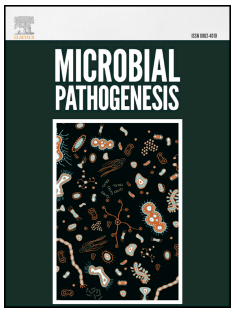
Accepted Manuscript



Vision for medicine: *Staphylococcus aureus* biofilm war and unlocking key's for anti-biofilm drug development

H.M. Manukumar, K.P. Rakesh, C.S. Karthik, P. Mallu, Hua-Li Qin, M. Yasser Hussein Eissa

PII: S0882-4010(18)30613-2

DOI: 10.1016/j.micpath.2018.07.002

Reference: YMPAT 3042

To appear in: *Microbial Pathogenesis*

Received Date: 6 April 2018

Revised Date: 2 July 2018

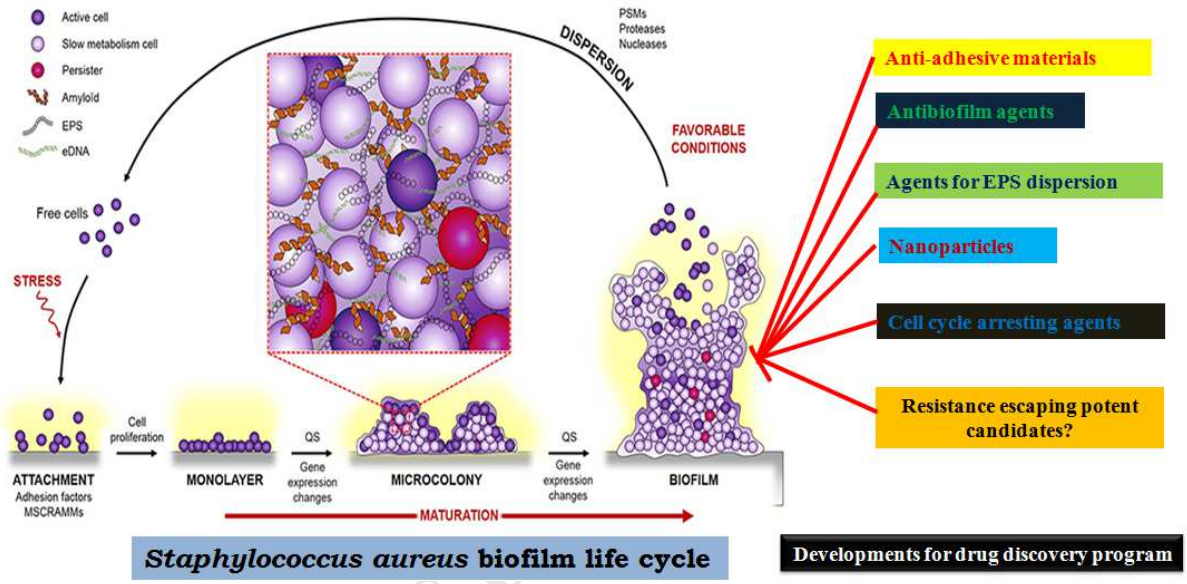
Accepted Date: 2 July 2018

Please cite this article as: Manukumar HM, Rakesh KP, Karthik CS, Mallu P, Qin H-L, Hussein Eissa MY, Vision for medicine: *Staphylococcus aureus* biofilm war and unlocking key's for anti-biofilm drug development, *Microbial Pathogenesis* (2018), doi: 10.1016/j.micpath.2018.07.002.

Graphical Abstract

Vision for medicine: *Staphylococcus aureus* biofilm war and unlocking key's for anti-biofilm drug development

H. M. Manukumar, K. P. Rakesh, C. S. Karthik, P. Mallu, Hua-Li Qin and M. Yasser Hussein Eissa,



Vision for medicine: *Staphylococcus aureus* biofilm war and unlocking key's for anti-biofilm drug development

H. M. Manukumar[1], K. P. Rakesh[2], C. S. Karthik[1], P. Mallu[1*], Hua-Li Qin[2]* and M. Yasser Hussein Eissa[3*]

[1]Department of Chemistry, Sri Jayachamarajendra College of Engineering, Mysuru-570 006, Karnataka, India.

[2]Department of Pharmaceutical Engineering, School of Chemistry, Chemical Engineering and Life Science, Wuhan University of Technology, 205 Luoshi Road, Wuhan, 430070, PR, China.

[3]Department of Biochemistry, Faculty of Applied Science College, University of Hajjah, Yemen.

***Corresponding authors**

Email: drmallu66@gmail.com, Mob: +91 9480057349

E-mail: qinhuali@whut.edu.cn, Fax: +86 27 87749300.

Email: issayasser16@gmail.com

Abstract:

The *Staphylococcus aureus* biofilm-associated burden is challenging to the field of medicine to eradicate or avoid it. Even though a number of *S. aureus* biofilm mechanisms understood and established the possible ways of biofilm formation but, still need to know more and require a development of new therapeutic strategies. In this viewpoint, we discuss the underlining biofilm mechanism, its existing systems as active therapeutic agents and as vehicles to transport drugs to the site of infection. The step-back in drug development is due to the emergence of antibiotic-resistant *S. aureus*. The understanding of bacteria/biofilms is an aspect that we likewise summarize for possible drug development for future as medicine against resistant *S. aureus* was viewed.

Keywords: *Staphylococcus aureus;* anti-biofilm; drug development; mechanism.

Introduction:

The micro-biota associated with human activity as both beneficiary and threat. Threaten due to widespread of antibiotic resistance in some microbe posing a grave effects on health of peoples globally through a multitude of ominous infections.[1] *Staphylococcus aureus* is one of the major human pathogen cause's mild superficial infections to severe life-threatening invasive infections to the human world resulting in significant morbidity and mortality.[2] The *S. aureus* grow on living or inert surfaces as biofilms, which is community having densely packed *S. aureus* cells surrounded with self-secreted matrix.[3] The biofilm play an important role in antibiotic drug resistance which leads to public threat globally.[4] In the past few decades, the number of efforts has been made in the medicinal chemistry through synthetic tailoring in a combinatorial fashion, to generate a large set of analogues as core scaffolds. Although the tremendous approaches have been fruitful, no new major class of antibiotics were invented between 1962 and 2000.[5] Therefore, to come up with new effective therapeutic agents, there is a need for aggressive efforts and it is imperative to discover novel synthetic entities for the microbial target is a big challenge to the medicinal chemistry.[6,7]

View on biofilm-relevance to human:

S. aureus belongs to the nosocomial opportunistic ESKAPE family of resistance pathogens includes *Enterococcus faecalis, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* sp., spread rapidly and challenges estimated to cause every year 10 million deaths and by 2050, the loss of productivity has been £100 was documented.[8,9] The *S. aureus* invade human immune system through its excellent protection strategies to cause antibiotic-resistant diseases. This characteristic is due to rapid proliferation and spread of unicellular organism colonizes body surfaces and persistent against stress conditions in tissues as multicellular aggregates called the matrix.[10] One of the reasons for *S. aureus* posse's great intrinsic resistance mechanism is due to acquired genes for encoding resistance determinants and also, resistance mediated through its

highly structured extracellular matrix biofilm possess 10-1,000-fold lower susceptibility to the vast number of antimicrobials. The genius escapes mechanism of *S. aureus* by biofilms during infection from potent antimicrobials, there are no approved drugs specifically for targeted biofilms in clinical trials to date.[11,12]

Number of etiologic biofilm formers involved in causing life-threatening disease by *S. aureus, S. epidermidis,* and *Pseudomonas aeruginosa* (prevalent) and *Klebsiella pneumoniae* and *Escherichia coli* (opportunistic pathogens). Among staphylococcal species, *S. aureus* and *S. epidermidis* are first and second positions followed by emerging pathogens such as *S. haemolyticus, S. capitis, S. hominis* and *S. warneri*.[13,14,15] The complex multicellular and multispecies involved biofilm nature was difficult to eliminate from the host defense machinery and with antibiotic therapy due to its tricky protective mechanisms played during biofilm formation. According to U.S. Center for Disease Control (CDC) report, to thirds of the major bacterial infections reported are due to resistant biofilm, which significantly causes the global burden on human health.[16,17]

What triggers biofilm life cycle?

The biofilm simply described as a multicellular consortium of *S. aureus* encased in self-produced extracellular polymeric substance (EPS) termed as a matrix.[18] Depending on strains and environmental factors, *S. aureus* shield by its self in matrix containing exopolysaccharides, proteins, teichoic acids, and/or extracellular DNA (eDNA) to protect from adverse stress conditions. These unfavorable conditions such as nutrient limitations/starvations, physical conditions or external attack trigger the formation of biofilm.[19-22] The biofilm formation is a complex mechanism in *S. aureus* to form a functional mature biofilm, is still an under investigations. But, based on *in vitro* research models *S. aureus* different steps in biofilm formation was classically described as 1. Initial attachment,

2. Cell aggregation and formation of multicellular layers, and 3. Biofilm maturation and detachment into single planktonic cells to begin new life cycle of biofilm.[23,]

Initial *S. aureus* attachment with surface takes place nonspecifically driven by electrostatic, hydrophobic and Lifshitz-Van der Waals forces by passive adsorption mechanism.[24] The success of attachment depends on the hydrophobicity of *S. aureus* cell surface and abiotic surfaces. This is mediated by protein autolysin (AtlA-137 kDa, whereas AtlE-148 kDa in *S. epidermidis* autolysin posses enzymatic function being peptidoglycan hydrolases and adhesin in nature) for biofilm process.[25] The *S. aureus* master execution triggered due to AtlA, is a glycine-tryptophane dipeptide repeats involved not only in surface adhesion and biofilm formation but also in internalization by host cell through its novel mechanism.[26] The second step of cell aggregation into multicellular layers through microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and intracellular adhesion.[27,28] In this phase progressive proliferation and maturation of biofilm occur and specific biofilm characteristics were developed. In the last phase, the biofilm encased, ruptured or cells were dispersed to initiate a planktonic form of *S. aureus* life, ready to start the journey for a new invasive phase is the final step of the *S. aureus* one biofilm life cycle.

EPS of biofilm supports *S. aureus* war

The *S. aureus* EPS play a vital role in biofilm formation via intracellular signaling molecules moderate many functions such as the production of virulence factors, physiology and adaptive to an antibiotic resistance mechanism.[16] Also, EPS involved in the channel for water and nutrients into inner components of the biofilm. The high metabolically active *S. aureus* cells present in the outer layer, the nongrowing dormant state cells in the centre are highly difficult to eradicate. Such category of cells is particularly survived against a broad range of antibiotics targeted for growing organisms.[17-19] But, intermediate cells susceptible to antibiotics are due to by the different physiologic state.

The cell-cell adhesion of *S. aureus* is triggered by production of polysaccharide intercellular adhesion (PIA) consists of linear β-1,6-linked glucosaminoylglycan mediates by *icaADBC* intercellular adhesion locus.[29] Even though, deletion of *ica* locus in *ica* independent biofilm pathway initiated for biofilm formation through harboring adhesive proteins called biofilm-associated protein (Bap) found anchored to *S. aureus* cell wall.[30,31] The Bap and another surface anchored protein SasG hold cells together by interacting with other cell surface proteins of neighboring cells.[32] The recent study explored that, the MRSA biofilm promoted by fibronectin-binding proteins (FnBPs) such as FnBPA and FnBPB.[33] The FnBPs are proteinatious in biofilm in presence of glucose and *S. aureus* can modulate its biofilm matrix depending on behavior to external stimuli.[34] Another protein called SasC and protein A in biofilm involved in cell aggregation which is important to investigate its actual role in immune defense to find a promising agent against biofilm in coming future.[35,36]

The origin of eDNA and its relationship with biofilm is an intensive investigation. Some reports say that the eDNA originates from cell lysis and helps in exchange of genetic material through plasmids, insertion sequences transposons and pathogenic islands of, so on.[37,38] In these conditions, virulent determinants and antibiotic resistance responsible elements are easily exchanged.

Antimicrobials v/s biofilm

The biofilm protected *S. aureus* eradication is a highly challenging task because of its tolerant towards conventional antibiotics shown by non-growing dormant cells in the biofilm matrix.[17,39] Many types of antimicrobials/agents are screened to understand the annihilate biofilms such as silver (Ag),[40] tert-butyl benzoquinone (TBBQ),[41] EDTA,[42] peptide IDR-1018,[43] etc. are in line with variable success rate through impairing *S. aureus* membrane, disturbing key enzymes, repressing adhesion proteins involved in cell-cell aggregation and by inhibiting protein biosynthesis important for biofilm formation.

Till now, the best enzyme therapies studied are deoxyribonuclease I (DNase I) and dispersion B (DspB). DNase I degrade the eDNA, which is structural components of the biofilm involved in giving stability to biofilm and DspB hydrolyses poly-(β-1,6)-N-acetylglucosamine (PNAG).[44] Biomedical sciences play a very important role to combating the biofilm-related infections through various approaches such as,

1. Development of anti-adhesive properties on biomaterial surfaces by coating biosurfactants polyamidoamine dendrimers and hydrophilic polymer brushes like poly(ethylene oxides) (PEO) and/or poly(ethylene glycerol) (PEG).[45,46]
2. Doping antimicrobial substances; antibiotics, disinfectants and bactericidal (Ag, Cu, Zn, NO, lysozyme, metal nanoparticles), disaggregating agents (DNase I, DspB, N-acetylcysteine), and antimicrobial peptides etc.[47]
3. Synergistic coatings of ant-adhesive and antimicrobials.[48-50]
4. Biofilm opposing material to support tissue integration; silver containing hydroxyapatite, bioglasses doped gold nanoparticles etc.[51,52]

How the *S. aureus* acquires resistance to the individual antibiotic is an untouched area in the antimicrobial therapy research. Even though, *S. aureus* is hidden inside the biofilm to protect against antibacterial agents also the biofilm matrix accessible to outside the environment through porous channels to run fluids. This interesting feature of biofilm is a promising target for anti-biofilm therapies. Some of the potent anti-biofilm molecules with possible mechanisms taking place to shut the *S. aureus* biofilm formation were detailed along with structures to understand the bacterial resistance and future drug discovery perspective **(Table 1** and **2, Fig. 1-4)**.

Table 1: The *S. aureus* targeted different anti-biofilm molecules.

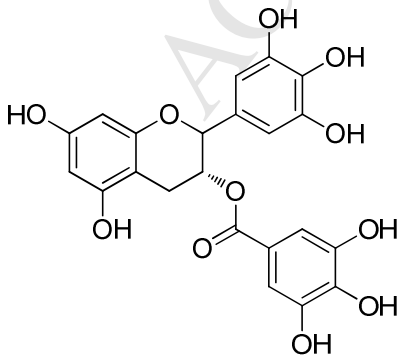
Sl. No.	Source	Anti-biofilm molecules	MIC/MBC/MBIC/IC$_{50}$ values

1	*Camellia sinesis* (Green tea)	Epigallocatech in gallate (EGCG)	MBC=64-1024 μg/ml
2	*Santolina oblongifolia, Alchemilla speciose, Tagetes lucida*	Esculetin	MIC >512 μg/ml
3	*Fragaria ananassa, Malus domestica*	Fisetin	MIC =64 μg/ml
4		Peptide 1018	
5	Produced by extra intestinal *E.coli* of Phylogenetic group B2 or D	CFT073 group-II capsular Polysaccharide (Serotype K2)	
6	*P. aeruginosa*	Pel polysaccharide	
7		Polymyxin B	MIC=158 μg/ml MBC=256 μg/ml
8	*Lactococcuslactis*	**Lantibiotics:** Nisin	
9	*Staphylococcus gallinarum* Tu3928	Gallidermin	MIC=0.5μg/ml
10	Human cationic host defense peptide	**Antimicrobial peptide (AMP):** LL-37	MIC=0.5 μg/ml
11	Synthetic analogue from Gaegurin 5	Lytic peptide (PTP-7)	MIC=2-16 μM
12	Derived from sushi-3 domain of Factor C, which is a LPS-sensitive serine protease of horseshoe crab coagulation cascade	Sushi peptides	
13	Cathelicidin derived peptide identified from porcine leukocytes	PMAP-23	
14	Isolated from the pig's small intestine	PR-39	MIC=0.94 μM
15	Derived from Buforin-I (stomach tissue of *Bufobufo gargarizans*)	Buforin-II	MIC=0.25-4.0 μg/ml
16	From cytoplasmic granules of bovine Neutrophils	Indolicidin	MIC=50 μg/ml
17		Pyrrhocoricin	IC_{50}<0.3 μM
18		**Chelating agents**: (a)Sodium citrate (b)Tetrasodiu m EDTA (c)Disodium- EDTA	MIC ≥0.5%
19	*Caesalpinia spinosa, Rhus semialata, Quercus infectoria, Rhus coriaria*	Tannic acid	
20		**Enzymes:** Deoxyribonuclease I, Glycoside hydrolase (dispersin B)	
21	A secondary lichen metabolite	Usnic acid	

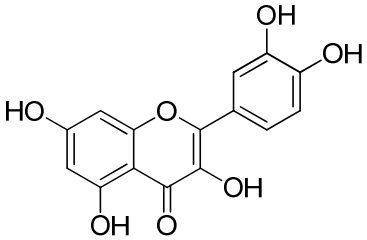
Table 2: The molecular mechanisms of different anti-biofilm candidates to *S. aureus*

Sl. No.	Molecules associated	Mechanism of action
1	Halogenated furanone compounds, Quercetin	Inhibition of AHL-mediated quorum sensing pathway
2	Peptide-1018, Peptide-1038	Inhibition of (p)ppGpp regulated stringent response
3	Deoxyribonuclease I and glycoside hydrolase dispersin B	Dispersion of Extracellular Polymeric Substance (EPS) of biofilm
4	Tannic acid, Endolysins (PlyC), Epigallocatechin gallate (EGCG)	Tannic acid, Endolysins (PlyC), Epigallocatechin gallate (EGCG)
5	Cyclic autoinducing peptide (AIP), Nuclease, extracellular proteases (eg. *sarA*, *sigB*, *Esp*), antiamyloid molecules (AA-861, parthenolides), DTyrosine, Ethyl-pyruvate	Biofilm disassembly
6	Polymyxin (B and E), Gramicidin S, Sushi peptides, PMAP-23	Neutralization/disaggregation of LPS
7	Lantibiotics (nisin, gallidermin), Lytic peptides (PTP-7), Sophorolipids, Polyhexamethylene biguanide, Chlorhexidine, Pentasilver hexaoxoiodate	Alteration of membrane Permeabilization
8	Pyrrhocoricin, Microcin B17	Inhibition of cell division or cell survival
9	Buforin II, PR-39, Indolicidin, LL-37, Bacteriocins, Cadexomer iodine, Mannosides, Pilicides	Inhibition of macromolecule synthesis and adhesion of cells
10	EPS273, Psl and Pel, K2, PAM galactan, A101, PslG, Polysaccharides of algae, plants and animals	Inhibition of biofilm by polysaccharides
11	LP 3134, LP 3145, LP 4010, LP 1062, ebselen, ebselen oxide Desformylflustra bromine	Inhibition of c-di-GMP signaling system
12	Analogs of FN075 and BibC6 of ring-fused 2-pyridones	Inhibition of curli biosynthesis

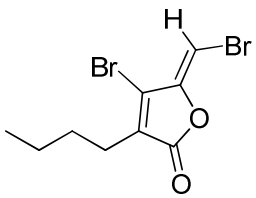
A



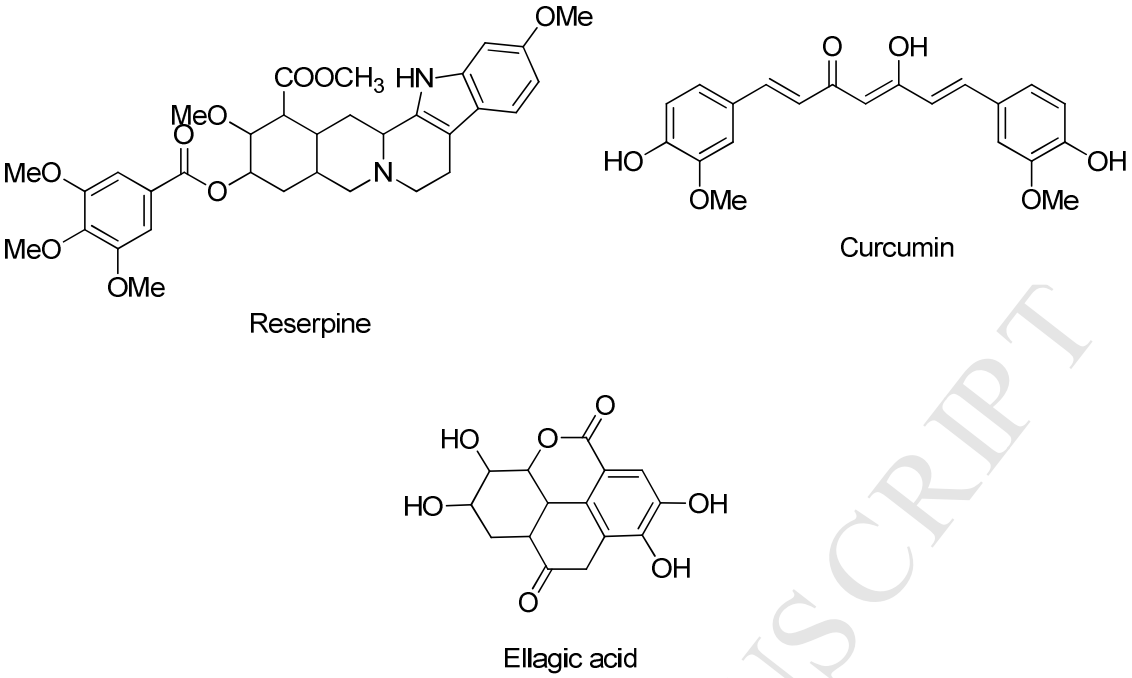
EGCG



Quercetin



Synthetic halogenated furanone



B

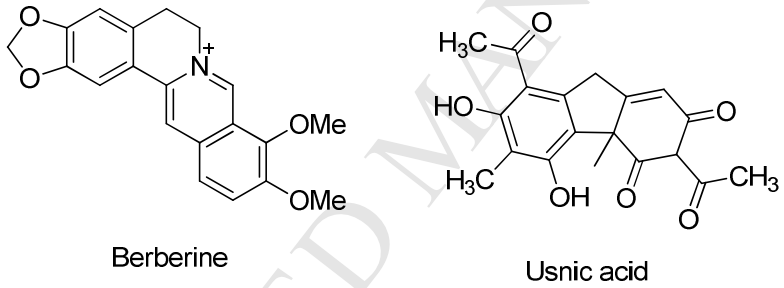


Figure 1: Structures of the anti-biofilm molecules that inhibit AHL-mediated quorum sensing (**A**) and structure of anti biofilm molecules that disassemble the biofilm (**B**).

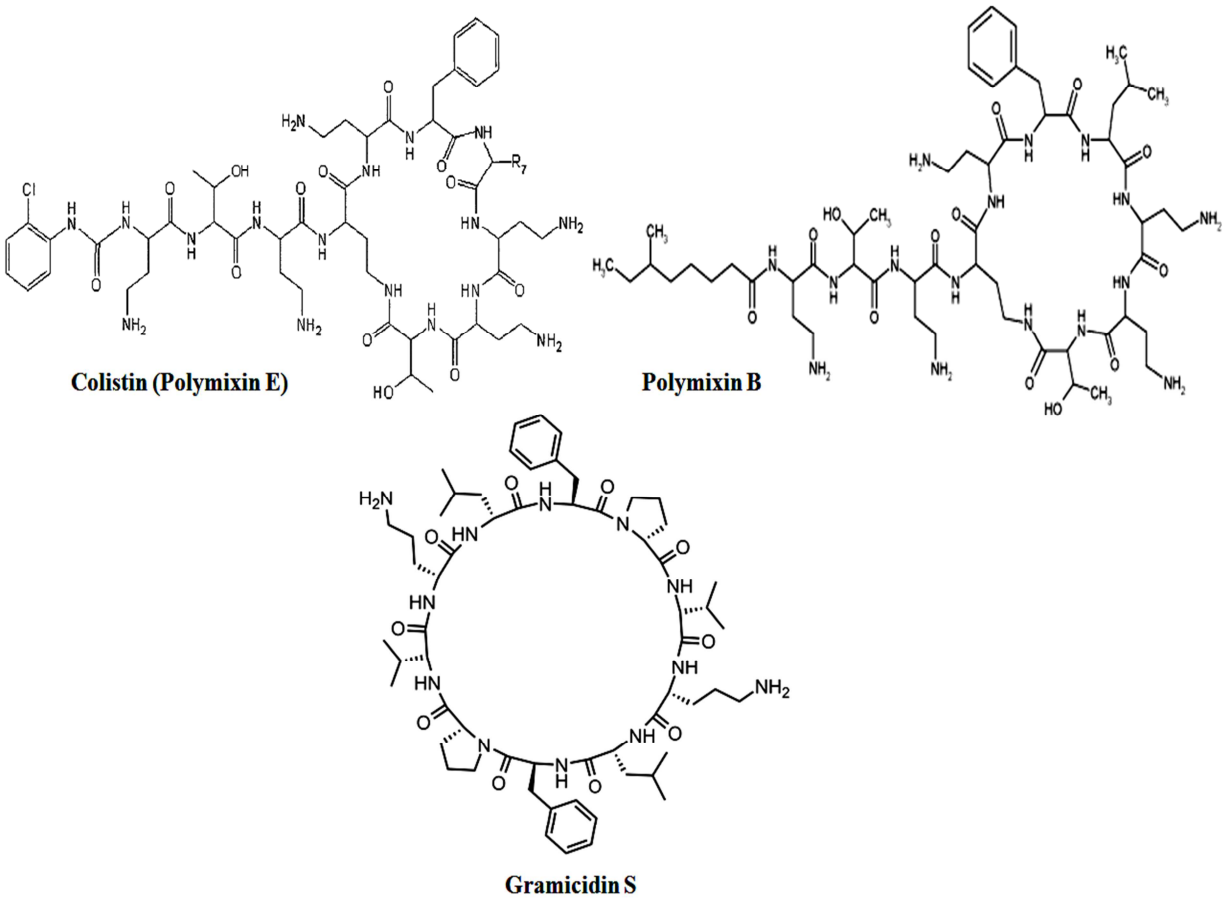


Figure 2: Structures of the anti-biofilm molecules that inhibit lipopolysachharides.

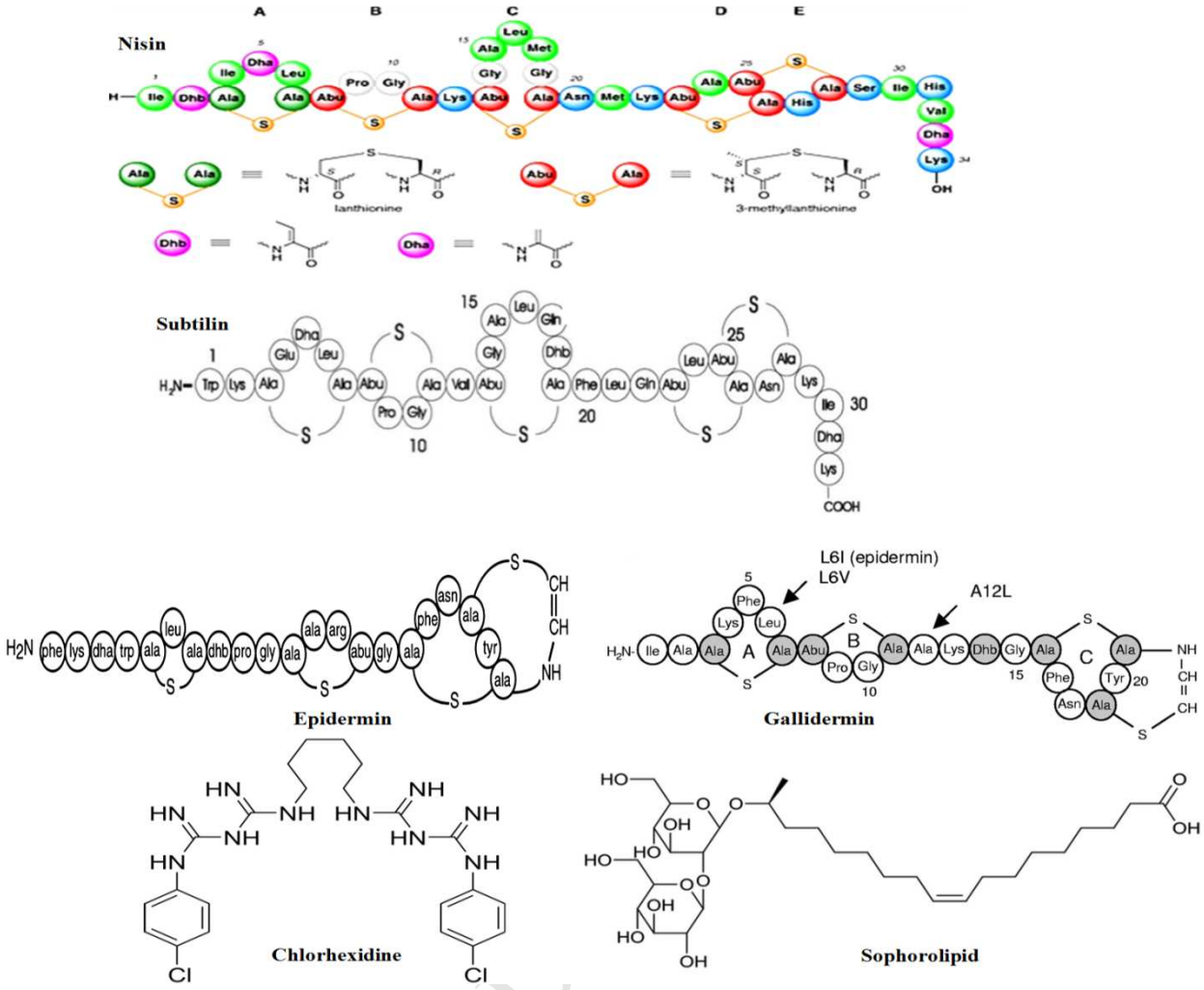


Figure 3: Structures of the anti-biofilm molecules that alter the membrane potential or membrane permeabilization.

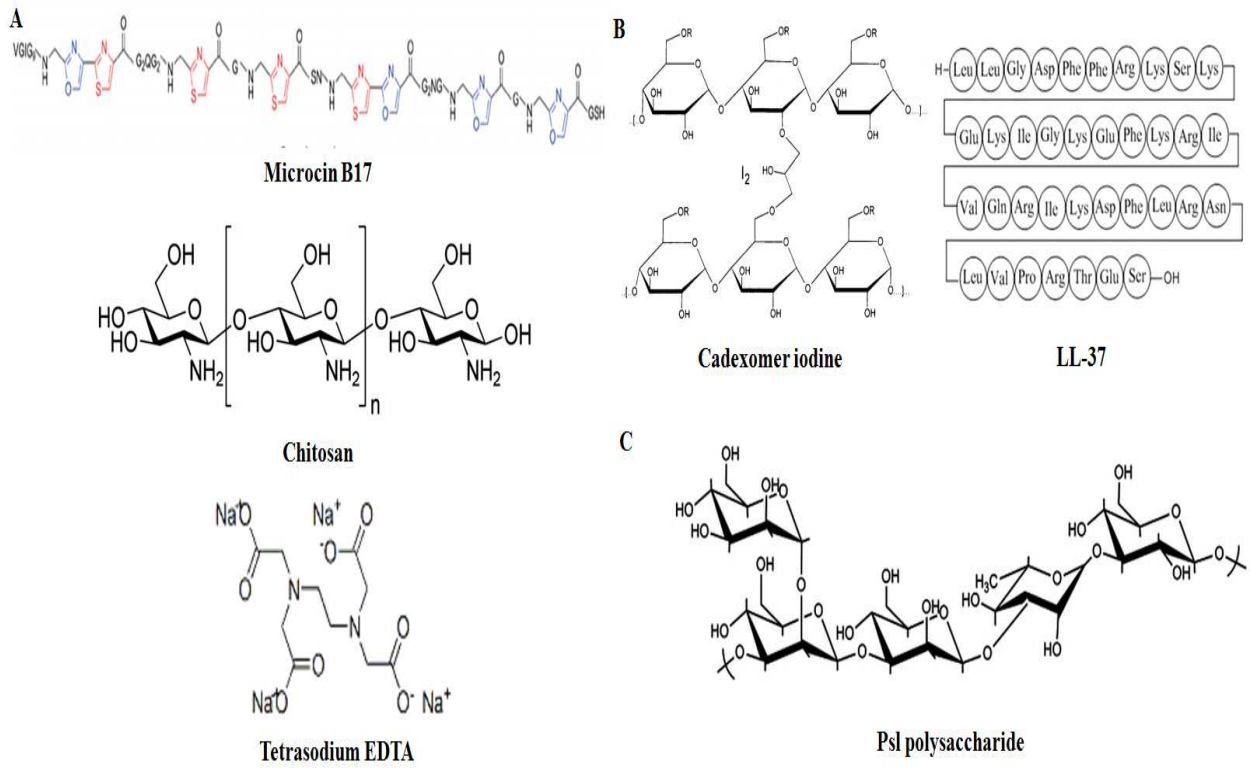


Figure 4: Structures of the anti-biofilm molecules that inhibit cell division and survival (A), structures of the anti-biofilm molecules that Inhibit adhesion molecule synthesis (B) and function and structures of the anti-biofilm molecules that inhibit polysaccharides (C).

Machineries of antibiotic resistance

A diverse nature of antibiofilm molecules has been discovered to inhibit biofilm formation against different targets **(Fig. 5)**. The *S. aureus* can be called as 'notorious' due to an ability to become resistant towards antibiotics to become successful strain such as MRSA. During the 1940s, the emergence of resistant *S. aureus* to antibiotic penicillin by expressing β-lactamase to hydrolyse the critical β-lactam bond and destroying the drug's antibacterial potency was observed. Further substitution of a natural aminoadipoyl chain of penicillin to bulkier moieties to become semisynthetic variants, they are not substrates for β-lactamase.[53,54] Methicillin is the first but being acid labile, which is superceded by acid stable isoxazoyl penicillin oxacillin. But, shortly after its introduction of methicillin resistance and MRSA has struck even though the term methicillin is no longer used.

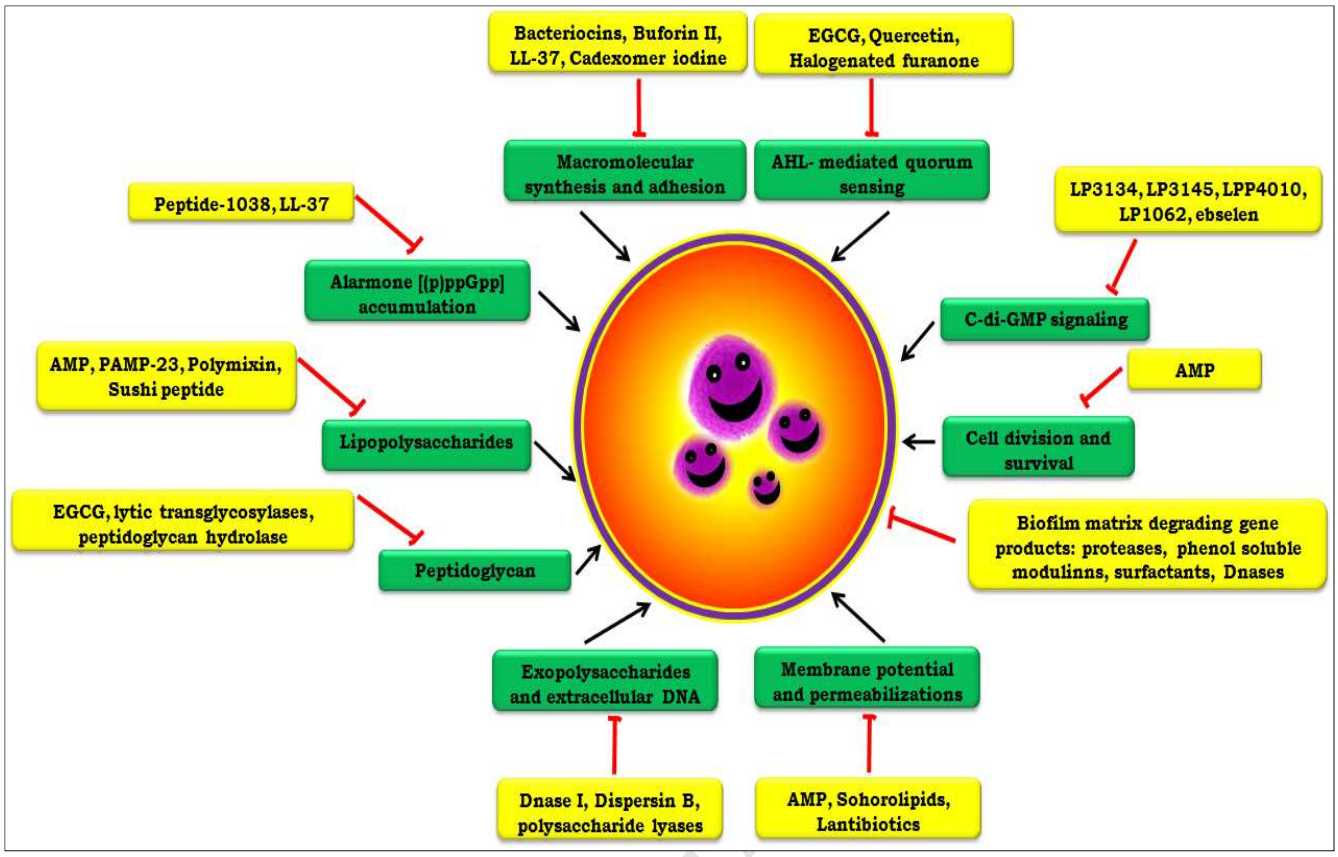


Figure 5: The schematic representation of *S. aureus* regulators (green) for biofilm formation and suitable inhibitors (yellow) targets as antibiofilm candidates.

The resistance was acquired by horizontal transfer of resistant determinants through following one of the mechanisms **(Fig. 6)**,[55] i) Drug efflux, ii) Enzymatic drug modification and inactivation, iii) Modifying drug binding sites by enzymes, iv) Displacing the drug to protect target and v) Acquiring drug-resistant targets by bypass mechanisms etc. The resistance can also through results of mutations such as i) Depression of the multi-drug resistance efflux pump, ii) Modifying drug target to prevent inhibitor from binding, and iii) Mutations altering the composition of cell wall/membrane to decrease the drug access to its target etc.

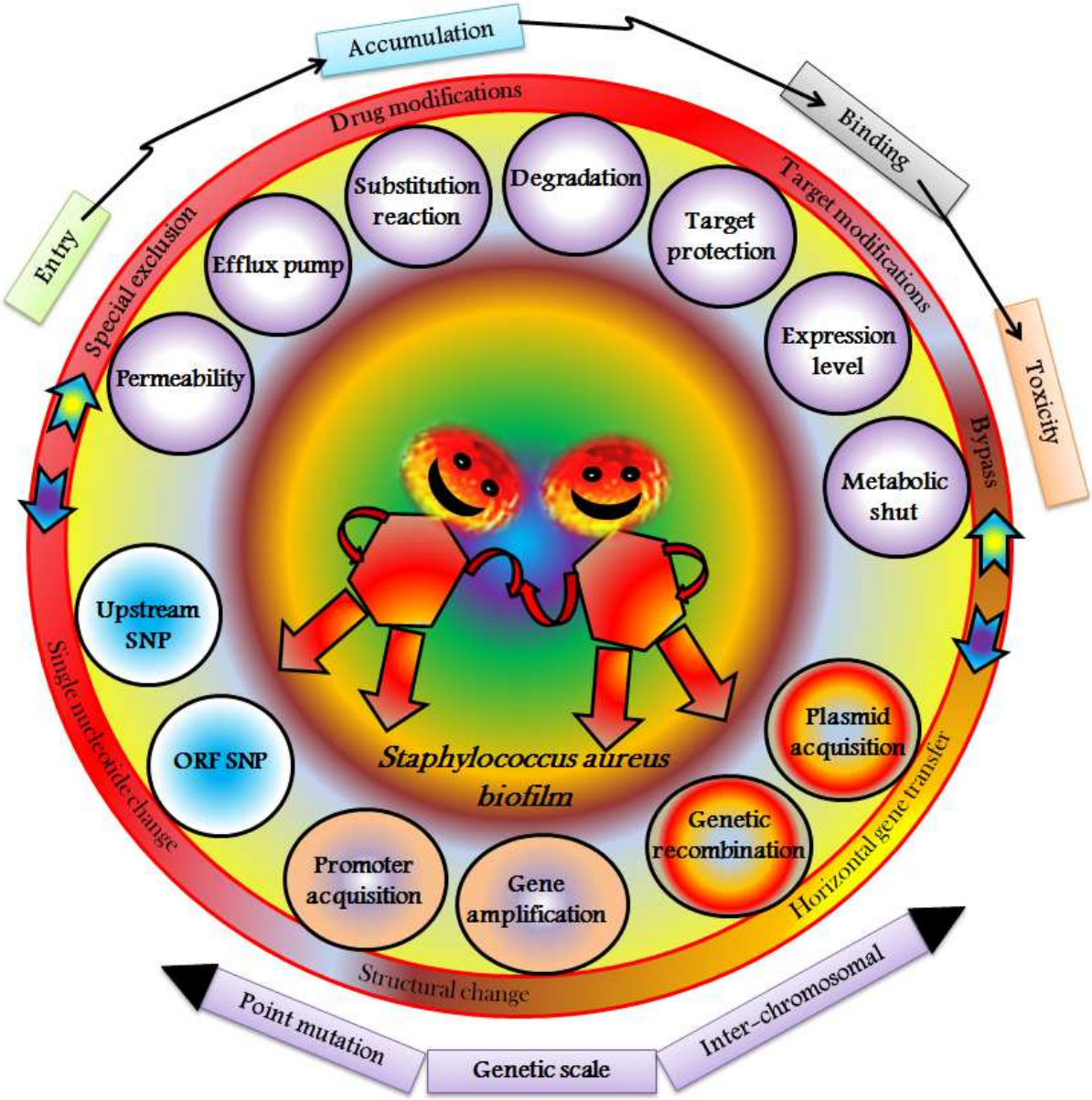


Figure 6: The biofilm resistance mechanism govern by the different machineries of *S. aureus*.

An imperative unlocking key mechanisms need to focus?

Currently a number of clinical investigations in drug discovery programs failures in designing target oriented candidates or in drug delivery stage. Till now the non-targeted drug molecules in clinical trials have the poor bioavailability,[56] quick excretions, and non-specific toxicity with adverse side effects.[57] The delivery of these drugs requiring larger dosages to achieve its desired site of action. These important pitfalls in traditional therapeutic strategies,

call for an urgent need for newer and promising approaches to achieving an improved therapeutic index of desired drug molecule of interest.

Many antimicrobial agents are specifically targeting the bacterial cell wall/cell membrane due to the presence of corresponding target residue on the surface of the bacterial cell, which allows it specific binding. This interesting strategy may works as specific molecules against bacteria rather they are used as an inhibitor at a lower dose. The utilization of vancomycin specifically bind to gram-positive bacteria via hydrogen bonding to N-acetylmuramic acid and N-acetlyglucosamine subunits in the cell wall, they can be used as molecular recognition of *S. aureus*.[58]

Daptomycin is a one of the most successful novel cyclic lipopeptide and very less toxic alternative to vancomycin for the treatment of Gram-positive pathogens including *S. aureus*.[59] The unique characteristic of daptomycin having hydrophilic core consists of 13-amino acid cyclic lipopeptide with decanoyl side chain (hydrophilic tail) exerts its effect by binding to the cell wall of the *S. aureus*, resulting in membrane depolarization and destruction.[60] Currently, an injectable solution Cubicin® is the only approved daptomycin formulation in the market. In this regard, there is a need for interest to develop some more promising formulation and delivery systems to enhance the effect of daptomycin against drug-resistant pathogens.[61,62] In this regard, developing molecular recognition determinants specifically to bacterial membrane targets through new technologies is an interesting area to escapes emergence of drug resistance problems in microorganisms.

Are these! or need much attention? for drug development

The development of desired candidates which is a specific affinity for pathogens and inherent destructive power can be called as “magic bullets”.[63] This worthy concept led to the development of newer non-sized drug carriers modified by targeting ligands referred as ‘active targeting drug delivery system’, which are used in tumour therapy. The main

limitation and difficulties in new drug designing against bacterial pathogens as new antibacterial agents such as existing antibiotics, toxicity to normal cells, rapid clearance from circulations and multi-drug resistance requires a pioneering urgent novel drug delivery strategy to address these existing and upcoming problems.[64,65]

The liposome-mediated antibacterial drug delivery system have been established by encapsulating potent hydrophilic/hydrophobic components to increase the solubility of the encapsulated drug and to enhance the promising action against both intracellular and extracellular pathogens.[66,67,68] The conventional liposomes can be further designed by engineering with a selectivity of the NPs to microorganisms by modifying surface potency. This improves the developed NPs to release drugs at infected sites, decreasing drug toxicity, reducing adverse side effects and increasing overall efficacy of the engineered liposome's.[69]

The unique specificity and higher sensitivity of enzymes can hold promise as a potential therapeutic candidate in the field of medicine.[70] Yet, the clinically important enzyme used as drugs is unknown and less common than the lower molecular weight drugs. This is due to the three important drawbacks such as poor stability, immunogenicity, and systemic toxicity. To address these global issues, the nanotechnology gains tremendous attention in many fields to solve the problem are arising/exciting.[71,72]

Perspectives in drug discovery

Since the late 1980s, a lot of exciting discovery programs executed for new synthetic classes of antimicrobial drug discovery, and their view is to combat notorious staphylococcal infections. During 1987, daptomycin lipopeptide is the last being discovered and suddenly a number of pharmaceutical companies have stopped the contribution for antibiotic discovery and development programs.[73] Among many, the main factor is getting resistance to developed drugs intern reducing its usage. Also, many drugs molecules introduced only for specific functions with expensive clinical trials, the regulatory bar set was too high and a big

investment in target-discovery programs parallel to structural biology did not show hoped-for breakthroughs.[74]

In certain infections caused by ESCAPE pathogens multi-drug resistance and the ability of bacteria to form biofilm to avoid antibiotics to penetrate, treatment options are indeed running out.[75,] This insight information clear that, need stewardship in antibiotic discovery imitations. In US GAIN Act initiated to encourage a number of small companies and research academic pioneering groups into discovery and development programs, to bring promising drug candidates towards clinical trials. We hypothesize that to account, this technology, the synthesis of novel nanoparticles using liposome's to boost the interest and make use of lipid nanoparticles as a carrier of enzyme-drugs to overcome the resistance problem in a number of diseases is promising approaches in the field of medicine and drug discovery fields.

Acknowledgements

We are grateful to SJCE, Mysuru and National Natural Science Foundation of China (Grant No. 21772150) and Wuhan University of Technology for financial support.

Transparency declaration

The author does not have any conflict of interest. The author did not receive any funding for this study.

References:

1. D. I. Andersson, and Hughes D, *FEMS Microbiol. Rev*, 2011, 35, 901-911.
2. P. N. Reddy, K. Srirama, and V. R. Dirisala, *Infect. Dis.* 2017, 10, https://doi.org/10.1177/1179916117703999.
3. H. Mu, J. Tang, Q. Liu, C. Sun, T. Wang, and J. Duan, *Sci. Rep.* 2016, 6, 18877.
4. M. Frieri, K. Kumar, and A. Boutin, *J. Inf. Public Health*. 2017, 10(4), 369-378.
5. M. A. Fischbach, and C. T. Walsh, *Science*. 2009, 325, 1089-1093.

6. D. M. Livermore, *J. Antimicrob. Chemother*., 2011, 66, 1941-1944.
7. A. Tripathi, et al. *J. Am. Chem. Soc*. 2014, 136, 1579-1586.
8. R. H. Sunenshine, et al. *Emerg. Infect. Dis*., 2007, 13, 97–103.
9. A. Parmar, R. Lakshminarayanan, A. Iyer, V. Mayandi, E. T. L. Goh, D. G. Lloyd, and A. Madder, *J. Med. Chem.* 2018, doi: 10.1021/acs.jmedchem.7b01634.
10. S. Furukawa, S. L. Kuchma, and G. A. O'Toole, *J. Bacteriol*., 2006, 188 (4), 1211-1217.
11. M. V. Ranall, M. S. Butler, M. A. Blaskovich, and M. A. Cooper, *Curr. Drug. Targets*., 2012, 13, 1375-1385.
12. O. C. Thomas Bjarnsholt, S. Molin, M. Givskov, and N. Høiby, *Nat. Rev. Drug Discov*., 2013, 12, 791–808.
13. C. R. Arciola, Y. H. An, D. Campoccia, M. E. Donati, and L. Montanaro, *Int. J. Artificial. Org.*, 2005, 28(11), 1091-1100.
14. C. Von Eiff, C. R. Arciola, L. Montanaro, K. Becker, and D. Campoccia, *Int. J. Artificial. Org.*, 2006, 29(4), 360-367.
15. D. Campoccia, L. Montanaro, L. Visai, T. Corazzari, C. Poggio, F. Pegreffi, and P. Speziale, *Int. J. Artificial. Org.*, 2010, 33(9), 575-581.
16. U. Romling, and C. Balsalobre, *J. Intern. Med*., 2012, 272 (6), 541-561.
17. C. de la Fuente-Núñez, F. Reffuveille, L. Fernandez, and R. E. W. Hancock, *Curr. Opin. Microbiol.*, 2013, 16 (5), 580-589.
18. H. C. Flemming, and J. Wingender, *Nat. Rev. Microbiol.*, 2010, 8 (9), 623-633.
19. P. S. Stewart, and J. W. Costerton, *Lancet.* 2001, 358 (9276), 135.
20. J. W. Costerton, L. Montanaro, and C. R. Arciola, *Int. J. Artif. Organs.*, 2005, 28(11), 1062e8.

21. N. Høiby, O. Ciofu, H. K. Johansen, Z. J. Song, C. Moser, P. O. Jensen, et al. *Int. J. Oral. Sci.*, 2011, 3, 55-65.

22. C. R. Arciola, D. Campoccia, S. Gamberini, M. E. Donati, V. Pirini, L. Visai, et al. *Biomaterials.* 2005, 26(33), 6530-6535.

23. D. Mack, P. Becker, I. Chatterjee, S. Dobinsky, J. K. Knobloch, G. Peters, et al. *Int. J. Med. Microbiol.*, 2004, 294, 203-212.

24. G. Legeay, F. Poncin-Epaillard, and C. R. Arciola, *Int. J. Artif. Organs.*, 2006, 29(4), 453-461.

25. S. J. Foster, *J. Bacteriol.*, 1995, 177, 5723-5725.

26. N. Hirschhausen, T. Schlesier, M. A. Schmidt, F. Götz, G. Peters, and C. Heilmann, C. *Cell. Microbiol*., 2010, 12, 1746-1764.

27. J. M. Patti, B. L. Allen, M. J. McGavin, and M. Höök, *Annu. Rev. Microbiol.*, 1994, 48, 585-617.

28. P. Speziale, G. Pietrocola, S. Rindi, M. Provenzano, G. Provenza, A. Di Poto et al. *Future. Microbiol.*, 2009, 4, 1337-1352.

29. C. Heilmann, O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack, and F. Götz, *Mol. Microbiol.*, 1996, 20, 1083-1091.

30. J. P. O'Gara, *FEMS Microbiol. Lett.*, 2007, 270, 179-188.

31. C. Cucarella, C. Solano, J. Valle, B. Amorena, I. Lasa, and J. R. Penadés, *J. Bacteriol.*, 2001, 83, 2888-2896.

32. J. A. Geoghegan, R. M. Corrigan, D. T. Gruszka, P. Speziale, J. P. O'Gara, J. R. Potts et al. *J. Bacteriol.*, 2010, 192, 5663-5673.

33. E. O'Neill, C. Pozzi, P. Houston, H. Humphreys, D. A. Robinson, A. Loughman et al. *J. Bacteriol*., 2008, 190, 3835-3850.

34. P. Houston, S. E. Rowe, C. Pozzi, E. M. Waters, and J. P. O'Gara, *Infect. Immun.*, 2011, 79, 1153-1165.

35. K. Schroeder, M. Jularic, S. M. Horsburgh, N. Hirschhausen, C. Neumann, A. Bertling, et al. *PLoS One.*, 2009, 4, e7567-7581.

36. N. Merino, A. Toledo-Arana, M. Vergara-Irigaray, J. Valle, C. Solano, E. Calvo, et al. *J. Bacteriol.*, 2009, 91, 832-843.

37. S. Molin, and T. Tolker-Nielsen, *Curr. Opin. Biotechnol.*, 2003, 14, 255-2561.

38. S. J. Sørensen, M. Bailey, L. H. Hansen, N. Kroer, and S. Wuertz, *Nat. Rev. Microbiol.*, 2005, 3, 700-710.

39. B. P. Conlon, S. E. Rowe, and K. Lewis, *Adv. Exp. Med. Biol.*, 2015, 831, 1-9.

40. J. A. Lemire, L. Kalan, N. Gugala, A. Bradu, and R. J. Turner, *Biofouling*., *2017, 33 (6),* 460−469.

41. N. Ooi, E. A. Eady, J. H. Cove, and A. J. O'Neill, *J. Antimicrob. Chemother*., 2016, 71 (7), 1841−1844.

42. E. Banin, K. M. Brady, and E. P. Greenberg, *Appl. Environ. Microbiol*., 2006, 72 (3), 2064−2069.

43. H. Hof, D. Bertsch, D. Passek, and R. Schwarz, *Urologe A*., *2017, 56 (2),* 167−171.

44. J. B. Kaplan, *Int. J. Artif. Organs*., *2009, 32(9),* 545-554.

45. C. R. Arciola, L. Radin, P. Alvergna, E. Cenni, and A. Pizzoferrato, *Biomaterials. 1993, 14(15),* 1161-1164.

46. K. G. Neoh, E. T. Kang, *ACS Appl. Mater. Interfaces*., 3 (8), 2808-2819.

47. D. Campoccia, L. Montanaro, P. Speziale, and C. R. Arciola, *Biomaterials. 2010, 31*, 6363-6377.

48. J. Fu, J. Ji, W. Yuan, and J. Shen, *Biomaterials. 2005, 26 (33),* 6684-6692.

49. S. J. Kang, D. H. Kim, T. Mishig-Ochir, and B. J. Lee, *Arch. Pharm. Res*., 2012, 35 (3), 409-413.

50. S. Gruenheid, and H. Le Moual, *FEMS Microbiol. Lett*., 2012, 330, 81-89.

51. D. Campoccia, C. R. Arciola, M. Cervellati, M. C. Maltarello, and L. Montanaro, *Biomaterials*. 2003, 24, 587-596.

52. T. P. Schaer, S. Stewart, B. B. Hsu, and A. M. Klibanov, *Biomaterials*. 2012, 33, 1245-1254.

53. S. J. Peacock, and G. K. Paterson, *Annu. Rev. Biochem*., 2015, 84, 577–601.

54. C. T. Walsh, and T. A. Wencewicz, Antibiotics: challenges, mechanisms, opportunities. washington, DC: ASM Press, 2016.

55. T. J. Foster, *Fems. Microbiology. Reviews*., 2017, 41 (3), 430-449.

56. S. Mitragotri, P. A. Burke and R. Langer, *Nat. Rev. Drug Discov*., 2014, 13, 655–672.

57. V. P. Torchilin, *Eur. J. Pharm. Sci*., 2000, 11, S81–S91.

58. A. Beiras-Fernandez, F. Vogt, R. Sodian and F. Weis, *Infect. Drug. Resist*., 2010, 3, 95–101.

59. J. N. Steenbergen, A. Jeff, G. M. Thorne and F. P. Tally, *J. Antimicrob. Chemother*., 2005, 55, 283–288.

60. X. L. Zhang, X. L. Huang, L. Deng, C. Li, G. J. Liao and Z. B. Chen, *Chin. J. Antibiot*., 2013, 38, 130–134.

61. J. R. Costa, N. C. Silva, B. Sarmento and M. Pintado, *Eur. J. Clin. Microbiol. Infect. Dis*., 2015, 34, 1255–1262.

62. C. Lagord, O. Kearins, G. Lawrence, H. Bishop, I. Monypenny and A. T. Bates, *Colomb. Méd*., 2008, 39, 291–5.

63. M. E. Davis, Z. G. Chen and D. M. Shin, *Nat. Rev. Drug Discov*., 2008, 7, 771–782.

64. R. A. Petros and J. M. DeSimone, *Nat. Rev. Drug Discov*., 2010, 9, 615–627.

65. G. W. Boswell, D. Buell and I. Ekersky, *J. Clin. Pharmacol*., 1998, 38, 583–592.

66. H. J. Kim and M. N. Jones, *J. Liposome. Res*., 2004, 14, 123–139.

67. C. O. Onyeji, C. H. Nightingale and M. N. Marangos, *Infection*. 1994, 22, 338–342.

68. Z. Drulis-Kawa and A. Dorotkiewicz-Jach, *Int. J. Pharm*., 2010, 387, 187–98.

69. R. Blumenthal, E. Ralston, P. Dragsten, L. D. Leserman and J. N. Weinstein, *Membr. Biochem*., 1982, 4, 283-303.

70. A. Kozubek, J. Gubernator, E. Przeworska and M. Stasiuk, *Acta. Biochim. Pol*., 2009, 47, 639-649.

71. S. M. McAllister, H. O. Alpar and M. R. Brown, *J. Antimicrob. Chemother*., 1999, 43, 203-210.

72. L. L. Silver, *Clin. Microbiol. Rev.*, 2011, 24, 71–109.

73. A. Zorzet, *Ups. J. Med. Sci.*, 2014, 119, 170–175.

74. J. N. Pendleton, S. P. Gorman and B. F. Gilmore, *Expert. Rev. Anti-Infe.* 2013, 11, 297–308.

75. K. J. Seung, S. Keshavjee and M. L. Rich, *Cold. Spring. Harb. Perspect. Med.*, 2015, 5, a017863.

Review Highlights

1. *Staphylococcus aureus* is one of the major human pathogen cause's mild superficial infections to severe life-threatening invasive infections.
2. The biofilm play an important role in antibiotic drug resistance which leads to public threat globally.
3. In this viewpoint, we discuss the underlining biofilm mechanism, its existing systems as active therapeutic agents and as vehicles to transport drugs to the site of infection.
4. The understanding of bacteria/biofilms is an aspect that we likewise summarize for possible drug development for future as medicine against resistant *S. aureus* was viewed.