Environmental and Experimental Biology (2021) 19: 121–130
http://doi.org/10.22364/eeb.19.12

Review

Bacterial biofilms: formation, advantages for community members, clinical implications, and antibiotic resistance

ISSN 2255-9582

Samia Aliane[1], Amina Meliani[2]

[1]Geo-environment and space development laboratory (LGEDE), University of Mustapha Stambouli, Mascara, Algeria
[2]Faculty of Nature and Life Sciences, University of Mustapha Stambouli, Mascara, Algeria

*Corresponding author, E-mail: samia.aliane@univ-mascara.dz

Abstract

Microbial biofilms offer several advantages to cells forming the biofilm community, such metabolic cooperation, genetic diversity, protection against environmental hostilities and a high resistance and tolerance potential. In addition, biofilm-forming bacteria present the most important cause of infection in human. Biofilm-assciociated infections are characterized by the development of biofilm in human tissues or medical devices, which makes these infections more difficult to cure and helps bacteria to acquire new pathogenesic features such as antibiotic resistance. Antibiotic resistance is considered as the most important threat for global health, which affects the treatment outcomes, costs, disease extention, and illness duration. In this review, we discuss an overview of biofilm formation, and advantages ensured to microbial cells. Furthermore, clinical implications of biofilms and antibiotic resistance profiles acquired are also highlighted.

Key words: antibiotic resistance, biofilm-forming bacteria, infections.
bbreviations: EPS, extracellular polymeric substances; VAP, ventilator-associated pneumonia.

Introduction

Microorganisms are universally capable of colonizing biotic and abiotic surfaces. This ability leads to the appearance of a structured organization known by the term "biofilm" (Davey, O'Toole 2000). Biofilms are structured communities of microorganisms attached to a surface and included in an extracellular matrix (Carpentier, Cer 1993; Flemming, Wuert 2019). Various definitions of the term biofilm have been reported, Costerton et al. (1999) proposed a basic meaning for biofilm: "polymeric matrix, adhered to inert or biotic surfaces". However, Tamilvanan et al. (2008) defined microbial biofilm as a microcosm irreversibly attached to biotic and abiotic surfaces as a simple or complex population. Both of these definitions include three major components: microorganisms, glycocalyx, and surface. Without one of these, development of a biofilm will not occur. Glycocalyx is the glue that brings to gether the biofilm. It is composed of a complex of exopolysaccharides of bacterial origin and trapped exogenous substances including nucleic acids, proteins, and minerals etc. (Tamilvanan 2010). This sessile mode offers numerous advantages to the microorganism community compared to the planktonic mode (Balaban et al. 2008).

Biofilms are characterized by a high heterogeneity, which can be associated to species and matrix components diversity (Charackilis et al. 1990; Balaban et al. 2008). The organization in "biofilm" is an adaptive, protective, and resistive response to different hostilities encountered in their environment. It should be noted that this bacterial differentiation involves a set of changes in their behavior, metabolism, expression of virulence factor, and resistance to host defenses (Davey, O'Toole 2000).

According to the World Health Organization (WHO), at present, antibiotic resistance is considered as one of the major problems that threatens public health (Pacios et al. 2020). The total number of deaths generated by this phenomenom is expected to rise to 10 million per year (Cattoir, Felden 2019). Antimicrobial resistance is defined as a natural phenomenom in which the developed bacteria resist the action of drugs, and make them ineffective (Annunziato 2019). The most important multidrug-resistant opportunistic microorganisms are species of the ESKAPE group: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa* and *Enterobacter* spp (Cattoir, Felden 2019).

The prevalence of multidrug-resistance organisms is increasing and associated with a high potential of mortality and morbidity in patients affected (Romandini et al. 2021). Moreover, infections caused by resistant organisms have an impact on treatment outcomes, costs, disease extention,

and their duration (Prestinaci et al. 2015; Mulu et al. 2017). Resistance genes affect the fitness of pathogens and their virulence. In addition, bacterial resistance in a pathogen can generate a delay in the administration of appropriate antibiotherapy and make the antibiotherapy toxic or inadequate (Cosgrove, Carmeli 2003).

Persistant infections caused by biofilm-growing bacteria can be associated with a device or a tissue. The biofilm persistance is associated with their multifactorial intrinsic tolerance toward antimicrobial agents and the immune system. The persistence of biofilm leads to reoccurrence of the infections and repeated antibiotic therapy (Ahmed et al. 2018). It should be noted that clinical biofilms are responsible for the main microbial infections in humans and it is considered as one of pathogens features in clinical strains that makes antibiotherapy more and more difficult with a remarkable reduction of host immunoresponse. It was described that the biofilm cells are more resistant to antibiotics than their counterparts (Madsen et al. 2012; Mittal et al. 2015; Lima et al. 2018; Sharma et al. 2019).

This review attempts to discuss the the different stages of biofilm formation and the advantages ensured to microbial cells. Clinical implications of biofilm are the most important interest in study of biofilms. It should be noted that many diseases and infections are associated with biofilms. Moreover, biofilm-forming bacteria acquire new traits such as antibiotic resistance, which causes antibiotic ineffectiveness and makes infections more and more difficult to cure.

Biofilm formation stages

The formation of a biofilm is a gradual structuring with high complexity, in which the microorganisms change their planktonic mode to acquire a new form (the sessile form). It has been assumed that the formation of a biofilm depends on the expression of specific genes (Sauer et al. 2004; Okada et al. 2005).

Biofilm can be formed on a wide variety of surfaces including living tissue, abiotic surfaces, and medical devices. Biofilm formation can be divided into four stages: (1) bacterial adhesion, (2) formation of microcolonies and colonization, (3) maturation of the biofilm and (4) detachment and dispersion of the biofilm (Muhsin et al. 2015; Asbury, Jazayeri 2018) (Fig. 1).

Bacterial adhesion

When bacteria are in proximity to certain surfaces or supports, they attach to surfaces in a reversible and irreversible manner, with reduced mobility. Bacteria can strongly adhere to a surface and to microorganisms previously adhered to the surface (Costerton et al. 1999; Haras 2005; Muhsin et al. 2015). Certain variables involved in this stage are related to the properties of the substratum, fluid and cells (Donlan 2002).

Initial transport and reversible adhesion to a surface can occur through sedimentation and Brownian movement of microorganisms. It should be highlighted that reversible attachment results in a balanced distribution between adherent cells and those in suspension (Tamilvanan 2010). This process is governed by non-covalent forces where the bacteria show rather weak Van der Walls type, electrostatic, hydrophobic, and steric interactions with the conditioned surface. The presence of locomotive structures at the cell surface, such as flagella, pili, proteins as well as extracellular polymeric substances (EPS), can be involved in bacterial adhesion (Donlan, Costerton 2002; Haras 2005; Muhsin et al. 2015; Asbury, Jazayeri 2018).

On the other hand, irreversible adhesion requires strong covalent interactions, due to the biomolecules present on the bacterial surface (exopolysaccharides, proteins, and lipopolysaccharides) (Haras 2005). Irreversible adhesion is time-dependent and requires the ability to express certain genes that allow microorganisms to acquire new adhesive structures. These structures are necessary for the establishment of bacterial interactions and thus for the structuration in biofilm (Khalilzadeh 2009).

The movement of individuals during bacterial adhesion

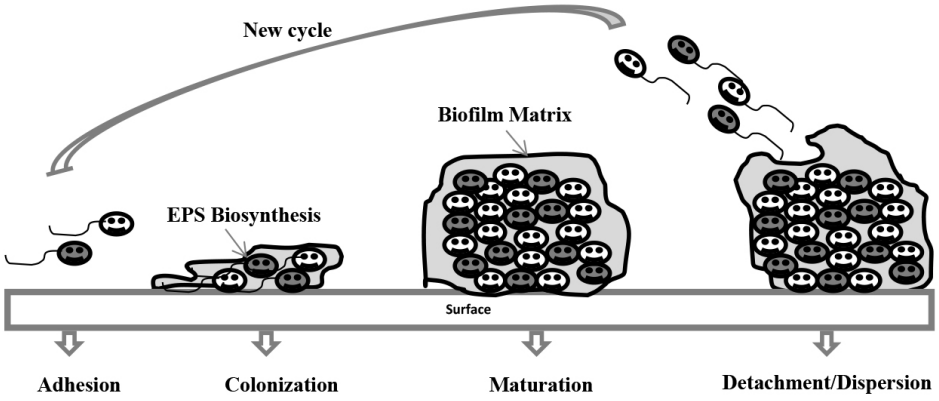

Fig. 1. Biofilm formation stages.

and subsequently the formation of the biofilm promotes the search for a niche favourable to their development, which involves different mechanisms of competition for resources and defense or flight from their predators (Ayé 2015).

Colonization

Once the bacteria are irreversibly adhered to a surface, the final stage of the biofilm formation process begins with the colonization of the surface. This stage is associated with bacterial growth and the expression of the mucoid phenotype, which is the origin of microcolonies. These microcolonies are considered as the basic unit of biofilm, a structural and biological entity with the ability to express specific immunogenicity (Tamilvanan 2010; Ayé 2015; Toyofuku et al. 2016).

Colonization can be divided into five crucial phases of biomass growth (Capdeville, Nguyen 1990; Khalilzadeh 2009): (1) latency phase includes initial adhesion with a period of bacterial adaptability to new living conditions; (2) acceleration phase during which an increase in biomass is observed as well as a high consumption of nutrients (nitrogen and others) associated with the adaptive responses; (3) linear accumulation phase refers to the maximum rates of production of biomass, proteins, and polysaccharides within the biofilm; (4) slowing phase conditioned by the nature and availability of the substrate; and (5) apparent stabilization phase in which a dynamic equilibrium is observed between the biomass loss (bacterial detachment and mortality) and bacterial persistence (Heydorn et al. 2000).

The level of colonization is multiparametric and depends on the properties of microorganisms, surface and environmental conditions (temperature, substrates, etc.) (Aye 2015). Morover, the trapping of planktonic cells in exopolysaccharides contributes to the formation of the biofilm; the exopolysaccharides constitute the framework of the biofilm and approximately 90% of its dry weight (Toyofuku et al. 2016). Surface colonization by a particular bacterium or species (primary colonizers) can influence the attachment of other bacteria (secondary colonizers) (Tamilvanan 2010).

Biofilm maturation

Within the biofilm, bacterial growth is reflected by exponential multiplication until reaching a three-dimensional structure (Doghri 2015). A complete biofilm has a rather complex architecture consisting of the inclusion of the bacteria of the biofilm in the EPS while generating microcolonies interspersed with less dense regions including water channels that are very permeable and transport both nutrients and waste (Tamilvanan 2010).

The maturation stage is divided into two phases. The first phase is characterized by genetic regulation, which allows the phenotypic differentiation of biofilm cells. The second is marked by active biosynthesis of the organic matrix exopolymers. This matrix occupies approximately 75 to 95% of the volume of the biofilm and production of the exopolymers is genetically controlled (Tamilvanan 2010; Doghri 2015).

Detachment and dispersion

Undoubtedly, there are times when it is advantageous for cells to escape the biofilm, the environmental challenge that limits bacterial growth in the biofilm (Petrova, Sauer 2016). It is suggested that the mature biofilm transcriptome is more similar to stationary phase cells than to exponential phase cultures. In addition, the biofilm matrix can prevent or at least deter cell leakage from deleterious conditions. Dispersal has the potential to promote the spread of bacteria in the environment and thus to exploit these processes to control harmful biofilms. Certain environmental conditions can influence the dispersion of the biofilm, including available nutrients, oxygen levels, and pH etc. (Goller, Romeo 2008).

The detachment of the biofilm has been recently described as the dispersion or the dissolution signifying the return to the planktonic form. It has been suggested that turbulent shear forces can lead to cell detachment from the biofilm and their subsequent transport to colonize new surfaces. This step constitutes a programme defect with quorum quenching regulation, which is induced by environmental stress (Costerton et al. 1999; Tamilvanan 2010; Muhsin et al. 2015).

Some studies have described various mechanisms that lead to the loss of cellular integrity, such as the depletion of oxygen within the thick biofilm, the hydrodynamic conditions creating shear forces, and changes and modifications in the composition of nutrients and thus their exhaustion (Sauer et al. 2002; Ayé 2015). It should be highlighted that the dispersion of the biofilm is part of the process of renewal of the microbial biomass (Khalilzadeh 2009).

The dispersive mechanism is caused by the release of extracellular enzymes, which work by degrading the extracellular matrix of the biofilm and releasing the bacteria retained inside the community, as well as by the disruption of non-covalent interactions between the components of the matrix by molecular amphipathic agents that reduce surface tension or the formation of cavities within biofilm caused by cell autolysis (Guilhen et al. 2017). The phenotypic characters are markedly affected by the dispersion of the biofilm. Dispersed cells have the ability to retain certain properties of the biofilm, such as resistance to antibiotics (Muhsin et al. 2015).

Dispersal of the biofilm virtually goes through three stages: first cells detach from the colony, and then these cells begin to translocate to new sites and attach to the new surface. It is also known that the dispersion is a consequence of two categories of mechanisms. Active dispersion depends on physical factors such as fluid shear force,

collision of solid particles with the biofilm, predation, and human intervention etc. and passive dispersion is induced by variations in environmental conditions like temperature and lack of oxygen etc. (Kaplan 2010; Toyofuku et al. 2016).

Biofilm advantages for bacteria

Several bacteria have showed the ability to switch between planktonic and biofilm modes. Although the floating form offers the bacteria quite a bit of growth, still the biofilm is the natural and dominant phenotype. Several advantages can be associated with this structuration (Rabin et al. 2015).

Biofilm as a metabolic strategy

The ability of the microorganisms to use the resources of the environment is ensured through metabolic cooperation. This process takes place between specialized cells forming a colony, and in biofilm-forming colonies that settle on a surface (Cavaliere et al. 2017). Morover, water channels present an efficient means to promote circulation and exchange of nutrients with the aqueous phase, making nutrients available and removing metabolites with toxic potential. Multispecific microcolonies can cooperate metabolically (Pandit et al. 2018). This cooperation is conditioned by proximity between species. For example, the degradation of complex organic matter into methane and carbon dioxide under anaerobic conditions involves at least three bacteria (fermentatives catabolize organic matter into acids for use by acetogenic, and methanogenic bacteria). Then the methanogens derive their energy from the conversion of acetate, carbon dioxide and hydrogen into methane (Kokare et al. 2009). Therefore, effective cooperations and mutual dependencies can evolve within a biofilm. In other words, the biofilm provides an ideal environment for establishing syntrophic relationships (Davey, O'Toole 2000).

When microbial cells organize as a community, the community members cooperate in order to share many benefits. Several cooperators produce a large variety of virulence factors, which are used in order to damage the host, known as "public goods". Among these products, there are enzymes involved in food resources digestion (for nutrients uptaking), surfactants which are implicated in bacterial motility, and siderophors used as iron uptaking molecules (Czàràn, Hoekstra 2009).

However, this type of interaction is not stable because uptake of each community member is selfish to suit their needs. The main problem of cooperation is that it helps the producer individual to increase its reproductive success and gene transmission to the next generations. In parallel, it reduces population productivity (West et al. 2006).

Since several species are cheaters, they thrive at the expense of cooperators. Their presence disrupts the cooperation. Their over-presence threatens the managing of the populations needs. It is also interested that studies have confirmed that a separated culture allows the cooperators to have a growth advantage over the cheaters, whereas in the mixed culture the cheaters exploit their cooperator partner and proliferate (Abisado et al. 2018). Individual cells secrete signalling molecules and when the concentration of these molecules reaches a threshold, the cells respond by modifying the target genes. Thus, bacteria can sense the local density, which allows cooperating their responses (Czàràn, Hoekstra 2009).

Biofilm as a genetic strategy

The structure of the biofilm, where cells are in close proximity, is likely to promote genetic exchange. Mechanisms involved in the process of genetic exchange in a biofilm include conjugation, transformation, and transduction (Houvion 2014). Horizontal gene transfer is very important for evolution and genetic diversity in microbial communities. The appearance and emergence of bacterial multidrug resistance, as well as the extensive use of antibiotics to improve animal growth and the use of genetically modified microorganisms in industrial processes has required the study of gene transfer (Davey, O'Toole 2000).

Reproductive fitness has an advantage in perpetuating the genetic material of an organism (Jefferson 2004). A few studies have presented arguments supporting the frequency of horizontal gene transfers in the sessile form as well as in the planktonic form. It was reported that horizontal genetic transfer of mobile plasmids allow them to persist as molecular parasites, while other genetic elements are transmitted vertically (Madsen et al. 2012).

The acquisition of the new genetic traits increases the probability of the population to copy their essential genes in order to become animate members of the biofilm. Many studies have showed that the gene expression of certain phenotypes is high within the biofilm compared to their counterparts. As an example, alginate production is increased four fold by biofilm cells (Pandit et al. 2018). More recently, a new study based on a comparative analysis of genome and proteome confirmed that several genes are exclusively expressed in biofilm mode; the same has been shown for biosynthesis of many proteins (Tang et al. 2019).

Biofilm as a protective strategy

Biofilm-resident bacteria are found to be more resistant than planktonic ones to various environmental aggressions such as UV irradiation, shear force, and changes in osmolarity, etc) (Mezoui 2016). Microorganisms are protected against a large number of harmful agents (UV, antibiotics and other antimicrobial agents). This is related not only to the formation of the extracellular matrix (Roy et al. 2018), which acts as an anion exchanger while preventing contact with antimicrobial substances and limits the diffusion of harmful substances from the environment to the biofilm (Prandit et al. 2018), but also to some physiological changes.

The biofilm EPS matrix provides homeostasis to the biofilm-residing bacteria. It should be highlighted that these EPS play structural and functional roles in the bacterial communities. This matrix constitutes a barrier, which prevents the penetration of certain antimicrobial agents by their action as anion exchangers, thereby preventing the diffusion of certain compounds found in the environment inside the biofilm. This characteristic is largely related to the nature of the agent and the EPS matrix. For example, this effect appears in the presence of aminoglycoside (a hydrophilic and positively charged antibiotic) (Kokare et al. 2008).

Biofilm as an adaptive and resistive strategy

We should return to the concept of the biofilm, which is cellular organization within a community. The emergence of primitive biofilms seems to have coincided with the first evidence of an evolutionary transition from unicellular to multicellular organization. This theory suggests that this transition is advantageous for survival in hostile environments. An environmental stress signal induces some genes involved in the adaptive response to have a resistant phenotypic expression to environmental challenges (de la Fuente-Nunez et al. 2013).

The development of resistance by the bacterial community allows having a dominant position in extreme environments. This property enhances the chance of both biofilm and planktonic cells to survive even in concerted attacks of metabolic poison. Two basic concepts highlight the resistance of bacteria to environmental stress. The first is the evolving functioning community and the second is the driving force of these communities that gives them an ambition to colonize different surfaces in permissive ecosystems (Costerton 2007).

When Canadian scientists examined the threat of air borne bacterial attack, they found that drought and ultraviolet irradiation abolished the presence of a planktonic bacterial cell culture within seconds. However, bacterial communities can survive in periodic drought and in the presence of ultraviolet radiation by embedding in their own matrix. Knowing that boiling water kills all bacterial cells except spores, nevertheless thriving biofilms can survive and persist in the Morning Glory pool in Yellow Stone Park, as a microbial community adapted to this constant heat stress in an extreme ecosystem (Staley, Konopka 1985; Costerton 2007).

A rather enormous bacterial biomass has been found in the depths of Lake Vostok (Christner et al. 2001; Costerton 2007), whose surface has been sealed by ice for millions of years. Extreme heat, extreme cold and drought are known as "extreme environments". Bacterial communities living in tidal, hot spring and dry or wet Antarctic ecosystems are known for lush growth and "high living", but any planktonic cells outside their perimeter will be lost (Costerton 2007).

Clinical implication of bacterial biofilms

Approximatively 80% of microbial infections in humans are associated with bacterial biofilms (Sharma et al. 2019), including device-associated and non device-associated infections (Jamal et al. 2018) (Table 1). Biofilm formation is one of pathogenesis traits in clinical strains, which is why biofilm-associated infections are more difficult to treat using antibiotherapy, particularly considering the reduction of the action of the host immune responses (Mittal et al. 2015; Lima et al. 2018). It should be noted that biofilm cells are more resistant to antibiotic than planktonic cells (Madsen et al. 2012).

The contamination of catheters and prosthesis induces biofilm formation with high tolerance to biocides and generates systemic infections. It is clear that bacterial cells included in biofilms are more resistant to immune reactions (Lebeaux, Ghigo 2012).

Bacteria-forming biofilm adhered to the uroepithelium can invade renal tissue causing pyelonephritis and be responsible for chronic bacterial prostatitis. It was reported that biofilm could adhere to catheters, highlighting that catheter-associated urinary tract infections is one of the most common care-associated infections around the world (Soto 2014).

Moreover, biofilms formed on medical implants (catherters, heart valves, contacts lenses, joint prosthesis, intra uterine devices, and dental unity) cause urinary tract and bloodstream infections. The treatment of these infections is based on the removal of medical implants, which increases the cost of treatment (Sharma et al. 2018).

An earler study has demonstrated biofilm formation in acute human wounds. It has been observed that *Staphylococcus aureus* biofilms were present in specimens isolated from patients with skin diseases like bullous impetigo, atopic dermatitis, and pemphigus foliaceus (Akiyama et al. 2003).

Mechanical ventilation is an artificial ventilation method used to ensure the maintenance of gas exchange essential in patients suffering from respiratory and metabolic weakness. This therapeutic support exposes patient to aquire ventilator-associated pneumonia (VAP). Biofilm formation by bacteria that cause VAP contributes to VAP pathogenesis and makes the antimicrobial therapy more difficult, increasing morbidity and mortality associated with this infection (Lima et al. 2017).

Periodontitis is an infection of the gums, in which soft tissue and that of bones supporting the teeth are damaged. It should be noted that it is caused by poor oral hygiene. The main causative agents are *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. These microorganisms can form biofilm on a variety of surfaces, especially mucosal surfaces in the oral cavity (Lamont, Jenkinson 1998; Jamal et al. 2018).

Table 1. Microbial species involved in biofilm-assiociated infections and their adherent surfaces

Bacterial species	Infection / disease	Surface	Reference
Burkholderia cepacia	Cystic fibrosis	Lungs	Murphy, Caraher 2015
Candida spp.	Dental caries	Tooth surface	Salehi et al. 2020
	Oral candidiasis		Salehi et al. 2020
Enterobacter faecalis	Urinary tract infections	Urinary tract	Ch'ng et al. 2018
	Wounds	Skin	Dahl et al. 2019
	Gastrointestinal infection	Gastrointestinal tract	Dahl et al. 2019
	Endocarditis	Heart chamber and valves	Dahl et al. 2019
Escherichia coli	Urinary tract infection	Urinary tract	Sharma et al. 2016
	Catheter-associated urinary tract infections	Catheter	Zafar et al. 2020
	Meningitis	Brain	Bergin et al. 2016
	Neonatal sepsis	Blood	Bergin et al. 2016
Haemophilis influenzae	Acute otitis media	Middle ear	Vermee et al. 2019
Klebsiella pneumoniae	Nosocomial infections		Seifi et al. 2016
	Urinary tract infection	Urinary tract	Sharma et al. 2019
	Pyogenic liver abscess	Liver	Sharma et al. 2019
	Hospital-aquired pneumonia	Lungs	Seifi et al. 2016
	Ventilator-associated pneumonia	Lungs	Seifi et al. 2016
	Bacteremia		Seifi et al. 2016
	Septicemia		Seifi et al. 2016
Mycobacterium tuberculosis	Tuberculosis	Lungs	Esteban, Garcia-Coca 2018
Pseudomonas aeruginosa	Device-related infections	Device surfaces	Maurice et al. 2018
	Cystic fibrosis	Lungs	Maurice et al. 2018
	Otitis media	Middle ear	Maurice et al. 2018
Staphylococcus aureus	Nosocomial infections	Device surfaces	Balaban et al. 2007
	Mortality		Zapotoczna et al. 2017
	Morbidity		Zapotoczna et al. 2017
	Device-related infection	Human tissues	Zapotoczna et al. 2017 Kong et al. 2018
Staphylococcus epidermidis	Device-related infection	Skin	Balaban et al. 2006
	Bacteremia	Mucous membranes	Fey, Olson 2010
	Endocarditis		França et al. 2016
Streptococcus mutans	Dental caries	Tooth surface	Salehi et al. 2020
Streptococcus pneumoniae	Acute otitis media	Middle ear	Vermee et al. 2019

Cystic fibrosis is defined as an autosomal recessive genetic condition caused by a mutation on human chromosome 7. Biofilm formation is considered as the main important patho-physiological features in *Pseudomonas aeruginosa*. It was reported that the pathophysiological conditions within the lung of a cystic fibrosis patient are favourable for the development of biofilm. Many studies have reported that the structuration in biofilm enhances the protection of the encased bacteria from antimicrobial agents and immunological responses of the host (Asbury, Jazayeri 2018).

Biofilm-mediated antimicrobial resistance

Heterogeneity in a bacterial community increases the survival chances. Bacterial growth and metabolic activity in biofilm are affected by nutrients and oxygen availability (Rodis et al. 2020). The biofilm physiochemical heterogeneity generating physiological heterogeneity leads to the appearance of sub-populations that are genetically similar but physiologically different, especially their tolerance to antibiotics. The frequent presence of different species in biofilm represents the origin of the biological heterogeneity (Lebeaux, Ghigo 2012).

Biofilm formation is recalcitrant to antibiotic treatment and the main cause of persistant infections by major clinical pathogens. Biofilm formation and cell entrapment in biofilm matrix enhance the resistance to antibiotics (Abebe 2020). The high resistance of biofilm to antibiotics has attracted the attention of clinical microbiologists (Fuente-Nunez et al. 2013). Several mechanisms have been proprosed to explain the remarkable resistance of biofilm forming-bacteria to antibiotics and phagocytosis (Li et al. 2020).

We have previously mentioned that the biofilm EPS matrix has a key role in biofilm structuration, and that it

acts as a barrier to different antibiotics. Biofilm tolerance to antibiotics increases through their inactivation by biofilm matrix, but some antibiotics that do not interact with EPS can penetrate through biofilms. Other reasons that inhibit antibiotic diffusion are antibiotic degradation by enzymes present in the biofilm matrix and antibiotic chelation by matrix components, which is why antibiotics can lose their ability to reach the target at an adequate concentration (Goel et al. 2021).

In a study of a *Pseudomonas aeruginosa* biofilm on a dialysis membrane, piperacillin diffusion into the biofilm was measured. This study showed that the *Pseudomonas aeruginosa* biofilm prevented antibiotic diffusion. However, in the same conditions, *Staphycoccus epidermidis* biofilm allowed rifampicin and vancomycin diffusion (Mah, O'Toole 2001).

It has been reported that there is a correlation between biofilm formation and PER1-β-lactamases production (Lee et al. 2007). The β-lactamases have the ability to inactivate the β-lactam antimicrobials by breaking the β-lactam ring, distrupting its amide bond, causing loss of antibacterial activity. Morover, antibiotic hydrolysis by β-lactamase activity involves ester bond formation between the active serine site of the β-lactamase enzyme and the β-lactam ring of the antibiotic (Rocha et al. 2019).

Structural modification of the antibiotic target is the process most involved in bacterial resistance to antibiotics. A study showed that *Staphylococcus aureus* strains have a high resistance to vancomycin due to substitution of dipeptide D-alanine-D-alanine by dipeptide D-alanine-D-lactate. This resistance can be transmited by plasmid transfer carrying the vanA operon of resistance to vancomycin, previously shown in *Enterococcus faecalis* (Daddi Oubekkaa 2012).

The most important traits of biofilm evolution are the higher degree of population diversity and the hypermutator process. The hypermutator phenotype in *Pseudomonas aeruginosa* biofilms was detected in chronic infections like cystic fibrosis where development of resistance during the exposure to antibiotics was observed (Ciofu et al. 2017; Uruén et al. 2020).

Bacteria within a biofilm develop different strategies in order to protect their cells from antibiotic stress, which is why the interaction between antibiotic and biofilm matrix can reduce their activites and their growth rates, casusing the antibiotic to lose its effectiveness and producing persister cells with high tolerance to antibiotics (Abebe 2020).

Persister cells are metabolically inert and high tolerant to antibiotics (Lee et al. 2016). Persisters have an important role in chronic infections and their presence indicates population heterogeneity, which is beneficial to the microbial population in fluctuating environments. It consititutes a survival strategy for microbial populations exposed to enviromental stress such as antibiotics (Posada et al. 2020).

The biofilm survival capacity depends on the formation of sub-populations of persisters, such as phenotypic variants tolerant to various stresses such as antibiotics. It should be noted that tolerance differs from resistance, because it is temporary and reversible. Another point to highlight is that a few cells can be transformed into persister cells in a isogenic population, and inversely, persisters can switch back to be the susceptible state (Carvalho et al. 2017).

Conclusions

In this review, we focused on biofilm development with its different steps, Biofilm formation is an important feature that allows bacteria to stratify metabolic and genetic cooperation, and offers them the ability to survive in hostile enviroments. Futhermor, the presence of biofilms in clinical environments is considered as the most significant threat to public health and the main cause for human infections. Biofilm-associated infections can be related to invasion of human tissues or medical contaminations. These infections make antibiotic therapy more and more difficult, and cause the reoccurrence of infection, and a significant antibiotic resistance profile.

References

Abebe G.M. 2020. The role of bacterial biofilm in antibiotic resistance, and food contamination. *Int. J. Microbiol.* 2020: 1705814.

Abisado R.G., Benomar S., Klaus J.R., Dendekar A.A., Chandler J.R. 2018. Bacterial quorum sensing and microbial community interactions. *mBio* 9: e02331-17.

Ahmed M.N., Porse A., Sommer M.O.A., Hoiby N., Ciofu O. 2018. Evolution of antibiotic resistance in biofilm and planktonic Pseudomonas aeruginosa populations exposed to subinhibitory levels of ciprofloxacin. *mBio* 62: e00320-18.

Akiyama H., Morizane S., Yamasaki O., Oono T., Iwatsuki K. 2003. Assessment of Streptococcus pyogenes microcolony formation in infected skin by confocal laser scanning microscopy. *J. Dermatol. Sci.* 32: 193–199.

Asbury J., Jazayeri J.A. 2018. The significance of *Pseudomonas aeruginosa* infection and biofilms to cystic fibrosis patients: The need for the development of new therapies. *Adv. Biotechnol. Microbiol.* 8: 555742.

Annunziato G. 2019. Strategies to overcome antimicrobial resistance (AMR) making use of non–essential target inhibitors: A review. *Int. J. Mol. Sci.* 20: 5844.

Ayé M. 2015. Demonstration of the quorum sensing communication system involving AHLs in marine bacteria isolated from the mediterranean. Ph.D Thesis, Toulon University, France.

Balaban N., Cirioni O., Giacometti A., Ghiselli R., Braunstein J., Silvestri C., Mocchegiani F., Saba V., Scalise G. 2007. Treatment of Staphylococcus aureus biofilm infection by the quorum sensing inhibitor RIP. *Antimicrob. Agents Chemother.* 51: 2226–2229.

Balaban N., Ren D., Givskov M., Rasmussen T.B. 2008. Introduction. In: Balaban N. (Ed.) *Control of Biofilm Infections by Signal Manipulation*. Springer Verlag, Berlin, pp. 1–11.

Bergin S.P., Thaden J., Ericson J.E., Cross H., Messina J., Clark R.H., Flower V.G., Benjamin D.K., Hornik C.P., Smith P.B. 2016. Neonatal *Escherichia coli* bloodstream infections: clinical, outcomes and impact of initial antibiotictherapy. *Pediatr. Infect. Dis. J.* 34: 933–936.

Bezoui M. 2016. Bacterial biofilms and its implications in human pathology. Ph.D Thesis, Université Mohammed V–Rabat University, Morocco.

Bogino P.C., Oliva M.M., Sorroche F.G., Giordano W. 2013. The role of bacterial biofilms and surface components in plant-bacterial associations. *Int. J. Mol. Sci.* 14: 15838–15859.

Capdeville B., Nguyen K.M. 1990. Kinetics and modeling of aerobic and anaerobic film. *Water Sci. Technol.* 22: 149–170.

Carpentier B., Cerf O. 1993. Biofilms and their consequences with particular reference to hygiene in the food industry. *J. Appl. Bacteriol.* 75: 499–511.

Carrett T.R., Bhakoo M., Zhang Z. 2008. Bacterial adhesion and biofilms on surfaces. *Progr. Nat. Sci.* 18: 1049–1056.

Carvalho G. 2017. Resilience to antibiotics of bacterial biofilms: Concepts, modeling and experimentation. Ph.D Thesis. Clermont Auvergne University, France.

Cattoir V., Felden B. 2019. Future antibacterial strategies: From basic consepts to clinical challenges. *J. Infect. Dis.* 220: 350–360.

Cavaliere M., Feng S., Soyer O.S., Jimenez J.I., 2017. Cooperation in microbial communities and their biotechnological applications. *Environ. Microbiol.* 8: 2949–2963.

Ch'ng J.H., Chong K.K.L., Kline K.A 2018. Biofilm-associated infection by enterococci. *Nat. Rev. Microbiol.* 17: 82–94.

Characklis W.G., Marshall K.C. 1990. Biofilms: a basis for an interdisciplinary approach. In: Characklis W.G., Marshall K.C. (Eds.) *Biofilms*. John Wiley and Sons, pp. 3–15.

Characklis W.G., McFeters G.A., Marshall L.C. 1990. Physiological ecology in biofilm systems. In: Characklis W.G., Marshall K.C. (Eds.) *Biofilms*. John Wiley and Sons, pp. 341–394.

Christner B.C., Mosley–Thompson E., Thompson L.G., Reeve JN. 2001. Isolation of bacteria and 16 S rDNAs from Lake Vostok accretion ice. *Environ. Microbiol.* 3: 570–578.

Ciofu O., Rojo-Molinero E., Macia M.D., Oliver A. 2017. Antibiotic treatment of biofilm infections. *APMIS* 125: 304–319.

Cosgrove S.E., Carmeli Y. 2003. The impact of antimicrobial resistance on health and economic outcomes. *Clin. Infect. Dis.* 36: 1433–1437.

Costerton J.W., Stewart P.S., Greenberg E.P. 1999. Bacterial biofilms: a common cause of persistent infections. *Science* 284: 1318–1322.

Costerton J.W. 2007. *The Biofilm Primer*. Springer, Berlin.

Czàràn T., Hoekstra R.F. 2009. Microbial communication, cooperation and cheating: Quorum sensing drives the evolution of cooperation in bacteria. *PLoS One* 4: 1–10.

Daddi Oubekka S. 2012. Reactional dynamic of antibiotics within Staphylococcus aureus biofilms: contribution of multimodal fluorescence microscopy. Ph.D Thesis, Paris Sud University.

Dahl A., Iversen K., Tonder N., Hoest N., Arpi M., Dalsgaard M., Chehri M., Soerensen L., Fanoe S., Junge S., Hoest U., Valeur N., Lauridsen T., Fosbol E., Hoi-Hansen T., Bruun N.E. 2019. Prevalence of infective endocarditis in *Enterococcus faecalis* bacteremia. *J. Am. Coll. Cardiol.* 74: 193–201.

Davey M.E., O'Toole G.A. 2000. Microbial biofilms: from ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* 64: 847–867

De la Fuente–Nunez C., Reffuveille F., Fernandez L., Hancock R.E.W. 2013. Bacterial biofilm development as a multicellular adaptation: Antibiotic resistance and new therapeutic strategies. *Curr. Opin. Microbiol.* 16: 580–589.

Doghri I. 2015. Molecular interaction between microoganisms within biofilms in the marine environment: demostration of antibiofilmbiomolecules. Ph.D.Thesis, La Rochelle University, France.

Donlan R.M., Costerton J.W. 2002. Biofilms: Survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* 15: 67–193.

Donlan R.M. 2002. Biofilms: microbial life on surfaces. *Emerg. Infect. Dis.* 8: 881–890.

Esteban J., Garcia-Coca M. 2018. Mycobacterium biofilms. *Front. Microbiol.* 8: 1–8.

Flemming HC., Wuertz S. 2019. Bacteria and archaea on Earth and their abundance in biofilms. *Nat. Rev. Microbiol.* 17: 247–260.

França A., Pier G.B., Vilanova M., Cerca N. 2016. Transcriptomic analysis of *Staphylococcus epidermidis* biofilm–released cells up interaction with human blood circulating immune cells and solube factors. *Front. Microbiol.* 7:1–6.

Goel N., Fatima S.W., Kumar S., Sinha R., Khare S.K. 2021. Antimicrobial resistance in biofilms: Exploring marine actinobacteria as a potential source of antibiotics and biofilm inhibitors. *Biotechnol. Rep.* 30: e00613.

Goller CC., Romeo T. 2008. Environmental influences on biofilm development. In: Romeo T. (Ed.) *Bacterial Biofilms*. Springer Verlag, Berlin, pp. 37–66.

Guilhen C., Forestier C., Balestrino D. 2017. Biofilm dispersal: multiple elaborate strategies for dissemination of bacteria with unique properties. *Mol. Microbiol.* 105: 188–210.

Haras D. 2005. Biofilm and material alterations : analysis of the phenomenon and prevention strategies. *Mater. Tech.* 93: 27–41.

Heydorn A., Nielsen A T., Hentzer M., Sternberg C., Givskov M., Ersbøll B.K., Molin S. 2000. Quantification of biofilm structures by the novel computer program comstat. *Microbiology* 146: 2395–2407.

Houvion E. 2014. Dental biofilm: composition, formation and properties. Ph.D Thesis, Lorraine University, France.

Jamal M., Ahmed W., Andleeb S., Jalil F., Imran M., Nawaz M.A., Ali M., Rafiq M., Kamil M.A. 2018. Bacterial biofilm and associated infections. *J. Chin. Med. Assoc.* 81: 7–11.

Jefferson K.K. 2004. What drives bacteria to produce a biofilm?. *FEMS Microbiol. Lett.* 236: 163–173.

Jones P.R., Cottrell M.T., Kirchman D.L., Dexter S.C. 2007. Bacterial community structure of biofilms on artificial surfaces in an Estuary. *Microb. Ecol.* 53: 153–162.

Kaplan J.B. 2010. Biofilm dispersal: Mechanisms, clinical implications, and potential therapeutic uses. *J. Dent. Res.* 93: 205–2018.

Khalilzadeh P. 2009. Biofilm formation *Pseudomonas aeruginosa*: Assessment of potential quorum sensing inhibitors. Ph.D Thesis, Paul Sabatier–Toulouse IIIUniversity, France.

Kokare C.R., Kadam S.S., Mahadi K.R., Khopade B.A. 2007. Studies on bioemulsifier production from marine *Streptomyces* sp. S1. *Indian J. Biotechnol.* 6: 78–84.

Kokare C.R., Chakraborthy S., Khopade A.N., Mahadik K.R. 2009. Biofilm: importance and applications. *Indian J. Biotechnol.* 8: 159–168.

Kong C., Chee C., Richter K., Thomas N., Rahman N.A., Nathan S. 2018. Suppression of *Staphylococcus aureus* biofilm formation and virulence by a benzimidazole derivative, UM–C132. *Sci.*

Rep. 8: 2758.

Kwiecinska–Pirog J., Bogiel T., Gospodarek E. 2013. Effects of ceftazidime and ciprofloxacin on biofilm formation in *Proteus mirabilis* rods. *Antibiotics* 66: 593–597.

Lamont J.R., Jenkison H.F. 2012. Life below the gum line: Pathogenic mechanisms of *Porphyromonas gingivalis*. *Microbiol. Mol. Biol. Rev.* 62: 1244–1263.

Lebeaux D., Ghigo J. 2012. Infections associées aux biofilms. *Med. Sci.* 28: 727–739.

Lee J., Pai H., Kim Y.K., Kim N.H., Eun B.W., Kang H.J., Park K.H., Choi E.H., Shin H.J., Kim E.C., Lee H.J., Ahn H.S. 2007. Control of extended spectrum beta–lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in children's hospital by changing antimicrobial agent usage policy. *J. Antimicrob. Chemother.* 60: 629–637.

Lee J.S., Giesler D.L., Gellad W.F., Fine M.F. 2016. Antibiotic therapy for adults hospitalized with community–acquired pneumonia: A systematic review. *JAMA* 315: 593–602.

Li Y., Xiao P., Wang Y., Hao Y. 2020. Mechanisms and control measures of mature biofilm resistance to antimicrobial agents in the clinical context. *ACS Omega* 5: 22684–22690.

Lima J.L.C., Alves L.R., Paz N.P., Rabelo M.A., Maciel M.A.V., Morais M.M.C. 2017. Analysis of biofilm production by clinical isolates of *Pseudomonas aeruginosa* from patients with v–associated pneumonia. *Braz. J. Infect. Dis.* 29: 310–316.

Lima J.L.C., Alves L.R., Jacomé P.R.L.A., Neto J.P.B., Maciel M.A.V., Morais M.M.C. 2018. Biofilm production by clinical isolates of *Pseudomonas aeruginosa* and structural changes in LasR protein of isolates non biofilm-producing. *Braz. J. Infect. Dis.* 22: 129–136.

Madsen J.S., Burmolle M., Hansen L.H., Sorensen S.J. 2012. The interconnection bewteen biofilm formation and horizontal genetransfer. *FEMS Immunol. Med. Microbiol.* 65: 183–195.

Mah T.F., O'Toole G.A. 2001. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol.* 9: 34–39.

Maurice N.M., Bedi B., Sadikot R.T. 2018. *Pseudomonas aeruginosa* biofilm: Host response and clinical implications in lung infections. *Am. J. Respir. Cell. Mol. Biol.* 58: 428–439.

Mittal S., Sharma M., Chaudhary U. 2015. Biofilm and multidrug resistance in uropathogenic *Escherichia coli*. *Pathog. Glob. Health* 109: 26–29.

Muhsin J., Ufaq T., Tahir H., Saadia A. 2015. Bacterial biofilm: its composition, formation and role in human infections. *Res. Rev. J. Microbiol. Biotechnol.* 4: 1–14.

Mulu W., Abera B., Yimer M., Ayele H., Abate D. 2017. Bacterial agents and antbiotic resistance profiles of infections from different sites that occurred among patients at Debre Markos Referral Hospital, Ethiopia: a cross-sectional study. *BMC Res. Notes* 10: 254.

Murphy M.P., Caraher E. 2015. Residence in biofilms allows *Burkholderia cepacia* complex (Bcc) bacteria to evade the antimicrobial activities of neutrophil–like dHL60 cells. *Pathog. Dis.* 73: ftv069.

Okada M., Sato I., Jeong Cho S., Iwata H., Nishio T., Dubnau D., Sakagami Y. 2005. Structure of the *Bacillus subtilis* quorum-sensing peptide pheromone ComX. *Nat. Chem. Biol.* 1: 23–24.

Pacios O., Blasco L., Bleriot I., Fernandez-Gracia L., Ambroa A., Lopez M., Bou G., Tomas M. 2020. Strategies to combat multi-drug resistant and persistant infectious deseases. *Antibiotics* 9: 65.

Pandit S., Sarode S., Chandrasekhar K. 2018. Fundamentals of bacterial biofilm: Present state of art. In: Kalia V.C. (Ed.): *Quorum Sensing and its Biotechnological Applications*. Springer Nature, Singapore, pp. 43–60.

Petrova O.E., Sauer K. 2016. Escaping the biofilm isprethat one way: desorption, detachment and dispersion. *Curr. Opin. Microbiol.* 30: 67–78.

Posada L., Acosta I., Rodriguez P., Huertas M.G., Zambrano M.M. 2020. Biofilm and persister cell formation variability in clinical isolates of *Klebsiella pneumoniae* in Colombia. *Univ. Sci.* 25: 545–571.

Prestinaci F., Petrizio P., Pantosti A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health* 109: 309–318.

Rabin N., Zheng Y., Opoku–Temeng C., Du X, Bonsu E., Sintim O.H. 2015. Biofilm formation mechanisms and targets for developing antibiofilm agents. *Future Med. Chem.* 7: 493–512.

Rocha F.R., Fehlberg L.C.C., Cordeiro–Moura J.R., Ramos A.C., Pinto V.T., Barbosa F.C.B. 2019. High frequency of extended–spectrum beta-lactamase–production *Klebsiella pneumoniae* nosocomial strains isolated from a teaching hospital in Brazil. *Microbiol. Drug. Resist.* 25: 909–914.

Rodis N., Tsapadikou V.K., Potsios C., Xaplanteri P. 2020. Resistance mechanisms in bacterial biofilm formations: A review. *J. Emerg. Intern. Med.* 4: 30.

Ramandini A., Pani A., Schenardi PA., Pattarino G.A.C., Giacomo C., Scaglione F. 2021. Antibiotic resistance in pediatric infections: Global emerging threats, predicting the near future. *Antibiotics* 10: 393.

Roy R., Tiwari M., Donelli G., Tiwari V. 2018. Strategies for combacting bacterial biofilms: a focus on anti–biofilm agents and their mechanismes of action. *Virulence* 9: 522–554.

Salehi B., Kregiel D., Mahady G., Sharifi-Rad J., Martins N., Rodrigues C.F. 2020. Managment of *Streptococcus mutans-Candida* spp. oral biofilms' infections: Paving the way for effective clinial interventions. *Clin. Med.* 9: 517.

Sauer K., Cullen M.C., Rickard A.H., Zeef L.A., Davies D.G., Gilbert P. 2004. Characterization of nutrient–induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. *Bacteriology* 186: 7312–7326.

Seifi K., Kazemian H., Heidari H., Rezagholizadeh F., Saee Y., Shirvani F., Houri H. 2016. Evaluation of biofilm formation among *Klebsiella pneumonia* isolates and molecular characterization by ERIC–PCR. *Jundishapur J. Microbiol.* 9: e30682.

Sharma G., Sharma S., Sharma P., Chandola D., Dang S., Gupta S., Gabrani R. 2016. *Escherichia coli* biofilm: development and therapeutic strategies. *Appl. Microbiol.* 121: 309–319.

Sharma C., Rokana N., Chandra M., Singh B.P., Gulhane R.D., Gill J.P.S., Rau P., Puniya A.K., Panwar H. 2018. Antimicrobial resistance: its surveillance, imapct, an alternative management strategies in dairy aminals. *Front. Vet. Sci.* 4: 237.

Sharma D., Misba L., Khan A.U. 2019. Antibiotics versus biofilm: an emerging battleground in microbial communities. *Antimicrob. Resist. Infect. Contr.* 8: 76.

Soto S.M. 2014. Importance of biofilm in urinary tract infections: new therapeutic approaches. *Adv. Biol.* 2014: 543974.

Staley J.T., Konopka A. 1985. Measurement of *in situ* activities of non photosynthetic microorganisms in aquatic and terrestrial habitats. *Annu. Rev. Microbiol.* 39: 321–346

Tamilvanan S., Venkateshan N., Ludwig A. 2008. The potential of lipid and polymer-based drug delivery carriers for eradicating biofilm consortia on device-related nosocomial infections. *J. Contr. Release* 128: 2–22 .

Tamilvanan S. 2010. *Biofilm Eradication and Prevention: A Pharmaceutical Approah to Medical Device Infections*. John Wiley & Sons, Hoboken.

Davey M.E., O'Toole G.A. 2000. Microbial biofilms: from ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* 64: 847–867

Tang T., Chen G., Guo A., Zhao L., Wang M., Lu C., Jiang Y., Zhang C. 2019. Comparative proteomic and genomic analyses of *Brucella abortus* biofilm and planktonic cells. *Mol. Med. Rep.* 21: 731–743.

Toyofuku M., Inaba T., Kiyokawa T., Obana N., Yawata Y., Nomura N. 2016. Environmental factors that shape biofilm formation. *Biosci. Biotechnol. Biochem.* 80: 7–12.

Uruén C., Chopo–Escuin G., Tommassen J., Mainar-Jaime R.C., Arenas J. 2020. Biofilms as promoters of bacterial antibiotic resistance and tolerance. *Antibiotics* 10: 3.

Vermee Q., Cohen R., Raymond J. 2019. Biofilm production by *Haemophilus influenzae* an *Streptococcus pneumoniae* isolated from the nasopharynx of children with acute otitis media. *BMC Infect. Dis.* 19: 44.

Wasfi R., Hamed S.M., Amer M.A., Fahmy L.I. 2020. *Proteus mirabilis* biofilm: development and therapeutic strategies. Front. Cell. Infect. Microbiol. 10: 1–14.

West S.A., Griffin A.S., Gardner A., Diggle S.P. 2006. Social evolution theory for microorganisms. *Nat. Rev. Microbiol.* 4: 597–607.

Zafar S., Hanif S., Akhtar H., Faryal R. 2019. Emergence of hypervirulent *K. pneumonia* causing complicated UTI in kidney stone patients. *Microb. Pathog.* 135: 103647.

Zafar M., Tauseef A., SouhaibAsghar M., Khan N., Farooqui N., Dawood M., Alam T., Naman D. 2020. *Escherichia coli*: a rare cause of meningitis in immuno-competent adult. *Commun. Hosp. Intern. Med. Perspect*. 10: 69–72

Zaptoczna M., Forde E., Hogan S., Humphreys H., O'Gara J.P., Fitzgerald-Hughes D., Devocelle M., O'Neill E. 2017. Eradication of *Staphylococcus aureus* biofilm infections using synthetic antimicrobial peptides. *Infect. Dis.* 16: 975–983.

Received 7 July 2021; received in revised form 24 July 2021; accepted 10 September 2021