WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 8, Issue 12, 379-388. Review Article ISSN 2277– 7105

A REVIEW ON ANTIBIOTIC RESISTANCE, BIOFILM FORMATION AND QUORUM SENSING

Chetan M. Jain* and Krutika S. Sonar

P. R. Pote Patil College of Pharmacy, Amravati.

Article Received on
29 August 2019,
Revised on 19 Sept. 2019,
Accepted on 09 Oct. 2019
DOI: 10.20959/wjpr201912-16058

***Corresponding Author**
Chetan M. Jain
P. R. Pote Patil College of Pharmacy, Amravati.

ABSTRACT

Resistance to antibiotics is observed when drug lower the effect against the disease causing bacteria and multiplication of bacteria is continues in presence of antimicrobial agents and these replication of bacteria in presence of antimicrobial agents is known as antimicrobial resistance. Microorganism are become resistance to bacterial infections by different mechanism especially based on chemical structure and mode of action of antibiotics such as Efflux pumps, Antibiotic inactivation, β-lactamases, Aminoglycoside modifying enzymes, Chloramphenicol acetyltransferases, Modifications of the Antibiotic Molecule. In these review article we have focused on antimicrobial resistance and also on biofilm and Quorum sensing. A microbial biofilm is group of cell of microorganism which are enclosed in a extracellular polymeric matrix (EPM) produced by the microorganism itself and attached to a living surface. In the process of biofilm formation the microorganism should attach to the surface. Bacteria are approach to surface by two forces such as attractive and repulsive. The formation of biofilm is mainly explained by three stages *Viz.* attachment, maturation and dispersion**.** Quorum sensing is the communication between the cells. Quorum sensing regulate the virulence factors like biofilm formation, motility, production of proteases. Hemolysin, pyocyanin, toxins and pigment production from the bacteria *Pseudomonas aeruginosa.* Here in these review we also discuss plants used as anti-QS agents.

INTRODUCTION

Resistance to antibiotics is observed when drug lower the effect against the disease causing bacteria and multiplication of bacteria is continues in presence of antimicrobial agents and these replication of bacteria in presence of antimicrobial agents is known as antimicrobial resistance.[1] In developing countries like India antibiotic resistance is become critical issue

because of easy availability and irrelevant use of antibiotics.[2] In day to day life in concern with the human health antibiotics become the most common medicine although 50% of antibiotics which are prescribed are not useful or not needed to cure the antimicrobial infection. Another reason for antibiotic resistance is use of antibiotics in agriculture or in food producing animals which boost the bulk of food. USFDA (United State Food Drug Administration) set the pathway on antibiotics used in food producing animals.[3,4]

The challenge of antibiotic resistance in microorganism is related all over the world with high morbidity and mortality. Conventional antibiotics are unable to multidrug resistance in Gram-positive and Gram -negative bacteria. The major challenge in today's era is to overcome on bacterial infection and prevention from the disease because irrational use of broad spectrum antibiotics, only few new antimicrobial agents is in pipeline.[5]

WHO published list of bacteria who developed the resistance against the new antibiotics to R&D on the basis of priority is given in table no 1.[6]

Table 1: WHO list for bacterial pathogens resistance to bacteria.

Sr.No.	Name of Bacterial Species	Name of resistant Antibiotic
Priority 1: Critical		
1.	*Acinetobacter baumannii*	Carbapenem-resistant
2.	*Pseudomonas aeruginosa*	Carbapenem-resistant
3.	*Enterobacteriaceae*	Carbapenem-resistant, 3rd generation cephalosporin-resistant
Priority 2: High		
4.	*Enterococcus faecium*	Vancomycin-resistant
5.	*Staphylococcus aureus*	Methicillin-resistant, Vancomycin intermediate resistant
6.	*Helicobacter pylori*	Clarithromycin-resistant
7.	*Campylobacter*	Fluoroquinolone-resistant
8.	*Salmonella spp*	Fluoroquinolone-resistant
9.	*Neisseria gonorrhoeae*	3rd generation cephalosporin- resistant, Fluoroquinolone-resistant
Priority 3: Medium		
10.	*Streptococcus pneumonia*	Penicillin-non-susceptible
11.	*Haemophilus influenza*	Ampicillin-resistant
12.	*Shigella spp*	Fluoroquinolone-resistant

Mechanism of Antimicrobial Resistance

In the era 20th century, resistance to antibiotics is not considered as to much problematic to treat the bacterial infections. But as time going on use of antibiotic is get increases and which results in treatment failure in case of bacterial infections. Microorganism are become

resistance to bacterial infections by different mechanism especially based on chemical structure and mode of action of antibiotics.[7] Following are some mechanism in which microorganisms develops their resistance.

Efflux pumps

The membrane protein known as efflux pump is expelling the antimicrobial agents from the cell and keeps it intracellular concentrations at low levels which results in decrease the permeability of outer membrane hence decreased antibiotic uptake. The reduced uptake and active efflux develop the resistance at low level. This mechanism of microbial resistance affects on all types antimicrobial agents such as macrolides, tetracyclines, and fluoroquinolones.[8]

Antibiotic inactivation

β-lactamases, aminoglycoside modifying enzymes, and chloramphenicol acetyltransferases (AACs) are the enzymes involved in this mechanism of antibiotic resistance.[9]

β-lactamases

This involved the hydrolysis of antimicrobial agents by the enzyme β-lactamase or modification in cellular permeability results in resistance in many bacteria because of heterogenous group of enzymes is present in β-lactamases.[7]

Aminoglycoside modifying enzymes

The structure of aminoglycoside antibiotics like streptomycin is complex which involved aminocyclitol nucleus (streptamine, 2-deoxystreptamine, or streptidine) linked to amino sugars through glycosidic bonds. This 2-deoxystreptamine nucleus or amino sugar is get modified at hydroxyl or amino group by Aminoglycoside modifying enzymes. Intrinsic and acquired mechanism is involved in resistance of aminoglycoside antibiotics.[10,11]

Chloramphenicol acetyltransferases

Hydroxyl group of chloramphenicol is acetylates by the enzyme Chloramphenicol Chloramphenicol acetyltransferases results in resistance to some Gram-positive, Gram-negative species of bacteria and some strains of *Haemophilus influenza* and this modification in chloramphenicol is unable to bind ribosomal 50S subunit.[12]

Modifications of the Antibiotic Molecule

This is very common mechanism of antibiotic resistance which observed in all class of antimicrobial agents. Target site of antibiotic is changes by spontaneous mutation of bacterial gene. For example antibiotics like rifamycins and is develop the antimicrobial resistance by mutation in enzyme RNA polymerase and similarly in case of quinolones by enzyme DNA gyrase.[13]

Protection of targets has been found to be a clinically relevant mechanism of resistance for several important antibiotics. Erythromycin ribosome methylase (erm) family of genes methylate 16S rRNA and alter the drug-binding site, thus preventing the binding of macrolides, lincosamines and streptogramins.[14]

BIOFILM

A microbial biofilm is group of cell of microorganism which are enclosed in a extracellular polymeric matrix (EPM) produced by the microorganism itself and attached to a living surface. All biofilm have some common characteristics such as production of extracellular polymeric substances (EPS) which are hydrated biopolymers secreted by bacteria.[15] Sessile community of microorganism are irreversibly adhere on interface.[13] The thickness of extracellular polymeric matrix is 0.2e1.0mm and not more than 10e30 nm. From the total volume of biofilm about 5e35% is occupied by the microorganism and remaining volume is of extracellular matrix. Extracellular matrix is mainly composed of protein and other constituents such as carbohydrates, DNA molecules, RNA, ions and water.[16]

Process of biofilm formation[17]

In the process of biofilm formation the microorganism should attach to the surface. Bacteria are approach to surface by two forces such as attractive and repulsive. The formation of biofilm is mainly explained by three stages *Viz.* attachment, maturation and dispersion**.**

1. Attachment

The attachment process is a two-stage process: initial reversible attachment and irreversible attachment. From these two attachment irreversible attachment can allow stronger physical or chemical shear forces. The movement of type IV pili (Tfp) and flagella is great significance in Initial attachment of biofilm. The affinity between the cells and surface is critical in flagella but type IV pili movement allow formation the micro colonies.

2. Maturation

During the biofilm formation the bacterial communities are talk to each other which is referred as specialized function of biofilm. In these stage of maturation proteins, DNA, polysaccharides are support to biofilm formation.

3. Dispersion

This stage is also the critical stage of biofilm formation. Dispersion of biofilm happens due to deficiency of nutrients, intense competition, outgrown population. It is observed within the complete bacteria or some part of bacteria.

How to Perform Biofilm Assay

Wash the biofilm three times with phosphate buffer saline (PBS) solution and stained the biofilm with 500 ul solution of crystal violet (2.3%) again rinse the stained biofilm five times with PBS solution. Transfer the strain in a petri plate and dissolve the crystal violet by using organic solvent ethanol. Then calculate the % biofilm formation against the untreated biofilm control sample. Then determine the concentration of crystal violet by OD 595 nm of 1:10 diluted samples.[18]

QUORUM SENSING

Quorum sensing is the communication between the cells. Quorum sensing regulate the virulence factors like biofilm formation, motility, production of proteases. Hemolysin, pyocyanin, toxins and pigment production from the bacteria *Pseudomonas aeruginosa.*[19] Population density mechanism which is present in bacteria is expressed by quorum sensing by using cell-cell communication system in bacteria. Quorum sensing is also respond and produce diffusible signals or secreted signals. These signals may vary according to type of bacteria. In most bacteria quorum sensing also changes virulence function which is important for pathogenesis.[20]

For the regulation of various physiological functions, Gram-negative and Gram-Positive bacteri uses quorum sensing or cell or density mechanism which is present within the bacteria. Autoinducers are produced which are responsible for production and detection of extracellular signaling molecules. The recent study of quorum sensing shows that it changes intra and inter species cell to cell communication which play an important role in enabling bacteria to develop complex community structure.[21] *Chromobacterium violaceum* is an gram negative bacterial species which widely distributed and with reponce to regulated gene

expression it produce pigment violacein. The recent study on *Chromobacterium violaceum* suggest that pigment violacein production is expressed by VioD., VioC, VioB, and VioA genes arranged in an operon by N-acyl homoserine lactone. *Chromobacterium violaceum* CV026 is biosensor strain of *Chromobacterium violaceum* which deficient in autoinducers synthase. If we add to strain *Chromobacterium violaceum* CV026 is helpful to understand the various mechanism of quorum sensing and also useful for performing biological assay of screening of Anti-QS agents.[22]

Languages for Cell-Cell Communication Within the Bacteria

Gram-negative bacterial species uses LuxI/LuxR languages for communication. It uses the autoinducers such as HSL (Homoserine lactone) whose synthesis is depends on LuxI and LuxR homologue encoding also known as transcriptional activator protein which is responsible for cognate HSL and induction correct outputs. As like gram-negative bacteria, gram-positive bacteria do not involve HSL as signals and LuxI/LuxR languages for cell-cell communication. In gram-positive bacteria, it secrete processed peptides signaling molecules through ATP-binding cassette exporters protein. These peptides signals are then greet by cognate two-component sensors kinase protein that interact with cytoplasmic response regulator proteins and this mechanism of communication is known as phosphorelay cascade.[21]

Anti-QS agents

The substances or agents used in inhibition of quorum sensing are known as Anti-QS agents. These agents were first describe in red marine algae known as Delisa pulchra and in Florida algae and in some plants. During this study it is found that plants produce autoinducers which mimics to perplexing the bacterial cell-cell communication system and it is also receive and respond to microbial signals.[23]

Inhibition of QS by Plants

The increased use of antibiotics leads to develop antibacterial resistance against various bacterial species. So in todays era it is necessary to develop alternative to conventional antibiotics or increased antibiotic activity by using various plants extracts or chemical constituents present in plants.

In plants mainly Traditional Chinese Medicines (TCM) mostly focused for Anti-QS activity. in reaserch article of Koh, et. al. selected the 10 TCM plants and studied for its anti-QS

activity by using *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PA01 strain. Out of ten, eight plants shows inhibition of QS activity of bacterial species. These plants include *Prunus armeniaca, Prunella vulgaris, Nelumbo nucifera, Panax notoginseng* (root and flower), *Punica granatum, Areca catechu, and Imperata cylindrica.*[24] Quave et. al. shows that extracts of three plants *viz. Ballota nigra, Castanea sativa* and *Sambucus ebulus* developed anti QS activity in pathogenic MRSA isolate which shows dose dependent response in production of δ-hemolysin.[25]

Chenia et. al. carried out their study on K. Africana extracts for Anti-QS activity which shows importance of these extracts in reducing virulence and pathogenicity of antimicrobial resistance of bacteria within the body (in-vivo).[26] Allison et. al. studied the effect on *Pseudomonas aeruginosa* virulenc factor and QS system of aq. extract of six medicinal plants such as *Conocarpus erectus, Chamaesyce hypericifolia, Callistemon viminalis, Bucida buceras, Tetrazygia bicolor,* and *Quercus Virginian* from that *Conocarpus erectus, Bucida buceras* and *Callistemon viminalis* inhibit the LasA protease, LasB elastase, Pyorevedin production and biofilm formation.[23]

Tan L.Y. et. al. studied on extract of Melicope lunu-ankenda in which acooording to them this extract inhibit the response of C. violaceum CV026 to N-hexanoylhomoserine lactone.[27] Jaramillo-Colorado et. al. studied on anti-QS activity of essential activity of essential oil, in that limonene carvone and citral (Geranial, naral*)* was found to be chemo type for *Lippia alba.*[28]

Stashenko et. al. also studied on Anti-QSactivity of some essential oil. In that they studied on three species of Piper for inhibitory effect on production of violacein pigment induced by N-hexanoyl homoserine lactone in C. violaceum CV026. The three species of piper such as *Piper bredemeyeri* shows IC50 for QS of 45.6 µg/ml, *Piper brachypodom* is 93.1µg/ml, and *Piper bogotence* 513.8 µg/ml. Hence piper species shows good anti-QS activity from the category of essential oil.[29] Musthafa K.S. et. al. studied on Anti-QS activity of edible plants and fruits against AHL (N-acylhomoserine lactone). In this study they identify as *Ananas comosus, Musa paradiciaca, Manilkara zapota* and *Ocimum sanctum* have N-acylhomoserine lactone inactivating compound which are used to alternative for antibiotic compounds to inhibit AHL-mediated bacterial infection.[30]

CONCLUSION

The challenge of antibiotic resistance in microorganism is related all over the world with high morbidity and mortality. Conventional antibiotics are unable to multidrug resistance in Gram-positive and Gram -negative bacteria. The major challenge in today's era is to overcome on bacterial infection and prevention from the disease because irrational use of broad spectrum antibiotics, only few new antimicrobial agents is in pipeline. WHO published the list of microorganism and their antibiotics resistance. According to literature different plants are used for inhibition of quorum sensing are discussed above in this review article.

REFERENCES

1. Zaman, S.B., Hussain, M.A., Nye, R., Mehta, V., Mamun, K.T. and Hossain, N., A review on antibiotic resistance: alarm bells are ringing. *Cureus*, 2017; *9*(6).
2. Kumar, S.G., Adithan, C., Harish, B.N., Sujatha, S., Roy, G. and Malini, A., Antimicrobial resistance in India: A review. *Journal of natural science, biology, and medicine*, 2013; *4*(2): 286.
3. Centers for Disease Control and Prevention (CDC, Antibiotic Resistance Threats in the United States, 2013; http://www.cdc.gov/drugresistance/threat-report-2013/) and the Public Health Agency of Canada (PLoS One, 2015; 10(4): 1-11).
4. Tang, K.L., Caffrey, N.P., Nóbrega, D.B., Cork, S.C., Ronksley, P.E., Barkema, H.W., Polachek, A.J., Ganshorn, H., Sharma, N., Kellner, J.D. and Ghali, W.A., Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *The Lancet Planetary Health*, 2017; *1*(8): e316-e327.
5. Frieri, M., Kumar, K. and Boutin, A., Antibiotic resistance. *Journal of infection and public health*, 2017; *10*(4): 369-378.
6. https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/
7. Byarugaba, D.K., Mechanisms of antimicrobial resistance. In *Antimicrobial Resistance in Developing Countries*, 2010; 15-26. Springer, New York, NY.
8. Džidić, S., Šušković, J. and Kos, B., Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. *Food Technology & Biotechnology*, 2008; *46*(1).
9. Kapoor, G., Saigal, S. and Elongavan, A., Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of anaesthesiology, clinical pharmacology*, 2017; *33*(3): 300.

10. Ramirez, M.S. and Tolmasky, M.E., Aminoglycoside modifying enzymes. *Drug Resistance Updates*, 2010; *13*(6): 151-171.
11. Garneau-Tsodikova, S. and Labby, K.J., Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. *Med Chem Comm*, 2016; *7*(1): 11-27.
12. Tolmasky, M.E., BACTERIAL RESISTANCE TO AMINOGLYCOSIDES AND BETA-LACTAMS: THE TN1331 Tn1331 TRANSPOSON PARADIGM. *Frontiers in Bioscience*, 2000; *5*: d20-29.
13. Lambert, P.A., Bacterial resistance to antibiotics: modified target sites. *Advanced drug delivery reviews*, 2005; *57*(10): 1471-1485.
14. Blair, J.M., Webber, M.A., Baylay, A.J., Ogbolu, D.O. and Piddock, L.J., Molecular mechanisms of antibiotic resistance. Nature reviews microbiology, 2015; 13(1): 42.
15. Ali Mohammed, M.M., Nerland, A.H., Al-Haroni, M. and Bakken, V., Characterization of extracellular polymeric matrix, and treatment of Fusobacterium nucleatum and Porphyromonas gingivalis biofilms with DNase I and proteinase K. *Journal of oral microbiology*, 2013; *5*(1): 20015.
16. Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M.A., Hussain, T., Ali, M., Rafiq, M. and Kamil, M.A., Bacterial biofilm and associated infections. *Journal of the Chinese Medical Association*, 2018; *81*(1): 7-11.
17. Rabin, N., Zheng, Y., Opoku-Temeng, C., Du, Y., Bonsu, E. and Sintim, H.O., Biofilm formation mechanisms and targets for developing antibiofilm agents. *Future medicinal chemistry*, 2015; *7*(4): 493-512.
18. Hong, Q., Dong, X., Chen, M., Sun, H., Hong, L., Wang, Y., Li, H. and Yu, Q., An in vitro and in vivo study of plasma treatment effects on oral biofilms. *Journal of oral microbiology*, 2019; *11*(1): 1603524.
19. Al-Haidari, R.A., Shaaban, M.I., Ibrahim, S.R. and Mohamed, G.A., Anti-quorum sensing activity of some medicinal plants. *African Journal of Traditional, Complementary and Alternative Medicines*, 2016; *13*(5): 67-71.
20. Abisado, R.G., Benomar, S., Klaus, J.R., Dandekar, A.A. and Chandler, J.R., Bacterial quorum sensing and microbial community interactions. mBio, 2018; e02331-17.
21. Bassler, B.L., How bacteria talk to each other: regulation of gene expression by quorum sensing. *Current opinion in microbiology*, 1999; *2*(6): 582-587.
22. Zhu, H., He, C.C. and Chu, Q.H., Inhibition of quorum sensing in Chromobacterium violaceum by pigments extracted from Auricularia auricular. *Letters in applied microbiology*, 2011; *52*(3): 269-274.

23. Allison Adonizio, Kok-Fai Kong, and Kalai Mathee., Inhibition of Quorum Sensing-Controlled Virulence Factor Production in Pseudomonas aeruginosa by South Florida Plant Extracts, Antimicrobial Agents and Chemotherapy, Jan, 2008; 198–203.
24. Koh, K.H. and Tham, F.Y., Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. *Journal of Microbiology, Immunology and Infection*, 2011; *44*(2): 144-148.
25. Quave, C.L., Plano, L.R. and Bennett, B.C., Quorum sensing inhibitors of Staphylococcus aureus from Italian medicinal plants. *Planta medica*, 2011; *77*(02): 188-195.
26. Chenia, H., Anti-quorum sensing potential of crude Kigelia africana fruit extracts. *Sensors*, 2013; 13(3): 2802-2817.
27. Tan, L.Y., Yin, W.F. and Chan, K.G., Silencing quorum sensing through extracts of Melicope lunu-ankenda. *Sensors*, 2012; 12(4): 4339-4351.
28. Jaramillo-Colorado, B., Olivero-Verbel, J., Stashenko, E.E., Wagner-Döbler, I. and Kunze, B., Anti-quorum sensing activity of essential oils from Colombian plants. *Natural product research*, 2012; 26(12): 1075-1086.
29. STASHENKO, E., Antiquorum sensing activity of essential oils isolated from different species of the genus Piper. *Vitae*, 2011; 18(1): 77-82.
30. Musthafa, K.S., Ravi, A.V., Annapoorani, A., Packiavathy, I.S.V. and Pandian, S.K., Evaluation of anti-quorum-sensing activity of edible plants and fruits through inhibition of the N-acyl-homoserine lactone system in Chromobacterium violaceum and Pseudomonas aeruginosa. *Chemotherapy*, 2010; *56*(4): 333-339.