Chapter 10
Synergism Between Quorum Sensing Inhibitors and Antibiotics: Combating the Antibiotic Resistance Crisis

Sahana Vasudevan, Shogan Sugumar Swamy, Gurmeet Kaur, S. Adline Princy, and P. Balamurugan

Abstract With the alarming increase in the antibiotic resistance, there is an immediate need for alternative therapeutic strategies to combat this ever-changing bacterial battle. Combinatorial therapies have gained attention owing to their multiple targeted actions. The use of antibiotic is inevitable and antibiotics in combinations have been in use to treat drug resistant infections. Nevertheless, the multidrug resistant strains have found their own mechanisms to surpass such combinatorial treatments. Quorum sensing (QS) inhibition is considered to be the silver lining but is yet to find its way to commercial use. Hence, to combat the antibiotic resistance crisis, the synergy of QSIs and antibiotics is one of the possible revolutionary approaches. In this chapter, we have highlighted the importance and need for the synergy approach with the successful *in vitro* and *in vivo* studies that can possibly be extended to the commercial use.

Keywords Biofilm · Antibiotic · Resistance · Quorum sensing inhibition · Synergy

10.1 Introduction

Antibiotics have been the wonder drugs since 1928 when Sir Alexander Fleming discovered Penicillin, from *Penicillium notatum,* which was a breakthrough in the field of medicines. By 1940, due to the extensive use of antibiotics, there was an accelerated development of antibiotic-resistant strains which C. Lee Ventola describes as 'The Antibiotic resistance crisis'. The major causes were the overuse and misuse of drugs for the up growth of the antibiotic-resistant strains (Ventola 2015).

S. Vasudevan · S. S. Swamy · G. Kaur · S. A. Princy · P. Balamurugan (✉)
Quorum Sensing Laboratory, Centre for Research in Infectious Diseases (CRID), School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur 613401, Tamil Nadu, India
e-mail: adlineprinzy@biotech.sastra.edu; balamurugan@scbt.sastra.edu

V. C. Kalia (ed.), *Biotechnological Applications of Quorum Sensing Inhibitors*,
https://doi.org/10.1007/978-981-10-9026-4_10

This is also attributed to improper sanitation, poor public health system and also irregular prescription (amr-review.org). The resistance is developed through bacterial evolution by spontaneous mutations making various drugs incompetent. Humans are affected either by direct contact or indirectly by the consumption of livestock, as antibiotics are used largely in animal feeds to prevent microbial infections. It is supported by the fact that 80% of antibiotics are used in livestock feed, in the US (Ventola 2015). Antibiotic resistance has also become an economic crisis, and many countries have employed various measures to overcome this crisis. It is estimated that by 2050, the death toll will exceed 10 million each year which extends to the cost of 100 trillion USD loss in output (amr-review.org). The main players involved are the pharmaceutical and biotechnology companies who are threatened by the resistance development. This crisis needs to be immediately addressed to avoid the economic loss of a nation.

Bacteria adopt numerous mechanisms to defend against the antimicrobials (Munita and Arias 2016). These mechanisms further ease the way for acquiring phenotypic and genotypic resistance to those antibiotics and make the pathogen to evolve as a superbug. Hence to overcome drug resistance, there is a pressing need for alternative therapeutic approaches. Among them, quorum sensing (QS) inhibition proves to be an encouraging strategy (Kalia and Purohit 2011), as these small molecules will not induce resistance (Gerdt and Blackwell 2014). Quorum sensing is the cell to cell communication, contributes to the defense mechanism and also produces virulence factors. Quorum sensing is attributed not only to the infectious diseases but also to different fields. Subsequently, quorum sensing inhibitors are reported to have varied applications (Kalia et al. 2014a; Kalia and Kumar 2015a). Though many quorum sensing inhibitors (QSIs) have been reported, the use of QSI alone in treatment has not been very successful (Sengupta et al. 2013) because of which commercialization of QSI is not in existence till now. A QSI can attenuate virulence of the pathogenic bacteria by targeting the QS signaling pathway. It is to be noted that QSI will not affect the bacterial growth, rather stop the pathogen to establish a community (Kalia 2013, 2015). A few research works claim that the host immune response will be sufficient enough to clear the bacteria after QS inhibition. However, in a real clinical setting, it may not be sufficient for the complete clearance of the high bacterial load, especially the ones which are in biofilm and may lead to re-emergence of the pathogen as resistant strains. Also to tackle the multidrug resistant strains, resensitization of resistant drugs has become the need of the hour. In spite of being considered as an alternative therapy to antibiotics, a few claims and theories state that bacteria might develop resistance to the QSIs which requires strong experimental evidence (Kalia et al. 2014b; Kalia and Kumar 2015b; Koul et al. 2016). Hence combinatorial treatment of QSI with antibiotics will be a promising approach, as target specific QSI will not pose survival stress and the antibiotic will aid in curbing the pathogenesis, at low concentrations.

10.2 Synergy Between QSIs and Antibiotics

10.2.1 Natural QSIs and Antibiotics

To date, many quorum sensing inhibitors have been tested along with conventionally used antibiotics that are listed in Table 10.1. Many naturally occurring QSIs are reported previously which were shown to have remarkable synergistic activity with conventional antibiotics. The following are a few reports which support the combinatorial action. A well-known QSI, furanone C-30, enhanced the susceptibility to tobramycin against *P. aeruginosa* biofilm (Hentzer et al. 2003). Fujita et al. (2005) have shown baicalein, a QSI compound from thyme leaves extract reduced the minimum inhibitory concentrations of tetracycline and other β-lactams antibiotics against Methicillin-resistant *Staphylococcus aureus* (MRSA). Also, they have proposed the possible mechanisms for the synergistic action: inhibition of penicillin-binding protein 2′(2a) by baicalein, and damage of peptidoglycan. A similar study was extended to vancomycin-resistant *Enterococcus* where the synergy with gentamicin was reported (Chang et al. 2007). Later, baicalein was shown to interfere with the transcriptional activator protein (TraR) of *P. aeruginosa* QS system, and also having combinatorial activity with ampicillin (Zeng et al. 2008).

Farnesol, a quorum signal of *Candida* sp. inhibited *S. epidermidis* biofilm in synergy with nafcillin and vancomycin (Pammi et al. 2011). Synergism between tobramycin and baicalin hydrate against *Burkholderia cenocepacia* was shown in a lung infected animal model (Brackman et al. 2011). It was also shown that the extracts of *Nymphaea tetragona* and antibiotics could be effective against drug-resistant Salmonella (Hossain et al. 2014). Synergistic efficacy of sub-MIC concentrations of curcumin with ceftazidime and ciprofloxacin against *P. aeruginosa* QS system was well documented (Roudashti et al. 2017). The synthetic derivatives of natural compounds have also been reported for QS inhibition activity. Zeng et al. (2011) investigated the combinatorial action of 14-alpha-lipoyl andrographolide (AL-1) and traditionally used antibiotics in inhibiting not only the *P. aeruginosa* biofilm but also the EPS and pyocyanin.

10.2.2 Synthetic QSIs and Antibiotics

Like natural QSIs, several synthetic small molecule inhibitors have been described to possess combinatorial action with conventional antibiotics with less or no toxicity. Balaban et al. (2003) have shown the synergistic actions of the RNAIII-inhibiting peptide (RIP) with conventional antibiotics in 100% clearance of graft-associated *S. epidermidis* infections *in vivo*, suggesting that RIP may be used to coat medical devices to prevent staphylococcal infections. The activity of 2-aminoimidazole/triazole conjugate with conventional antibiotics promoted the biofilm dispersion and along with resensitization of MRSA and multi-drug resistant *Acinetobacter*

Table 10.1 Synergistic action of quorum sensing inhibitors and antibiotics

Quorum sensing inhibitors	Proposed mechanism of quorum sensing inhibition	Concentration of QSI	Antibiotics	Target organism	Effective synergistic action	References
RNAIII-inhibiting peptide	Phosphorylation inhibition of target of RNAIII activating protein (TRAP)	10 μg/ml	Cefazolin	*Staphylococcus epidermidis*	>10–100-fold bacterial clearance *in vivo*	Balaban et al. (2003)
			Rifampin			
			Imipenem			
			Levofloxacin			
			Teicoplanin			
			Mupirocin			
			Quinupristin			
			Dalfopristin			
Furanone C-30	Interference in Acyl-homoserine lactone signaling	10 μM	Tobramycin	*Pseudomonas aeruginosa*	Two to three orders of magnitude more sensitive in bacterial clearance in biofilm	Hentzer et al. (2003)
Baicalein	Efflux pump inhibition	25 μg/ml	β-lactam antibiotics tetracycline	Methicillin-resistant *Staphylococcus aureus*	Reduction in MIC of tetracycline in MRSA strain OM584, from 128 μg/ml to 0.06 μg/ml	Fujita et al. (2005)
	NA	32 μg/ml	Gentamicin	Vancomycin-resistant *Enterococcus*	Two orders of magnitude higher activity in reduction of bacterial growth	Chang et al. (2007)
	Interference in quorum sensing system, transcription activator protein (TraR)	200 mM	Ampicillin	*Pseudomonas aeruginosa*	NA	Zeng et al. (2008)

Dispersin B	Dispersal of matured biofilm	20–600 μg/ml	Cefamandole nafate	*Staphylococcus epidermidis*	NA	Donelli et al. (2007)
2-Aminoimidazole/ triazole conjugates	Inhibition and dispersal of biofilm	2.6–325 nM	Novobiocin	*Acinetobacter baumannii* and *Staphylococcus aureus*	Three orders magnitude increase of biofilm dispersion	Rogers et al. (2010)
			Colistin			
			Tobramycin			
			Novobiocin			
			Tobramycin etc.,			
Baicalin hydrate	Acyl-homoserine lactone QS signaling	100 μM	Tobramycin	*Burkholderia cenocepacia*, *Burkholderia multivorans* and *Pseudomonas aeruginosa*	Significant decrease in biofilm as compared to antibiotic alone	Brackman et al. (2011)
Cinnamaldehyde		250 μM	Clindamycin	*Pseudomonas aeruginosa* and *Staphylococcus aureus*		
Hamamelitannin		250 μM	Vancomycin	*Staphylococcus aureus*		
14-Alpha-lipoyl andrographolide (AL-1)	Interference in QS system	0.5 mM	Azithromycin	*Pseudomonas aeruginosa*	Significant inhibition of biofilm, EPS and pyocyanin compared to antibiotic alone	Zeng et al. (2011)
			Ciprofloxacin Fosfomycin			
			Streptomycin			
			Gentamicin			
Lactonase	Increasing antibiotic susceptibility of the biofilm	0.3 units	Ciprofloxacin	*Pseudomonas aeruginosa*	5–6 folds reduction in minimum biofilm eradication concentration	Kiran et al. (2011)
			Gentamicin			

(continued)

Table 10.1 (continued)

Quorum sensing inhibitors	Proposed mechanism of quorum sensing inhibition	Concentration of QSI	Antibiotics	Target organism	Effective synergistic action	References
Nanosilver	Antibacterial and antibiofilm	0.25 – 2 μg/mL	Ampicillin	*Escherichia coli*	Reduction of antibiotic concentration	Hwang et al. (2012)
			Chloramphenicol	*Pseudomonas aeruginosa*		
			Kanamycin	*Staphylococcus aureus*		
				Streptococcus mutans		
Silver nanocolloids	Antiquorum and antibacterial	10 μg/mL	Amoxicillin	*Escherichia coli*	Increase in antagonistic activity	Arunkumar et al. (2013)
			Methicillin	*Pseudomonas aeruginosa*		
			Ampicillin	*Staphylococcus aureus*		
Isobutyl-DPD	Inhibiting the genotypic QS responses and dispersal of preformed biofilm	40 and 100 μM	Gentamicin	*Escherichia coli*	Reduction in biofilm thickness by more than 80% for *E. coli* and 90% for *P. aeruginosa*	Roy et al. (2013)
Phenyl DPD			Gentamicin	*Pseudomonas aeruginosa*		
Fimbrolide	Antivirulent and anti-biofilm agents	NA	NO hybrids	*Pseudomonas aeruginosa*	Increased biofilm inhibition at picomolar to nanomolar levels	Kutty et al. (2013)
Biogenic Silver nanoparticles	Antibacterial and antibiofilm agent	0.1–1 μg/mL	Ampicillin	*Pseudomonas aeruginosa*	Enhanced antibacterial activity	Gurunathan et al. (2014)
			Chloramphenicol	*Shigella flexineri*		
			Erythromycin	*Staphylococcus aureus*		
			Gentamicin	*Streptococcus pneumoniae*		
			Tetracycline			
			Vancomycin			
Citrate-capped silver nanoparticles	Biofilm inhibition and eradication	0.156–10 μg/mL	Aztreonam	*Pseudomonas aeruginosa*	Synergistic antimicrobial activity	Habash et al. (2014)

Mycofabricated Silver nanoparticles	Antibiofilm agent	25 µg/mL	Tobramycin	*Pseudomonas aeruginosa*	100% killing of biofilm cells	Singh et al. (2015)
4-(Benzylamino) cyclohexyl2-hydroxycinnamate	SarA inhibitor	15 and 65 µg/mL	Gentamicin	*Staphylococcus aureus*	Significant clearance of *Staphylococcus aureus in vivo* at lower concentration of gentamicin	Balamurugan et al. (2015)
Biogenic Silver nanoparticles (Fungus derived)	Antimicrobial and antibiofilm	1–128 µg/mL	Amikacin	*Escherichia coli*	Synergistic antibacterial activity	Barapatre et al. (2016)
			Kanamycin	*Pseudomonas aeruginosa*		
			Oxytetracycline	*Staphylococcus aureus*		
			Streptomycin			
2,3-Pyrazine dicarboxylic acid	LuxO modulator	25 µM	Chloramphenicol Doxycycline	*Vibrio cholerae*	Significant reduction in *Vibrio cholerae* growth	Hema et al. (2016)
			Erythromycin Tetracycline			
1, 3-Disubstituted Ureas	ComA inhibitors	0.23–15 µM	Sodium fluoride	*Streptococcus mutans*	Reduced the fluoride concentration to 31.25 ppm	Kaur et al. (2016, 2017)

baumannii (Rogers et al. 2010). Roy et al. (2013) have shown the clearance of preformed biofilm by DPD derivatives with a significant increase by 80 and 90% for *E. coli* and *P. aeruginosa* respectively. In another study, the combined use of 2,3-Pyrazine dicarboxylic acid and conventional antibiotics against *V. cholerae* showed a significant reduction in growth (Hema et al. 2016). The combined use of 1,3-disubstituted urea derivatives specifically targeted against the ComA (a bacteriocin associated ABC transporter) of *Streptococcus mutans* and sodium fluoride showed a significant inhibition of growth and biofilm (Kaur et al. 2016). Interestingly, in this case, the results showed a remarkable decrease in the fluoride concentration to 31.25–62.5 ppm (~1000 ppm is presently used in toothpaste formulations). Additionally, 1,3-di-m-tolylurea (DMTU) which is a 1,3-disubstituted ureas derivative along with fluoride was capable of reducing dental caries *in vivo* which was evident from the macroscopic observations and pathological studies (Kaur et al. 2017). Similarly, combinatorial treatments showed promising activity in animal models as shown in Balamurugan et al. (2015). In this study, a significant reduction in gentamicin concentration was reported with SarA (a quorum regulator) targeted QS inhibitor, 4-(benzylamino)cyclohexyl 2-hydroxycinnamate against *Staphylococcus aureus* associated with gestational urinary tract infection.

10.2.3 Quorum Quenching Enzymes and Antibiotics

Enzymes such as acyl homoserine lactone (AHL) lactonases and acylases have also shown potent quorum quenching activities and are found in diverse set of organisms which makes them more versatile to explore (Kalia et al. 2011; Huma et al. 2011; Kalia 2014; Kumar et al. 2015; Koul and Kalia 2017). Interestingly, quorum quenching enzymes also have a significant role in the combinatorial approaches to biofilm treatment. Donelli et al. (2007) have shown the mature biofilm dispersal by dispersin B (β-*N*-Acetylglucosaminidase) thereby enhancing the antibiotic activity against adherent cells on polyurethane surfaces. Similarly, the lactonase enzyme eradicated biofilm and increased the susceptibility to antibiotics ciprofloxacin and gentamicin against *Pseudomonas aeruginosa* biofilm (Kiran et al. 2011).

10.2.4 Nanoparticles and Antibiotics

In recent times, metal nanoparticles have been explored extensively for antimicrobial activities against multidrug resistant pathogens (Bose and Chatterjee 2015; Dobrucka and Długaszewska 2015; Szweda et al. 2015; Deng et al. 2016; Wan et al. 2016). As an advancement, quorum sensing inhibitory as well as antibiofilm activity of nanoparticles have also been reported (Agarwala et al. 2014; Gurunathan et al. 2014; Ahiwale et al. 2017). The combination of the nanoparticles and antibiotics is proven to have potential synergistic activities enhancing the efficiency of the

antibiotic. Silver nanoparticles are the most widely used metal nanoparticle for the antibacterial application which is extended to the combinatorial action with antibiotics. Mycofabricated silver nanoparticles were utilized along with tobramycin which improved the clearing of biofilm cells by facilitating the efficient penetration of the antibiotic (Singh et al. 2015). Gurunathan et al. (2014) have shown the antibiofilm activity of biogenic silver particles with different antibiotics against a wide range of Gram-positive and Gram-negative bacteria. Similar results were obtained for the combination of nano-silver and antibiotics (Hwang et al. 2012). Silver nanocolloids were shown to have effective combinatorial action with the conventionally used antibiotics against *S. aureus*, *E. coli* and *P. aeruginosa* (Arunkumar et al. 2013). The antimicrobial activity of aztreonam has been synergistically enhanced in the presence of citrate-capped silver nanoparticles against *P. aeruginosa* biofilms (Habash et al. 2014). Green synthesized silver nanoparticles from lignin-degrading fungus, *Aspergillus flavus* and *Emericella nidulans*, having antibiofilm activity showed synergistic antimicrobial activity against Gram-positive and Gram-negative bacteria (Barapatre et al. 2016).

Recently, Ilk et al. (2017), have encapsulated the quorum sensing inhibitor, kaempferol, in chitosan nanoparticles, and have shown the increased stability and QSI activity. Hence, the combined use of antibiotics with nanoparticles and QSIs with nanoparticles would be an exciting new choice of treatment in alternative therapies.

10.2.5 Combined Toxicity of Antibiotics and QSI

Even though the combined use of QSIs and antibiotic reduces the concentration of antibiotics, studies on assessing the combined toxicity are limited currently. A recent QSAR-based mechanistic study has assessed the combined toxicity of antibiotics and QSIs against *E. coli*. They have reported the toxicity effects of commonly used antibiotics such as sulfonamides, β–lactams and tetracyclines, and some potential QSIs (including furanone, pyrrolidones, and pyrroles). The eight QSIs taken showed an additive or antagonistic effect in combination with sulphonamides and had antagonistic effects with β-lactams and tetracyclines (Wang et al. 2017).

10.3 Synergy Mechanism

From the above examples, it can be clearly seen that the combinatorial use of QSI and antibiotics work in such a way that the QSI will inhibit/eradicate biofilm formation as well as virulence factors which will favor the effective functioning of antibiotics (Figs. 10.1a and 10.1b). As explained in the mechanism (Figs. 10.1a and 10.1b), QSI targets the quorum sensing pathway thereby reducing the production of QS signals and virulence factors. This interference in the virulence then paves a way for the antibiotics to complete their action in reduced dosage. If the QSI has biofilm

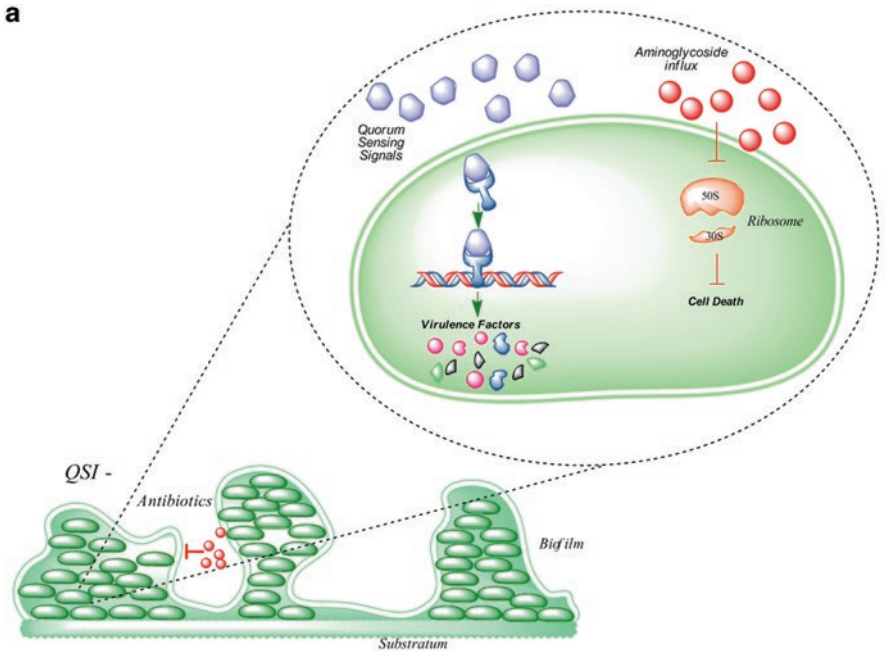

Fig. 10.1a Treatment of biofilm with antibiotics (aminoglycoside) and without quorum sensing inhibitor, QSI−. In the absence of QSI, the mature biofilm impose an antibiotic diffusion barrier for antibiotics. As the antibiotics are unable to enter the biofilm cells and bind to the 30s ribosomal unit, cell death is inhibited even at higher concentrations

disruption activity, then the combination with antibiotics will have an enhanced effect in biofilm dispersal (QSI action) by limiting the antibiotic diffusion barrier as well as the bacterial clearance (antibiotic action).

10.4 Methods of Measurement

In order to evaluate the synergistic action of the drugs, different models have been proposed. When the drugs are given in combination, the resulting effect can be either equal to, greater or less than the corresponding individual drugs' effect, which is termed as additive, synergism or antagonism respectively (Yang et al. 2014). In the case of QSI and antibiotics combination, the desired outcome is synergy rather than additive effect. QSI and antibiotics have independent targets and both the agents should have mutual participation in bringing out the desired effect. Thus, the interaction between a QSI and antibiotics is best explained in terms of synergy.

The most common assays that are carried out to understand synergy are checkerboard assay and time-kill assays (TKA). The checkerboard assay data are analyzed

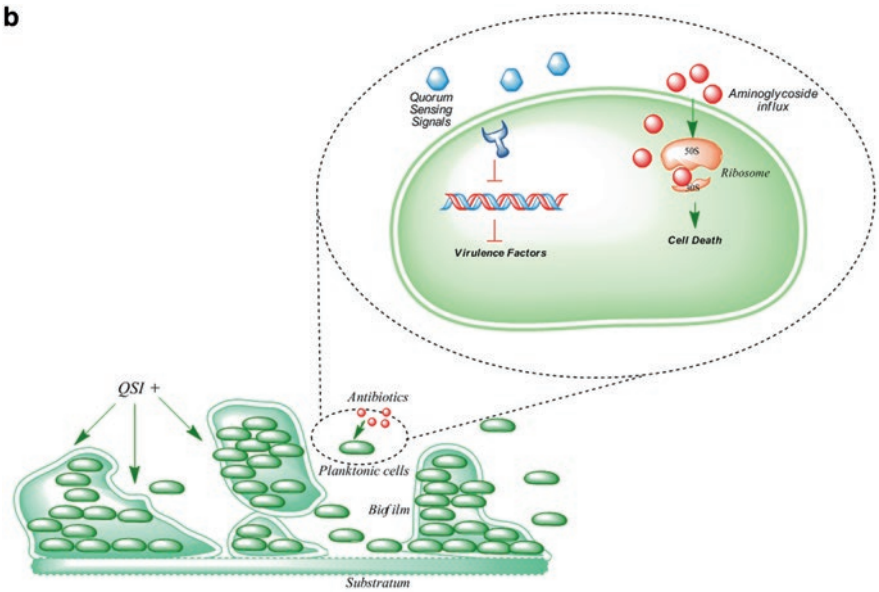

Fig. 10.1b Treatment of biofilm with antibiotics (aminoglycoside) and with quorum sensing inhibitor, QSI+. In the presence of QSI has disrupted the biofilm thereby paving a way for the antibiotic to enter the cell and complete its action. In this case, at low concentrations, the aminoglycoside is able to bind with 30s ribosomal subunit and interfere with the protein translational process and thereby causing cell death

using fractional inhibitory concentration (FIC). The data obtained from TKA gives the rate of killing in addition to the optimum concentration (Doern 2014).

10.4.1 Fractional Inhibitory Concentration Index (FICI)

The effect of the combinatorial treatment can be interpreted as synergistic, indifferent or antagonistic based on the non-parametric method, FICI (Kaur et al. 2016; Subramaniam et al. 2014). The following are the formulae to calculate the FICI of the drug interaction.

Let us consider the two drugs, A and B,

$$\mathrm{FICI} = \mathrm{FIC}_{\mathrm{A}} + \mathrm{FIC}_{\mathrm{B}}$$

Where,

$$\mathrm{FIC}_{\mathrm{A}} = \left(\text{MIC of A in combination}\right) / \left(\text{MIC of A alone}\right)$$

$$\mathrm{FIC}_{\mathrm{B}} = \left(\text{MIC of B in combination}\right) / \left(\text{MIC of B alone}\right)$$

FICI ≤0.5 denotes Synergy; 0.5 < FICI <4denotes indifference or absence of interaction; FICI >4 denotes antagonism.

10.4.2 Bliss Independence Model

Synergism is a mutually non-exclusive action of two drugs leading to the enhanced inhibitory activity. Thus, application of independence probability theory, Bliss model explains the synergistic action of the two drugs be informational (Yang et al. 2014). This model is particularly useful when QSI with antibiofilm activity is given in combination with the antibiotics.

The bliss independence model or BI theory (Goldoni and Johansson 2007; Sun et al. 2008) is described by the following equations.

$$I_i = \left(I_A + I_B\right) - \left(I_A X I_B\right) \tag{10.1}$$

Where,

Ii = predicted inhibition percentage of A and B.

I_A = experimental inhibition percentage of A (alone).

I_B = experimental inhibition percentage of B (alone).

$$I = 1\text{-}E \tag{10.2}$$

Where,

E = growth percentage

Equation 10.3 is obtained by substituting (10.2) in (10.1)

$$E_i = E_A X E_B \tag{10.3}$$

Where,

E_i = predicted growth percentage of A and B.

E_A = observed growth percentage of A

E_B = observed growth percentage of B.

Interaction (ΔE) is given by the formula (10.4):

$$\Delta E = E_{predicted} - E_{observed} \tag{10.4}$$

By the nonparametric approach described by Prichard et al. (1991,1993), E_A and E_B are obtained directly from the experimental data. With the obtained results, the interpretations are as follows: ΔE – positive (synergy) and ΔE – negative (antagonism).

With the previous studies, both the above said models correlated with each other (Barapatre et al. 2016; Kaur et al. 2016; Hema et al. 2016). Response surface methodology can also be used to understand the synergistic pattern of the above synergy. Thus these methods give a mathematical validation to the synergistic activity of the QSIs and antibiotics.

10.5 Conclusion

Combinatorial therapies are currently used in the treatment of complex diseases like cancer. The idea behind the combinatorial therapy is to target different molecular mechanisms thereby disarming the proliferation of the infectious diseases. Antibiotics are broad spectrum and require a very high dosage for the treatment of multidrug resistant strains. On the other hand, QSIs are target specific which works at very low concentrations without inducing a survival stress. With the aforementioned examples, it can be clearly seen that the use of these antibiotics with QSIs potentiates significant synergistic action and is a thoughtful way to combat the overuse of antibiotics.

10.6 Opinion

Currently, a broad range of antibiotics is used to scale down the infections. It is well known that continuous administration of antibiotics leads to resistance development. Therefore, a cocktail of antibiotics will also eventually lead to more resistant strains causing a disastrous epidemic. On the contrary, the use of QSI may not contribute to resistance development but it makes the bacteria more susceptible to antibiotics at low levels. This way the bacteria are exposed to the minimal level of antibiotics such that they are unable to trigger the evolution of resistant strains. Thus, the combinatorial use of antibiotics with quorum sensing inhibitors uplifts the condition of the antibiotics resistance crisis and also the market per se.

References

Agarwala M, Choudhury B, Yadav RN (2014) Comparative study of antibiofilm activity of copper oxide and iron oxide nanoparticles against multidrug resistant biofilm forming uropathogens. Indian J Microbiol 54:365–368. https://doi.org/10.1007/s12088-014-0462-z

Ahiwale SS, Bankar AV, Tagunde S, Kapadnis BP (2017) A bacteriophage mediated gold nanoparticle synthesis and their antibiofilm activity. Indian J Microbiol 57:188–194. https://doi.org/10.1007/s12088-017-0640-x

Arunkumar M, Mahesh N, Balakumar S, Sivakumar R, Priyadharshni S (2013) Antiquorum sensing and antibacterial activity of silver nanoparticles synthesized by mutant *Klebsiella pneumoniae* MTCC 3354. Asian J Chem 25:9961–9964. https://doi.org/10.14233/ajchem.2013.15754

Balaban N, Giacometti A, Cirioni O, Gov Y, Ghiselli R, Mocchegiani F, Viticchi C, Del Prete MS, Saba V, Scalise G, Dell'Acqua G (2003) Use of the quorum-sensing inhibitor RNAIII-inhibiting peptide to prevent biofilm formation in vivo by drug-resistant *Staphylococcus epidermidis*. J Infect Dis 187:625–630. https://doi.org/10.1086/345879

Balamurugan P, Hema M, Kaur G, Sridharan V, Prabu PC, Sumana MN, Princy SA (2015) Development of a biofilm inhibitor molecule against multidrug resistant *Staphylococcus aureus* associated with gestational urinary tract infections. Front Microbiol 6:832. https://doi.org/10.3389/fmicb.2015.00832

Barapatre A, Aadil KR, Jha H (2016) Synergistic antibacterial and antibiofilm activity of silver nanoparticles biosynthesized by lignin-degrading fungus. Bioresour Bioprocess 3:8. https://doi.org/10.1186/s40643-016-0083-y

Bose D, Chatterjee S (2015) Antibacterial activity of green synthesized silver nanoparticles using Vasaka (*Justicia adhatoda* L.) leaf extract. Indian J Microbiol 55:163–167. https://doi.org/10.1007/s12088-015-0512-1

Brackman G, Cos P, Maes L, Nelis HJ, Coenye T (2011) Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. Antimicrob Agents Chemother 55:2655–2661. https://doi.org/10.1128/AAC.00045-11

Chang PC, Li HY, Tang HJ, Liu JW, Wang JJ, Chuang YC (2007) *In vitro* synergy of baicalein and gentamicin against vancomycin resistant *Enterococcus*. J Microbiol Immunol Infect 40:56–61

Deng H, McShan D, Zhang Y, Sinha SS, Arslan Z, Ray PC, Yu H (2016) Mechanistic study of the synergistic antibacterial activity of combined silver nanoparticles and common antibiotics. Environ Sci Technol 50:8840–8848. https://doi.org/10.1021/acs.est.6b00998

Dobrucka R, Długaszewska J (2015) Antimicrobial activities of silver nanoparticles synthesized by using water extract of *Arnicae anthodium*. Indian J Microbiol 55:168–174. https://doi.org/10.1007/s12088-015-0516-x

Doern CD (2014) When does 2 plus 2 equal 5? A review of antimicrobial synergy testing. J Clin Microbiol 52:4124–4128. https://doi.org/10.1128/JCM.01121-14

Donelli G, Francolini I, Romoli D, Guaglianone E, Piozzi A, Ragunath C, Kaplan JB (2007) Synergistic activity of dispersin B and cefamandole nafate in inhibition of staphylococcal biofilm growth on polyurethanes. Antimicrob Agents Chemother 51:2733–2740. https://doi.org/10.1128/AAC.01249-06

Fujita M, Shiota S, Kuroda T, Hatano T, Yoshida T, Mizushima T, Tsuchiya T (2005) Remarkable synergies between baicalein and tetracycline, and baicalein and beta-lactams against methicillin resistant *Staphylococcus aureus*. Microbiol Immunol 49:391–396. https://doi.org/10.1111/j.1348-0421.2005.tb03732.x

Gerdt JP, Blackwell HE (2014) Competition studies confirm two major barriers that can preclude the spread of resistance to quorum-sensing inhibitors in bacteria. ACS Chem Biol 9:2291–2299. https://doi.org/10.1021/cb5004288

Goldoni M, Johansson C (2007) A mathematical approach to study combined effects of toxicants in vitro: evaluation of the Bliss independence criterion and the Loewe additivity model. Toxicol Vitro 21:759–769. https://doi.org/10.1016/j.tiv.2007.03.003

Gurunathan S, Han JW, Kwon DN, Kim JH (2014) Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. Nanoscale Res Lett 9:373–390. https://doi.org/10.1186/1556-276X-9-373

Habash MB, Park AJ, Vis EC, Harris RJ, Khursigara CM (2014) Synergy of silver nanoparticles and aztreonam against *Pseudomonas aeruginosa* PAO1 Biofilms. Antimicrob Agents Chemother 58:5818–5830. https://doi.org/10.1128/AAC.03170-14

Hema M, Princy SA, Sridharan V, Vinoth P, Balamurugan P, Sumana M (2016) Synergistic activity of quorum sensing inhibitor, pyrizine-2-carboxylic acid and antibiotics against multi-drug resistant *V. cholerae*. RSC Adv 6:45938–45946. https://doi.org/10.1039/C6RA04705J

Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P, Manefield M (2003) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J 22:3803–3815. https://doi.org/10.1093/emboj/cdg366

Hossain MA, Park JY, Kim JY, Suh JW, Park SC (2014) Synergistic effect and antiquorum sensing activity of *Nymphaea tetragona* (water lily) extract. Bio Med Res Int 2014:562173. https://doi.org/10.1155/2014/562173

Huma N, Shankar P, Kushwah J, Bhushan A, Joshi J, Mukherjee T, Raju SC, Purohit HJ, Kalia VC (2011) Diversity and polymorphism in AHL-lactonase gene (*aiiA*) of *Bacillus*. J Microbiol Biotechnol 21:1001–1011. https://doi.org/10.4014/jmb.1105.05056

Hwang IS, Hwang JH, Choi H, Kim KJ, Lee DG (2012) Synergistic effects between silver nanoparticles and antibiotics and the mechanisms involved. J Med Microbiol 61:1719–1726. https://doi.org/10.1099/jmm.0.047100-0

Ilk S, Sağlam N, Özgen M, Korkusuz F (2017) Chitosan nanoparticles enhances the anti-quorum sensing activity of kaempferol. Int J Biol Macromol 94:653–662. https://doi.org/10.1016/j.ijbiomac.2016.10.068

Kalia VC, Purohit HJ (2011) Quenching the quorum sensing system: potential antibacterial drug targets. Crit Rev Microbiol 37:121–140. https://doi.org/10.3109/1040841X.2010.532479

Kalia VC, Raju SC, Purohit HJ (2011) Genomic analysis reveals versatile organisms for quorum quenching enzymes: acyl-homoserine lactone-acylase and –lactonase. Open Microbiol J 5:1–13. https://doi.org/10.2174/1874285801105010001

Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31:224–245. https://doi.org/10.1016/j.biotechadv.2012.10.004

Kalia VC, Kumar P, Pandian SK, Sharma P (2014a) Chapter 15: biofouling control by quorum quenching. In: Kim SK (ed) Hb_25 Springer handbook of marine biotechnology. Springer, Berlin, pp 431–440

Kalia VC, Wood TK, Kumar P (2014b) Evolution of resistance to quorum-sensing inhibitors. Microb Ecol 68:13–23. https://doi.org/10.1007/s00248-013-0316-y

Kalia VC (2014) In search of versatile organisms for quorum-sensing inhibitors: acyl homoserine lactones (AHL)-acylase and AHL-lactonase. FEMS Microbiol Lett 359:143. https://doi.org/10.1111/1574-6968.12585

Kalia VC (2015) Microbes: the most friendly beings? In: Kalia VC (ed) Quorum sensing vs quorum quenching: a battle with no end in sight. Springer, New Delhi, pp 1–5. ISBN 978-81-322-1981-1. https://doi.org/10.1007/978-81-322-1982-8_1

Kalia VC, Kumar P (2015a) Potential applications of quorum sensing inhibitors in diverse fields. In: Kalia VC (ed) Quorum sensing vs quorum quenching: a battle with no end in sight. Springer, New Delhi, pp 359–370. https://doi.org/10.1007/978-81-322-1982-8_29

Kalia VC, Kumar P (2015b) The Battle: quorum-sensing inhibitors versus evolution of bacterial resistance. In: Kalia VC (ed) Quorum sensing vs quorum quenching: a battle with no end in sight. Springer India, New Delhi, pp 385–391. https://doi.org/10.1007/978-81-322-1982-8_31

Kaur G, Balamurugan P, Uma Maheswari C, Anitha A, Princy SA (2016) Combinatorial effects of aromatic 1, 3-disubstituted Ureas and fluoride on in vitro inhibition of *Streptococcus mutans* biofilm formation. Front Microbiol 7:861. https://doi.org/10.3389/fmicb.2016.00861

Kaur G, Balamurugan P, Princy SA (2017) Inhibition of the quorum sensing system (ComDE Pathway) by aromatic 1,3-di-m-tolylurea (DMTU): cariostatic effect with fluoride in wistar rats. Front Cell Infect Microbiol 7:313. https://doi.org/10.3389/fcimb.2017.00313

Kiran S, Sharma P, Harjai K, Capalash N (2011) Enzymatic quorum quenching increases antibiotic susceptibility of multidrug resistant *Pseudomonas aeruginosa*. Iran J Microbiol 3:1–12

Koul S, Prakash J, Mishra A, Kalia VC (2016) Potential emergence of multi-quorum sensing inhibitor resistant (MQSIR) bacteria. Indian J Microbiol 56:1–18. https://doi.org/10.1007/s12088-015-0558-0

Koul S, Kalia VC (2017) Multiplicity of quorum quenching enzymes: a potential mechanism to limit quorum sensing bacterial population. Indian J Microbiol 57:100–108. https://doi.org/10.1007/s12088-016-0633-1

Kumar P, Koul S, Patel SKS, Lee JK, Kalia VC (2015) Heterologous expression of quorum sensing inhibitory genes in diverse organisms. In: Kalia VC (ed) Quorum sensing vs quorum quenching: a battle with no end in sight. Springer, New Delhi, pp 343–356. https://doi.org/10.1007/978-81-322-1982-8_28

Kutty SK, Barraud N, Pham A, Iskander G, Rice SA, Black DS, Kumar N (2013) Design, synthesis, and evaluation of fimbrolide-nitric oxide donor hybrids as antimicrobial agents. J Med Chem 56:9517–9529. https://doi.org/10.1021/jm400951f

Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectr 4. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Pammi M, Liang R, Hicks JM, Barrish J, Versalovic J (2011) Farnesol decreases biofilms of *Staphylococcus epidermidis* and exhibits synergy with nafcillin and vancomycin. Pediatr Res 70:578–583. https://doi.org/10.1203/PDR.0b013e318232a984

Prichard MN, Prichard LE, Baguley WA, Nassiri MR, Shipman C (1991) Three-dimensional analysis of the synergistic cytotoxicity of ganciclovir and zidovudine. Antimicrob Agents Chemother 35:1060–1065. https://doi.org/10.1128/AAC.35.6.1060

Prichard MN, Prichard LE, Shipman C (1993) Strategic design and three-dimensional analysis of antiviral drug combinations. Antimicrob Agents Chemother 37:540–545. https://doi.org/10.1128/AAC.37.3.540

Rogers SA, Huigens RW, Cavanagh J, Melander C (2010) Synergistic effects between conventional antibiotics and 2-aminoimidazole-derived antibiofilm agents. Antimicrob Agents Chemother 54:2112–2118. https://doi.org/10.1128/AAC.01418-09

Roudashti S, Zeighami H, Mirshahabi H, Bahari S, Soltani A, Haghi F (2017) Synergistic activity of sub-inhibitory concentrations of curcumin with ceftazidime and ciprofloxacin against *Pseudomonas aeruginosa* quorum sensing related genes and virulence traits. World J Microbiol Biotechnol 33:50. https://doi.org/10.1007/s11274-016-2195-0

Roy V, Meyer MT, Smith JA, Gamby S, Sintim HO, Ghodssi R, Bentley WE (2013) AI-2 analogs and antibiotics: a synergistic approach to reduce bacterial biofilms. Appl Microbiol Biotechnol 97:2627–2638. https://doi.org/10.1007/s00253-012-4404-6

Sengupta S, Chattopadhyay MK, Grossart HP (2013) The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol 4:47. https://doi.org/10.3389/fmicb.2013.00047

Singh BR, Singh BN, Singh A, Khan W, Naqvi AH, Singh HB (2015) Mycofabricated biosilver nanoparticles interrupt *Pseudomonas aeruginosa* quorum sensing systems. Sci Rep 5:13719. https://doi.org/10.1038/srep13719

Subramaniam S, Keerthiraja M, Sivasubramanian A (2014) Synergistic antibacterial action of β-sitosterol-D-glucopyranoside isolated from Desmostachya bipinnata leaves with antibiotics against common human pathogens. Rev Bras Farm 24:44–50. https://doi.org/10.1590/0102-695X20142413348

Sun S, Li Y, Guo Q, Shi C, Yu J, Ma L (2008) *In vitro* interactions between tacrolimus and azoles against *Candida albicans* determined by different methods. Antimicrob Agents Chemother 52:409–417. https://doi.org/10.1128/AAC.01070-07

Szweda P, Gucwa K, Kurzyk E, Romanowska E, Dzierżanowska-Fangrat K, Jurek AZ, Kuś PM, Milewski S (2015) Essential oils, silver nanoparticles and propolis as alternative agents against

fluconazole resistant *Candida albicans, Candida glabrata* and *Candida krusei* clinical isolates. Indian J Microbiol 55:175–183. https://doi.org/10.1007/s12088-014-0508-2

Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. Pharm Ther 40:277

Wan G, Ruan L, Yin Y, Yang T, Ge M, Cheng X (2016) Effects of silver nanoparticles in combination with antibiotics on the resistant bacteria *Acinetobacter baumannii*. Int J Nanomedicine 11:3789. https://doi.org/10.2147/IJN.S104166

Wang D, Shi J, Xiong Y, Hu J, Lin Z, Qiu Y (2017) A QSAR-based mechanistic study on the combined toxicity of antibiotics and quorum sensing inhibitors against *Escherichia coli*. J Hazard Mater 341:438–447. https://doi.org/10.1016/j.jhazmat.2017.07.059

Yang H, Novick SJ, Zhao W (2014) Drug combination synergy. In: Zhao W, Yang H (eds) Drug statistical methods in drug combination studies. CRC Press, Boca Raton, pp 17–40. ISBN 978-14-822-1674-5

Zeng Z, Qian L, Cao L, Tan H, Huang Y, Xue X, Shen Y, Zhou S (2008) Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 79:119–126. https://doi.org/10.1007/s00253-008-1406-5

Zeng X, Liu X, Bian J, Pei G, Dai H, Polyak SW, Song F, Ma L, Wang Y, Zhang L (2011) Synergistic effect of 14-alpha-lipoyl andrographolide and various antibiotics on the formation of biofilms and production of exopolysaccharide and pyocyanin by *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 55:3015–3017. https://doi.org/10.1128/AAC.00575-10