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MINI-REVIEW

Alternative strategies for the application of aminoglycoside antibiotics against the biofilm-forming human pathogenic bacteria

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Abstract

Aminoglycosides are one of the common classes of antibiotics that have been widely used for treating infections caused by pathogenic bacteria. The mechanism of bactericidal action by aminoglycosides is well-known, by which it terminates the cytoplasmic protein synthesis. However, the potentials of aminoglycosides become hindered when facing the evolution of bacterial resistance mechanisms. Among multiple resistance mechanisms displayed by bacteria against antibiotics, the formation of biofilm is the mechanism that provides a barrier for antibiotics to reach the cellular level. Bacteria present in the biofilm also get protection against the impact of host immune responses, harsh environmental conditions, and other antimicrobial treatments. Hence, with the multifaceted resistance developed by biofilm-forming pathogenic bacteria, antibiotics are therefore discontinued for further applications. However, the recent research developed several alternative strategies such as optimization of the active concentration, modification of the environmental conditions, modification of the chemical structure, combinatorial application with other active agents, and formulation with biocompatible carrier materials to revitalize and exploit the new potential of aminoglycosides. The present review article describes the above mentioned multiple approaches and possible mechanisms for the application of aminoglycosides to treat biofilm-associated infections.

Keywords Aminoglycosides · Antibiotics · Bacteria · Biofilm · Immobilization · Pathogens

Introduction

Since its introduction in 1944, aminoglycoside such as streptomycin, neomycin, gentamicin, and tobramycin has been a crucial class of antibiotics for treating a wide spectrum of human pathogenic Gram-positive and Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, etc.) (Henry-Stanley et al. 2014; Krause et al. 2016; Labby and Garneau-Tsodikova 2013). The structure of aminoglycosides is composed of a 2-deoxystreptamine (2-DOS) ring linked to several amino-modified sugars, giving rise to their polycationic nature (Mingeot-Leclercq et al. 1999). The chemical structures of different types of aminoglycosides are given in Fig. 1. The polycationic nature causes aminoglycosides to target the negatively charged nucleic acid of bacterial cells as the major site of bactericidal action (Chittapragada et al. 2009). By interacting with the outer membrane and utilizing the energy-dependent phase I, aminoglycosides arrive at the protein synthesis system in the bacterial cellular cytosol (Taber et al. 1987). There, the antibiotic utilizes the energy-dependent phase II to irreversibly bind to the 16S ribosomal RNA (rRNA) at its A site of 30S subunit of bacterial ribosome, resulting in (1) misreading of codon, (2) terminating the peptide elongation by inhibiting tRNA translocation from the A to the P site, and (3) interfering the mobility of the ribosomal subunit and thus producing malfunctioning or nonfunctioning (immature) protein (Kotra et al. 2000; Walter et al. 1999). These proteins upon binding to the cell wall and membrane would disrupt the structure, thus allowing the rapid entry of more antibiotics into bacterial cells (Davis 1987).

Unfortunately, similar to several other antibiotics, the misuse and overuse of aminoglycosides over a long time have caused numerous bacteria to emerge resistant strains against the antibiotics (Li and Webster 2018; Mulani et al. 2019; Perez-Rodriguez and Mercanoglu Taban 2019). Up to the

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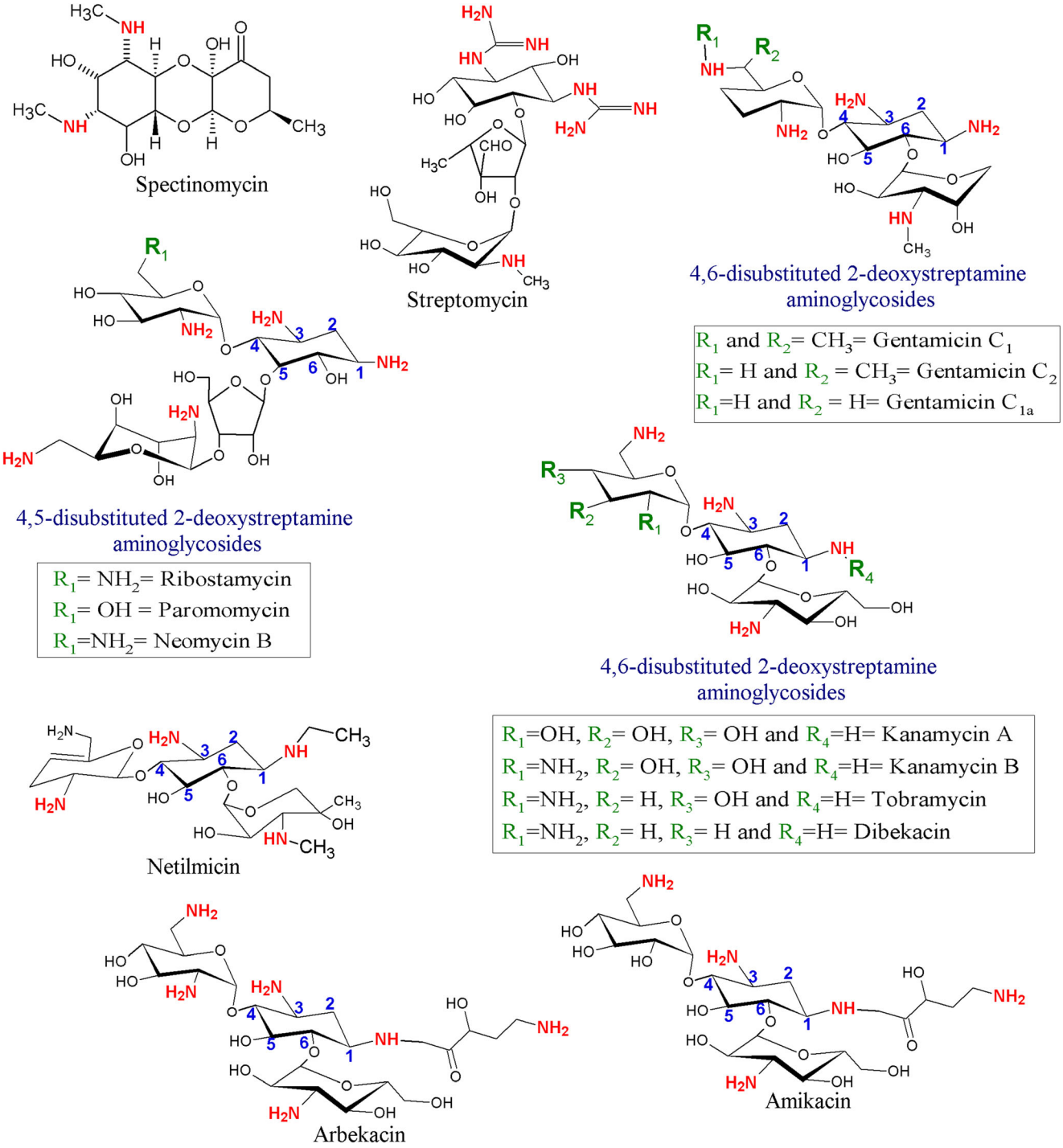

Fig. 1 Chemical structure of different aminoglycoside antibiotics

present, these traits are known to be genuinely similar across antibiotic classes, which include modifying enzymes, ribosomal mutation, cell wall permeability, and biofilm formation (Pang et al. 2019; Peterson and Kaur 2018) (Fig. 2). The production of enzymes that modify the antibiotics chemistry has been the most extensively studied mechanism of bacterial resistance to aminoglycosides (Ramirez and Tolmasky 2010; Zarate et al. 2018). These enzymes which are *N*-acetyltransferases, *O*-nucleotidyltransferases, and *O*-phosphotransferases target specific –NH_2 and –OH groups of the aminoglycosides, causing the antibiotics to poorly bind to the ribosomes and thus reducing the antibacterial efficacy (Garneau-Tsodikova and Labby 2016; Shakil et al. 2008). In addition to aminoglycosides being modified themselves, their binding site which is the 16S rRNA of the 30S ribosomal subunit can be mutated or methylated for deactivation

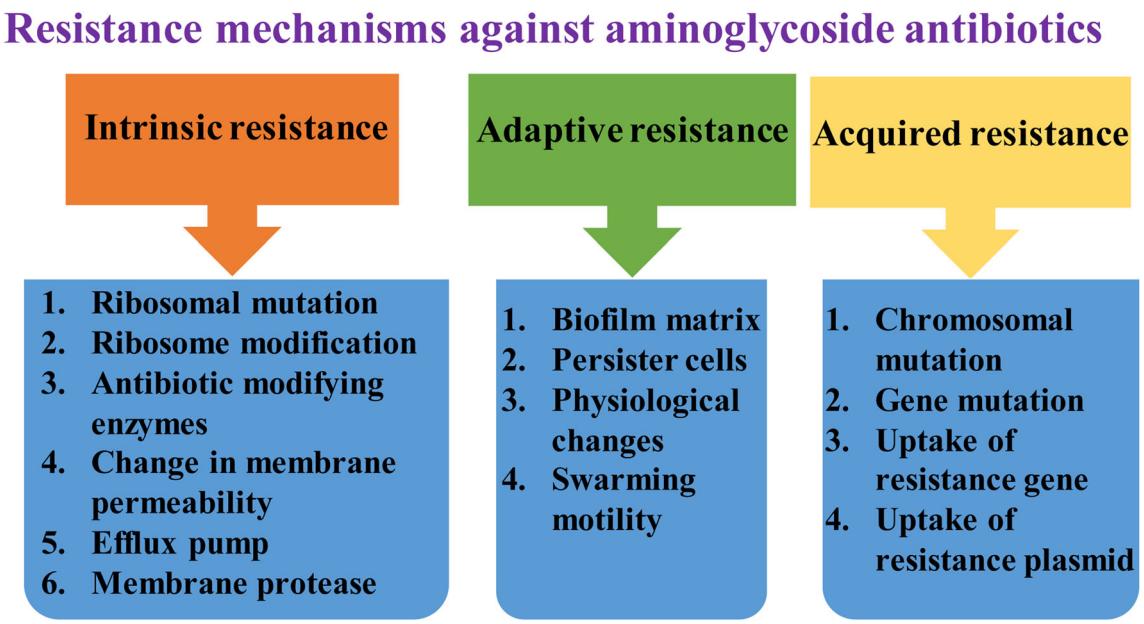

Fig. 2 Different resistance mechanisms in bacteria against aminoglycosides [information obtained from the literature (Garneau-Tsodikova and Labby 2016; Lin et al. 2015; Olivares et al. 2013)]

(Krause et al. 2016). Resistance can also be derived from modifications in the bacterial membrane to reduce the aminoglycosides' permeability. Previous studies have shown that mutations of genes encoding for the components of the cell wall or cellular membranes such as lipopolysaccharides, porins, and efflux pumps disrupt the electrostatic interaction with aminoglycosides, thereby limiting their entry into bacterial cells (Garneau-Tsodikova and Labby 2016; Morita et al. 2012a, b). Similarly, the drug's permeability across the bacterial membrane is also reduced by the formation of biofilm. Biofilm is defined as a mucoid matrix made of polysaccharides, proteins, and nucleic acid which is produced by a bacterial community adhering onto biotic (e.g., host organs, damaged tissues) or abiotic (e.g., medical devices) surfaces and displaying resistance to the extreme of the surrounding environment (e.g., host immune response, adverse conditions, and antimicrobial therapies). Such extreme capability of resistance is known to be attributed to (1) the increasing frequency of mutation and (2) horizontal transfer of resistant gene(s) among the bacterial population and between different species (i.e. intra- and interspecies biofilm) (Giaouris et al. 2015; Hoiby et al. 2010). Currently, most pathogenic bacteria in humans have employed biofilm formation as one of the resistance mechanisms against several antibiotics, causing numerous chronic infections (e.g., chronic wounds, urinary tract infections, tuberculosis, and dental caries) and hospital-acquired infections (e.g., catheter- and ventilator-associated infections) which could last up to a lifetime (Chen and Wen 2011; Cole et al. 2014; Di Domenico et al. 2017; Esteban and Garcia-Coca 2017; Hoiby et al. 2010). Furthermore, biofilm formation also causes a tremendous burden in the food industry through food spoilage and foodborne diseases, as the biofilm-forming pathogenic bacteria can also colonize on the surface of food processing and preservation facilities (Bai and Rai 2011; Bridier et al. 2015; Chmielewski and Frank 2003; Gopu et al. 2015). Resistance against aminoglycosides in biofilm-forming bacteria is majorly regulated by the nucleic acid (extracellular DNA, e-DNA) (Chiang et al. 2013). The e-DNA component of the biofilm matrix was proposed to hinder the penetration of aminoglycosides by (1) acidifying the biofilm environment, (2) chelating with positively charged drugs through electrostatic interaction, (3) modifying the outer membrane permeability, and (4) initiating surface protection (Das et al. 2010; Mulcahy et al. 2008; Wilton et al. 2016). Other barriers against aminoglycoside access include (1) the multicellular organization within the biofilm which mediates the metabolism rate and nutrient availability across the cell layers, (2) persister subpopulation which remains dormant throughout the antimicrobial treatment, (3) horizontal transfer of resistance gene(s), and (4) environmental stresses (Fraud and Poole 2011; Sato et al. 2018; Yu et al. 2018). Furthermore, the use of certain aminoglycosides at low concentrations (sub-inhibitory concentration) also contributed to bacterial resistance through diverse mechanisms (Aka and Haji 2015; Ranieri et al. 2018). Combining with other cellular-level resistance mechanisms mentioned earlier, biofilm formation poses a tremendous challenge for the use of aminoglycosides in current antimicrobial therapies. The present review paper firstly explains how biofilm formation and other mechanisms are involved in aminoglycoside resistance, and summarizes several alternative approaches that are currently conducted to improve the use of aminoglycosides in treating biofilm-

forming pathogenic bacteria. Furthermore, some future perspectives are also proposed for extending the applications of aminoglycosides in the long term.

Emergence of the aminoglycoside antibiotic-resistant bacterial strain and possible mechanism of resistance

For biofilm-forming pathogenic bacteria, biofilm formation adds to their diverse mechanisms to resist aminoglycoside activity. Besides the efflux pumps, degrading enzymes, and membrane impermeability which are common resistance mechanisms against other antibiotics, the bacteria develop several mechanisms to specifically resist against aminoglycoside activities (Vestergaard et al. 2018; Westbrock-Wadman et al. 1999). Three major types of resistance mechanisms such as intrinsic, adaptive, and acquired have been explained in Fig. 2. These mechanisms which have been developed by both Gram-negative and Gram-positive (Garneau-Tsodikova and Labby 2016; Lin et al. 2015) are summarized as follows:

1. Presence of aminoglycoside-modifying enzymes causing *O*-adenylylation, *O*-phosphorylation, or *N*-acetylation of amine or hydroxyl groups by specific enzymes of aminoglycoside molecule at different locations (Ramirez et al. 2013).
2. Mutation of 16S rRNA-encoded gene or ribosomal proteins (Hobbie et al. 2006; Springer et al. 2001).
3. Methylation of 16S rRNA (Galimand et al. 2005).
4. Riboswitch, which is present on the leader DNAs encoded by acetyltransferase- and adenyltransferase-encoding genes, senses the binding of aminoglycoside and activates aminoglycoside resistance (Jia et al. 2013).
5. Reduction in aminoglycoside permeability through the outer membrane or their transport through the inner membrane (Vestergaard et al. 2018).
6. Export by efflux pumps (Westbrock-Wadman et al. 1999).
7. Magnet et al. (2003) showed that the aminoglycoside resistance also occurs as a result of tight binding with the altered aminoglycoside acetyltransferase.
8. Shielding of extracellular DNA present in the biofilms (Chiang et al. 2013; Wilton et al. 2016).

Biofilm-forming and virulence factors producing properties of aminoglycosides: contribution of aminoglycosides toward pathogenesis

The attempts to reduce the concentrations of antibiotics to below their minimum inhibitory concentrations (sub-MIC) as a solution for lowering selective pressure that resulted from overuse and misuse of these drugs have so far fallen behind (Wistrand-Yuen et al. 2018). Recent studies have reported multiple adverse effects of using aminoglycosides at sub-MIC in antimicrobial therapies. Firstly, the low concentration of drugs may face several difficulties upon penetration through the bacterial cell membrane and biofilm matrix such as (1) unexpected and uncontrollable loss of drug concentration which leads to low accumulation and limited drug activity and (2) instability against environmental changes and resistant responses during circulation in the bacterial system or biofilm matrix (Tseng et al. 1972). For instance, a study conducted by Bhattacharya et al. (2017) found that exposure to gentamicin at the sub-MIC level has triggered *S. aureus* to generate reactive oxygen species (ROS), thus becoming more resistant to antibiotics treatment. Secondly, the low dose of aminoglycosides is often associated with frequent administration route, which would trigger the bacteria to develop resistance over a long period. Finally, due to the complex organization of bacteria, it is exceedingly challenging for a small amount of conventional aminoglycosides to encounter multiple resistance mechanisms all at once. Taking the bacterial biofilm formation as an example, this multidrug resistance mechanism is the result of complicated signal sensing and regulatory networks and is essentially processed along with the production of numerous virulence factors. Thus, biofilm responds to aminoglycoside activity at sub-MIC through extremely various means (Kaplan 2011). For example, as it is widely known that elevation of the bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) second messenger level plays a determining role throughout the establishment and dispersal of biofilm, sub-MIC of aminoglycosides which elevates the c-di-GMP level would trigger biofilm formation. Tobramycin at sub-MIC has promoted the dense biofilm formation in *P. aeruginosa* by suppressing the expression of aminoglycoside response regulator gene (*arr*) that regulates the c-di-GMP production while elevating the expression of a multitude of biofilm-associated genes (Hoffman et al. 2005; Linares et al. 2006). Tobramycin at this level also upregulated the expression of other essential factors of biofilm formation such as e-DNA, quorum sensing, and iron uptake (Tahrioui et al. 2019). In contrast, biofilm establishment in *Escherichia coli* was resulted from sub-MIC of fluoroquinolone enhancing the c-di-GMP level and stresses (Boehm et al. 2009). In addition to c-di-GMP, bacterial biofilm formation is also linked with type VI and type III protein secretion systems as they are all under the regulation of Gac/Rsm regulatory pathway. Thus, by genuinely triggering the type VI protein secretion systems, the presence of kanamycin at the sub-MIC level has promoted *P. aeruginosa* biofilm formation (Jones et al. 2013).

Another target of sub-MIC aminoglycoside triggering bacterial biofilm formation is toward the components of extracellular polysaccharide substrates (EPS) such as

exopolysaccharides (alginate and teichoic acids), extracellular proteins (protease, adhesins, lectins, and surface appendages), lipids (lipopolysaccharides and biosurfactants), and e-DNA. The important role of EPS in hindering the penetration of antimicrobial drugs which has been described in the previous section is also targeted by sub-MIC aminoglycosides. A previous study conducted by Szczuka et al. (2017) has shown that erythromycin, fluoroquinolone, and tigecycline upregulated several biofilm-related genes such as poly-*N*-acetylglucosamine gene (*ica*) and sigma factor (*sigB*) in *Staphylococcus epidermidis,* thereby promoting the attachment of biofilm-forming cells and production of teichoic acids, adhesins, and e-DNA. Expression of the Wyz membrane transporter system in *E. coli* was upregulated proportionally with colonic acid polysaccharide by the presence of sub-MIC of streptomycin, thereby enhancing biofilm formation (Kumar and Ting 2016). Although the curli fimbriae production and biofilm formation by *E. coli* were reduced by amikacin, this reduction appeared insignificant and highly dependent on the bacterial lifestyle (Wojnicz and Tichaczek-Goska 2013).

It is well-known that virulence properties play an equally important role as biofilm formation itself in biofilm-associated infections significantly contributes to bacterial pathogenesis and is closely linked to antibiotic resistance (Schroeder et al. 2017). In the establishment stage of biofilm, virulence properties function in sensing, translocating, and properly attaching to desirable surfaces to allow the switch from planktonic (free-floating) to sessile states (flagellar-mediated swimming and swarming, pili-mediated twitching motilities, and surface adhesins). During the development and maturation of biofilm, virulence properties are performed through initiating reactive oxygen damage, disrupting erythrocytes, sequestering the iron, releasing toxins, and damaging the host proteins (Schroeder et al. 2017). In general, virulence properties are regulated by "quorum sensing" (QS), which is a cell-to-cell communication network (Khan et al. 2019a). Recently, several reports have shown that by triggering either the bacterial virulence properties directly or indirectly through their QS regulation system, sub-MICs of aminoglycosides can also promote biofilm formation. For example, in the presence of kanamycin at the sub-MIC level, *N*-acyl-L-homoserine lactones signaling molecules of the QS system were upregulated, leading to the increasing level of biofilm formation, chitinase production, and flagellar activity (Liu et al. 2013). Previously, sub-MIC of tobramycin increased QS activity, e-DNA production, and iron acquisition, causing dense biofilm formation (Tahrioui et al. 2019). *P. aeruginosa* virulence factors, along with the surface charge, hydrophobicity, and adhesins, were also significantly affected by streptomycin activity, thus forming high biofilm biomass (Kumar and Ting 2016). Overall, the sub-MIC of several conventional aminoglycosides have been incapable of inhibiting the growth and biofilm formation of a wide range of Gram-negative and Gram-positive bacteria. In the attempts to reintroduce these treatments, several alternative strategies by combining multiple drugs (i.e., combination strategy) or using nonantibiotic potentiator/adjuvants/materials appear to be highly favorable at the present.

Alternative strategies for the application of aminoglycosides

Due to the downfalls in preventing the human pathogenic bacteria from forming biofilm and causing biofilm-associated infections, the application of conventional aminoglycoside monotherapy has been discontinued. To revitalize the aminoglycoside efficacy to catch up with such rapid rate of emerging resistance, several directions have been proposed: (1) widening the spectrum of aminoglycoside activities toward multiple targets, (2) shifting the aminoglycoside target from "killing" to "weakening," and (3) potentiating the aminoglycoside activity by using additional materials (e.g., nonantibiotic compounds and nanocarriers) or by exploiting the active concentrations (e.g., sub-MIC) and modifying chemical structure. Up to the present, each direction has brought about promising results in biofilm inhibition by targeting various aspects of biofilm formation from development stages, signaling and regulation systems, biofilm architecture to the alongside virulence properties of a wide range of human pathogenic bacteria. Although the pharmacokinetics and interactions between combined drugs or compounds highly require further studies and discovery for new alternative strategies should remain ongoing, the significant achievements from modern alternative strategies toward biofilm formation of human pathogenic bacteria give rise to the future possibility of combating biofilm-associated infections. Table 1 presents a list of aminoglycoside antibiotics which showed antimicrobial activity at their active concentration.

Optimization of subinhibitory concentration and environmental factors of aminoglycosides

Although conventional aminoglycoside drugs have been reported to induce resistance in various bacteria, several improvement approaches such as (1) combining the sub-MIC of multiple drugs, (2) shifting their application to antivirulence or anti-QS (quorum quenching) strategies, and (3) optimizing the bacterial culture environment in terms of temperature, pH, and media types have recently been introduced and set a promising future for aminoglycosides.

In the first approach, combination strategies between different aminoglycosides and between aminoglycoside and other antibiotics have shown effectiveness in maintaining the antimicrobial effect at a lower dose of individual drugs. For

Table 1 Aminoglycosides either alone or in combination with other antibiotic or antimicrobial agents used for treating the biofilm-forming pathogenic bacteria

Aminoglycosides	Pathogenic bacteria	Active concentration	Mechanism of inhibition	References
Amikacin and fosfomycin	*Pseudomonas aeruginosa*	NA	Fosfomycin upon the combination with amikacin has supported the aminoglycoside to suppress the growth and biofilm formation of *P. aeruginosa* in a rat model by performing cell wall-degrading function, thereby improving the drug penetration through the bacterial cell wall and biofilm matrix	Cai et al. (2009)
Arbekacin	Methicillin-resistant *Staphylococcus aureus* (MRSA)	0.3–10 mg/kg	It showed effective eradication of biofilm of MRSA as studied in the rat	Yoshikawa et al. (2004)
Arbekacin and fosfomycin	MRSA	Arbekacin (0.1 mg/kg) and fosfomycin (100 mg/kg)	The combination showed a synergistic effect on the eradication of biofilm formed by the MRSA in rat	Morikawa et al. (2005)
Gentamicin	*S. aureus*, *Escherichia coli*, and *P. aeruginosa*	5 mg/mL	The alkaline condition and basic amino acid L-arginine potentiated the efficacy of gentamicin against Gram-positive and Gram-negative bacteria both in vitro and in vivo	Lebeaux et al. (2014)
Gentamicin	*P. aeruginosa*	½-MIC	The sub-MIC of gentamicin significantly inhibited the formation of biofilm of *P. aeruginosa* on immobilized fibronectin	Gagniere and Di Martino (2004)
Gentamicin and streptomycin	*S. aureus*	Gentamicin (5 μg/mL) and streptomycin (32 μg/mL)	These antibiotics inhibited the formation of biofilm when incubated in 1/3 or 1× diluted tryptic soy broth	Henry-Stanley et al. (2014)
Kanamycin with ceftiofur, amoxicillin, colistin sulfate, lincomycin, clarithromycin, and berberine	*S. aureus*	Berberine and lincomycin (1/16–1/2 MIC)	The combination of an aminoglycoside with other antibiotics or other antimicrobial agents inhibited bacterial biofilm formation. This combination also effectively inhibited several biofilm-related gene expressions	Yang et al. (2017)
Netilmicin	*P. aeruginosa*	32 mg/L	Biofilm-forming cell became susceptible against netilmicin	Cernohorska and Votava (2008)
Spectinomycin	*E. coli* ATCC 33456 pEGFP[†]	40 ppm	The biofilm inhibition occurred at the minimum biofilm preventive concentration	O'Connell et al. (2006)
Streptomycin	*Acinetobacter baumannii*	Sub-MIC	The sub-MIC of streptomycin inhibited the QS-regulated genes. Apart from inhibition of QS signaling molecule production, the sub-MIC of streptomycin also antagonized these molecules and attenuated the motility property of *A. baumannii*	Saroj and Rather (2013)
Streptomycin	*P. aeruginosa*	Sub-MIC	The sub-MIC of streptomycin in alkaline TSB media inhibited the formation of biofilm. The eradication of mature biofilm was also found by streptomycin at different concentrations. Sub-MIC of streptomycin attenuated virulence properties such as virulence factor production and motility properties. The inhibition of these phenotypic properties by sub-MIC of streptomycin was also confirmed at the gene expression level	Khan et al. (2019b)
Tobramycin	*P. aeruginosa* PAO1	Varied concentrations (from 1.9 to 6.3 μg/mL) with respect to time of incubation	Exhibited killing effect to bacterial cells during agar diffusion antimicrobial susceptibility testing as a result of switching the cell lifestyle from planktonic to sessile forms. The bacterial cells that aggregated in the sessile lifestyle (young biofilm) were rapidly killed by tobramycin inside the inhibition zone	Hoiby et al. (2019)
Tobramycin	*P. aeruginosa* PAO1	[1× MIC, 1/10× MIC, and 1/100× MIC] and 160–2560 mg/mL	A significant biofilm inhibition by tobramycin at a tested concentration (1× MIC, 1/10× MIC, and 1/100× MIC). Its higher concentration (160–2560 mg/mL), also eradicated the matured biofilm	Dosler and Karaaslan (2014)
Tobramycin	*E. coli*	2 μg/mL	Effective in eliminating the biofilms	Ceri et al. (1999)
Tobramycin	*P. aeruginosa*	16 and 256 mg/L	It inhibited the formation of biofilm at 16 mg/L and eradicated of preformed mature biofilm at 256 mg/L on cultured human cystic fibrosis (CF) airway cells	Anderson et al. (2013)

Table 1 (continued)

Aminoglycosides	Pathogenic bacteria	Active concentration	Mechanism of inhibition	References
Tobramycin and clarithromycin	*P. aeruginosa*	Tobramycin (0.5–1 μg/mL) and clarithromycin (256–512 μg/mL)	The combination has synergistically inhibited bacterial growth and biofilm formation and also eradicated the pre-existing mature biofilm of *P. aeruginosa*	Ghorbani et al. (2017)

NA not available

† Nonpathogenic bacteria

example, the combinations of kanamycin with ceftiofur, amoxicillin, colistin sulfate, lincomycin, clarithromycin, and berberine at their sub-MICs have effectively inhibited biofilm formation in *S. aureus* by suppressing several biofilm-related genes (Yang et al. 2017). The synergistic effects between tobramycin and clarithromycin or ceftazidime have exhibited high antibacterial and antibiofilm activities on *P. aeruginosa* and also eradicated the pre-existing mature biofilm (Ghorbani et al. 2017; Kapoor and Murphy 2018).

In the second approach, sub-MIC of aminoglycosides are used to target the virulence properties and QS of the biofilm-forming bacteria to disarm their biofilm formation as well as their pathogenesis (Fleitas Martinez et al. 2018; Fong et al. 2018). As the virulence properties and QS are nonessential to bacterial survival, their attenuation would reduce selection pressure for resistant strains as compared to conventional antimicrobial drugs, thus reducing the potential of resistance evolution (Dickey et al. 2017; Rasko and Sperandio 2010). The combination of curcumin and sub-MIC of gentamicin has synergistically downregulated the expression of QS-related genes, biofilm formation, and motility of *P. aeruginosa* (Bahari et al. 2017). Similarly, expression of the genes encoding for QS signaling molecules of *Acinetobacter baumannii* was also reduced by the presence of streptomycin at sub-MIC (Saroj and Rather 2013). Furthermore, the antivirulence activity of sub-MIC aminoglycosides can be further improved when they are incorporated with nonantibiotic compounds (e.g., bioactive compounds, small molecules, antimicrobial peptides) or delivered by nanocarriers, of which a detailed discussion shall be present in the following section of this review paper. Despite being highly potential to combat bacterial biofilm formation, antivirulence strategy yet requires further optimization work due to three main reasons (Fleitas Martinez et al. 2019). Firstly, the dynamics of their production/regulation are extremely varied throughout different stages of biofilm formation and different bacterial species (Dickey et al. 2017). Secondly, besides phenotypic tests and gene expression, the effective screening and diagnosis techniques that give out fast and precise results about virulence properties of various biofilm-forming bacteria remain under research (Ashrafudoulla et al. 2019; Tukenmez et al. 2019). Finally, the clinical application of sub-MICs of aminoglycoside as antivirulence agents onto animal models or infectious human patients requires long-term and complicated research and development and social approval (Fleitas Martinez et al. 2019; Maura et al. 2017).

In the third approach, the antibacterial and antibiofilm activities of aminoglycosides at sub-MIC are improved by optimizing the culture environment, including temperature, pH, and culture types. Due to its polycationic nature, the aminoglycoside activity was reported to be highly influenced by these environmental factors. For instance, changes in pH and the concentration of the nutrients of the culture media have affected the permeability of gentamicin through *S. aureus* biofilm as these conditions supported the electrostatic interaction between the positively charged aminoglycoside drug and the negatively charged bacterial biofilm components such as exopolysaccharides and e-DNA (Henry-Stanley et al. 2014). A similar observation was obtained when streptomycin at sub-MIC was applied to the biofilm of *P. aeruginosa* (Khan et al. 2019b). The drug performed optimal inhibitory activity to the bacterial biofilm formation and virulence factor production under the culture conditions of alkaline pH, 35 °C, and TSB/LB media (Khan et al. 2019b). In the current situation where in-depth studies about the underlying mechanisms of these effects remain lacking, the reported results provide insights for the future development of a new antibiofilm strategy.

Immobilization and combinatorial application of aminoglycosides

During the past few decades, frequent administration of a single antibiotic at high doses and frequency termed as monotherapy has resulted in resistance emergence in bacteria (Gonzalez 3rd and Spencer 1998; Traugott et al. 2011). Particularly, several human pathogenic bacteria have developed multiple mechanisms to resist a majority of commonly used antibiotics (multidrug resistance); thus, alternative therapeutic approaches are in urgent need. Under such circumstances, combinatory approaches which involve combining (1) two or more antibiotics and (2) conventional antibiotics with one or more nonantibiotic compounds have been used to improve the antimicrobial efficacy of monotherapies (Eom et al. 2016; Kim et al. 2017; Tyers and Wright 2019). Several combinations used in this approach are summarized in Table 2.

Depending on the specific nature and function of individuals, combinations of antibiotics help to broaden the spectrum of antibiotic activity while lowering their doses and toxicity. For example, amikacin and isepamicin have been combined with fosfomycin to synergistically suppress the growth and biofilm formation of *P. aeruginosa* in the rat model (Cai et al. 2009). The mechanism was proposed that fosfomycin has performed the cell wall-degrading function, which improved the limited penetration of aminoglycosides through the bacterial cell wall and biofilm matrix (Cai et al. 2009). The combination of tobramycin and clarithromycin applied to biofilm-forming isolates of *P. aeruginosa* has exhibited antibiofilm effectiveness against a significant number of tested isolates (Ghorbani et al. 2017).

Recently, the combinations of antibiotics with the nonantibiotic compounds that are often referred to as "antibiotic adjuvants" have been extensively exploited (Douafer et al. 2019). These compounds are varied in sources (e.g., natural compounds, chemical compounds, plant phytochemicals, small molecules, and antimicrobial peptides) and functions (e.g., QS inhibitor, efflux pump inhibitor, drug uptake promoter, and antivirulent agent) (Wright 2016). Gentamicin upon mixing with chitosan polysaccharide has been improved in permeability through the biofilm architecture of *L. monocytogenes*, *Listeria welshimeri*, and *Listeria innocua*, thereby exhibiting inhibition and eradication activities toward the biofilm of the three *Listeria* bacteria (Mu et al. 2014). Combinations of a QS inhibitor called quercetin with tobramycin, levofloxacin, and amikacin exerted significant killing effects on *P. aeruginosa* biofilm cells, as the combination was added with anti-quorum-sensing activity of quercetin (Vipin et al. 2019). By combining with chitosan-A polycationic biopolymer, the antibiofilm activity of streptomycin toward Gram-positive bacteria has been significantly enhanced due to higher drug permeability and stronger ionic interaction with negatively charged biofilm constituents (Zhang et al. 2013). Similarly, conjugation with chitosan-oligosaccharide, which is low molecular weight chitosan and also well-known for its antimicrobial and antibiofilm activities, has supported the streptomycin efficacy in biofilm dispersal and inactivation of efflux pump and exopolysaccharide production (Li et al. 2019). On the other hand, the antimicrobial peptides (AMPs) such as G10KHc and GL13K targeted the *P. aeruginosa* cell membrane structure to synergistically elevate the penetration of tobramycin; thus, the drug activity against the bacterial biofilm was significantly improved (Dosler and Karaaslan 2014; Eckert et al. 2006; Hirt and Gorr 2013). Colistin is another AMP that has been combined with a wide range of antibiotics to treat biofilm-forming bacteria. The combinatory therapy using colistin combined with tobramycin has effectively killed the *P. aeruginosa* biofilm cells without causing adverse reactions when applied for mice models and cystic fibrosis patients (Herrmann et al. 2010). Besides, combinations of aminoglycosides (gentamicin, tobramycin, and streptomycin) with chemical compounds such as triclosan and nitric oxide have effectively eradicated the established *P. aeruginosa* biofilm and eliminated the persistent cells living within (Barraud et al. 2006; Maiden et al. 2018). Rhamnolipid as a membrane-acting agent induces the uptake of aminoglycosides inside the bacterial without the involvement of proton motive force, thereby it potentiates the bactericidal properties of aminoglycosides (Radlinski et al. 2019; Yarlagadda and Wright 2019). A similar eradicating effect was achieved when the combinations of aminoglycosides with plant phytochemicals such as (1) gentamicin with oleanolic acid or (2) tobramycin with tannic acid and gallic acid were applied to *A. baumannii* and *S. aureus* biofilms, respectively (Dong et al. 2018; Shin and Park 2015). Overall, with numerous significances in improving and potentiating the antibiofilm activity of aminoglycosides mentioned above, the combinatory strategies can be considered as highly helpful for drug use in the long run.

Nanoformulation of aminoglycosides

One of the major challenges of conventional antibiotic approaches is the undesirable loss of antibiotic concentration upon penetration through the bacterial cell membrane/cell wall/biofilm matrix. The insufficient amount of antimicrobial drugs, therefore, requires administering at a high frequency and high dose, which is most likely to result in in vivo toxicity, bacterial resistance, and tremendous economic burden (Allahverdiyev et al. 2011; Van Giau et al. 2019). The advanced development of nanotechnology during the past few years has revitalized this limitation of conventional antibiotic therapies. Numerous studies have recognized the tremendous benefits of encapsulating and grafting of antibiotics into nanomaterials, including (1) controlled release with minimized concentration leakage and (2) stability against the bacterial clearance responses (Baptista et al. 2018). Nanomaterials are extremely diverse in terms of source, production methods, compatibility, and functions, allowing them to carry different classes of antibiotics. Several types of nanomaterials such as liposomes, hydrogel, film, smart surface, and nanoparticles have been used for aminoglycoside encapsulation/immobilization and showed significant improvement in the drug's activity (Jijie et al. 2017). Such diversity in nanocarrier types and antibacterial/antibiofilm actions is highly promising for the control of biofilm formation and the emergence of multidrug resistance in the future (Abed and Couvreur 2014).

A liposome is a universal lipid-based colloidal vesicle which has been used to deliver both hydrophilic and hydrophobic antibiotics for a long time (Langner and Kral 1999). Having similar physiochemical properties as a bacterial cell membrane, liposome nanoformulation easily fuses through

Table 2 Combinatorial application, immobilization, and nanoformulation of aminoglycosides

Antibiotics	Carrier molecules or active agents	Pathogenic bacteria	Mode of actions	References
Amikacin	Ethylenediaminetetraacetic acid (EDTA)	*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, and *Enterobacter cloacae*	Combination of amikacin and EDTA results in the synergistic eradication of biofilm formed by Gram-negative bacteria	Lebeaux et al. (2015)
Amikacin	Hyaluronan	*P. aeruginosa*, *Listeria monocytogenes*, and *Staphylococcus aureus*	The conjugate showed effective eradication of intracellular bacteria with reduced dose requirements. The conjugates effectively allow the entry of amikacin inside the cell as a result of binding with CD44 r eceptor present on the macrophage	Wang et al. (2018b)
Gentamicin	EDTA	*S. aureus*, *S. epidermidis*, *E. coli*, or *P. aeruginosa*	This combination has broad-spectrum biofilm-eradicating properties against pathogenic bacteria as evidenced by in vivo study in the rat model. EDTA acts as an adjuvant to potentiate the biofilm eradication property of gentamicin	Chauhan et al. (2012)
Gentamicin	Plumbagin	*P. aeruginosa*	The sub-MIC of gentamicin and plumbagin combination showed effective synergistic biofilm inhibition and the eradication of mature biofilm. This combination also attenuates several virulence properties such as protease activity, production of virulence factors, and motilities. Such attenuation of virulence properties was also confirmed by the inhibition of virulent gene expression	Gupta et al. (2017)
Gentamicin	Protein P128	*S. aureus*	The combination of gentamicin with a peptidoglycan-degrading protein P128 results in a synergistic way of biofilm inhibition	Nair et al. (2016)
Gentamicin	Alginate	*P. aeruginosa*, *E. coli*, and *S. aureus*	The covalently joined gentamicin and alginate showed effective antimicrobial activity. The mechanism of antimicrobial might be due to the electrostatic interaction between positively charged ions of the conjugate and negatively charged cell membrane	Kondaveeti et al. (2018)
Gentamicin	Chitosan	*P. aeruginosa*, *S. aureus*, and *E. coli*	The electrostatic interaction and hydrogen bonding between the conjugate and membrane protein resulted in cell death	Liu et al. (2017), Yan et al. (2019)
Gentamicin	Gold nanoparticles	*S. epidermidis* and *Staphylococcus haemolyticus*	The efficacy of gentamicin against these bacteria increased as a result of conjugation with gold nanoparticle	Roshmi et al. (2015)
Gentamicin	Curcumin	*P. aeruginosa*	The sub-MIC of gentamicin in combination with curcumin showed synergistic antimicrobial activity. This combination also inhibits motility properties such as twitching and swarming. Furthermore, this combination inhibits the formation of biofilm. The expression of QS regulatory rhlI/rhlR and lasI/lasR genes were also inhibited by this combination	Bahari et al. (2017)

Table 2 (continued)

Antibiotics	Carrier molecules or active agents	Pathogenic bacteria	Mode of actions	References
Gentamicin, neomycin, and kanamycin	Silica nanoparticles	*B. cereus*, *S. aureus*, *E. coli*, *S. enterica Typhimurium*, and kanamycin-resistant *E. coli*	The conjugation of antibiotic with silica nanoparticle results in the effective antimicrobial activity without causing cytotoxic effect	Agnihotri et al. (2015)
Kanamycin	Gold nanoparticle	Gram-negative and Gram-positive bacteria	The bacterial cell death occurred as a result of rupturing the membrane followed by the leakage of cytoplasmic content	Payne et al. (2016)
Kanamycin and amikacin	Bile acid such as deoxycholic acid and ursodeoxycholic acid	*S. aureus*	These complexes showed bactericidal and concentration-dependent inhibition of biofilm formation and dispersion of mature biofilm. Both bile acid and the deoxycholic acid act as a carrier for the transport of kanamycin and amikacin	Giovagnoli et al. (2017)
Netilmicin/tobramycin/gentamicin/amikacin	Hordenine	*P. aeruginosa*	Combination of hordenine with aminoglycosides showed an effective biofilm inhibition as well as eradication of preformed mature biofilm	Zhou et al. (2018a, b)
Streptomycin	Chitosan-magnetic nanoparticle	Gram-positive, Gram-negative bacteria, and *Mycobacterium tuberculosis*	The loaded streptomycin to the magnetic nanoparticle acted as an antimicrobial agent. Due to the magnetic nature of the nanocomposites, it can be also used for the diagnosis of microorganism using the imaging techniques	El Zowalaty et al. (2015)
Streptomycin	Streptomycin-loaded starch nanoparticle incorporated to chitosan	*E. coli* and *Bacillus subtilis*	Streptomycin-loaded starch nanoparticles acted as a potent antimicrobial agent with sustained release of the streptomycin	Hari and Nair (2016)
Streptomycin	Chitosan	*L. monocytogenes*, *S. aureus*, and *Salmonella Typhimurium*	The covalently coupled chitosan and streptomycin showed bactericidal activity toward intracellular bacteria. It was capable of eliminating endocytic or endosomal escaped bacteria as a result of direct contact between the bacterial cell and streptomycin antibiotic	Mu et al. (2016b)
Streptomycin	Chitosan-oligosaccharide	*P. aeruginosa*	Eradicates the mature biofilm of *P. aeruginosa.* The possible mechanism for the susceptibility of the cells against the conjugates might be due to the suppression of MexX–MexY drug efflux pump and downregulation of biofilm exopolysaccharide synthesis	Li et al. (2019)
Streptomycin	Thymol and cinnamaldehyde	*L. monocytogenes* and *S. Typhimurium*	The combination of streptomycin with thymol and cinnamaldehyde showed a synergistic effect against *L. monocytogenes*, whereas the combination of streptomycin with cinnamaldehyde and eugenol showed synergy against *S.* Typhimurium. These combinations were also effective in the eradication of mature biofilm of both bacteria	Liu et al. (2015)
Streptomycin	Chitosan	*S. aureus*, *L. monocytogenes*, and *Enterococcus faecalis*	Exhibited antibiofilm as well as bactericidal effects toward the Gram-positive pathogenic bacteria. The polycationic nature of the chitosan resulted in the interaction with the component of the biofilm matrix that efficiently delivered streptomycin antibiotic	Zhang et al. (2013)
Streptomycin	Chitosan and gold nanoparticle	*P. aeruginosa*, *S. Typhimurium*, *L. monocytogenes*, and *S. aureus*	Chitosan–streptomycin gold nanoparticles (CA NPs) showed antibiofilm activity and damaged the established mature biofilm of Gram-negative bacteria. Furthermore, CA NPs also showed the killing of dispersed biofilm cell as well as growth inhibition of Gram-positive bacteria	Mu et al. (2016a)

Table 2 (continued)

Antibiotics	Carrier molecules or active agents	Pathogenic bacteria	Mode of actions	References
Streptomycin	Chitosan-magnetic nanoparticle	Methicillin-resistant *S. aureus*	Nanoformulation of streptomycin resulted in controlled release at the site of action with effective antibacterial activity	Hussein-Al-Ali et al. (2014)
Tobramycin	Liposome	*S. epidermidis*	Immobilization of tobramycin to liposomes which effectively inhibited the bacterial growth. The loaded tobramycin was released as a result of bacterial membrane interaction with liposome membrane	Mourtas et al. (2015)
Tobramycin	Low-intensity and low-frequency ultrasound	*E. coli*	Showed synergistic bactericidal activity to the multidrug-resistant *E. coli* biofilm cells. The combination of ultrasound and tobramycin also altered the morphological structure (reduced thickness and loosened structure) of biofilms	Hou et al. (2019)
Tobramycin	Low-frequency vibration	*P. aeruginosa*	The low frequency of vibration promotes the efficacy of sub-MIC tobramycin against the biofilm cells	Bandara et al. (2014)
Tobramycin	Azteronam	*P. aeruginosa*	Sequential treatment of tobramycin and aztreonam combination resulted in the effective reduction of viable cells and biofilm biomass	Rojo-Molinero et al. (2016)
Tobramycin	PEGylation	*P. aeruginosa*	The PEGylation of tobramycin increased the penetration across the mucus as studied using the mucus barrier biofilm model. The PEGylated tobramycin showed effective antimicrobial activity against biofilm cells	Bahamondez-Canas et al. (2018)
Tobramycin	DJK-5 (chemically synthesized peptide)	*P. aeruginosa*	This combination showed effective in the biofilm inhibition on the plastic surface as well as 3-dimensional lung epithelial cells	Crabbe et al. (2017)
Tobramycin	N-(2-pyrimidyl) butanamide	*P. aeruginosa*	The QS inhibitor, i.e., N-(2-pyrimidyl) butanamide showed synergistic biofilm inhibition in combination with tobramycin	Furiga et al. (2015)
Tobramycin	Linolenic acid	*P. aeruginosa*	The combination inhibits the formation of biofilm in a synergistic way via the quorum sensing system. This combination also inhibits several virulence properties such as motility property, protease activity, and production of virulence factors.	Chanda et al. (2017)
Tobramycin	ALX-109 (lactoferrin and hypothiocyanite)	*P. aeruginosa*	ALX-109 in combination results in the effective biofilm inhibition as well as disruption of established mature biofilm of *P. aeruginosa*, which was grown on cystic fibrosis airway epithelial cells	Moreau-Marquis et al. (2015)

this barrier to successfully deliver their containing drugs, providing beneficial pharmacokinetics, selectivity, and biodistribution which were expected to overcome drug resistance in bacteria, especially the human pathogenic ones (Alipour and Suntres 2014; Drulis-Kawa and Dorotkiewicz-Jach 2010). For example, tobramycin (Tob) to which *P. aeruginosa* has developed resistance was chemically encapsulated in polyethylene glycol (PEG)ylated-liposome to form Tob–PEG conjugated structure. The conjugate has shown an increase in stability and antibacterial and antibiofilm efficacy as compared to individual Tob (Du et al. 2015). Similarly, by encapsulating aminoglycosides (amikacin, gentamicin, and tobramycin) into a liposome, the permeability of the drug through the bacterial cell membrane was significantly increased, thereby performing more active inhibitory effect to *P. aeruginosa* growth (Alipour and Suntres 2014). On the other hand, gentamicin encapsulated in liposome was further stabilized with positively charged lysozyme enzyme to elevate the drug delivery and interaction with negatively charged biofilm constituents of Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*), thus significantly increasing the inhibition and disruption efficacy toward the biofilm formed by both bacteria (Hou et al. 2017). Despite these achievements, the use of liposome has currently become less favorable due to their instability against physical conditions (e.g., heat, storage temperature, and oxidation) and high-cost production. Further

optimization work is demanded to improve and extend the liposome activities for future clinical applications (Drulis-Kawa and Dorotkiewicz-Jach 2010).

"Smart" surfaces that have been adopted in combating biofilm formation recently are varied in design and antibiofilm functions (Li et al. 2018). Although the antibiofilm activity of "smart" aminoglycoside surfaces has remained unexploited, their significances in physiochemical properties and antibacterial activity against *S. epidermidis*, *E. coli*, *P. aeruginosa*, and *S. aureus* recorded in vitro and in vivo has provided an insight to the potential applications of these surfaces (Hu et al. 2017).

Nanoparticles (NPs) which are defined as having at least one dimension less than 100 nm and synthesized from metal (i.e., metal-based/metallic/inorganic NPs) or polysaccharides (i.e., polysaccharide-based/polymeric/organic NPs) are extensively applied in drug delivery systems (Jeevanandam et al. 2018; Khan et al. 2017, 2018). Due to their large surface-area-to-volume ratio, small size, controlled release, diverse biological activities, stability, and minimized toxicity, NPs easily pass through the cell membrane and the biofilm matrix to effectively deliver their carry-on drug to the targeted infectious site with minimal concentration loss (Aderibigbe 2017; Andonova 2017; Javaid et al. 2018; Liu et al. 2008; Salouti and Ahangari 2014). Up to the present, aminoglycosides have been loaded onto these delivery systems either externally (as a coating or a stabilizing/capping/reducing agent) or internally (encapsulation). In the former case, it was proposed that the capping and reducing properties of the drugs allowed the drug adsorption onto the NPs' surface (Shah et al. 2014). In return, the NPs which are capped/reduced by antibiotics are less likely to form aggregates and had increasing antibacterial activity (Gad El-Rab et al. 2018; Shedbalkar et al. 2014). For instance, by conjugation to gold NPs in the form of reducing/capping agents, kanamycin was rapidly delivered into the cytosol and effectively exerted a bactericidal effect on *S. epidermidis* and *E. aerogenes* (Payne et al. 2016). Streptomycin and kanamycin were employed as reducing agents along with sodium borohydride to synthesize antibiotic-conjugated gold NPs, which exhibited antibacterial activity against *S. aureus*, *Micrococcus luteus*, and *E. coli* and had high stability against heat, UV light, and long-term storage at room temperature (Bhattacharya et al. 2012). Hybrid nanoformulation of silica oxide (SiO_2) and gentamicin exhibited (1) antibacterial and antibiofilm activities to methicillin-resistant *S. aureus* (MRSA) and (2) eradication and destructive activities to *E. coli* established biofilm structure (Mosselhy et al. 2018). Silica NPs which were synthesized using aminoglycosides (gentamicin, kanamycin, and neomycin) actively inhibited the growth of resistant bacterial strains without causing cytotoxicity (Agnihotri et al. 2015). Due to the synergism with aminoglycoside capping agent, the synthesized silver NPs exhibited higher antibacterial activity against *E. coli* and *S. aureus* than those which were capped with citrate or SDS (Kora and Rastogi 2013). In the latter case where aminoglycosides are encapsulated into the NPs, the permeability of the drug through the bacterial cell membrane or biofilm matrix, their control release, and their stability are improved, thus remaining active inside the living systems for a longer period (Deacon et al. 2015). Gentamicin loaded to gold NPs was able to inhibit the growth and biofilm formation as well as eradicated the preformed mature biofilm of *P. aeruginosa*, *L. monocytogenes*, and *E. coli* without causing cytotoxicity to macrophages (Mu et al. 2016c). By loading tobramycin onto small-sized citrate-capped silver NPs to treat *P. aeruginosa* biofilm formation, the NPs further potentiated the disruption effect of tobramycin toward the bacterial biofilm matrix and cell membrane (Habash et al. 2017). Likewise, the *S. aureus* cell membrane and biofilm were also targeted by the chitosan/Fe_3O_4@poly (ethylene glycol) (PEG)-gentamicin NPs, where the electrostatic interaction between gentamicin, protonated chitosan, and PEG aided the drug entry through the bacterial membrane, while the magnetic force of Fe_3O_4 NPs allowed the drug penetration through the bacterial preformed biofilm (Wang et al. 2018a). Besides, loading into nanocarriers was also found to affect the rate of drug release, as, in the case of gentamicin being loaded onto cysteine and glutathione-capped gold NPs, the addition of gentamicin enhanced the antibacterial efficacy of the synthesized NPs against *S. aureus*. Furthermore, the drug remained actively releasing for two more days, which was attributed to the neutral environmental pH supporting the binding of nanocarrier and biofilm polysaccharides (Perni and Prokopovich 2014). As the combination strategies of aminoglycosides are becoming highly favorable, the combinations were proposed to enhance their "killing" and biofilm inhibitory effectiveness upon co-delivery by NPs, as the drug conjugates can penetrate more rapidly through the cell membrane and biofilm matrix. For instance, the chitosan–streptomycin conjugates which had previously shown improved antibacterial and antibiofilm activities were used to synthesize gold NPs (Mu et al. 2016c). With the aid of gold NPs, the conjugates readily crossed the biofilm and cell membrane barriers, thus (1) actively inhibiting biofilm formation and eradicating preformed biofilm of *P. aeruginosa* and (2) exerting a bactericidal effect to both Gram-positive and Gram-negative bacteria (*L. monocytogenes*, *S. aureus*, *S. Typhimurium*, and *E. coli*) (Mu et al. 2016a).

In addition to metal-based NPs, polymeric NPs have also been used as a nanocarrier for aminoglycosides. In comparison to metal-based NPs, the polymeric NPs provide several different advantages in terms of minimal toxicity, biocompatibility, biodegradability, and environmental friendliness (El-Say and El-Sawy 2017). Tobramycin binding to alginate, which has been functionalized with DNase I and then encapsulated into chitosan NPs, was stably and effectively delivered

and exhibited antibacterial activity against *P. aeruginosa* in the lungs of cystic fibrosis-infected patients (Deacon et al. 2015). Amikacin was loaded into poly-D,L-lactide-co-glycolide (PLGA)-based NPs and was readily delivered through a biofilm matrix to perform both antibiofilm and antibacterial activities to *P. aeruginosa* planktonic and biofilm cells without causing cytotoxicity (Sabaeifard et al. 2017). As tobramycin was reported as forming a weak bonding with the PLGA NPs, the aminoglycoside was firstly combined with dioctyl sulfosuccinate and then loaded onto PLGA NPs, which resulted in the sustainable antibacterial activity against *P. aeruginosa* (Hill et al. 2019). Polymeric NPs were also capable of co-delivering the combination of nitric oxide (NO) and gentamicin across the biofilm matrix of *P. aeruginosa* (Nguyen et al. 2016). The release of NO and gentamicin has effectively eradicated the bacterial mature biofilm and killed the dispersed biofilm cells (Nguyen et al. 2016). The chemistry used for the conjugation or nanoformulation of aminoglycosides has been explained in several literature (Agnihotri et al. 2015; Kondaveeti et al. 2018; Liu et al. 2017; Mugabe et al. 2006b; Rukholm et al. 2006; Yan et al. 2019). In most of the cases, the conjugation of aminoglycoside with other molecules/agents involved carbodiimide chemistry (Kondaveeti et al. 2018; Liu et al. 2017; Perni and Prokopovich 2014). For example, the synthesis of aminoglycoside–metal NPs comprises two steps: the first step involves the synthesis of metal NPs by using a reducing agent and the second step involves the conjugation of aminoglycoside via condensation reaction in the presence of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) (Perni and Prokopovich 2014). Another example for conjugation of aminoglycoside with chitosan involved the following step reactions: in the first step, chitosan gets oxidized by periodate which results in the C_2–C_3 bond cleavage and formation of the aldehyde group. In the second step, aminoglycoside conjugates with the aldehyde group of oxide chitosan via the Schiff base reaction (Yan et al. 2019). Similarly, for the encapsulation of aminoglycoside into liposome, the methodology included the dehydration–rehydration vesicle method (Alhariri et al. 2017; Kirby and Gregoriadis 1984; Mugabe et al. 2006a), where liposome was prepared by mixing 1,2-dipalmitoylsn-glycero-3-phosphocholine (DPPC) and cholesterol in the molar ratio of 2:1. The prepared vesicles were mixed with aminoglycosides and freeze dried followed by the rehydration of the mixture. The rehydrated mixture was ready to use after washing with phosphate buffer. A representative example of the chemical reaction (chemistry of conjugation) used for the covalent conjugation of gentamicin with different materials such as chitosan/alginate or nanoformulation with metallic or polymeric nanoparticles is explained in Fig. 3.

Application of chemically modified form of the aminoglycosides

Although their activity toward the bacterial biofilm structure has remained limitedly reported, the large diversity of forms and synthesis methods of chemically modified aminoglycosides has been extremely significant (Thamban Chandrika and Garneau-Tsodikova 2018). Modifying the currently available aminoglycosides provides the advantage of improving certain characteristics of the drugs within a shorter time period as compared to searching and developing a new drug (Bera et al. 2016). A few typical examples could be aminoglycoside derivatives, antibacterial amphiphilic aminoglycoside (AAG), and aminoglycoside-derived cationic amphiphilic drug. Firstly, synthetic derivatives of aminoglycosides such as plazomicin and netilmicin are some new generation of aminoglycosides that have been discovered in recent years. Since the $–NH_2$ group majorly determines the aminoglycoside activity, modifications in their position and number which have given rise to plazomicin, netilmicin, and numerous modified aminoglycosides have been performed (Zarate et al. 2018). In most cases, they exhibited antibacterial activity to various human pathogens, including those which are referred to as "multidrug resistant" and have biofilm-forming ability (Cox et al. 2018; Landman et al. 2011; Noone 1984; Reyes et al. 2011). Secondly, AAG such as naphthylalkyl amine is a structurally modified form of neamine that has shifted to a new target site—the bacterial outer membrane and/or lipopolysaccharide and exhibited electrostatic interaction to destabilize the bacterial cells (Sautrey et al. 2014). Thirdly, various aminoglycoside-derived cationic amphiphilic drugs have also shown effective antibacterial activity against a wide range of biofilm-forming Gram-positive and Gram-negative bacteria (Benhamou et al. 2015). Overall, based on their active antibacterial potentials to a wide spectrum of biofilm-forming bacteria, the chemically modified aminoglycosides can be considered as a promising alternative for further application on biofilm inhibition approaches.

Conclusion and future perspectives

The major challenge to combination strategies in developing a new drug relies on complex antibiotic pharmacology. Searching for the precise treatment concentrations and duration for a single antibiotic is already difficult. In order to achieve the target for two compounds that are synergistically conjugative in dynamics and pharmacokinetics to maintain and encourage drug development, single agents may also be required for clinical trials. The toxicology of each agent, as well as the combination, must also be carefully investigated,

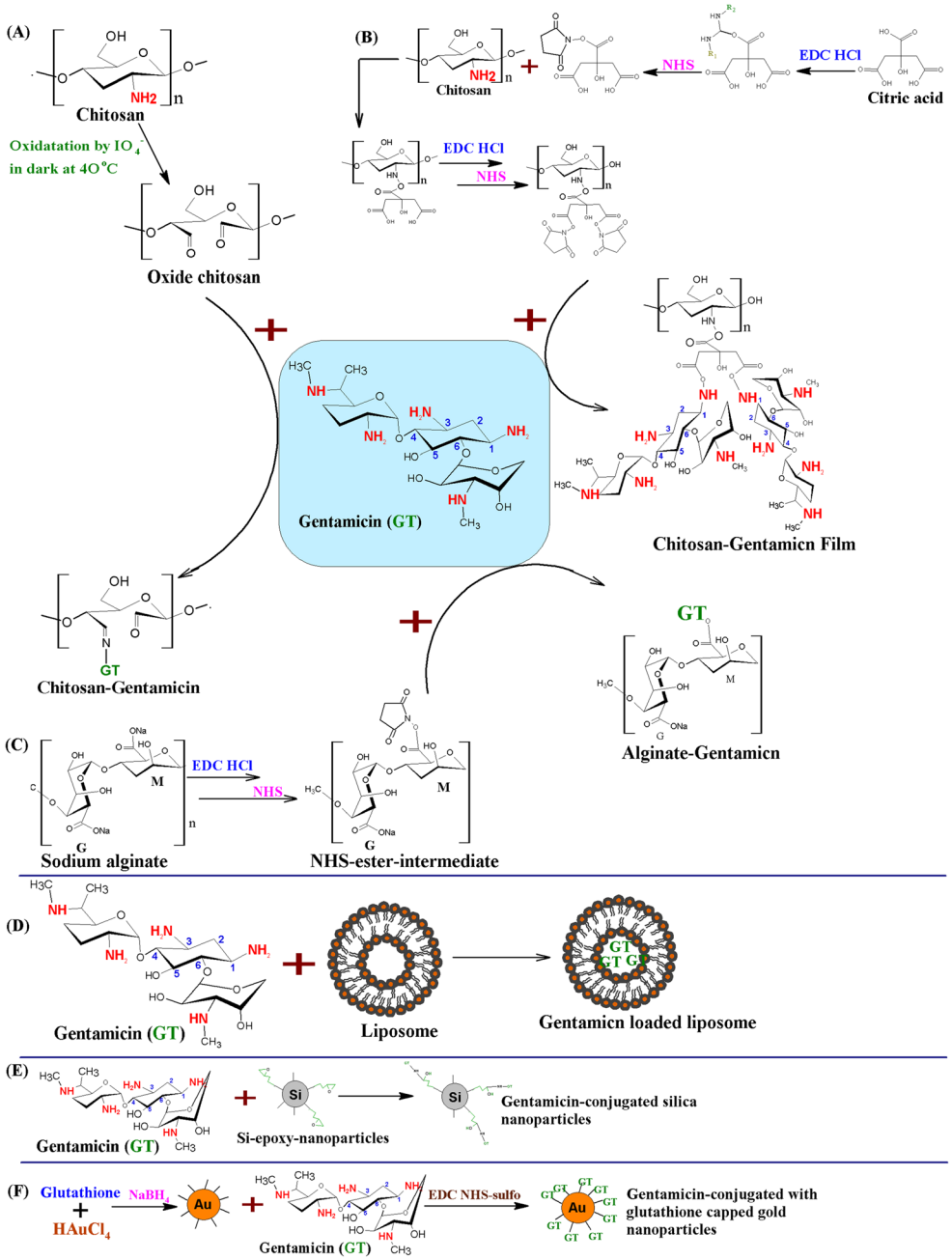

Fig. 3 Chemical reaction used for the conjugating of gentamicin with other active agents and nanoformulation with metallic/polymeric nanomaterials [information obtained from the literature (Agnihotri et al. 2015; Kondaveeti et al. 2018; Liu et al. 2017; Mugabe et al. 2006b; Rukholm et al. 2006; Yan et al. 2019)]. **a** Conjugation of gentamicin to chitosan followed by two steps. In the first step, chitosan gets oxidized by periodate which results in the C_2–C_3 bond cleavage and formation of the aldehyde group. In the second step, gentamicin conjugated with the aldehyde group of oxide chitosan via the Schiff base reaction (Yan et al. 2019). **b** Formation of a chitosan–gentamicin film by carbodiimide chemistry (Liu et al. 2017). Firstly, chitosan film formed by air drying of chitosan solution; secondly, generation of amide and carboxyl group by citric acid on the surface of chitosan; and thirdly, covalent grafting of gentamicin to the available carboxyl group of chitosan via the help of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as cross-linker. **c** Synthesis of the alginate–gentamicin conjugate by carbodiimide chemistry (Kondaveeti et al. 2018). **d** Encapsulation of gentamicin in a liposome (Rukholm et al. 2006). **e** Functionalized gentamicin-conjugated silica nanoparticles. First, synthesis of silica nanoparticles; second, generation of epoxy groups with the help of 3-glycidyloxypropyltrimethoxysilane; and third, functionalization with gentamicin (Agnihotri et al. 2015). **f** Synthesis of gentamicin-conjugated gold nanoparticles. In the first step, glutathione-capped gold nanoparticles are synthesized, and in the second step, the gentamicin is conjugated via condensation reaction upon EDC and NHS presence (which are involved in the activation of carboxyl group on the glutathione capping agent) (Perni and Prokopovich 2014)

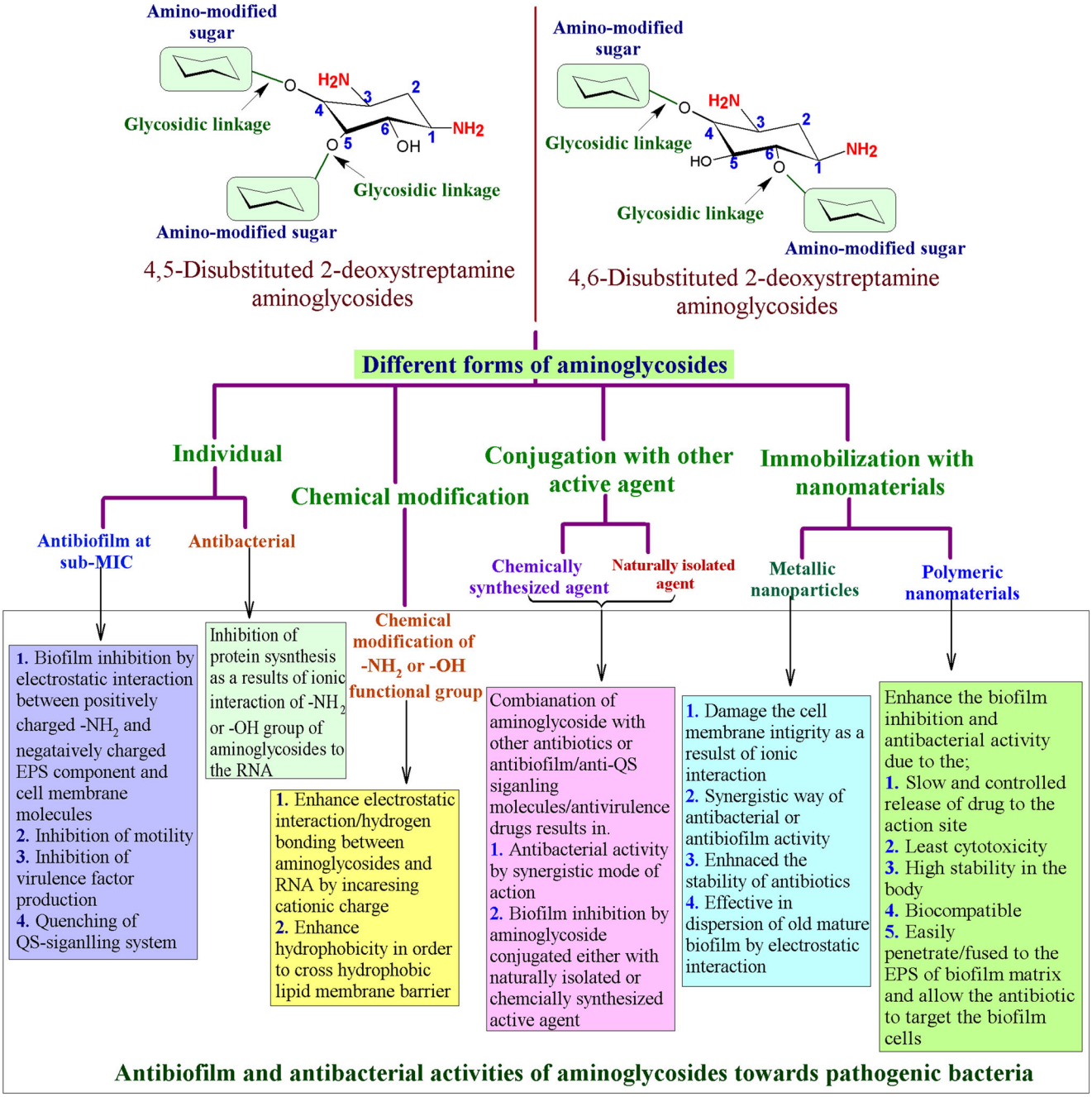

Fig. 4 Different strategies employed for treating biofilm-forming pathogenic bacteria by free form or conjugate forms of aminoglycoside antibiotics

whether unexpected drug–drug interactions take place. Developing combination therapy is more complex than monotherapy. Nevertheless, under the situation where monotherapy with single-target drugs has led to rapid resistance, the new single agents have shown efficacy during the past years despite that all antibiotics are at risk of being compromised by increasing resistance level.

Throughout the past few decades, knowledge about biofilm formation and other associated virulence properties has been extensively advanced. With the current situation where human pathogenic bacteria have vastly developed biofilm formation and with the production of virulence factors to resist a majority of conventional aminoglycosides, the discovery of an alternative control strategy is now highly urgent. Some of the up-to-date strategies to improve and develop aminoglycoside antibiofilm activity have been reviewed in the present paper, including (1) exploitation of the antivirulence potentials of aminoglycosides at the sub-MIC level, (2) immobilization/encapsulation of aminoglycosides to various types of nanocarriers, and (3) modifications in the chemical structure of aminoglycosides. The detailed mechanisms of antibiofilm and antibacterial activity of different forms of aminoglycosides (either free forms or in conjugation/

nanoformulation forms) have been explained in detail in Fig. 4. Although these strategies have actively controlled bacterial biofilm in various means such as preventing biofilm formation, disrupting the pre-existing mature biofilm, or attenuating the expression and regulation of virulence properties, extensive studies are demanded to take place in the future in order to achieve higher control over bacterial biofilm in the long term. Some suggestions are presented as follows:

1. The antibiofilm activity of some aminoglycosides should be studied at the molecular level.
2. The use of aminoglycoside combinations should be carefully examined and changed if necessary to prevent new resistance emergence.
3. The adjuvants that are used to potentiate aminoglycoside activity should also be studied for their profiles and activities.
4. With the vast number of new antibiofilm and antivirulence strategies being developed, other aminoglycosides should also be exploited for their potentials using all the summarized alternative strategies.
5. Optimization work toward the culture environment and storage conditions should be paid more attention.
6. The options of aminoglycoside adjuvants and nanocarriers should continuously be extended and advanced.
7. Co-delivery between aminoglycosides with other antibiotics or bioactive compounds is recommended to improve the drug activity, especially when the drug and its nanocarriers are weakly bonded.
8. The antibiotic adjuvants/enhancers which are either naturally or chemically synthesized must positively interact with its conjugated antibiotics without causing side effects or antagonistic effects to the antibiotics.
9. For clinical trials, an appropriate schedule of applications should be constructed carefully and specifically to the aminoglycosides used.
10. Studies of resistant genes must be conducted for more specific understandings about the internal driving force of resistant responses.
11. The antibiofilm activity of newly synthesized aminoglycosides demands more exploitation.
12. The applications of aminoglycosides in inhibiting human pathogenic bacteria must be carefully maintained and regulated by responsible authorities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Abed N, Couvreur P (2014) Nanocarriers for antibiotics: a promising solution to treat intracellular bacterial infections. Int J Antimicrob Agents 43(6):485–496. https://doi.org/10.1016/j.ijantimicag.2014.02.009

Aderibigbe BA (2017) Metal-based nanoparticles for the treatment of infectious diseases. Molecules 22(8). https://doi.org/10.3390/molecules22081370

Agnihotri S, Pathak R, Jha D, Roy I, Gautam HK, Sharma AK, Kumar P (2015) Synthesis and antimicrobial activity of aminoglycoside-conjugated silica nanoparticles against clinical and resistant bacteria. New J Chem 39(9):6746–6755. https://doi.org/10.1039/C5NJ00007F

Aka ST, Haji SH (2015) Sub-MIC of antibiotics induced biofilm formation of *Pseudomonas aeruginosa* in the presence of chlorhexidine. Braz J Microbiol 46(1):149–154. https://doi.org/10.1590/S1517-838246120140218

Alhariri M, Majrashi MA, Bahkali AH, Almajed FS, Azghani AO, Khiyami MA, Alyamani EJ, Aljohani SM, Halwani MA (2017) Efficacy of neutral and negatively charged liposome-loaded gentamicin on planktonic bacteria and biofilm communities. Int J Nanomedicine 12:6949–6961. https://doi.org/10.2147/IJN.S141709

Alipour M, Suntres ZE (2014) Liposomal antibiotic formulations for targeting the lungs in the treatment of *Pseudomonas aeruginosa*. Ther Deliv 5(4):409–427. https://doi.org/10.4155/tde.14.13

Allahverdiyev AM, Kon KV, Abamor ES, Bagirova M, Rafailovich M (2011) Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. Expert Rev Anti-Infect Ther 9(11):1035–1052. https://doi.org/10.1586/eri.11.121

Anderson GG, Kenney TF, Macleod DL, Henig NR, O'Toole GA (2013) Eradication of *Pseudomonas aeruginosa* biofilms on cultured airway cells by a fosfomycin/tobramycin antibiotic combination. Pathog Dis 67(1):39–45. https://doi.org/10.1111/2049-632X.12015

Andonova V (2017) Synthetic polymer-based nanoparticles: intelligent drug delivery systems. In: Boreddy S.R. Reddy (ed) Acrylic polymers in healthcare, IntechOpen. https://doi.org/10.5772/intechopen.69056

Ashrafudoulla M, Mizan MFR, Park H, Byun KH, Lee N, Park SH, Ha SD (2019) Genetic relationship, virulence factors, drug resistance profile and biofilm formation ability of *Vibrio parahaemolyticus* isolated from mussel. Front Microbiol 10:513. https://doi.org/10.3389/fmicb.2019.00513

Bahamondez-Canas TF, Zhang H, Tewes F, Leal J, Smyth HDC (2018) PEGylation of tobramycin improves mucus penetration and antimicrobial activity against *Pseudomonas aeruginosa* biofilms in vitro. Mol Pharm 15(4):1643–1652. https://doi.org/10.1021/acs.molpharmaceut.8b00011

Bahari S, Zeighami H, Mirshahabi H, Roudashti S, Haghi F (2017) Inhibition of *Pseudomonas aeruginosa* quorum sensing by subinhibitory concentrations of curcumin with gentamicin and azithromycin. J Glob Antimicrob Resist 10:21–28. https://doi.org/10.1016/j.jgar.2017.03.006

Bai AJ, Rai VR (2011) Bacterial quorum sensing and food industry. Compr Rev Food Sci Food Saf 10(3):183–193. https://doi.org/10.1111/j.1541-4337.2011.00150.x

Bandara HM, Harb A, Kolacny D Jr, Martins P, Smyth HD (2014) Sound waves effectively assist tobramycin in elimination of *Pseudomonas aeruginosa* biofilms in vitro. AAPS PharmSciTech 15(6):1644–1654. https://doi.org/10.1208/s12249-014-0200-1

Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M, Fernandes AR (2018) Nano-strategies to fight multidrug resistant bacteria—"a battle of the titans". Front Microbiol 9:1441. https://doi.org/10.3389/fmicb.2018.01441

Barraud N, Hassett DJ, Hwang SH, Rice SA, Kjelleberg S, Webb JS (2006) Involvement of nitric oxide in biofilm dispersal of *Pseudomonas aeruginosa*. J Bacteriol 188(21):7344–7353. https://doi.org/10.1128/JB.00779-06

Benhamou RI, Shaul P, Herzog IM, Fridman M (2015) Di-*N*-methylation of anti-gram-positive aminoglycoside-derived membrane disruptors improves antimicrobial potency and broadens spectrum to gram-negative bacteria. Angew Chem Int Ed Engl 54(46):13617–13621. https://doi.org/10.1002/anie.201506814

Bera S, Mondal D, Palit S, Schweizer F (2016) Structural modifications of the neomycin class of aminoglycosides. MedChemComm 7(8):1499–1534. https://doi.org/10.1039/C6MD00079G

Bhattacharya D, Saha B, Mukherjee A, Ranjan Santra C, Karmakar P (2012) Gold nanoparticles conjugated antibiotics: stability and functional evaluation. Nanosci Nanotechnol 2(2):14–21. https://doi.org/10.5923/j.nn.20120202.04

Bhattacharya G, Dey D, Das S, Banerjee A (2017) Exposure to subinhibitory concentrations of gentamicin, ciprofloxacin and cefotaxime induces multidrug resistance and reactive oxygen species generation in meticillin-sensitive *Staphylococcus aureus*. J Med Microbiol 66(6):762–769. https://doi.org/10.1099/jmm.0.000492

Boehm A, Steiner S, Zaehringer F, Casanova A, Hamburger F, Ritz D, Keck W, Ackermann M, Schirmer T, Jenal U (2009) Second messenger signalling governs *Escherichia coli* biofilm induction upon ribosomal stress. Mol Microbiol 72(6):1500–1516. https://doi.org/10.1111/j.1365-2958.2009.06739.x

Bridier A, Sanchez-Vizuete P, Guilbaud M, Piard JC, Naitali M, Briandet R (2015) Biofilm-associated persistence of food-borne pathogens. Food Microbiol 45(Pt B):167–178. https://doi.org/10.1016/j.fm.2014.04.015

Cai Y, Fan Y, Wang R, An MM, Liang BB (2009) Synergistic effects of aminoglycosides and fosfomycin on *Pseudomonas aeruginosa in vitro* and biofilm infections in a rat model. J Antimicrob Chemother 64(3):563–566. https://doi.org/10.1093/jac/dkp224

Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A (1999) The Calgary biofilm device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol 37(6):1771–1776

Cernohorska L, Votava M (2008) Antibiotic synergy against biofilm-forming *Pseudomonas aeruginosa*. Folia Microbiol (Praha) 53(1):57–60. https://doi.org/10.1007/s12223-008-0008-z

Chanda W, Joseph TP, Padhiar AA, Guo X, Min L, Wang W, Lolokote S, Ning A, Cao J, Huang M, Zhong M (2017) Combined effect of linolenic acid and tobramycin on *Pseudomonas aeruginosa* biofilm formation and quorum sensing. Exp Ther Med 14(5):4328–4338. https://doi.org/10.3892/etm.2017.5110

Chauhan A, Lebeaux D, Ghigo JM, Beloin C (2012) Full and broad-spectrum in vivo eradication of catheter-associated biofilms using gentamicin-EDTA antibiotic lock therapy. Antimicrob Agents Chemother 56(12):6310–6318. https://doi.org/10.1128/AAC.01606-12

Chen L, Wen YM (2011) The role of bacterial biofilm in persistent infections and control strategies. Int J Oral Sci 3(2):66–73. https://doi.org/10.4248/IJOS11022

Chiang WC, Nilsson M, Jensen PO, Hoiby N, Nielsen TE, Givskov M, Tolker-Nielsen T (2013) Extracellular DNA shields against aminoglycosides in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 57(5):2352–2361. https://doi.org/10.1128/AAC.00001-13

Chittapragada M, Roberts S, Ham YW (2009) Aminoglycosides: molecular insights on the recognition of RNA and aminoglycoside mimics. Perspect Medicin Chem 3:21–37. https://doi.org/10.4137/PMC.S2381

Chmielewski RAN, Frank JF (2003) Biofilm formation and control in food processing facilities. Compr Rev Food Sci Food Saf 2(1):22–32. https://doi.org/10.1111/j.1541-4337.2003.tb00012.x

Cole SJ, Records AR, Orr MW, Linden SB, Lee VT (2014) Catheter-associated urinary tract infection by *Pseudomonas aeruginosa* is mediated by exopolysaccharide-independent biofilms. Infect Immun 82(5):2048–2058. https://doi.org/10.1128/IAI.01652-14

Cox G, Ejim L, Stogios PJ, Koteva K, Bordeleau E, Evdokimova E, Sieron AO, Savchenko A, Serio AW, Krause KM, Wright GD (2018) Plazomicin retains antibiotic activity against most aminoglycoside modifying enzymes. ACS Infect Dis 4(6):980–987. https://doi.org/10.1021/acsinfecdis.8b00001

Crabbe A, Liu Y, Matthijs N, Rigole P, De La Fuente-Nunez C, Davis R, Ledesma MA, Sarker S, Van Houdt R, Hancock RE, Coenye T, Nickerson CA (2017) Antimicrobial efficacy against *Pseudomonas aeruginosa* biofilm formation in a three-dimensional lung epithelial model and the influence of fetal bovine serum. Sci Rep 7:43321–43313. https://doi.org/10.1038/srep43321

Das T, Sharma PK, Busscher HJ, van der Mei HC, Krom BP (2010) Role of extracellular DNA in initial bacterial adhesion and surface aggregation. Appl Environ Microbiol 76(10):3405–3408. https://doi.org/10.1128/AEM.03119-09

Davis BD (1987) Mechanism of bactericidal action of aminoglycosides. Microbiol Rev 51(3):341–350

Deacon J, Abdelghany SM, Quinn DJ, Schmid D, Megaw J, Donnelly RF, Jones DS, Kissenpfennig A, Elborn JS, Gilmore BF, Taggart CC, Scott CJ (2015) Antimicrobial efficacy of tobramycin polymeric nanoparticles for *Pseudomonas aeruginosa* infections in cystic fibrosis: formulation, characterisation and functionalisation with dornase alfa (DNase). J Control Release 198:55–61. https://doi.org/10.1016/j.jconrel.2014.11.022

Di Domenico EG, Farulla I, Prignano G, Gallo MT, Vespaziani M, Cavallo I, Sperduti I, Pontone M, Bordignon V, Cilli L, De Santis A, Di Salvo F, Pimpinelli F, Lesnoni La Parola I, Toma L, Ensoli F (2017) Biofilm is a major virulence determinant in bacterial colonization of chronic skin ulcers independently from the multidrug resistant phenotype. Int J Mol Sci 18(5). https://doi.org/10.3390/ijms18051077

Dickey SW, Cheung GYC, Otto M (2017) Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. Nat Rev Drug Discov 16(7):457–471. https://doi.org/10.1038/nrd.2017.23

Dong G, Liu H, Yu X, Zhang X, Lu H, Zhou T, Cao J (2018) Antimicrobial and anti-biofilm activity of tannic acid against *Staphylococcus aureus*. Nat Prod Res 32(18):2225–2228. https://doi.org/10.1080/14786419.2017.1366485

Dosler S, Karaaslan E (2014) Inhibition and destruction of *Pseudomonas aeruginosa* biofilms by antibiotics and antimicrobial peptides. Peptides 62:32–37. https://doi.org/10.1016/j.peptides.2014.09.021

Douafer H, Andrieu V, Phanstiel O, Brunel JM (2019) Antibiotic adjuvants: make antibiotics great again! J Med Chem. https://doi.org/10.1021/acs.jmedchem.8b01781

Drulis-Kawa Z, Dorotkiewicz-Jach A (2010) Liposomes as delivery systems for antibiotics. Int J Pharm 387(1–2):187–198. https://doi.org/10.1016/j.ijpharm.2009.11.033

Du J, Bandara HM, Du P, Huang H, Hoang K, Nguyen D, Mogarala SV, Smyth HD (2015) Improved biofilm antimicrobial activity of polyethylene glycol conjugated tobramycin compared to tobramycin in *Pseudomonas aeruginosa* biofilms. Mol Pharm 12(5):1544–1553. https://doi.org/10.1021/mp500846u

Eckert R, Brady KM, Greenberg EP, Qi F, Yarbrough DK, He J, McHardy I, Anderson MH, Shi W (2006) Enhancement of antimicrobial activity against *Pseudomonas aeruginosa* by coadministration of G10KHc and tobramycin. Antimicrob Agents Chemother 50(11): 3833–3838. https://doi.org/10.1128/AAC.00509-06

El Zowalaty ME, Hussein Al Ali SH, Husseiny MI, Geilich BM, Webster TJ, Hussein MZ (2015) The ability of streptomycin-loaded chitosan-coated magnetic nanocomposites to possess antimicrobial and antituberculosis activities. Int J Nanomedicine 10:3269–3274. https://doi.org/10.2147/IJN.S74469

El-Say KM, El-Sawy HS (2017) Polymeric nanoparticles: promising platform for drug delivery. Int J Pharm 528(1–2):675–691. https://doi.org/10.1016/j.ijpharm.2017.06.052

Eom SH, Kang SK, Lee DS, Myeong JI, Lee J, Kim HW, Kim KH, Je JY, Jung WK, Kim YM (2016) Synergistic antibacterial effect and antibacterial action mode of chitosan-ferulic acid conjugate against methicillin-resistant *Staphylococcus aureus*. J Microbiol Biotechnol 26(4):784–789. https://doi.org/10.4014/jmb.1511.11046

Esteban J, Garcia-Coca M (2017) *Mycobacterium* biofilms. Front Microbiol 8:2651. https://doi.org/10.3389/fmicb.2017.02651

Fleitas Martinez O, Rigueiras PO, Pires ADS, Porto WF, Silva ON, de la Fuente-Nunez C, Franco OL (2018) Interference with quorum-sensing signal biosynthesis as a promising therapeutic strategy against multidrug-resistant pathogens. Front Cell Infect Microbiol 8:444. https://doi.org/10.3389/fcimb.2018.00444

Fleitas Martinez O, Cardoso MH, Ribeiro SM, Franco OL (2019) Recent advances in anti-virulence therapeutic strategies with a focus on dismantling bacterial membrane microdomains, toxin neutralization, quorum-sensing interference and biofilm inhibition. Front Cell Infect Microbiol 9:74. https://doi.org/10.3389/fcimb.2019.00074

Fong J, Zhang C, Yang R, Boo ZZ, Tan SK, Nielsen TE, Givskov M, Liu XW, Bin W, Su H, Yang L (2018) Combination therapy strategy of quorum quenching enzyme and quorum sensing inhibitor in suppressing multiple quorum sensing pathways of *P. aeruginosa*. Sci Rep 8(1):1155. https://doi.org/10.1038/s41598-018-19504-w

Fraud S, Poole K (2011) Oxidative stress induction of the MexXY multidrug efflux genes and promotion of aminoglycoside resistance development in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 55(3):1068–1074. https://doi.org/10.1128/AAC.01495-10

Furiga A, Lajoie B, El Hage S, Baziard G, Roques C (2015) Impairment of *Pseudomonas aeruginosa* biofilm resistance to antibiotics by combining the drugs with a new quorum-sensing inhibitor. Antimicrob Agents Chemother 60(3):1676–1686. https://doi.org/10.1128/AAC.02533-15

Gad El-Rab SMF, Halawani EM, Hassan A (2018) Formulation of ceftriaxone conjugated gold nanoparticles and their medical applications against extended-spectrum beta-lactamase producing bacteria and breast cancer. J Microbiol Biotechnol 28(9):1563–1572. https://doi.org/10.4014/jmb.1711.11037

Gagniere H, Di Martino P (2004) Effects of antibiotics on *Pseudomonas aeruginosa* NK125502 and *Pseudomonas fluorescens* MF0 biofilm formation on immobilized fibronectin. J Chemother 16(3):244–247. https://doi.org/10.1179/joc.2004.16.3.244

Galimand M, Lambert T, Courvalin P (2005) Emergence and dissemination of a new mechanism of resistance to aminoglycosides in Gram-negative bacteria: 16S rRNA methylation. Euro Surveill 10(1): E050127 2. https://doi.org/10.2807/esw.10.04.02626-en

Garneau-Tsodikova S, Labby KJ (2016) Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. MedChemComm 7(1):11–27. https://doi.org/10.1039/C5MD00344J

Ghorbani H, Memar MY, Sefidan FY, Yekani M, Ghotaslou R (2017) In vitro synergy of antibiotic combinations against planktonic and biofilm *Pseudomonas aeruginosa*. GMS Hyg Infect Control 12: Doc17. https://doi.org/10.3205/dgkh000302

Giaouris E, Heir E, Desvaux M, Hebraud M, Moretro T, Langsrud S, Doulgeraki A, Nychas GJ, Kacaniova M, Czaczyk K, Olmez H, Simoes M (2015) Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. Front Microbiol 6:841. https://doi.org/10.3389/fmicb.2015.00841

Giovagnoli S, Pietrella D, Barberini L, Santi C, Carotti A, di Michele A, Ricci M (2017) Reshaping antibiotics through hydrophobic drug-bile acid ionic complexation enhances activity against *Staphylococcus aureus* biofilms. Int J Pharm 528(1–2):144–162. https://doi.org/10.1016/j.ijpharm.2017.06.008

Gonzalez LS 3rd, Spencer JP (1998) Aminoglycosides: a practical review. Am Fam Physician 58(8):1811–1820

Gopu V, Meena CK, Shetty PH (2015) Quercetin influences quorum sensing in food borne bacteria: in-vitro and in-silico evidence. PLoS One 10(8):e0134684. https://doi.org/10.1371/journal.pone.0134684

Gupta P, Sarkar A, Sandhu P, Daware A, Das MC, Akhter Y, Bhattacharjee S (2017) Potentiation of antibiotic against *Pseudomonas aeruginosa* biofilm: a study with plumbagin and gentamicin. J Appl Microbiol 123(1):246–261. https://doi.org/10.1111/jam.13476

Habash MB, Goodyear MC, Park AJ, Surette MD, Vis EC, Harris RJ, Khursigara CM (2017) Potentiation of tobramycin by silver nanoparticles against *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 61(11). https://doi.org/10.1128/AAC.00415-17

Hari N, Nair AJ (2016) Development and characterization of chitosan-based antimicrobial films incorporated with streptomycin loaded starch nanoparticles. New Horiz Transl Med 3(1):22–29. https://doi.org/10.1016/j.nhtm.2016.04.002

Henry-Stanley MJ, Hess DJ, Wells CL (2014) Aminoglycoside inhibition of *Staphylococcus aureus* biofilm formation is nutrient dependent. J Med Microbiol 63(Pt 6):861–869. https://doi.org/10.1099/jmm.0.068130-0

Herrmann G, Yang L, Wu H, Song Z, Wang H, Hoiby N, Ulrich M, Molin S, Riethmuller J, Doring G (2010) Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm *Pseudomonas aeruginosa*. J Infect Dis 202(10):1585–1592. https://doi.org/10.1086/656788

Hill M, Cunningham RN, Hathout RM, Johnston C, Hardy JG, Migaud ME (2019) Formulation of antimicrobial tobramycin loaded PLGA nanoparticles via complexation with AOT. J Funct Biomater 10(2). https://doi.org/10.3390/jfb10020026

Hirt H, Gorr SU (2013) Antimicrobial peptide GL13K is effective in reducing biofilms of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 57(10):4903–4910. https://doi.org/10.1128/AAC.00311-13

Hobbie SN, Pfister P, Bruell C, Sander P, Francois B, Westhof E, Bottger EC (2006) Binding of neomycin-class aminoglycoside antibiotics to mutant ribosomes with alterations in the A site of 16S rRNA. Antimicrob Agents Chemother 50(4):1489–1496. https://doi.org/10.1128/AAC.50.4.1489-1496.2006

Hoffman LR, D'Argenio DA, MacCoss MJ, Zhang Z, Jones RA, Miller SI (2005) Aminoglycoside antibiotics induce bacterial biofilm formation. Nature 436(7054):1171–1175. https://doi.org/10.1038/nature03912

Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35(4):322–332. https://doi.org/10.1016/j.ijantimicag.2009.12.011

Hoiby N, Henneberg KA, Wang H, Stavnsbjerg C, Bjarnsholt T, Ciofu O, Johansen UR, Sams T (2019) Formation of *Pseudomonas aeruginosa* inhibition zone during tobramycin disk diffusion is due to transition from planktonic to biofilm mode of growth. Int J Antimicrob Agents 53(5):564–573. https://doi.org/10.1016/j.ijantimicag.2018.12.015

Hou Y, Wang Z, Zhang P, Bai H, Sun Y, Duan J, Mu H (2017) Lysozyme associated liposomal gentamicin inhibits bacterial biofilm. Int J Mol Sci 18(4). https://doi.org/10.3390/ijms18040784

Hou Y, Yang M, Jiang H, Li D, Du Y (2019) Effects of low-intensity and low-frequency ultrasound combined with tobramycin on biofilms of extended-spectrum beta-lactamases (ESBLs) *Escherichia coli*. FEMS Microbiol Lett 366(3). https://doi.org/10.1093/femsle/fnz026

Hu J, Quan Y, Lai Y, Zheng Z, Hu Z, Wang X, Dai T, Zhang Q, Cheng Y (2017) A smart aminoglycoside hydrogel with tunable gel degradation, on-demand drug release, and high antibacterial activity. J Control Release 247:145–152. https://doi.org/10.1016/j.jconrel.2017.01.003

Hussein-Al-Ali SH, El Zowalaty ME, Hussein MZ, Ismail M, Webster TJ (2014) Synthesis, characterization, controlled release, and antibacterial studies of a novel streptomycin chitosan magnetic nanoantibiotic. Int J Nanomedicine 9:549–557. https://doi.org/10.2147/IJN.S53079

Javaid A, Oloketuyi SF, Khan MM, Khan F (2018) Diversity of bacterial synthesis of silver nanoparticles. BioNanoScience 8(1):43–59. https://doi.org/10.1007/s12668-017-0496-x

Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK (2018) Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol 9:1050–1074. https://doi.org/10.3762/bjnano.9.98

Jia X, Zhang J, Sun W, He W, Jiang H, Chen D, Murchie AI (2013) Riboswitch control of aminoglycoside antibiotic resistance. Cell 152(1–2):68–81. https://doi.org/10.1016/j.cell.2012.12.019

Jijie R, Barras A, Teodorescu F, Boukherroub R, Szunerits S (2017) Advancements on the molecular design of nanoantibiotics: current level of development and future challenges. Mol Syst Des Eng 2(4):349–369. https://doi.org/10.1039/C7ME00048K

Jones C, Allsopp L, Horlick J, Kulasekara H, Filloux A (2013) Subinhibitory concentration of kanamycin induces the *Pseudomonas aeruginosa* type VI secretion system. PLoS One 8(11):e81132. https://doi.org/10.1371/journal.pone.0081132

Kaplan JB (2011) Antibiotic-induced biofilm formation. Int J Artif Organs 34(9):737–751. https://doi.org/10.5301/ijao.5000027

Kapoor P, Murphy P (2018) Combination antibiotics against *Pseudomonas aeruginosa*, representing common and rare cystic fibrosis strains from different Irish clinics. Heliyon 4(3):e00562. https://doi.org/10.1016/j.heliyon.2018.e00562

Khan I, Saeed K, Khan I (2017) Nanoparticles: properties, applications and toxicities. Arab J Chem. https://doi.org/10.1016/j.arabjc.2017.05.011

Khan F, Khan MM, Kim YM (2018) Recent progress and future perspectives of antibiofilm drugs immobilized on nanomaterials. Curr Pharm Biotechnol 19(8):631–643. https://doi.org/10.2174/1389201019666180828090052

Khan F, Javaid A, Kim YM (2019a) Functional diversity of quorum sensing receptors in pathogenic bacteria: interspecies, intraspecies and interkingdom level. Curr Drug Targets 20(6):655–667. https://doi.org/10.2174/1389450120666181123123333

Khan F, Lee JW, Pham DTN, Lee JH, Kim HW, Kim YK, Kim YM (2019b) Streptomycin mediated biofilm inhibition and suppression of virulence properties in *Pseudomonas aeruginosa* PAO1. Appl Microbiol Biotechnol 104:799–816. https://doi.org/10.1007/s00253-019-10190-w

Kim JH, Yu D, Eom SH, Kim SH, Oh J, Jung WK, Kim YM (2017) Synergistic antibacterial effects of chitosan-caffeic acid conjugate against antibiotic-resistant acne-related bacteria. Mar Drugs 15(6). https://doi.org/10.3390/md15060167

Kirby C, Gregoriadis G (1984) Dehydration-rehydration vesicles: a simple method for high yield drug entrapment in liposomes. Bio/Technology 2(11):979–984. https://doi.org/10.1038/nbt1184-979

Kondaveeti S, Bueno PVA, Carmona-Ribeiro AM, Esposito F, Lincopan N, Sierakowski MR, Petri DFS (2018) Microbicidal gentamicin-alginate hydrogels. Carbohydr Polym 186:159–167. https://doi.org/10.1016/j.carbpol.2018.01.044

Kora AJ, Rastogi L (2013) Enhancement of antibacterial activity of capped silver nanoparticles in combination with antibiotics, on model gram-negative and gram-positive bacteria. Bioinorg Chem Appl 2013:871097. https://doi.org/10.1155/2013/871097

Kotra LP, Haddad J, Mobashery S (2000) Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. Antimicrob Agents Chemother 44(12):3249–3256. https://doi.org/10.1128/aac.44.12.3249-3256.2000

Krause KM, Serio AW, Kane TR, Connolly LE (2016) Aminoglycosides: an overview. Cold Spring Harb Perspect Med 6(6). https://doi.org/10.1101/cshperspect.a027029

Kumar A, Ting YP (2016) Streptomycin favors biofilm formation by altering cell surface properties. Appl Microbiol Biotechnol 100(20):8843–8853. https://doi.org/10.1007/s00253-016-7793-0

Labby KJ, Garneau-Tsodikova S (2013) Strategies to overcome the action of aminoglycoside-modifying enzymes for treating resistant bacterial infections. Future Med Chem 5(11):1285–1309. https://doi.org/10.4155/fmc.13.80

Landman D, Kelly P, Backer M, Babu E, Shah N, Bratu S, Quale J (2011) Antimicrobial activity of a novel aminoglycoside, ACHN-490, against *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from New York City. J Antimicrob Chemother 66(2):332–334. https://doi.org/10.1093/jac/dkq459

Langner M, Kral TE (1999) Liposome-based drug delivery systems. Pol J Pharmacol 51(3):211–222

Lebeaux D, Chauhan A, Letoffe S, Fischer F, de Reuse H, Beloin C, Ghigo JM (2014) pH-mediated potentiation of aminoglycosides kills bacterial persisters and eradicates in vivo biofilms. J Infect Dis 210(9):1357–1366. https://doi.org/10.1093/infdis/jiu286

Lebeaux D, Leflon-Guibout V, Ghigo JM, Beloin C (2015) *In vitro* activity of gentamicin, vancomycin or amikacin combined with EDTA or l-arginine as lock therapy against a wide spectrum of biofilm-forming clinical strains isolated from catheter-related infections. J Antimicrob Chemother 70(6):1704–1712. https://doi.org/10.1093/jac/dkv044

Li B, Webster TJ (2018) Bacteria antibiotic resistance: new challenges and opportunities for implant-associated orthopedic infections. J Orthop Res 36(1):22–32. https://doi.org/10.1002/jor.23656

Li X, Wu B, Chen H, Nan K, Jin Y, Sun L, Wang B (2018) Recent developments in smart antibacterial surfaces to inhibit biofilm formation and bacterial infections. J Mater Chem B 6(26):4274–4292. https://doi.org/10.1039/C8TB01245H

Li R, Yuan X, Wei J, Zhang X, Cheng G, Wang ZA, Du Y (2019) Synthesis and evaluation of a chitosan oligosaccharide-streptomycin conjugate against *Pseudomonas aeruginosa* biofilms. Mar Drugs 17(1). https://doi.org/10.3390/md17010043

Lin J, Nishino K, Roberts MC, Tolmasky M, Aminov RI, Zhang L (2015) Mechanisms of antibiotic resistance. Front Microbiol 6:34. https://doi.org/10.3389/fmicb.2015.00034

Linares JF, Gustafsson I, Baquero F, Martinez JL (2006) Antibiotics as intermicrobial signaling agents instead of weapons. Proc Natl Acad Sci U S A 103(51):19484–19489. https://doi.org/10.1073/pnas.0608949103

Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z (2008) Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev 60(15):1650–1662. https://doi.org/10.1016/j.addr.2008.09.001

Liu Z, Wang W, Zhu Y, Gong Q, Yu W, Lu X (2013) Antibiotics at subinhibitory concentrations improve the quorum sensing behavior of *Chromobacterium violaceum*. FEMS Microbiol Lett 341(1):37–44. https://doi.org/10.1111/1574-6968.12086

Liu Q, Niu H, Zhang W, Mu H, Sun C, Duan J (2015) Synergy among thymol, eugenol, berberine, cinnamaldehyde and streptomycin against planktonic and biofilm-associated food-borne pathogens. Lett Appl Microbiol 60(5):421–430. https://doi.org/10.1111/lam.12401

Liu Y, Ji P, Lv H, Qin Y, Deng L (2017) Gentamicin modified chitosan film with improved antibacterial property and cell biocompatibility. Int J Biol Macromol 98:550–556. https://doi.org/10.1016/j.ijbiomac.2017.01.121

Magnet S, Smith TA, Zheng R, Nordmann P, Blanchard JS (2003) Aminoglycoside resistance resulting from tight drug binding to an altered aminoglycoside acetyltransferase. Antimicrob Agents Chemother 47(5):1577–1583. https://doi.org/10.1128/aac.47.5.1577-1583.2003

Maiden MM, Hunt AMA, Zachos MP, Gibson JA, Hurwitz ME, Mulks MH, Waters CM (2018) Triclosan is an aminoglycoside adjuvant for eradication of *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 62(6). https://doi.org/10.1128/AAC.00146-18

Maura D, Drees SL, Bandyopadhaya A, Kitao T, Negri M, Starkey M, Lesic B, Milot S, Deziel E, Zahler R, Pucci M, Felici A, Fetzner S, Lepine F, Rahme LG (2017) Polypharmacology approaches against the *Pseudomonas aeruginosa* MvfR regulon and their application in blocking virulence and antibiotic tolerance. ACS Chem Biol 12(5):1435–1443. https://doi.org/10.1021/acschembio.6b01139

Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM (1999) Aminoglycosides: activity and resistance. Antimicrob Agents Chemother 43(4):727–737

Moreau-Marquis S, Coutermarsh B, Stanton BA (2015) Combination of hypothiocyanite and lactoferrin (ALX-109) enhances the ability of tobramycin and aztreonam to eliminate *Pseudomonas aeruginosa* biofilms growing on cystic fibrosis airway epithelial cells. J Antimicrob Chemother 70(1):160–166. https://doi.org/10.1093/jac/dku357

Morikawa K, Nonaka M, Yoshikawa Y, Torii I (2005) Synergistic effect of fosfomycin and arbekacin on a methicillin-resistant *Staphylococcus aureus*-induced biofilm in a rat model. Int J Antimicrob Agents 25(1):44–50. https://doi.org/10.1016/j.ijantimicag.2004.07.015

Morita Y, Tomida J, Kawamura Y (2012a) MexXY multidrug efflux system of *Pseudomonas aeruginosa*. Front Microbiol 3:408. https://doi.org/10.3389/fmicb.2012.00408

Morita Y, Tomida J, Kawamura Y (2012b) Primary mechanisms mediating aminoglycoside resistance in the multidrug-resistant *Pseudomonas aeruginosa* clinical isolate PA7. Microbiology 158(Pt 4):1071–1083. https://doi.org/10.1099/mic.0.054320-0

Mosselhy DA, He W, Hynonen U, Meng Y, Mohammadi P, Palva A, Feng Q, Hannula SP, Nordstrom K, Linder MB (2018) Silica-gentamicin nanohybrids: combating antibiotic resistance, bacterial biofilms, and in vivo toxicity. Int J Nanomedicine 13:7939–7957. https://doi.org/10.2147/IJN.S182611

Mourtas S, Diamanti G, Foka A, Dracopoulos V, Klepetsanis P, Stamouli V, Spiliopoulou I, Antimisiaris SG (2015) Inhibition of bacterial attachment on surfaces by immobilization of tobramycin-loaded liposomes. J Biomed Nanotechnol 11(12):2186–2196

Mu H, Guo F, Niu H, Liu Q, Wang S, Duan J (2014) Chitosan improves anti-biofilm efficacy of gentamicin through facilitating antibiotic penetration. Int J Mol Sci 15(12):22296–22308. https://doi.org/10.3390/ijms151222296

Mu H, Liu Q, Niu H, Sun Y, Duan J (2016a) Gold nanoparticles make chitosan–streptomycin conjugates effective towards Gram-negative bacterial biofilm. RSC Adv 6(11):8714–8721. https://doi.org/10.1039/C5RA22803D

Mu H, Niu H, Wang D, Sun F, Sun Y, Duan J (2016b) Chitosan conjugation enables intracellular bacteria susceptible to aminoglycoside antibiotic. Glycobiology 26(11):1190–1197. https://doi.org/10.1093/glycob/cww079

Mu H, Tang J, Liu Q, Sun C, Wang T, Duan J (2016c) Potent antibacterial nanoparticles against biofilm and intracellular bacteria. Sci Rep 6:18877. https://doi.org/10.1038/srep18877

Mugabe C, Azghani AO, Omri A (2006a) Preparation and characterization of dehydration-rehydration vesicles loaded with aminoglycoside and macrolide antibiotics. Int J Pharm 307(2):244–250. https://doi.org/10.1016/j.ijpharm.2005.10.005

Mugabe C, Halwani M, Azghani AO, Lafrenie RM, Omri A (2006b) Mechanism of enhanced activity of liposome-entrapped aminoglycosides against resistant strains of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 50(6):2016–2022. https://doi.org/10.1128/AAC.01547-05

Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR (2019) Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol 10:539. https://doi.org/10.3389/fmicb.2019.00539

Mulcahy H, Charron-Mazenod L, Lewenza S (2008) Extracellular DNA chelates cations and induces antibiotic resistance in *Pseudomonas aeruginosa* biofilms. PLoS Pathog 4(11):e1000213. https://doi.org/10.1371/journal.ppat.1000213

Nair S, Desai S, Poonacha N, Vipra A, Sharma U (2016) Antibiofilm activity and synergistic inhibition of *Staphylococcus aureus* biofilms by bactericidal protein P128 in combination with antibiotics. Antimicrob Agents Chemother 60(12):7280–7289. https://doi.org/10.1128/AAC.01118-16

Nguyen TK, Selvanayagam R, Ho KKK, Chen R, Kutty SK, Rice SA, Kumar N, Barraud N, Duong HTT, Boyer C (2016) Co-delivery of nitric oxide and antibiotic using polymeric nanoparticles. Chem Sci 7(2):1016–1027. https://doi.org/10.1039/c5sc02769a

Noone P (1984) Sisomicin, netilmicin and dibekacin. A review of their antibacterial activity and therapeutic use. Drugs 27(6):548–578. https://doi.org/10.2165/00003495-198427060-00003

O'Connell HA, Kottkamp GS, Eppelbaum JL, Stubblefield BA, Gilbert SE, Gilbert ES (2006) Influences of biofilm structure and antibiotic resistance mechanisms on indirect pathogenicity in a model polymicrobial biofilm. Appl Environ Microbiol 72(7):5013–5019. https://doi.org/10.1128/AEM.02474-05

Olivares J, Bernardini A, Garcia-Leon G, Corona F, Sanchez M, Martinez JL (2013) The intrinsic resistome of bacterial pathogens. Front Microbiol 4:103. https://doi.org/10.3389/fmicb.2013.00103

Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z (2019) Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnol Adv 37(1):177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013

Payne JN, Waghwani HK, Connor MG, Hamilton W, Tockstein S, Moolani H, Chavda F, Badwaik V, Lawrenz MB, Dakshinamurthy R (2016) Novel synthesis of kanamycin conjugated gold nanoparticles with potent antibacterial activity. Front Microbiol 7:607. https://doi.org/10.3389/fmicb.2016.00607

Perez-Rodriguez F, Mercanoglu Taban B (2019) A state-of-art review on multi-drug resistant pathogens in foods of animal origin: risk factors and mitigation strategies. Front Microbiol 10:2091. https://doi.org/10.3389/fmicb.2019.02091

Perni S, Prokopovich P (2014) Continuous release of gentamicin from gold nanocarriers. RSC Adv 4(94):51904–51910. https://doi.org/10.1039/c4ra10023a

Peterson E, Kaur P (2018) Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol 9:2928. https://doi.org/10.3389/fmicb.2018.02928

Radlinski LC, Rowe SE, Brzozowski R, Wilkinson AD, Huang R, Eswara P, Conlon BP (2019) Chemical induction of aminoglycoside

uptake overcomes antibiotic tolerance and resistance in *Staphylococcus aureus*. Cell Chem Biol 26(10):1355–1364 e4. https://doi.org/10.1016/j.chembiol.2019.07.009

Ramirez MS, Tolmasky ME (2010) Aminoglycoside modifying enzymes. Drug Resist Updat 13(6):151–171. https://doi.org/10.1016/j.drup.2010.08.003

Ramirez MS, Nikolaidis N, Tolmasky ME (2013) Rise and dissemination of aminoglycoside resistance: the aac(6′)-Ib paradigm. Front Microbiol 4:121. https://doi.org/10.3389/fmicb.2013.00121

Ranieri MR, Whitchurch CB, Burrows LL (2018) Mechanisms of biofilm stimulation by subinhibitory concentrations of antimicrobials. Curr Opin Microbiol 45:164–169. https://doi.org/10.1016/j.mib.2018.07.006

Rasko DA, Sperandio V (2010) Anti-virulence strategies to combat bacteria-mediated disease. Nat Rev Drug Discov 9(2):117–128. https://doi.org/10.1038/nrd3013

Reyes N, Aggen JB, Kostrub CF (2011) In vivo efficacy of the novel aminoglycoside ACHN-490 in murine infection models. Antimicrob Agents Chemother 55(4):1728–1733. https://doi.org/10.1128/AAC.00862-10

Rojo-Molinero E, Macia MD, Rubio R, Moya B, Cabot G, Lopez-Causape C, Perez JL, Canton R, Oliver A (2016) Sequential treatment of biofilms with aztreonam and tobramycin is a novel strategy for combating *Pseudomonas aeruginosa* chronic respiratory infections. Antimicrob Agents Chemother 60(5):2912–2922. https://doi.org/10.1128/AAC.00196-16

Roshmi T, Soumya KR, Jyothis M, Radhakrishnan EK (2015) Effect of biofabricated gold nanoparticle-based antibiotic conjugates on minimum inhibitory concentration of bacterial isolates of clinical origin. Gold Bull 48(1):63–71. https://doi.org/10.1007/s13404-015-0162-4

Rukholm G, Mugabe C, Azghani AO, Omri A (2006) Antibacterial activity of liposomal gentamicin against *Pseudomonas aeruginosa*: a time-kill study. Int J Antimicrob Agents 27(3):247–252. https://doi.org/10.1016/j.ijantimicag.2005.10.021

Sabaeifard P, Abdi-Ali A, Gamazo C, Irache JM, Soudi MR (2017) Improved effect of amikacin-loaded poly(D,L-lactide-co-glycolide) nanoparticles against planktonic and biofilm cells of *Pseudomonas aeruginosa*. J Med Microbiol 66(2):137–148. https://doi.org/10.1099/jmm.0.000430

Salouti M, Ahangari A (2014) Nanoparticle based drug delivery systems for treatment of infectious diseases. In: Ali DS (ed) Application of nanotechnology in drug delivery, IntechOpen. https://doi.org/10.5772/58423.

Saroj SD, Rather PN (2013) Streptomycin inhibits quorum sensing in *Acinetobacter baumannii*. Antimicrob Agents Chemother 57(4):1926–1929. https://doi.org/10.1128/AAC.02161-12

Sato Y, Unno Y, Ubagai T, Ono Y (2018) Sub-minimum inhibitory concentrations of colistin and polymyxin B promote *Acinetobacter baumannii* biofilm formation. PLoS One 13(3):e0194556. https://doi.org/10.1371/journal.pone.0194556

Sautrey G, Zimmermann L, Deleu M, Delbar A, Souza Machado L, Jeannot K, Van Bambeke F, Buyck JM, Decout JL, Mingeot-Leclercq MP (2014) New amphiphilic neamine derivatives active against resistant *Pseudomonas aeruginosa* and their interactions with lipopolysaccharides. Antimicrob Agents Chemother 58(8):4420–4430. https://doi.org/10.1128/AAC.02536-13

Schroeder M, Brooks BD, Brooks AE (2017) The complex relationship between virulence and antibiotic resistance. Genes (Basel) 8(1). https://doi.org/10.3390/genes8010039

Shah M, Badwaik V, Kherde Y, Waghwani HK, Modi T, Aguilar ZP, Rodgers H, Hamilton W, Marutharaj T, Webb C, Lawrenz MB, Dakshinamurthy R (2014) Gold nanoparticles: various methods of synthesis and antibacterial applications. Front Biosci (Landmark Ed) 19:1320–1344

Shakil S, Khan R, Zarrilli R, Khan AU (2008) Aminoglycosides versus bacteria—a description of the action, resistance mechanism, and nosocomial battleground. J Biomed Sci 15(1):5–14. https://doi.org/10.1007/s11373-007-9194-y

Shedbalkar U, Singh R, Wadhwani S, Gaidhani S, Chopade BA (2014) Microbial synthesis of gold nanoparticles: current status and future prospects. Adv Colloid Interf Sci 209:40–48. https://doi.org/10.1016/j.cis.2013.12.011

Shin B, Park W (2015) Synergistic effect of oleanolic acid on aminoglycoside antibiotics against *Acinetobacter baumannii*. PLoS One 10(9):e0137751. https://doi.org/10.1371/journal.pone.0137751

Springer B, Kidan YG, Prammananan T, Ellrott K, Bottger EC, Sander P (2001) Mechanisms of streptomycin resistance: selection of mutations in the 16S rRNA gene conferring resistance. Antimicrob Agents Chemother 45(10):2877–2884. https://doi.org/10.1128/AAC.45.10.2877-2884.2001

Szczuka E, Jablonska L, Kaznowski A (2017) Effect of subinhibitory concentrations of tigecycline and ciprofloxacin on the expression of biofilm-associated genes and biofilm structure of *Staphylococcus epidermidis*. Microbiology 163(5):712–718. https://doi.org/10.1099/mic.0.000453

Taber HW, Mueller JP, Miller PF, Arrow AS (1987) Bacterial uptake of aminoglycoside antibiotics. Microbiol Rev 51(4):439–457

Tahrioui A, Duchesne R, Bouffartigues E, Rodrigues S, Maillot O, Tortuel D, Hardouin J, Taupin L, Groleau M-C, Dufour A, Déziel E, Brenner-Weiss G, Feuilloley M, Orange N, Lesouhaitier O, Cornelis P, Chevalier S (2019) Extracellular DNA release, quorum sensing, and PrrF1/F2 small RNAs are key players in *Pseudomonas aeruginosa* tobramycin-enhanced biofilm formation. NPJ Biofilms Microbiomes 5(1):15. https://doi.org/10.1038/s41522-019-0088-3

Thamban Chandrika N, Garneau-Tsodikova S (2018) Comprehensive review of chemical strategies for the preparation of new aminoglycosides and their biological activities. Chem Soc Rev 47(4):1189–1249. https://doi.org/10.1039/c7cs00407a

Traugott KA, Echevarria K, Maxwell P, Green K, Lewis JS 2nd (2011) Monotherapy or combination therapy? The *Pseudomonas aeruginosa* conundrum. Pharmacotherapy 31(6):598–608. https://doi.org/10.1592/phco.31.6.598

Tseng JT, Bryan LE, Van den Elzen HM (1972) Mechanisms and spectrum of streptomycin resistance in a natural population of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2(3):136–141. https://doi.org/10.1128/aac.2.3.136

Tukenmez H, Edstrom I, Ummanni R, Fick SB, Sundin C, Elofsson M, Larsson C (2019) *Mycobacterium tuberculosis* virulence inhibitors discovered by *Mycobacterium marinum* high-throughput screening. Sci Rep 9(1):26. https://doi.org/10.1038/s41598-018-37176-4

Tyers M, Wright GD (2019) Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol 17(3):141–155. https://doi.org/10.1038/s41579-018-0141-x

Van Giau V, An SSA, Hulme J (2019) Recent advances in the treatment of pathogenic infections using antibiotics and nano-drug delivery vehicles. Drug Des Devel Ther 13:327–343. https://doi.org/10.2147/DDDT.S190577

Vestergaard M, Nohr-Meldgaard K, Ingmer H (2018) Multiple pathways towards reduced membrane potential and concomitant reduction in aminoglycoside susceptibility in *Staphylococcus aureus*. Int J Antimicrob Agents 51(1):132–135. https://doi.org/10.1016/j.ijantimicag.2017.08.024

Vipin C, Mujeeburahiman M, Saptami K, Arun AB, Rekha PD (2019) Synergistic interactions of quercetin with antibiotics against biofilm associated clinical isolates of *Pseudomonas aeruginosa* in vitro: 601336. https://doi.org/10.1101/601336%JbioRxiv

Walter F, Vicens Q, Westhof E (1999) Aminoglycoside-RNA interactions. Curr Opin Chem Biol 3(6):694–704

Wang X, Deng A, Cao W, Li Q, Wang L, Zhou J, Hu B, Xing X (2018a) Synthesis of chitosan/poly (ethylene glycol)-modified magnetic nanoparticles for antibiotic delivery and their enhanced anti-

biofilm activity in the presence of magnetic field. J Mater Sci 53(9): 6433–6449. https://doi.org/10.1007/s10853-018-1998-9

Wang Z, Qiu Y, Hou C, Wang D, Sun F, Li X, Wang F, Yi H, Mu H, Duan J (2018b) Synthesis of hyaluronan-amikacin conjugate and its bactericidal activity against intracellular bacteria in vitro and in vivo. Carbohydr Polym 181:132–140. https://doi.org/10.1016/j.carbpol.2017.10.061

Westbrock-Wadman S, Sherman DR, Hickey MJ, Coulter SN, Zhu YQ, Warrener P, Nguyen LY, Shawar RM, Folger KR, Stover CK (1999) Characterization of a *Pseudomonas aeruginosa* efflux pump contributing to aminoglycoside impermeability. Antimicrob Agents Chemother 43(12):2975–2983

Wilton M, Charron-Mazenod L, Moore R, Lewenza S (2016) Extracellular DNA acidifies biofilms and induces aminoglycoside resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 60(1):544–553. https://doi.org/10.1128/AAC.01650-15

Wistrand-Yuen E, Knopp M, Hjort K, Koskiniemi S, Berg OG, Andersson DI (2018) Evolution of high-level resistance during low-level antibiotic exposure. Nat Commun 9(1):1599. https://doi.org/10.1038/s41467-018-04059-1

Wojnicz D, Tichaczek-Goska D (2013) Effect of sub-minimum inhibitory concentrations of ciprofloxacin, amikacin and colistin on biofilm formation and virulence factors of *Escherichia coli* planktonic and biofilm forms isolated from human urine. Braz J Microbiol 44(1): 259–265. https://doi.org/10.1590/S1517-83822013000100037

Wright GD (2016) Antibiotic adjuvants: rescuing antibiotics from resistance. Trends Microbiol 24(11):862–871. https://doi.org/10.1016/j.tim.2016.06.009

Yan T, Li C, Ouyang Q, Zhang D, Zhong Q, Li P, Li S, Yang Z, Wang T, Zhao Q (2019) Synthesis of gentamicin-grafted-chitosan with improved solubility and antibacterial activity. React Funct Polym 137: 38–45. https://doi.org/10.1016/j.reactfunctpolym.2019.01.013

Yang B, Lei Z, Zhao Y, Ahmed S, Wang C, Zhang S, Fu S, Cao J, Qiu Y (2017) Combination susceptibility testing of common antimicrobials in vitro and the effects of sub-MIC of antimicrobials on *Staphylococcus aureus* biofilm formation. Front Microbiol 8: 2125. https://doi.org/10.3389/fmicb.2017.02125

Yarlagadda V, Wright GD (2019) Membrane-active rhamnolipids overcome aminoglycoside resistance. Cell Chem Biol 26(10):1333–1334. https://doi.org/10.1016/j.chembiol.2019.09.015

Yoshikawa Y, Morikawa K, Nonaka M, Torii I (2004) Effect of arbekacin on a methicillin-resistant *Staphylococcus aureus*-induced biofilm in a rat model. J Infect Chemother 10(5):268–273. https://doi.org/10.1007/s10156-004-0336-0

Yu W, Hallinen KM, Wood KB (2018) Interplay between antibiotic efficacy and drug-induced lysis underlies enhanced biofilm formation at subinhibitory drug concentrations. Antimicrob Agents Chemother 62(1). https://doi.org/10.1128/AAC.01603-17

Zarate SG, De la Cruz Claure ML, Benito-Arenas R, Revuelta J, Santana AG, Bastida A (2018) Overcoming aminoglycoside enzymatic resistance: design of novel antibiotics and inhibitors. Molecules 23(2). https://doi.org/10.3390/molecules23020284

Zhang A, Mu H, Zhang W, Cui G, Zhu J, Duan J (2013) Chitosan coupling makes microbial biofilms susceptible to antibiotics. Sci Rep 3: 3364. https://doi.org/10.1038/srep03364

Zhou JW, Hou B, Liu GY, Jiang H, Sun B, Wang ZN, Shi RF, Xu Y, Wang R, Jia AQ (2018a) Attenuation of *Pseudomonas aeruginosa* biofilm by hordenine: a combinatorial study with aminoglycoside antibiotics. Appl Microbiol Biotechnol 102(22):9745–9758. https://doi.org/10.1007/s00253-018-9315-8

Zhou JW, Luo HZ, Jiang H, Jian TK, Chen ZQ, Jia AQ (2018b) Hordenine: a novel quorum sensing inhibitor and antibiofilm agent against *Pseudomonas aeruginosa*. J Agric Food Chem 66(7):1620–1628. https://doi.org/10.1021/acs.jafc.7b05035