound Care, 2014. The Essentials. A clinical source book for health care professionals HMP Communications, LLC.
44. Percival, S.L., W. Slone, S. Linton, T. Okel, L. Corum, J.G. Thomas, 2011. The antimicrobial efficacy of a silver alginate dressing against a broad spectrum of clinically relevant wound isolates. International Wound Journal, 8: 237-243.
45. Smith, T.L., M.D. Michele, R. Kenneth, M.D. Wilcox, P.H. Cosme Cruz, V. Michael, V. Lancaster, Barbara Robinson-Dunn, Fred C. Tenover, Marcus J. Zervos, Jeffery D. Band, Elizabeth White, Willian R. Jarvis. 1999. Emergence of vancomycin resistance in Staphylococcus aureus. The New England Journal of Medicine, 340: 493-501.
46. Wang, L., M. Bassiri, R. Najafi, J. Yang, W. Hwong, E. Barati, B. Belisle, C. Celeri and M.C. Robson. 2007. Hypochlorous acid as a potential wound care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. Journal of Burns and Wounds, 6: e5.
47. Kiamco, M.M., H.M. Zmuda, A. Mohamed, D.R. Call, Y.S. Raval, R. Patel and H. Beyenal, 2019. Hypochlorous-acid-generating electrochemical scaffold for treatment of wound biofilms. Scientific Reports, 9: 2683.
48. Knox, W.E., P.K. Stumpf, D.E. Green and V.H. Auerbach, 1948. The inhibition of sulfhydryl enzymes as the basis of the bactericidal action of chlorine. The Journal of Bacteriology, 55: 451.
49. Barrette, W.C., J.M. Albrich and J.K. Hurst, 1987. Hypochlorous acid-promoted loss of metabolic energy in *Escherichia coli*. Infection and Immunity, 55: 2518.
50. Rani, S.A., R. Hoon, R. Najafi, B. Khosrovi, L. Wang and D. Debabov, 2014. The *in vitro* antimicrobial activity of wound and skin cleansers at nontoxic concentrations. Advances in Skin and Wound Care, 27: 65-69.
51. Ramey, D.W. and H. Kinde, 2015. Commercial and homemade extremely dilute hypochlorous acid solutions are bactericidal against *Staphylococcus aureus* and *Escherichia coli In vitro*. Journal of Equine Veterinary Science, 35: 161-164.
52. McKenna, S.M. and K.J. Davies, 1988. The inhibition of bacterial growth by hypochlorous acid: Possible role in the bactericidal activity of phagocytes. The Biochemical Journal, 254: 685.
53. CRC, 1978. Handbook of Chemistry and Physics. Chemical Rubber Company Handbook of Chemistry and Physics.
54. Jones, K.E., N.G. Patel, M.A. Levy, A. Storeygard, D. Balk, J.L. Gittleman and P. Daszak, 2008. Global trends in emerging infectious diseases. Nature, 451: 990-993.
55. Cooper, R. and K. Kirketerp-Moller, 2018. Non-antibiotic antimicrobial interventions and antimicrobial stewardship in wound care. Journal of Wound Care, 27: 355-377.
56. Naseri-Nosar, M. and Z.M. Ziora, 2018. Wound dressings from naturally-occurring polymers: A review on homopolysaccharide-based composites. Carbohydrate Polymers, 189: 379-398.
57. Graf, C., D.L.J. Vossen, A. Imhof and A. Van Blaaderen, 2003. A general method to coat colloidal particles with silica. Langmuir, 19: 6693-6700.
58. Zhang, L., F.X. Gu, J.M. Chan, A.Z. Wang, R.S. Langer and O.C. Farokhzad, 2008. Nanoparticles in medicine: therapeutic applications and developments. Clinical Pharmacology and Therapeutics, 83: 761-769.

59. Chaudhry, Q., M. Scotter, J. Blackburn, B. Ross, A. Boxall, L. Caste, Aitken R, Watkins R. 2008. Applications and implications of nanotechnologies for the food sector. Food Additives and Contaminants. Part A: Chemistry, Analysis, Control, Exposure and Risk Assessment, 25: 241-258.
60. Mohler, J.S., W. Sim, M.A.T. Blaskovich, M.A. Cooper and Z.M. Ziora, 2018. Silver bullets: A new lustre on an old antimicrobial agent. Biotechnology Advances, 36: 1391-1411.
61. Sim, W., R.T. Barnard, M.A.T. Blaskovich and Z.M. Ziora, 2018. Antimicrobial silver in medicinal and consumer applications: A patent review of the past decade (2007–2017). Antibiotics, 7: 93.
62. Martinez-Gutierrez, F., P.L. Olive, A. Banuelos, E. Orrantia, N. Nino, E.M. Sanchez, F. Ruiz, H. Bach and Y. Av-Gay, 2010. Synthesis, characterization and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. Nanomedicine, 6: 681-688.
63. Martinez-Gutierrez, F., E.P. Thi, J.M. Silverman, C.C. de Oliveira, S.L. Svensson, A. Vanden Hoek, E.M. Sanchez, N.E. Reiner, E.C. Gaynor, E.L. Pryzdial, E.M. Conway, E. Orrantia, F. Ruiz, Y. Av-Gay and H. Bach, 2012. Antibacterial activity, inflammatory response, coagulation and cytotoxicity effects of silver nanoparticles. Nanomedicine, 8: 328-336.
64. Rai, M., A. Yadav and A. Gade, 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv, 27(1): 76-83.
65. Lansdown, A.B., 2002. Silver. I: Its antibacterial properties and mechanism of action. Journal of Wound Care, 11: 125-130.
66. Brayner, R., R. Ferrar-Iliou, N. Brivois, S. Djediat, M.F. Benedetti and F. Fievet, 2006. Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Letters, 6: 866-870.
67. Pasquet, J., Y. Chevalier, J. Pelletier, E. Couval, D. Bouvier and M.A. Bolzinger, 2014. The contribution of zinc ions to the antimicrobial activity of zinc oxide. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 457: 263-274.
68. Brunner, T.J., P. Wick, P. Manser, P. Spohn, R.N. Grass, L.K. Limbach, A. Bruinink and W.J. Stark, 2006. *In vitro* cytotoxicity of oxide nanoparticles: comparison to asbestos, silica and the effect of particle solubility. Environmental Science and Technology, 40: 4374-4381.
69. Zhang, L., Y. Ding, M. Povey and D. York, 2008. ZnO nanofluids–A potential antibacterial agent. Progress in Natural Science, 18: 939-944.
70. Jenkinson, H.F., 2011. Beyond the oral microbiome. Environmental Microbiology, 13: 3077-3087.
71. Baker, J.L., B. Bor, M. Agnello, W.Shi and X. He, 2017. Ecology of the Oral Microbiome: Beyond Bacteria. Trends in Microbiology, 25: 362-374.
72. Elias, S. and E. Banin, 2012. Multi-species biofilms: living with friendly neighbors. FEMS Microbiology Reviews, 36: 990-1004.
73. Shakerimoghaddam, A., E.A. Ghaemi and A. Jamalli, 2017. Zinc oxide nanoparticle reduced biofilm formation and antigen 43 expressions in uropathogenic *Escherichia coli*. Iranian Journal of Basic Medical Sciencs, 20: 451-456.
74. Bhattacharyya, P., B. Agarwal, M. Goswami, D. Maiti, S. Baruah and P. Tribedi, 2018. Zinc oxide nanoparticle inhibits the biofilm formation of *Streptococcus pneumoniae*. Antonie Van Leeuwenhoek, 111(1): 89-99.
75. Kim, B., D. Kim, D. Cho and S. Cho, 2003. Bactericidal effect of TiO_2 photocatalyst on selected foodborne pathogenic bacteria. Chemosphere, 52: 277-281.
76. Matsunaga, T., R. Tomoda, T. Nakajima and H. Wake, 1985. Photoelectrochemical sterilization of microbial cells by semiconductor powders. FEMS Microbiology Letters, 29: 211-214.
77. Yadav, S. and G. Jaiswar, 2016. Review on Undoped/Doped TiO_2 Nanomaterial; Synthesis and Photocatalytic and Antimicrobial Activity. Journal of Chinese Chemical Society, 64: 103-116.
78. Chorianopoulos, N.G., D.S. Tsoukleris, E.Z. Panagou, P. Falaras and G.J. Nychas, 2011. Use of titanium dioxide (TiO_2) photocatalysts as alternative means for Listeria monocytogenes biofilm disinfection in food processing. Food Microbiology, 28: 164-170.
79. Khan, S.T., J. Ahmad, M. Ahamed, J. Musarrat and A.A. Al-Khedhairy, 2016. Zinc oxide and titanium dioxide nanoparticles induce oxidative stress, inhibit growth and attenuate biofilm formation activity of *Streptococcus mitis*. Journal of Biological Inorganic Chemistry, 21: 295-303.
80. Jesline, A., N.P. John, P.M. Narayanan, C. Vani and S. Murugan, 2015. Antimicrobial activity of zinc and titanium dioxide nanoparticles against biofilm-producing methicillin-resistant *Staphylococcus aureus*. Applied Nanosciencs, 5: 157-162.

81. Kim, C.H., E.S. Lee, S.M. Kang, De Josselin, E. De Jong and B.I. Kim, 2017. Bactericidal effect of the photocatalystic reaction of titanium dioxide using visible wavelengths on *Streptococcus mutans* biofilm. Photodiagnosis and Photodynamic Therapy, 18: 279-283.
82. Lopes, F.S., J.R. Oliveira, J. Milan, J.P.B. Machado, V.J. Trava-Airoldi, A.O. Lobo and F.R. Marciano, 2017. Biomineralized diamond-like carbon films with incorporated titanium dioxide nanoparticles improved bioactivity properties and reduced biofilm formation. Mater Science Engineering C - Materials for Biological Applications, 81: 373-379.
83. Zasloff, M., 1987. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms and partial cDNA sequence of a precursor. Proceedings of the National Academy of Sciences. U.S.A., 84: 5449-5453.
84. Gutsmann, T., S.O. Hagge, A. David, S. Roes, A. Bohling, M.U. Hammer and U. Seydel, 2005. Lipid-mediated resistance of Gram-negative bacteria against various pore-forming antimicrobial peptides. Journal of Endotoxin Research, 11: 167-173.
85. Freudenberg, M.A., S. Tchaptchet, S. Keck, G. Fejer, M. Huber, N. Schutze, B. Beutler and C. Galanos, 2008. Lipopolysaccharide sensing an important factor in the innate immune response to Gram-negative bacterial infections: benefits and hazards of LPS hypersensitivity. Immunobiology, 213: 193-203.
86. Glaser, L., 1973. Bacterial cell surface polysaccharides. Annual Review of Biochemistry, 42: 91-112.
87. Sahl, H.G., R.W. Jack and G. Bierbaum, 1995. Biosynthesis and biological activities of lantibiotics with unique post-translational modifications. European Journal of Biochemistry, 230: 827-853.
88. Guani-Guerra, E., T. Santos-Mendoza, S.O. Lugo-Reyes and L.M. Teran, 2010. Antimicrobial peptides: General overview and clinical implications in human health and disease. Clinical Immunology, 135: 1-11.
89. Zapotoczna, M., E. Forde, S. Hogan, H. Humphreys, J.P. O'Gara, D. Fitzgerald-Hughes, M. Devocelle and E. O'Neill, 2017. Eradication of *Staphylococcus aureus* biofilm infections using synthetic antimicrobial peptides. Journal of Infectious Diseases, 215: 975-983.
90. Augustin, M., T. Ali-Vehmas and F. Atroshi, 2004. Assessment of enzymatic cleaning agents and disinfectants against bacterial biofilms. Biofouling, 7: 55-64.
91. Longhi, C., G.L. Scoarughi, F. Poggiali, A. Cellini, A. Carpentieri, L. Seganti, P. Pucci, A. Amoresano, P.S. Cocconcelli, M. Artini, J.W. Costerton and L. Selan, 2008. Protease treatment affects both invasion ability and biofilm formation in *Listeria monocytogenes*. Microbial Pathogenesis, 45: 45-52.
92. Schindler, C.A. and V.T. Schuhardt, 1965. Purification and properties of lysostaphin-a lytic agent for *Staphylococcus aureus*. Biochimica et Biophysica Acta, 97: 242-250.
93. Dakheel, K.H., R.A. Rahim, V.K. Neela, J.R. Al-Obaidi, T.G. Hun, M.N.M. Isa and K. Yusoff, 2019. Genomic analyses of two novel biofilm-degrading methicillin-resistant Staphylococcus aureus phages. BMC Microbiology, 19: 114.
94. Stewart, P.S. and J.W. Costerton, 2001. Antibiotic resistance of bacteria in biofilms. Lancet., 358: 135-138.
95. Hoffman, L.R., D.A. D'argenio, M.J. MacCoss and Z. Zhang, 2005. Aminoglycoside antibiotics induce bacterial biofilm formation. Nature, 436: 1171.
96. Rohwer, F., 2003. Global phage diversity. Cell., 113: 141.
97. Breitbart, M., L. Wegley, S. Leeds, T. Schoenfeld and F. Rohwer, 2004. Phage community dynamics in hot springs. Applied Environmental Microbiology, 70: 1633-1640.
98. Filee, J., F. Tetart, C.A.Suttle and H. Krisch, 2005. Marine T_4-type bacteriophages, a ubiquitous component of the dark matter of the biosphere. Proceedings of National Academy of Sciences. U.S.A., 102: 12471-12476.
99. Pincus, N.B., J.D. Reckhow, D. Saleem, M.L. Jammeh, S.K. Datta and I.A. Myles, 2015. Strain specific phage treatment for *Staphylococcus aureus* infection is influenced by host immunity and site of infection. PLoS One, 10: e0124280.
100. O'Flaherty, S., R.P. Ross and A. Coffey, 2009. Bacteriophage and their lysins for elimination of infectious bacteria. FEMS Microbiology Reviews, 33: 801-819.

101. Keary, R., O. McAuliffe, R.P. Ross, C. Hill, J. O'Mahony and A. Coffey, 2014. Genome analysis of the Staphylococcal temperate phage DW2 and functional studies on the endolysin and tail hydrolase. Bacteriophage, 4: e28451.
102. Rodriguez-Rubio, L., B. Martinez, D.M. Donovan, A. Rodriguez and P. Garcia, 2013. Bacteriophage virion-associated peptidoglycan hydrolases: potential new enzybiotics. Critical Reviews in Microbiology, 39: 427-434.
103. Zhang, L., X. Li, L. Hu, M. Cheng, F. Xia, P. Gong, P. Gong, B. Wang, J. Ge, H. Zhang, R. Cai, Y. Wang, C. Sun, X. Feng, L. Lei, W. Han and J. Gu, 2016. LysGH15 kills Staphylococcus aureus without being affected by the humoral immune response or inducing inflammation. Scientific Reports, 6: 29344.