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Manon Lang, André Carvalho, Zeynep Baharoglu, Didier Mazel. Aminoglycoside uptake, stress, and potentiation in Gram-negative bacteria: new therapies with old molecules. *Microbiology and Molecular Biology Reviews*, 2023, 10.1128/membr.00036-22 . pasteur-04334889

**HAL Id: pasteur-04334889**

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Submitted on 11 Dec 2023

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# Aminoglycoside uptake, stress and potentiation in Gram-negative bacteria: new therapies with old molecules.

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## Table of contents

Summary .....	3
Introduction .....	3
I - Methods for monitoring AG uptake.....	5
I – 1. Indirect methods.....	5
I – 2. Labeled drugs.....	5
II - Mechanism of aminoglycoside entry from the external environment into the cytoplasm.....	6
II – 1. AGs can pass through the Gram-negative bacterial envelope.....	6
II – 2. Reaching the target: Initial binding to the outer membrane.....	7
II – 3. Crossing the outer membrane.....	8
II – 4. Energy-dependent Phase for aminoglycoside transport across the inner membrane .....	9
II – 4. 1. The EDPI (Energy Dependent Phase I): slow uptake in the cytoplasm. ....	9
II – 4. 2. The EDPII (Energy Dependent Phase II): fast cytoplasmic uptake prior to cell death..	11
II – 5. Active transport through the inner membrane.....	12
III. Aminoglycoside uptake and stress .....	14
III – 1. Aminoglycosides and heat shock response .....	14
III – 2. Aminoglycosides and envelope stress .....	15
III – 3. Aminoglycosides and oxidative stress response .....	16
IV – Potentiation of aminoglycosides .....	16
IV – 1. Potentiation as a strategy to enhance antibiotic efficacy .....	16
IV – 2. The use of aminoglycosides in modern medicine .....	17
IV – 3. Enhancing uptake for aminoglycoside potentiation. ....	17
IV – 4. PMF-dependent potentiation of aminoglycoside uptake.....	17
IV -5. PMF-independent potentiation of aminoglycoside uptake .....	19
Conclusion and perspectives .....	20
Acknowledgements. ....	22
References: .....	25

## Summary

Aminoglycosides (AGs) are long-known molecules successfully used against Gram-negative pathogens. While their use declined with the discovery of new antibiotics, they are now classified as critically important molecules because of their effectiveness against multi-drug resistant bacteria. While they can efficiently cross the Gram-negative envelope, the mechanism of AG entry is still incompletely understood, although this comprehension is essential for the development of new therapies in the face of the alarming increase in antibiotic resistance. Increasing antibiotic uptake in bacteria is one strategy to enhance effective treatments. This review aims first to consolidate old and recent knowledge about AG uptake; second to explore the connection between AG-dependent bacterial stress and drug uptake; and finally, to present new strategies of potentiation of AG uptake for more efficient antibiotic therapies. In particular, we emphasize on the connection between sugar transport and AG potentiation.

## Introduction

Since their introduction in the 1940s, antibiotics have played a major role in the development of modern medicine and have saved many lives. However, bacterial resistance to treatment has been observed since the first administrations during the Second World War (1), and it has only increased since then. After decades of use, the World Health Organization warns that if nothing is done by 2050, millions of lives could be lost in the battle against pathogenic bacteria. A study published in 2022 (2) reports on the impact of multi-antibiotic resistant (MDR) bacteria on mortality: in 2019, 1.27 million deaths were directly due to MDR bacterial infections. Of these deaths, nearly 75% were related to 6 pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Additionally, studies show the significant proportion of hospital infections related to Gram-negative bacteria (3–6).

This review focuses on the use a specific class of antibiotics, the aminoglycosides (AGs), against Gram-negative bacteria. AGs are “old” molecules. The first member of AGs, streptomycin, was discovered in 1944 by Albert Schatz and isolated from *Streptomyces griseus* (Albert Schatz *et al.*, 1944). The discovery of this major antimicrobial earned Professor Selman Waksman the Nobel Prize in Medicine in 1952. Despite their efficacy, one limitation of the use of aminoglycosides is their side effects on patients. Nephrotoxicity and ototoxicity are observed in variable proportions depending on the study, averaging between 3% and 15% of cases, although these figures remain difficult to determine as they depend on the patient and the type of infections (8, 9). AGs use decreased with the approval of new treatments such as fluoroquinolones or cephalosporins, but they are now experiencing renewed interest because of their broad-spectrum efficiency and the development of semi-synthetic derivatives (10). AGs are now classified as a critically important class of antimicrobials by the WHO (11), particularly with the arrival of plazomycin, the latest AG approved in the United States (2018) since amikacin in 1981. Plazomycin is active against Multi-Drug Resistant (MDR) Enterobacteriaceae, including those resistant to carbapenemases (12).

AGs can be classified into 4 groups based on the core structure as illustrated in Table 1 (13). They are composed of an amino-sugar core structure (14). The amino- moieties are most often protonated under biological conditions, which confers them a highly positive charge at neutral pH, making these molecules basic and hydrophobic. These properties influence their transport across the bacterial membranes.

AGs act primarily by disrupting protein synthesis, because they bind the ribosome on the 16S ribosomal RNA, near the decoding site (15, 16). The 4,5 and 4,6-substituted AGs (Table 1) additionally bind the 23S ribosomal RNA (17), impeding ribosome assembly (18) and recycling (17, 19, 20). Consequently, these AGs have been associated with an overall slowing of translation (20, 21). Importantly, AGs are bactericidal, which makes them an exception among antibiotics targeting translation. Other antibiotics targeting the ribosome, such as chloramphenicol or tetracycline, prevent translation initiation and show a bacteriostatic action (22). On the other hand, AGs do not stop translation, but they cause mistranslation (23–27). AG-dependent translation errors have recently been shown to cluster together (27). As soon as a translation error occurs, others follow, leading to a protein synthesis catastrophe, which has implications in terms of lethality but also AG uptake, as we will detail below (EDPII).

The primary target of the AGs is thus the ribosome. However, AGs' mode of action, and how they exert their bactericidal effect is still incompletely understood and sometimes under debate. As synthetically illustrated in (28), first understanding of AG lethality was that mistranslation would lead to insertion of misfolded proteins to the membrane and eventually membrane disruption (29, 30). Reactive oxygen species (ROS) have later been proposed as key players in AG-induced cell death (30), while iron-sulfur cluster biosynthesis was shown to be involved in AG lethality through increased uptake in *E. coli*, in a ROS-independent way (31). Moreover, these mechanisms are not necessarily mutually exclusive and may even depend on each other. For example, membrane disruption was shown to lead to ROS production (30, 32). Additional complexity stems from the fact that not all bacterial species susceptible to AGs bear the same pathways for response to oxidative, envelope and Fe-S stresses. AG mode of action is thus complex and available data indicates that there are many factors at play.

Several resistance mechanisms to AGs have been described, primarily involving enzymes that modify and inactivate AG molecules, through acetylation, adenylation or phosphorylation (14). Another resistance mechanism involves alteration of the target site, the ribosome, through mutations in the 30S ribosomal subunit or methylation of the AG binding site. Although this second mechanism also confers high levels of resistance, it may be associated with a fitness cost in the absence of AGs, with a general alteration of the ribosome functional structure. The third resistance mechanism involves reducing intracellular AG concentrations, which can be achieved by decreasing uptake or increasing efflux (33, 34). In a study on 1349 clinical isolates including ESKAPE pathogens, *E. coli*, *Proteus spp.*, *Providencia stuartii* and *Serratia marcesens*, it was found that when resistance was not due to AG inactivation enzymes, it was attributed to a decrease in their ability to transport AGs inside the cell. Although the molecular mechanisms have not been addressed in the corresponding study, it was observed that such altered transport was responsible for 90% of resistance to amikacin (35). It would be thus important to determine the exact mechanisms by which AGs enter the bacterial cells, in order to understand resistance mechanisms and potentially the means to counteract them.

Despite their long history of use, the complete understanding of AG uptake and action remains incomplete. Ongoing research continues to uncover new insights into their transport into bacterial cells and the mechanisms underlying their bactericidal effects. This review aims to consolidate both the long-standing and recent discoveries pertaining to AG entry.

We will first review the methods available for quantifying AG uptake in bacteria, as these have been crucial in understanding the mechanisms involved in their entry through the bacterial envelope. We then focus on how AGs cross the Gram-negative double membrane barrier to enter bacterial cells, including recent discoveries on active transport mediated by carbohydrate transporters. Subsequently,

we will explore the relationship between AG-induced cellular stress and its impact on further AG uptake. Finally, based on our understanding of AG uptake, we will discuss possible potentiation mechanisms for enhancing the efficacy of AG therapies.

## **I - Methods for monitoring AG uptake**

Several methods have been developed to quantify the entry of AG into bacterial cells, and to construct and consolidate the current model of AG uptake.

### **I – 1. Indirect methods**

Initially, indirect methods based on evaluating antimicrobial action were employed to assess intracellular AG uptake. The supernatant from bacterial cultures treated with AGs was collected and used to treat naive bacteria. The uptake by the initial batch of AG-treated culture was determined by measuring the concentration of AG remaining in the supernatant. This evaluation was performed by comparing the inhibition rate after treatment with the supernatant to the inhibition rate observed with the initial drug concentration (36). A similar approach was used to assess intracellular drug concentrations by measuring the inhibition rate after treatment with a lysate of bacteria previously exposed to the antibiotics (37). Quantification of AG in bacterial lysate can also be performed with high-performance liquid chromatography (HPLC) (38, 39).

More recently, the remaining amount of tobramycin in the supernatant of treated *P. aeruginosa* cell was evaluated with electroanalytic quantification through tobramycin binding on electrodes, which characterizes its electrochemical behavior through voltammetry, redox potentials, and pH dependence. In this assay, the decrease in the electrochemical current corresponds to consumption of tobramycin (40). This has the advantage to discriminate sensitive and various degrees of resistant bacteria, in the biological sample and not only in laboratory media and appeared as an interesting alternative for clinical antibiotic susceptibility testing.

Although these indirect approaches can provide valuable information, they cannot be employed to directly assess the penetration of the drug into the bacterial cell, and especially at the single cell level.

### **I – 2. Labeled drugs**

The first labelling of AG molecules was accomplished using radioactivity. Various radiolabeled AG drugs have been developed, with tritium-3H (3H-gentamicin, 3H-tobramycin, or 3H-amikacin for example) which has the advantage of being a very low-penetration  $\beta$  emitter (41) ; with Iode 125 (42), Carbon 14 (43, 44), or with technetium 99m (45). Radioactive molecules offer the advantage of sensitive detection, but their use is not always practical as it involves a considerable investment by the laboratory, and they cannot be used in microscopy for localizing the antibiotics. Moreover, radioactive isotopes could be subject to passive adsorption on bacteria (42).

Subsequently, fluorescent labeling techniques were developed to measure AG concentrations in bacteria. One such method is the automated fluorescence immunopolarization assay, which involves competition between gentamicin in the test sample and fluorescein-labeled gentamicin on sheep anti-gentamicin serum (42). Compared to radiolabeled AG entry measurement, this method is less sensitive (threshold of 300  $\mu\text{g/ml}$ ) and requires a higher inoculum, although the transport characteristics are similar. As a result, this method is more suitable for monitoring AG concentration in patient fluids rather than bacterial cells (46).

The use of Texas red-AG conjugate molecules offers advantages as they exhibit a similar range of minimum inhibitory concentration (MIC) compared to unconjugated gentamicin (47). They have been tested in *E. coli* (48, 49), *A. baumannii* (50, 51), and *P. aeruginosa* (52, 53). However, the Texas red dye itself can penetrate the cell, making it difficult to differentiate between the uptake of Texas red alone and the Texas red-AG conjugate.

Kanamycin labeled with fluorescent CFDA-SE [5,(6)-carboxyfluorescein diacetate, succinimidyl ester] has been developed for microscopy studies (54). However, the dyes from the aromatic carboxylic acids family, including CFDA-SE, can also permeate cells and exhibit pH-dependent properties (55).

An alternative approach involves labeling neomycin with cyanine fluorophores Cy5 and Cy3, which retains the properties of aminoglycosides for uptake and activity (56). Neomycin-Cy5 has been employed for uptake study in *V. cholerae* (57–59), *E. coli* and *P. aeruginosa* (60). By coupling neomycin with Cy5, it becomes possible to visualize the precise localization of the antibiotic, which can be found in the periplasm or cytoplasm depending on the duration of treatment, antibiotic concentration, and the species (56).

## II - Mechanism of aminoglycoside entry from the external environment into the cytoplasm

In this part, we describe how AGs can bind to and cross the outer membrane, to enter the periplasm, before passing the inner membrane to reach the cytoplasm. We include long-known and recent discoveries on non-specific and specific transport of these molecules through the Gram-negative double membrane. Note that the majority, if not all of the studies of AG entry into the bacterial cell has been in the context of exponentially growing cells.

### II – 1. AGs can pass through the Gram-negative bacterial envelope

The outer membrane of Gram-negative bacteria consists of an asymmetric bilayer of phospholipids associated with a layer of lipopolysaccharides (LPS). The LPS displays an outwardly directed O-antigen, an oligosaccharide (sugar) core, and lipid A. The composition of O-antigen varies according to the bacterial species. Lipid A is composed of a disaccharide linked to fatty acids (61). This arrangement of the outer membrane forms a highly hydrophobic lipid bilayer which contains embedded pores that act as a selective filter for exogenous compounds (62).

The periplasmic space between the outer and the inner membranes contains a layer of peptidoglycan, which provides mechanical and osmotic protection to the cell. The inner membrane is composed of a phospholipid bilayer embedded with proteins, including the respiratory chain responsible for generating the proton motive force and enabling ATP synthesis.

The hydrophobicity the outer membrane acts as a barrier against the external environment. This constitutes a challenge for antibiotics that need to reach the periplasm or the cytoplasm to exert their action. Several antibiotics can hence be only used against Gram-positive bacteria. One such example is vancomycin, which targets peptidoglycan synthesis. Both Gram-negative and Gram-positive bacteria synthesize peptidoglycan. However, despite its high efficiency against Gram-positive bacteria, vancomycin is generally ineffective against Gram-negative bacteria because it cannot cross the Gram-negative outer membrane (63).

AGs are one class of antibiotics which can cross both membranes and are efficiently used against Gram-negative bacteria. In the 1960s, the entry of AGs into the Gram-negative bacterial cell was proposed to occur as follows. First, a small amount of the antibiotic enters by passive diffusion through both membranes. Since AGs target translation, their entry affects protein synthesis, among

which membrane proteins, and eventually causes a deterioration of membrane integrity, and further AG entry. Then, after a lag, increased entry leads to death (64). This model was further developed in the 1980s, where most of the discoveries were made, that led to the currently accepted uptake model (see (65) for a comprehensive review). The entry of AGs into the cytoplasm is now described as primarily dependent on the proton motive force, and divided into 3 phases: 1) a slow linear concentration-dependent entry up to a plateau; 2) a second phase of linear entry, referred to as Energy Dependent Phase I (EDPI) ; 3) a rapid entry which saturates after several minutes, just before cell death, referred to as Energy Dependent Phase II (EDPII) (66–69).

Adding to this model, new discoveries have been made regarding AG uptake through outer membrane porins, and a specific entry mechanism at the inner membrane through sugar transporters, as detailed later in this review.

## II – 2. Reaching the target: Initial binding to the outer membrane

AGs are small hydrophobic molecules that can bind to the LPS of the outer membrane. Similar to the polymyxin family of antibiotics, AGs binds to the cell through electrostatic interactions between the positively charged groups of the antibiotic and negatively charged components of the outer membrane (70). The binding sites for polymyxin on the outer membrane can be antagonized by the presence of cations, indicating that polymyxin B and cations compete for binding sites on the cells (71). Interestingly, a polymyxin-resistant *E. coli* also exhibits lower outer membrane binding of the AG gentamicin (72). This competition between polymyxin and an AG suggests that both antibiotics may share the same binding sites on the outer membrane (73). It was observed in *P. aeruginosa*, that the addition of cations, such as magnesium and polyamines, raises the minimal inhibitory concentration (MIC) of AGs by preventing their adsorption to the outer membrane (74). However, addition of cations after AG exposure cannot not rescue viability, demonstrating the irreversible binding of AGs (75). Also note that addition of magnesium (as well as changing other parameters of the external environment) may have pleiotropic effects including binding to the outer membrane, changing metabolism and gene expression, impacting ribosome stability. Such experiments should thus be interpreted with caution.

The initial entry phase through the outer membrane occurs rapidly upon the addition of AGs, and the rate of uptake is influenced by AG concentration (67, 76, 77). Remarkably, initial observations in *P. aeruginosa* showed that 30 seconds of incubation with an AG was sufficient to result in a 312-fold increase in the intracellular concentration of the AG compared to the external medium (74). This binding and transport across the outer membrane does not appear to require energy, as indicated by the fact that following a cold treatment that inactivates energy-dependent transport, AG binding to the outer membrane remained possible (78).

It is possible to decrease the binding of AGs to the outer membrane. AGs form strong hydrogen bonds with the lipids of the LPS (79, 80). Altering the LPS can thus, in some cases, decrease the binding and the susceptibility to AGs as observed in *P. aeruginosa* (81, 82), in *E. coli* (83), and others (84). External agents can also alter the binding of AGs to the outer membrane. One illustrative example is observed in *P. aeruginosa*, with the outer membrane protein OprH. OprH binds and occupies the magnesium cross-bridging sites of the LPS (85), thus masking these cationic sites on the outer membrane where AGs bind (73, 86–88). Magnesium starvation induces the expression of OprH (89), highlighting how environmental factors can significantly impact the effectiveness of AGs.



### II – 3. Crossing the outer membrane

**Outer membrane disruption.** Once bound to the outer membrane of bacteria, AGs are able to permeabilize it before reaching the periplasmic space. The hydrophobic fluorescent dye 1-N-phenylnaphthylamine was used to observe disruption of the outer membrane by AGs. This dye can only penetrate the Gram-negative cell upon outer membrane permeabilization. Upon treatment with AGs the dye could enter inside *P. aeruginosa* cells, demonstrating the permeabilization effect of AGs (90). Such outer membrane permeabilization could even be observed in a setup where AGs were prevented from reaching the cytoplasm, indicating that the action of AGs on the outer membrane is independent of their action in the cytoplasm (87). In fact, AGs insert themselves into the bacterial lipid bilayer and initiate a threshold response, ultimately leading to surface disruption (91).

Importantly, this disruption occurs without altering the width or fluidity of the membrane, but it does induce membrane disorder and increase lipid dynamics (92). This results in rapid leakage of cytoplasmic materials (75). For example, lethal exposure to AGs in *P. aeruginosa* has been found to cause a decrease of 34% in total proteins. Moreover, even a short gentamicin treatment was observed to lead to removal of outer membrane constituents (67). Formation of small transient holes were also observed in the same study conducted by Martin and Beveridge.

**Implication of porins.** In addition to a general membrane disruption, non-specific transport through porins has been proposed as an uptake mechanism for AGs at the outer membrane, similar to what has been observed for other antibiotics, such as  $\beta$ -lactams (93, 94). Porin function does not require an energy supply such as ATP. While porins are often non-specific for the substrates they transport in *Enterobacteriaceae*, some porins may exhibit selectivity, such as LamB for maltose and maltodextrins (95) or ScrY for sucrose (96). The exclusion size of porins varies among bacteria, and in *E. coli*, it is estimated to range between 600 and 800 Daltons. Thus, porins are capable of transporting small, hydrophilic solutes and proteins, and they also serve as signal transduction relays. In some cases, AGs were even proposed to induce their own passage through porin opening as exemplified with the MscL mechanosensitive channel which bears an attachment site for streptomycin (97, 98).

AGs can diffuse through pores formed by porins, at a rate as rapid as that of hexoses and disaccharides, except for pores formed by LamB (93). Another indirect evidence of the interaction between AGs and porins comes from the observation that kanamycin affects the intrinsic tryptophan fluorescence of the OmpF porin, indicating direct binding (99, 100). In *E. coli*, it has been estimated that approximately 10-20 kanamycin molecules per second can pass through OmpF and OmpC. Permeation to AGs, including kanamycin, gentamicin, and amikacin, is also possible through ChiP (chitoporin) at a rate of approximately 3 molecules per second. However, LamB maltoporin does not show significant permeation, consistent with previous observations (101, 102).

On the other hand, there is limited direct *in vivo* evidence regarding the transport of AGs by porins, as few porin-deficient mutants that are resistant to AGs have been isolated. Most porin-deficient mutants showed no change in susceptibility to AGs (93, 99, 103). A  $\Delta ompF$  (also called *tolF*) strain was identified as resistant to AGs (103), however, this effect could also be indirect, as the loss of OmpF alters the protein composition of the outer membrane (104). Later, it was shown that a single deletion of either *ompF* or *ompC* did not affect susceptibility to AGs, but the double mutant did (101). This suggests that these two porins may act in a redundant way and compensate for each other's function in AG entry. Additionally, the probability of occurrence and selection of a double mutation is low, which could explain why the involvement of porins is mainly identified using specific experimental setups. An *E. coli* strain resistant to both polymyxin and AGs was isolated, and exhibited reduced porin

levels, suggesting that decreased permeability to both compounds may be a result of reduced porin abundance (105). However, a more recent study on a knockout strain lacking all 40 porins in *P. aeruginosa* did not demonstrate increased resistance to AGs (106), indicating that even if AGs are transported by porins, this may not be the primary route of entry into bacterial cells.

In addition to studying AG uptake in porin deletion mutants, overexpression studies have also been conducted. This is relevant because the expression of porins is usually inducible and they may not be expressed at high levels under normal growth conditions. For example, PhoE is induced under phosphate limitation, and the expression of LamB, OmpC, and OmpF is regulated by variations in the physicochemical environment (osmolarity, temperature) (107). It has been shown that only 5% of the population produces open channels for the OprF porin (108). Therefore, under non-induced conditions, the expression of porins may not be sufficient to facilitate significant uptake of AGs, resulting in negligible impact on the minimum inhibitory concentration (MIC). However, there have been instances of AG transport by porins, such as OprB in *P. aeruginosa*, which increases susceptibility to AGs when overexpressed (60). It is important to mention that, to the best of our knowledge, no studies have been conducted on AG uptake under conditions where OmpC and OmpF porins are artificially overexpressed *in vivo*. Moreover, the overexpression of OmpC in *E. coli* has been shown to inhibit the translation of other porins, namely LamB and OmpA (109). This indicates that the expression of different porins can be intertwined, and could potentially minimize any effect on AG susceptibility phenotypes upon overexpression of a given porin. This factor should be considered when investigating the impact of multiple porins on antibiotic uptake using overexpression techniques.

Importantly, a species-specific effect on AG recognition by porins should not be overlooked. For example, while overexpression of LamB in *E. coli* did not enhance sensitivity to AGs (60), AG passage through LamB appears to be possible in *V. cholerae* (59). This may further complicate the identification of specific porins responsible for AG transport.

In conclusion, AGs can cross the outer membrane through electrostatic binding and membrane disruption, and probably also through non-specific transport by porins.

## II – 4. Energy-dependent Phase for aminoglycoside transport across the inner membrane

Once AGs have crossed the outer membrane, they accumulate in the periplasmic space and need to traverse the inner membrane to enter the cytoplasm, where they can target the ribosome. This uptake process occurs in two distinct energy-dependent phases, which have been a topic of debate, leading to different conclusions among different research groups (68, 76, 110). However, the role of the membrane potential in facilitating AGs' crossing of the inner membrane is widely acknowledged (65, 68, 111–113).

We describe below the details of this process.

### II – 4. 1. The EDPI (Energy Dependent Phase I): slow uptake in the cytoplasm.

**The proton-motive force, PMF.** The PMF relies on both membrane potential ( $\Delta\psi$ ) and  $\Delta\text{pH}$ .

The membrane potential ( $\Delta\psi$ ) is generated by the respiratory complexes. The respiratory chain consists of a set of protein complexes located at the inner membrane, responsible for the oxidative phosphorylation of ADP to produce ATP, i.e. energy. Unlike eukaryotes, the bacterial respiratory chain exhibits diversity depending on the species, allowing for the utilization of various electron donors and acceptors. Despite their diversity, respiratory chains share a common organization: a dehydrogenase receives electrons from a donor, and gets oxidized. The electrons are

then transferred to a co-factor, which becomes reduced, and pass through several co-factors (which are subsequently re-oxidized) until they reach a final acceptor, which is oxygen in the case of aerobic respiration.  $\Delta\text{pH}$  depends on the proton concentration on either side of the membrane (114). Respiratory chain complexes are capable of coupling their redox reactions with the transfer of protons across the membrane to balance charges, thereby establishing an electrochemical gradient generated by proton pumps. This potential is referred to as the proton motive force (PMF). Thus, the PMF is tightly linked with energy production, and the uptake of AGs depends on the PMF, (111–113), as described below.

**The EDPI.** A threshold membrane potential must be reached in order to initiate AG transport. In *E. coli* cells, AG uptake occurs when  $\Delta\psi$  is between -107 and -125 mV (115). As an indication, in *E. coli*, the  $\Delta\psi$  value is -105 mV during fermentation, -130 mV in anaerobic conditions (116), and ranges from -220 mV at the beginning of the exponential phase to -140 mV at the end under aerobic conditions (117, 118). On the other hand,  $\Delta\text{pH}$  is measured as -117 mV during fermentation, -144 mV in anaerobic conditions, and -160 mV in aerobic conditions (116).

This implies that the amount of AGs that can enter the bacterial cells varies with growth conditions: AGs are less effective in an environment with low pH (119, 120) and also less effective against anaerobic or facultative anaerobic bacteria that have a reduced PMF. For example, bacteria with anaerobic nitrate respiration are resistant to higher AG doses (121). Mutants of *E. coli* with defective respiratory chains exhibit reduced AG entry and susceptibility, indicating that a deficiency in cellular respiration hinders AG accumulation within the cell. Therefore, reduced AG uptake and susceptibility may be observed when the respiratory chain is altered, as it happens in mutants for NADH-dehydrogenase (31, 122), cytochrome oxidase (123), succinate dehydrogenase (124), or heme biosynthesis (which is a cofactor of the respiratory chain) (111, 125–127). Recent literature also indicates that the PMF-dependent uptake of AGs is hindered in mutants deficient for iron sulfur (Fe-S) cluster machineries of the respiratory chain (31). Thus, mutations altering iron sulfur cluster biogenesis can also have an impact on AG susceptibility.

**Inhibition of the EDPI.** The inhibition of the EDPI uptake phase is possible and can be achieved through the inhibition of membrane potential, by uncoupler, also called protonophores. These are compounds that dissipate the gradient of protons and decouple it from oxidative phosphorylation of ADP to ATP. Uncouplers include dinitrophenol (DNP), carbonyl cyanide m-chlorophenylhydrazone (CCCP), KCN, oxamic acid, sodium arsenate, and sodium azide (31, 128, 129). Moreover, nitric oxide and nitrite, by binding to terminal oxidases, can halt respiration (130–132). Other inhibitors such as N-ethylmaleimide (76, 128), p-chloromercuribenzoate (sulfhydryl reagents) (129), and low temperature (128, 133) have also been shown to be effective.

The direct role of ATP in AG transport was addressed in an *unc* mutant (F-ATPase), in which ATP production is uncoupled from the electron transport and the PMF. Treatment with the protonophore CCCP dissipates  $\Delta\psi$ , and leads to a decrease in ATP levels in wild-type conditions, but the ATP content of the cell remains unchanged in the *unc* mutant. CCCP treatment could still abolish AG uptake in the *unc* mutant despite normal ATP levels, suggesting that ATP alone does not have a direct role in the transport of AG (134, 135). AG uptake does not depend on the levels of intracellular ATP, as a tenfold reduction in ATP content does not modify AG uptake (136). In the same line, the use of the ATPase inhibitor DCCD does not change the rate of AG uptake in the Gram-positive *S. aureus* (77), but no similar experiment was performed in Gram-negative bacteria.

In an interesting unpublished observation, Miller and Dougherty observed that AG uptake could occur in CCCP-treated cells lacking  $\Delta\psi$  (as also observed in Muir et al., 1985 on anaerobic cells

or Nielsen, 1978), but only after a lag phase (their personal communication to Taber in Taber et al., 1987), which probably corresponds to the resumption of bacterial growth and restoration of intracellular ATP levels (134). They hypothesized that this could be attributable to the induction of an alternative uptake pathway, such as an active transport mechanism that relies on ATP rather than membrane potential  $\Delta\Psi$ . This may be one of the first instances where the idea of active AG transport, e.g. through ATP-binding cassette (ABC) transporters, was mentioned (see I-5).

In summary, during EDPI, a small amount of AGs cross the inner membrane through PMF-dependent passive diffusion and this amount is concentration dependent.

#### II – 4. 2. The EDPII (Energy Dependent Phase II): fast cytoplasmic uptake prior to cell death.

The second phase of energy-dependent entry, known as EDPII, relies on the action of AGs on protein synthesis. As mentioned previously, AGs induce translation errors (23–25, 27), leading to the production of non-functional, misfolded, or truncated proteins. Recent research has demonstrated the presence of error clusters following AG treatment, with an error rate within these clusters that is 60,000 times higher than the predicted random error rate. AGs can remain bound to the ribosome over multiple protein elongation cycles, inducing consecutive errors and causing proteotoxic stress and protein aggregation (27). Furthermore, AGs stimulate the accumulation of reactive oxygen species (ROS) (139, 140), which are also associated with the misfolding of mistranslated proteins (141), further exacerbating proteotoxic stress (see part III below).

How mistranslation primes AG entry during EDPII is still unclear, although it is widely accepted that poorly translated membrane proteins can create pores and facilitate the entry of AGs into the cell (29, 65, 142, 143), in a so-called leakage pathway. This, in turn, disrupts various cellular processes and ultimately leads to cell death due to the irreversible nature of absorption (29). It has been proposed that after protein mistranslation, rapid proteolysis occurs, trapping the AGs inside the cell and contributing to its irreversible action (144).

**Inhibition of the EDPII.** Protein synthesis and interaction with the ribosome are necessary to initiate EDPII (145). Inhibiting AG binding to the ribosome prevents EDPII. A specific example can be found in an *rpsL* (Ribosomal Protein S12) mutant, which lacks the high-affinity ribosomal binding site for streptomycin (146, 147). In this mutant, the second phase of uptake for the AG streptomycin cannot occur, demonstrating the essential role of ribosomal binding of AGs in EDPII.

Reduced ribosomal activity has also been associated with a decrease in EDPII, likely due to a reduced amount of mistranslated proteins: for example, chloramphenicol supplementation, which halts protein synthesis, during or before treatment with AGs, hinders AG uptake (128) by blocking EDPII, not EDPI (148–150). Therefore, protein production is required for both the initiation and continuation of EDPII (145).

Although this EDPII is not directly linked to respiration or alteration of the PMF, this step is still referred to as energy-dependent because it is prevented by uncouplers (as described above), and requires the presence of the PMF (76, 115). This energy-dependency is inconsistent with a simple leakage pathway resulting from membrane damage but rather suggests the involvement of energized channels in the passage of AGs. Furthermore, a possible link between the protein translation machinery (essential for EDPII) and the PMF (essential for EDPI/EDPII) cannot be excluded. For example, PMF drives the translocation and resolution of protein folding problems in the inner membrane (Brundage et al., 1990; Schiebel et al., 1991; Zhu et al., 2022). However, no studies have provided substantial evidence to support such a hypothesis.

During EDPII, a large amount of AGs enter the bacterial cytoplasm following mistranslation of membrane proteins. This phase necessitates the presence of a membrane potential.

## II – 5. Active transport through the inner membrane

In addition to unspecific uptake during EDPII due to membrane damage, the possibility of active transport of AGs through the inner membrane has been proposed and debated for half a century. In 1978, Holtje observed that streptomycin induces the active transport of polyamines and competes with them for entry into the cell. He proposed that streptomycin could be actively recognized and carried by the polyamine transporters (129). Since then, new insights invalidated this hypothesis. Firstly, it was observed that polyamine uptake is inhibited during amino acid depletion in a stringent strain of *E. coli* but not in a relaxed strain (151). A stringent strain constitutively activates the stringent response. We now know that AGs also induce the stringent response (152), which could explain the observed "competitive" effect with polyamine uptake. Additionally, polyamines have the ability to displace gentamicin from the ribosome *in vitro* (153), suggesting that the competition between AGs and polyamines may be due to their opposed effects on the ribosome. In *P. aeruginosa*, polyamines have been shown to increase the minimum inhibitory concentration (MIC) of several antibiotics, including AGs, but this effect was found to be linked to LPS perturbation and not to an active transporter (154).

Another observation supporting the hypothesis of active transport of AGs arose from the finding that AGs accumulate in *E. coli* when chloramphenicol is present, provided that the AG is added prior to chloramphenicol treatment. Initially, this observation was interpreted as evidence of the AG inducing an active transport system (66). Subsequent studies showed that active protein synthesis is necessary for AG accumulation (EDPII), only during the early stages of treatment. Current theory suggests that the requirement for protein synthesis during early AG accumulation can be explained by the fact that, at later stages, some AGs have already entered the cell (EDPI) and induced a certain amount of misfolded proteins, thereby initiating EDPII (147). However, this does not rule out the potential involvement of active transport in AG entry (128).

Active transporters of nutrients in bacteria usually exhibit both compound selectivity and size selectivity. For example, in contrast to the AG dihydrostreptomycin, its adenylated derivative does not enter *E. coli* cells. This disparity could be attributed to charge modifications or to the size of the modified molecule being too large to pass through an active transporter. These findings support the notion of transport occurring via specific carriers (155). Another supportive fact came from a study using N-Ethylmaleimide, which is a compound known to inactivate several transporters with thiol groups in *E. coli*, without affecting the growth rate or the PMF. Similar to lactose, melibiose, and proline, the uptake of the AG tobramycin is hindered when N-Ethylmaleimide is added. This suggests that AGs may be transported by proteins with accessible thiol groups on the external face of the cytoplasmic membrane (76).

A recent study suggests that AGs are transported by amino acid carriers in *E. coli*. This hypothesis is based on the fact that deletion of the *gcvB* sRNA, which represses amino acid transporters, increases sensitivity to several AGs (156). Moreover, AG import was reduced in the *btuD* ATP-binding subunit mutant of the vitamin B12 transporter, which was attributed to an active transport of the AG in *Lysobacter* (54), but this has not yet been further studied.

Finally, recent studies have suggested that AG transport occurs through carbohydrate transporters, regulated by carbon catabolite repression (CCR). CCR is a regulatory mechanism in bacteria that controls the transport and utilization of carbon sources. It ensures efficient utilization of

the most favorable carbon source, e.g. glucose for *E. coli*, before utilizing secondary carbon sources (157). Thus, in the presence of the preferred carbon source, bacteria repress the transport and utilization of alternative carbon sources. The repression is mediated by catabolite repression protein, such as the cAMP receptor protein (CRP), in complex with cyclic AMP (cAMP). The specific mechanisms vary between bacterial species, as different organisms may have their own set of regulatory proteins and signaling pathways involved in CCR.

Although sugar utilization related genes have sometimes been detected (158, 159), the vast majority of high-throughput studies of resistant/persistent populations usually identify and study PMF and membrane related mutants (e.g. electron transport and oxidative respiration) (160–162). Initial data leading to the hypothesis of a link between sugar transporters and AG uptake came from the observation in *E. coli*, that glucose supplementation reduces the rate of uptake of the AGs (129). These findings suggested a connection between AG uptake and CCR. Recent research in *V. cholerae* (59) and other Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *A. baumannii*) (60) has demonstrated that AGs are actually transported by a diverse array of sugar transporters. In the case of *V. cholerae*, high-throughput studies have identified the involvement of a small non-coding RNA called *ctrR* in AG susceptibility. Inactivation of *ctrR* leads to increased tolerance to AGs (59). A homologue of *ctrR* has in parallel been identified in *V. tasmaniensis* (163), and it has been shown to interact with mRNAs of carbohydrate utilization and transport genes, resulting in the stabilization of these transcripts. In both species, upregulation of this small RNA leads to an increased number of the corresponding transporters.

This discovery highlighted the connection between carbon source utilization and AG uptake. Even though no *ctrR* homolog was identified in other genera, this mechanism of AG uptake by sugar transporters is shared among other Gram-negative bacteria. Indeed, at least 11 transporters have been confirmed as involved in AG specific susceptibility and uptake when overexpressed in *E. coli*. They are primarily located at the inner membrane (PTS and ABC transporters) and are non-preferential sugar transporters, induced by the CRP-cAMP complex, upon glucose limitation. Thus, AG uptake seems to be regulated by CCR and *de facto*, under the control of environmental factors. Moreover, other small regulatory RNAs can regulate sugar transport, such as Spot 42 (*spf*) in  $\gamma$ -proteobacteria (164, 165), but their involvement in AG susceptibility has not been tested.

The recognition of AGs by sugar transporters could be attributed to the sugar moiety of AGs (60). However, there are still unresolved questions regarding the relationship between this active transport mechanism and the PMF, as the PMF may play a direct or indirect role in powering or regulating active sugar transporters (166–168). Moreover, several active transport systems, such as lactose permease, are proton-driven transporters (169), and their functionality can be inhibited by the uncoupler CCCP, which is also an inhibitor of AG entry (170). These recent findings raise the question of the extent to which the dependence on membrane potential is connected to active transport systems that are coupled to proton transport.

In summary, once AGs have reached the periplasm, there is an initial slight increase in entry known as the energy dependent phase I (EDPI), followed by a plateau, and then a rapid increase in entry referred to as energy dependent phase II (EDPII). The latter is dependent on the production of proton motive force by cellular respiration, which drives the uptake process (68). In addition, specific transport of AGs through carbohydrate transporters has also been demonstrated. Figure 1 summarizes the current AG uptake model in Gram-negative bacteria.

Once AGs have entered the cytoplasm, the uptake process becomes irreversible, as the molecules cannot exit the cell even when the proton gradient is artificially removed using a protonophore to dissipate the membrane potential (68).

### III. Aminoglycoside uptake and stress

Once AGs have entered inside the bacterial cell, they can exert their action primarily by targeting the ribosome, but also, and consequently, by triggering various stresses, some of which can also influence their further uptake.

Bacterial stress responses are important molecular mechanisms bacteria deploy to survive and adapt to a variety of stressful conditions and challenges, including exposure to antibiotics. Indeed, as part of the everlasting arms race among microorganisms, antibiotics have been well known stressors for bacteria with several studies showing how the presence of these compounds, sometimes even at very low concentrations, induce different bacterial stress responses (171–177). Activation of these mechanisms can enhance bacterial survival in the presence of antibiotics by either modulating the accumulation of antibiotics inside the cell or by interacting with the factors required for their efficacy. In this section we will briefly review how AGs trigger different bacterial stress responses and how these affects AG entry.

#### III – 1. Aminoglycosides and heat shock response

As previously mentioned, after their entry in the cell, the primary target of aminoglycosides is the 16S rRNA at the 30S small subunit of the ribosome (143, 178). The binding of AGs to the ribosome's 16S rRNA, specifically at the tRNA acceptor A site (aminoacyl site), disrupts the proofreading mechanism responsible for accurately discriminating between cognate and non-cognate tRNAs during protein synthesis (179–183). Consequently, ribosomes bound by AGs result in a greater occurrence of erroneous amino acid incorporation during protein synthesis (27) which in turn leads to the synthesis of proteins with a higher propensity to misfold. The presence of misfolded proteins in the cell is the main stress signal triggering the heat shock response (184), characterized by the induction of a set of heat shock proteins (HSPs) that help protect cells from the damaging effects of misfolded proteins. Establishing the link between AGs and misfolded proteins, several studies have shown the induction of heat shock genes and the importance of several chaperones and proteases in both gram positive and gram negative bacteria exposed to these antibiotics (30, 176, 185–190). For example, microarray analysis of *Pseudomonas aeruginosa* exposed to lethal concentrations of the AG tobramycin revealed the induction of several heat shock genes including a Lon-type protease important for this activation (176). Similarly, a study on the proteomic response of *P. aeruginosa* to different classes of antibiotics, also showed a higher abundance of the chaperones DnaK/GrpE and GroESL, in cells treated with tobramycin or gentamicin (191). Moreover, the induction of these chaperones and other heat shock genes have equally been observed in streptomycin-treated *Acinetobacter baumannii* (192) and *E. coli* (174), and tobramycin treated *V. cholerae* (193, 194).

Misfolded proteins have long been suggested to be key factors in AG lethality because several of these misfolded proteins would be inserted in the membrane, forming pores and disrupting membrane stability (29, 195, 196). This would in turn lead to the PMF-dependent second phase of AG uptake (EDPII) as described above. How adaptative can heat shock response activation be in this process?

It is almost intuitive that stressors and conditions that could induce heat shock genes in bacterial cells, independently of aminoglycoside treatment, would lead to expression of chaperones

and proteases which in turn would limit the amount of misfolded proteins inserted in the membrane and thus temporarily increase AG tolerance once in presence of these drugs. This was in fact shown to be case, with numerous studies reporting that overexpression of products of the heat shock machinery, such as chaperones and/or proteases, increased tolerance and led to short-term adaptation to AGs in different species (176, 185, 186, 189, 192). Moreover, some of these studies provide evidence of reduced membrane depolarization during the first hours of aminoglycoside treatment upon heterologous expression of different heat shock proteins in *E. coli*, which highlights the link between heat shock response and EDPII phase of AG uptake (185, 186).

However, even though it is clear that AGs induce the heat shock response in bacteria, the consequences of such response on further aminoglycoside uptake and lethality are not yet totally understood and may vary in different contexts. Indeed, a recent study challenges some of these previous observations, showing that a pre heat shock induction of *E. coli* cells could also potentiate AG lethality through enhancing of the PMF (197).

### III – 2. Aminoglycosides and envelope stress

Misfolded proteins generated during AG treatment may have as target both the inner and outer membranes of the cell. As previously said, insertion and accumulation of these aberrant proteins at bacterial membranes are thought to generate envelope stress leading to entry of more AGs. This is sensed by bacteria which trigger several envelope stress responses (ESRs) to mitigate such stress (198, 199). Thus, it is perhaps not surprising that aminoglycoside susceptibility has been often linked to the activation of some of these ESRs in many Enterobacteriaceae. One example of a component of the ESR tightly linked to aminoglycoside susceptibility is the Cpx.

The Cpx is a well-known two-component system (TCS) composed of a sensor protein CpxA that senses misfold protein insertion in the inner membrane and the periplasm; and the regulator CpxR which regulates many genes including proteases responsible for cleavage of the aberrant proteins (200). The first observations on the link between the Cpx system and aminoglycoside susceptibility are not new, with a 70's study showing that mutations in *cpx* genes are associated with changes in AG resistance levels (201). More recently, in *E. coli* it was shown a *cpxR* mutant leads to increased sensitivity to aminoglycosides (202) and in *S. enterica* *cpxAR* system activation have also been shown to increase AG resistance (203). Moreover, *cpxP*, a member of the *cpx* regulon, was found to be highly induced by the aminoglycoside gentamicin in *E. coli*, in a process dependent on *cpx* system (204). The Cpx system is also important for the AG tolerant phenotype of a *V. cholerae*  $\Delta$ *ravA-viaA* mutant (124). The RavA-ViaA complex of *E. coli* is known to modulate AG sensitivity (205–207) but mechanisms are still elusive.

Because the *cpx* regulon is complex and involves several genes, the role of the *cpx* system on AG susceptibility may be highly pleiotropic. For example, the activation of the Cpx response in *E. coli* leads to a reduction in the transcription of genes involved in the electron transport chain (202) and more recently it was also observed in *E. coli* that the Cpx stress response is involved in the turnover of respiratory chain proteins (208). These observations thus suggest an indirect role of *cpx* system on the metabolic state of the cell, which in turn may affect membrane polarization and consequently aminoglycoside uptake.

Similar to the *cpxAR* system in *E. coli*, aminoglycoside resistance in *Pseudomonas aeruginosa* has been linked to the AmgRS two-component system (TCS) (187, 209). One of the primary effects of the AmgRS system activation is the upregulation of specific genes involved in aminoglycoside resistance, such as several membrane proteases like FtsH and HtpX (187). The mechanism of intrinsic



aminoglycoside resistance through the AmgRS are proposed to be similar to that of Cpx in *E. coli*, i.e., efficient degradation of aberrant misfolded membrane proteins and maintenance of envelope homeostasis.

Another TCS of high importance in AG resistance in *E. coli* is the EnvZ/OmpR system. This system, which can be activated by several environmental cues, is known to control the relative levels of the outer membrane proteins OmpC and OmpF to adjust membrane permeability and maintain cell envelope stability. As AGs can passively diffuse through these OMP (as previously discussed in this review) (101), it is likely that EnvZ/OmpR system ends up by affecting AG uptake.

Finally, TCS systems linked with uptake or response to AGs are still being discovered, such as the Zra system which was proposed to be a functional homologue of Cpx in *E. coli* (210) and a Zra/Cpx hybrid system newly discovered in *V. cholerae* (124).

### III – 3. Aminoglycosides and oxidative stress response

Oxidative stress in bacteria occurs due to excessive production of reactive oxygen species (ROS) (211). ROS are highly reactive molecules derived from oxygen and include superoxide anions (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (•OH), which can react with nearly all biomolecules in the cell including proteins and ribonucleic acids. ROS are naturally byproducts of aerobic metabolism because leakage of electrons from the electron transport chain can react with oxygen. To cope with the presence of ROS, bacteria encode several proteins capable of detoxifying the cell from these molecules (212). However, external factors such as antibiotics can also induce ROS production in bacteria and generate excessive ROS, overwhelming the antioxidant machinery of the cell. For example, because of AG-induced mistranslation, misfolded membrane proteins are thought to generate ROS through disruption of membrane integrity and alterations in the PMF (195). Indeed, some studies have reported a link between AGs and ROS formation in different bacterial species (139, 195) and others have shown that overproduction of ROS scavengers increase AG resistance (188). It is also important to note that not only misfolded proteins induce ROS production, but also that misfolded proteins may be themselves more prone to oxidation by ROS (188, 213). Indeed, excessive levels of ROS cause post-translational modification of specific amino acids and side chains which in turn affects folding chemistry and can result in protein aggregation (214, 215).

Thus, from the moment of the initial uptake until the collapse of proteostasis, AGs are involved in the activation of several bacterial stress responses that interplay and dictate the fate of AGs uptake and their efficiency.

## IV – Potentiation of aminoglycosides

### IV – 1. Potentiation as a strategy to enhance antibiotic efficacy

In response to the lack of new antibiotic molecules, researchers have been exploring alternative approaches for the treatment of bacterial infections (216–218). One promising strategy lays in methods improving the efficacy of existing antibiotics (219). One such approach is antibiotic potentiation, which involves combining an antibacterial agent with a non-active agent (molecule, physical treatment, or chemical treatment) to enhance the effectiveness of the antibiotic. This concept has been classified into two types by (220): class I adjuvants/potentiators that act directly on the pathogen by inhibiting active resistance mechanisms (e.g., modifying enzymes or efflux pumps) or passive resistance mechanisms (e.g., targeting the membrane barrier or biofilm formation); and class

II adjuvants that enhance the host's ability to eliminate the pathogen. It's important to note that this review does not discuss potentiation of the lethal effect of aminoglycosides that are independent of uptake. We will focus below on class I adjuvants that have been proposed as potentiators of AGs.

#### IV – 2. The use of aminoglycosides in modern medicine

AGs are crucial molecules in the fight against infectious diseases due to their broad spectrum of action, targeting aerobic Gram-negative bacteria, *Staphylococci*, and Gram-positive bacilli. However, they are ineffective against *Streptococci*, *Pneumococci*, and anaerobic bacteria. AGs are prescribed in humans for prophylaxis during surgery or as part of the treatment for various infections, including urinary tract infections, pyelonephritis, abscesses, septicemia, pneumonia, and endocarditis (221). The primary AGs used in hospital settings are gentamicin, amikacin, and tobramycin (222). Initially, these antibiotics were administered in multiple doses until the effectiveness of single doses was demonstrated, attributed to their prolonged post-antibiotic effect resulting from irreversible binding to ribosomes (223). This is conditioned by a normal renal function (224, 225). The administration of multiple doses is however still necessary for specific infections such as endocarditis, pediatric treatment, or in synergistic combinations with other antibiotics such as  $\beta$ -lactams, colistin, fosfomycin, or glycopeptides (222).

#### IV – 3. Enhancing uptake for aminoglycoside potentiation.

Once administered in the human body, the antibiotic reaches a peak concentration that gradually decreases over time. Thus, the choice of the dosage protocol for a given antibiotic is determined by its pharmacokinetic and pharmacodynamic parameters. AGs exert their effects in a concentration-dependent manner, and the dosing strategy for these molecules aims to achieve a higher peak concentration ( $C_{max}$ ) in relation to the minimum inhibitory concentration (MIC) (Figure 2). The probability of a clinical response, indicating treatment efficacy, has been shown to be strongly correlated with the dose administered and the peak concentration ( $C_{max}/MIC$ ) (226). However, the occurrence of adverse effects (227), limits the flexibility in therapeutic options. Generally, a dose at least 10 times higher than the MIC is recommended to ensure effective treatment and to minimize the risk of selecting antibiotic-resistant mutations (228, 229). This means that, in the case of AGs, increasing the intracellular drug uptake in bacteria increases the peak at  $C_{max}$  and enhances the likelihood of treatment efficacy. Recently, Webster and Shepherd (28) provided a comprehensive review of the environmental and metabolic factors that can influence the efficacy of AGs. Some of these factors act as booster of AG entry into cells and can be exploited to potentiate AGs and improve their effectiveness in killing bacterial pathogens. This becomes particularly relevant in the context of rising antimicrobial resistance (AMR), where each effective molecule plays a crucial role.

#### IV – 4. PMF-dependent potentiation of aminoglycoside uptake

In the case of AGs, the literature suggests that the modes of potentiation primarily involve increasing drug entry by stimulating the PMF. Various approaches have been tested and published to enhance aminoglycoside uptake by increasing PMF.

As mentioned earlier, PMF is generated by respiration, and also depends on pH. Thus, modifying respiration, energy metabolism or pH can be a strategy to increase PMF and, consequently, drug uptake. The use of bacterially metabolizable compounds, such as amino-acids, nucleosides and sugars, provides the advantage of reduced toxicity in humans. Below are examples of the proposed use of such compounds to potentiate AGs.

Regarding pH, the use of basic molecules such as L-arginine or L-lysine has been shown to potentiate AGs against persistent cells of *A. baumannii* (230), *S. aureus*, *E. coli*, and *P. aeruginosa* (231). Interestingly, lung epithelial cells have been found to secrete host metabolites such as succinate and glutamate, which also increase intracellular pH (232), and contact between *P. aeruginosa* and mouse epithelial cells sensitizes the bacteria to AGs (233).

Activating respiration by fumarate inhibits the formation of persistent *P. aeruginosa* cells (53, 234). On the other hand, the biosynthetic pathways of specific amino acids, such as serine, glycine, glutamine, tryptophan, threonine, and alanine, were found to potentiate AG uptake by *E. coli* persisters by influencing the cellular energy state (49). In the same line, a study on lab-evolved *E. coli* revealed that AG-resistant clones had low levels of metabolites involved in amino-acid metabolism which fuels the energy metabolism (235). However, this effect was not specific to AGs (236). Adenosine has been shown to enhance the effectiveness of various antibiotic families, including AGs, against persister bacteria, through increase of PMF, and suppression of stress responses (237). NADH plays a central role in energy metabolism and respiration. Promoting NADH production directly leads to increased PMF (238). Metabolites such as glutamate, L-tryptophan or alanine have been shown to increase NADH production, thus AG uptake, re-sensitizing resistant bacteria or reducing persister cell formation (49, 239–242). Alanine's role in AG sensitivity has also been associated to ROS production (243).

In a similar way to the above mentioned amino-acids, some sugars have also been shown to increase AG uptake through increased NADH. These include D-ribose in *Salmonella* (244), fructose in *Edwardsiella tarda* biofilms and persister cells (245); glucose in cefoperazone/sulbactam resistant *Pseudomonas* (246), and fructose in resistant *Salmonella enteritidis* (247).

One promising carbohydrate potentiator of AGs is mannitol. Potentiation of AGs using mannitol stimulates PMF and leads to eradication of persistent *E. coli* cells and biofilms (48), and of persistent *Pseudomonas* cells (248). Importantly, such a potentiating effect of mannitol can lead to the use of effective concentrations below the threshold of renal toxicity (249).

But other carbohydrates can also potentiate AGs without stimulating the PMF, because they can regulate the expression of carbohydrate transporters, which internalize AGs. One promising strategy of potentiation comes from the search for molecules capable of increasing the number of sugar transporters at the membrane (60) identifying uridine and other nucleosides as metabolites that increase the expression of sugar transporters. Supplementation with uridine increases AG uptake into *E. coli* and improves the efficiency of AGs, without changing the PMF. However, it is important to maintain the PMF for this potentiating effect to be observed (60), likely because its presence is necessary to power the sugar transporters.

Alternative approaches to metabolites have also been proposed as potentiators of AGs, such as heat shock which, similarly to AGs, can induce proteotoxic stress, protein misfolding and ROS formation. A synergistic effect is thus almost expected between high temperature and AGs. However, high temperature appeared also to boost the PMF in *E. coli* stationary phase cells, and facilitate AG uptake (197).

The use of n-butanol has demonstrated effectiveness in rapidly eradicating persistent cells of *S. aureus*, as well as various Gram-negative and Gram-positive species within a short period (1 minute). This potentiation effect of n-butanol is PMF-dependent since it is abolished by CCCP, although PMF is not modified, and still relies on the action of AGs on the ribosome, as it is abolished by the presence of a ribosomal mutation (250). However, implementing this type of potentiation strategy would be challenging due to concerns regarding toxicity of such an alcoholic compound.

**Synergy between AG and other drugs for the uptake of AGs.** In addition to potentiators devoid of any bactericidal action when administered alone, AG uptake can also be increased due to synergistic effect with other drugs (251). The majority of these examples involve boosting of PMF by the co-administered drug. This is the case for tigecycline, an inhibitor of protein synthesis (252), the silver-containing antimicrobial "AGXX" (253), or even anticancer drugs such as vincristine or vinblastine (254), which are indole derivatives. Another indole derivative, 4-fluoroindole, not only acts on PMF, but also possibly inhibits the efflux pump MexXY-OprM (255). One PMF boosting adjuvant was recently identified through an approach based on the idea that antibiotic-producing bacteria could also produce adjuvants: the actinomycete product VentA (for venturicin A) potentiates gentamicin against multidrug-resistant clinical isolates (256). Agents that target the bacterial cell wall, such as  $\beta$ -lactam antibiotics, have also been shown to facilitate AG entry (257–259). The combination of AGs with penicillin, for example, has proven to be effective and is currently used in clinical therapies. The synergistic effect between penicillin and AGs can be suppressed by electron transport inhibitors (260), indicating that penicillin also enhances AG entry through PMF-dependent uptake into the cytoplasm.

#### IV -5. PMF-independent potentiation of aminoglycoside uptake

PMF-independent potentiation of uptake primarily involves strategies that enhance AGs uptake by destabilizing the outer membrane and modulating porins, or through EDPII.

One mechanism is believed to occur through physical perturbations applied to the cell membrane, and the inhibition of cellular responses to such stress. For instance, hypotonic shock has been proposed to potentiate AGs entry through the mechanosensitive MscL porin, which is activated in response to membrane destabilization (261). Cold shock also significantly increases the entry of AGs in *P. aeruginosa* in a skin infection model in mice (262). Rifampicin was found to interact with the *Pseudomonas* AmgRS two-component system (mentioned earlier), counteracting the stress response against AG-induced membrane damage (263).

Potentiators that act on AG export through efflux pumps have also been described (264). For example meropenem, which inhibits the MexXY-OprM efflux system in *P. aeruginosa*, increases sensitivity to AGs (265).

Other potentiators act on protein synthesis and potentiate EDPII, like silver (266). While the precise mechanism is not yet fully understood, silver is believed to destabilize the membrane, bypassing the EDPI process and increasing the entry of AGs into the cytoplasm. This requires EDPII, i.e. the production of non-functional proteins. Alternatively, the action may occur directly at the level of the translating ribosome, leading to the generation of more misfolded/non-functional proteins and consequently increasing entry (266). Silver has actually been observed to interact with various bacterial cellular processes, ultimately leading to membrane permeability (267). In a *C. elegans* model, silver nanoparticles reduced the MIC of amikacin by 22-fold without toxicity against the host (268).

Certain potentiation pathways are specific to Gram-positive bacteria and cannot be exploited in Gram-negative bacteria. For instance, rhamnolipids increase PMF-independent entry of AGs in *S. aureus* and other Gram-positive bacteria by altering membrane charges, fluidity and permeability. However, it is ineffective against *E. coli* (150).

The process of developing a new molecule and bringing it to market can span over 20 years, involving extensive research, clinical trials, and production setup, incurring significant costs. Potentiating antibiotics by enhancing the entry of existing antibiotics represents a rapid, effective, and cost-efficient alternative to the development of new molecules.

## Conclusion and perspectives

In the current scenario, the rise in antibiotic resistance is anticipated to result in up to 50 million deaths per year within the next 30 years, leading to significant economic implications such as increased treatment costs and work absences, in addition to the obvious public health concerns (269). Gram-negative bacteria are associated with high lethality: for example, in the case of MDR *P. aeruginosa* septicemia, the rate of associated death is 67%. For a soft tissue infection with *K. pneumoniae* necrotizing hyper-virulent strain, it is from 25% to 47% (270). Concurrently, pharmaceutical companies are progressively disengaging from antibiotic research due to the high development costs and limited financial returns associated with them (271).

Therefore, the need for new treatments has become imperative. A comprehensive review by Walesch and colleagues provides insights into the present and future of antimicrobial development (272). Discovering new active compounds faces numerous challenges, including the isolation of molecules from non-cultivable organisms, the necessity for novel mechanisms of action to overcome pathogen resistance, concerns regarding toxicity and lower efficacy in humans compared to animal models, as well as the difficulty of crossing the bacterial outer membrane. Alternatively, improving existing antibiotics through structural modifications, targeted delivery, and potentiation or combination therapies is another avenue (273).

Understanding the different mechanisms by which AGs enter bacterial cells can contribute to the development of new treatments, as exemplified by several promising studies on AG potentiation. Among ongoing antimicrobial projects, 8% involve potentiation approaches (274). Most of the potentiators currently being developed are  $\beta$ -lactamase inhibitors, such as those from the diazabicyclooctane family, which are currently undergoing clinical trials (275). In terms of improving antibiotic uptake, SPR741, a novel polymyxin derivative, has shown the ability to enhance the penetration of macrolides or glycopeptides in Gram-negative bacteria and is currently in clinical trials for systemic use (276, 277).

AGs uptake now appears to be tightly linked to carbon sources utilization, as demonstrated by numerous studies that show the relevance of using sugars to stimulate PMF, and the role of active sugar transporters in AG transport. This can pave the way for future research on AG uptake through specific transporters, and have implications in terms of potentiation.

**From a fundamental perspective**, further research is needed to establish the profiles of carbon source related regulation (CCR) in different Gram-negative pathogens and the identification, for each species of interest, of sugar transporters that also transport AGs. This would enhance our understanding of the conditions that lead to increased uptake of AGs in a variety of bacterial species.

The regulation of AG transporters can be species-specific, particularly regarding the CRP-cAMP dependent carbon catabolite regulation (CCR). CCR is well-characterized *E. coli*, and the regulator CRP is highly conserved, but the regulatory mechanisms can vary among different species (278), as different bacterial species are specialized in their own natural environment. This means that, although it would be ideal to identify a universal molecule to increase the number of AG transporters, the use of a particular sugar would not necessarily produce the same outcome in every species. For instance, bacteria belonging to the order *Pseudomonadales*, including the genera *Pseudomonas* and *Acinetobacter*, exhibit a diverse range of metabolic capabilities. Unlike for Enterobacteria, glucose does not play a central role for them. Instead, they preferentially utilize amino acids or succinate as carbon sources, which repress the enzymes involved in glucose utilization. CCR in *Pseudomonadales* is thus referred to as "inverted" (279). Unlike for *E. coli* (280, 281), the levels of cAMP in *P. aeruginosa* and *P.*

*putida* remain relatively constant regardless of the growth conditions (282), and does not alter CCR (283).

Transcriptional regulators of CCR can also be unexpectedly different among different species. In *Pseudomonas*, while the transcriptional regulator Vfr shares 67% identity with CRP (284), the functional homologue of CRP in *P. aeruginosa* is in fact, not Vfr (285), but Crc (286–289). Consequently, understanding the impact of sugar transporters and different carbon sources on AG uptake can be more challenging in *P. aeruginosa*.

Additional regulatory layers even complicate the complete understanding of regulation of sugar transporters, as observed with the example of *Vibrio* spp, where the *ctrR* a non-coding RNA is involved in regulation of the abundance of sugar transporters, resulting in increased AG uptake (59). Although no sequence homologue of this RNA has been found in other species, one cannot exclude the existence of such RNA related regulation. One indication is that in *Pseudomonadales*, CCR involves the RNA chaperone Hfq and the small RNAs CrcZ and CrcY (290–293).

While the regulatory mechanisms can be complex, sugar transporters may be able to transport AGs in numerous, if not all, bacterial organisms, and they can easily be identified through overexpression strategies (60).

**From a clinical perspective**, the use of sugars as potentiators of AGs could be a promising strategy due to the expected innocuity of these metabolizable compounds, and the wealth of previous clinical studies on the (non-)toxicity of these molecules in the human body. In fact, mannitol and uridine, described above as potentiators of AGs, are already used in clinics for other indications. Mannitol is currently used for managing cerebral edema or for kidney protection (294). In addition, numerous studies have been conducted to compare the pharmacokinetic and dynamic properties, as well as side effects, considering different modes of administration, for compounds such as uridine, used in cancer therapies and rheumatology, and ribose, used as dietary supplement (e.g. (295–297). This offers the possibility of potentiating AGs using existing treatments, with new applications.

In several countries, including France, there is the provision for the use of drugs for purposes other than those specified in their Marketing Authorization, particularly when no therapeutic alternatives are available. This approach, known as drug repositioning, often applies to older drugs. With drug repositioning, the initial steps of assessing toxicity and tolerance in humans have already been conducted, eliminating the need for repetition. Similarly, "off-label" use of drugs is not prohibited in the United States. This rationale has also been applied to the development of treatments for Covid-19. For instance, the combination of the drugs remdesivir (originally developed for Ebola) and diltiazem (an anti-hypertensive) could provide significant benefits to Covid-19 patients (298). Given the concerning rise of antibiotic resistance, this approach is gaining increasing interest among scientists. For AG, potentiators have been identified among various molecules that are already approved and available on the market for treating other diseases, as described above.

These investigations help to reduce costs and time-to-market by leveraging existing knowledge and data, thereby minimizing the need for extensive clinical trials.

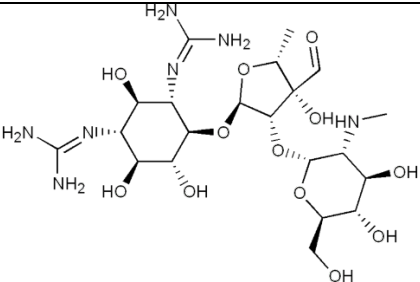
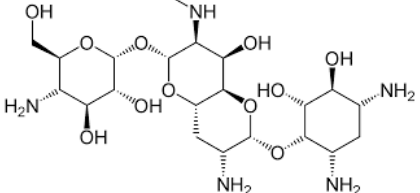
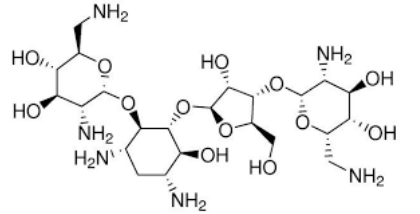
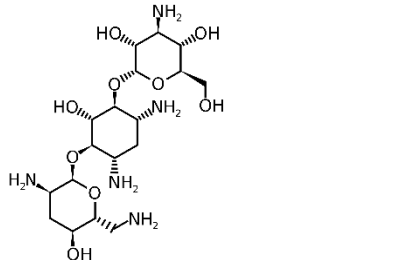
In addition to the advantage conferred by the use of potentiators together with known antibiotics in the context of drug repurposing, potentiation also offers the possibility of a novel treatment, while avoiding the necessity of identifying new bacterial targets for new antibiotics. Potentiation can also allow for bypassing bacterial resistance mechanisms, through efflux compensation or saturation of antibiotic inactivating enzymes, like it is the case for a widely used antibiotic *Augmentin*, which combines the  $\beta$ -lactam amoxicillin with a  $\beta$ -lactamase inhibitor. AGs are particularly suitable for potentiation through enhanced uptake, as their efficacy is closely tied to their

concentration within bacteria. While the use of AGs is limited due to their side effects (299, 300), increasing the intracellular concentration of AGs in bacteria could enable the administration of lower doses and mitigate side effects in humans. The study of potentiation largely focuses on stimulating the PMF or CCR, both of which are highly conserved mechanisms. This suggests broad spectrum efficacy, thus simplifying patient recruitment compared to pathogen-specific drugs.

Considering the escalating threat of antibiotic-resistant infections, AGs could play a crucial role in the effective treatment, and potentiation appears to be a fast and efficient approach of unlocking the full potential of these molecules.

#### Acknowledgements.

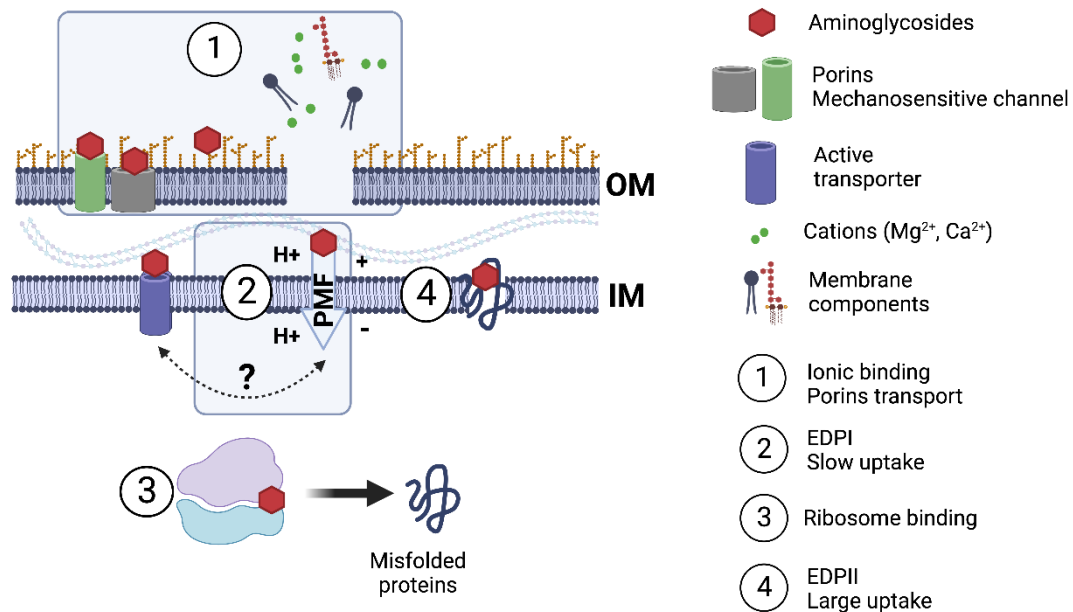
This work was supported by the Institut Pasteur, the Centre National de la Recherche Scientifique (CNRS-UMR 3525), the Fondation pour la Recherche Médicale (FRM Grant No. EQU202103012569) and by the French Government's Investissement d'Avenir program Laboratoire d'Excellence 'Integrative Biology of Emerging Infectious Diseases' [ANR-10-LABX-62-IBEID]. AC was funded by Institut Pasteur PTR 245-19. Figures were created with BioRender.com.

Group	Examples	Molecule
No core (streptidine core)	<u>Streptomycin</u>	
Mono-substitued deoxystreptamine	<u>Apramycin</u>	
4,5-disubstitued deoxystreptamine	<u>Neomycin</u> Ribostamycin Paromomycin	
4,6-disubstitued deoxystreptamine	Kanamycin Gentamicin Amikacin <u>Tobramycin</u> Plazomycin	

**Table 1:** Classification of aminoglycosides according to their core structure and examples.

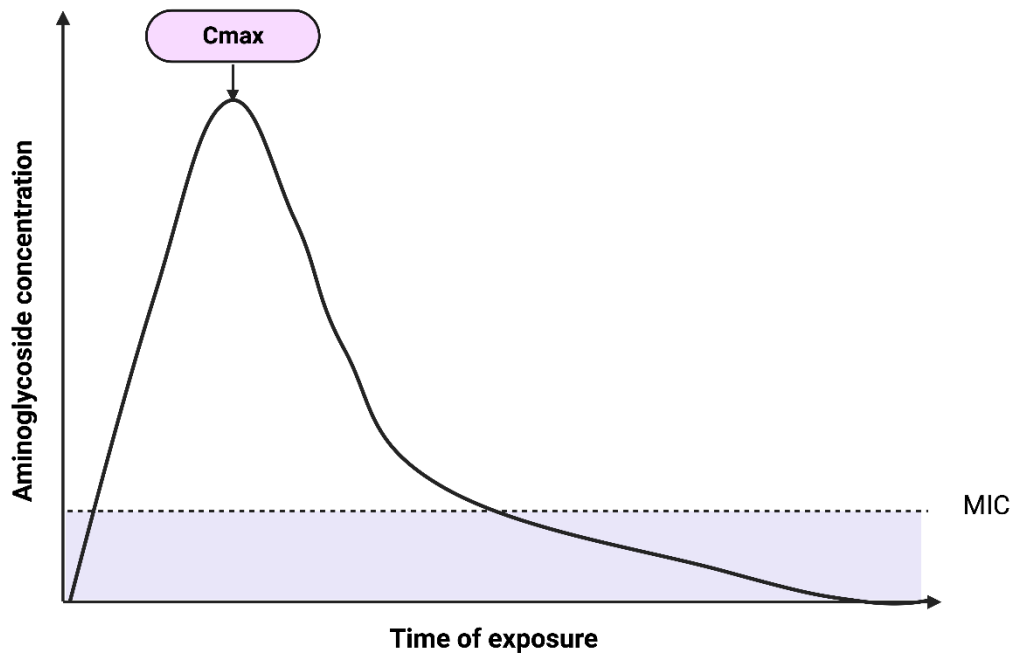
The molecule that is represented is underlined. Adapted from Krause *et al.*, 2016.

## Figure legends



**Figure 1: Model of AG entry in Gram-negative bacteria.** 1) Electrostatic binding. The interaction between AG and the outer membrane induces its destabilization. Entry through porins is possible. 2) Energy Dependent Phase I. Slow uptake, proton motive force dependent. Active transporters (notably carbohydrates transporters) are also able to carry AG. The link between active transporters and the role of the PMF in AG entry remains to be fully elucidated. 3) Ribosome binding. AG induce protein mistranslations. 4) Energy Dependent Phase II. Misfolded protein induces membrane permeability and large uptake, preceding cells death. “Active transporter” corresponds to carbohydrate transporters and others.





**Figure 2: Pharmacokinetic and pharmacodynamic parameters of aminoglycosides concentration.** The probability of clinical response is correlated with the dose administered (peak  $C_{max}/MIC$ ), rather than the time of exposure.

## References:

1. Rammelkamp CH, Maxon T. 1942. Resistance of *Staphylococcus aureus* to the Action of Penicillin. *Exp Biol Med* 51:386–389.
2. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, Han C, Bisignano C, Rao P, Wool E, Johnson SC, Browne AJ, Chipeta MG, Fell F, Hackett S, Haines-Woodhouse G, Kashef Hamadani BH, Kumaran EAP, McManigal B, Agarwal R, Akech S, Albertson S, Amuasi J, Andrews J, Aravkin A, Ashley E, Bailey F, Baker S, Basnyat B, Bekker A, Bender R, Bethou A, Bielicki J, Boonkasidecha S, Bukosia J, Carvalho C, Castañeda-Orjuela C, Chansamouth V, Chaurasia S, Chiurchiù S, Chowdhury F, Cook AJ, Cooper B, Cressey TR, Criollo-Mora E, Cunningham M, Darboe S, Day NPJ, De Luca M, Dokova K, Dramowski A, Dunachie SJ, Eckmanns T, Eibach D, Emami A, Feasey N, Fisher-Pearson N, Forrest K, Garrett D, Gastmeier P, Giref AZ, Greer RC, Gupta V, Haller S, Haselbeck A, Hay SI, Holm M, Hopkins S, Iregbu KC, Jacobs J, Jarovsky D, Javanmardi F, Khorana M, Kissoon N, Kobeissi E, Kostyanev T, Krapp F, Krumkamp R, Kumar A, Kyu HH, Lim C, Limmathurotsakul D, Loftus MJ, Lunn M, Ma J, Mturi N, Munera-Huertas T, Musicha P, Mussi-Pinhata MM, Nakamura T, Nanavati R, Nangia S, Newton P, Ngoun C, Novotney A, Nwakanma D, Obiero CW, Olivás-Martinez A, Olliaro P, Ooko E, Ortiz-Brizuela E, Peleg AY, Perrone C, Plakkal N, Ponce-de-Leon A, Raad M, Ramdin T, Riddell A, Roberts T, Robotham JV, Roca A, Rudd KE, Russell N, Schnall J, Scott JAG, Shivamallappa M, Sifuentes-Osornio J, Steenkeste N, Stewardson AJ, Stoeva T, Tasak N, Thaiprakong A, Thwaites G, Turner C, Turner P, van Doorn HR, Velaphi S, Vongpradith A, Vu H, Walsh T, Waner S, Wangrangsimakul T, Wozniak T, Zheng P, Sartorius B, Lopez AD, Stergachis A, Moore C, Dolecek C, Naghavi M. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 399:629–655.
3. Aliyu S, Smaldone A, Larson E. 2017. Prevalence of multidrug-resistant gram-negative bacteria among nursing home residents: A systematic review and meta-analysis. *Am J Infect Control* 45:512–518.
4. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, Colomb-Cotinat M, Kretzschmar ME, Devleeschauwer B, Cecchini M, Ouakrim DA, Oliveira TC, Struelens MJ, Suetens C, Monnet DL, Strauss R, Mertens K, Struyf T, Catry B, Latour K, Ivanov IN, Dobрева EG, Tambic Andrašević A, Soplek S, Budimir A, Paphitou N, Žemlicková H, Schytte Olsen S, Wolff Sönksen U, Märtin P, Ivanova M, Lyytikäinen O, Jalava J, Coignard B, Eckmanns T, Abu Sin M, Haller S, Daikos GL, Gikas A, Tsiodras S, Kontopidou F, Tóth Á, Hajdu Á, Guólaugsson Ó, Kristinsson KG, Murchan S, Burns K, Pezzotti P, Gagliotti C, Dumpis U, Liuimiene A, Perrin M, Borg MA, de Greeff SC, Monen JC, Koek MB, Elstrøm P, Zabicka D, Deptula A, Hryniewicz W, Caniça M, Nogueira PJ, Fernandes PA, Manageiro V, Popescu GA, Serban RI, Schréterová E, Litvová S, Štefkovicová M, Kolman J, Klavs I, Korošec A, Aracil B, Asensio A, Pérez-Vázquez M, Billström H, Larsson S, Reilly JS, Johnson A, Hopkins S. 2019. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis* 19:56–66.
5. Jatzkowski S, Rimek D, Popp A, Schmidt N, Reuss A. 2020. Prevalence of multiresistant gram-negative bacteria in inhabitants of long-term care facilities in 2019 in Thuringia. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 63:1454–1459.
6. Siwakoti S, Subedi A, Sharma A, Baral R, Bhattarai NR, Khanal B. 2018. Incidence and outcomes of multidrug-resistant gram-negative bacteria infections in intensive care unit from Nepal- a prospective cohort study. *Antimicrob Resist Infect Control* 7:114.
7. Albert Schatz, Elisabeth Bugie, Selman Waksman. 1944. Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-Positive and Gram-Negative Bacteria. *Proc Soc Exp Biol Med* 55:66–69.

8. Craig WA. 2011. Optimizing Aminoglycoside Use. *Crit Care Clin* 27:107–121.
9. Foster J, Tekin M. 2016. Aminoglycoside induced ototoxicity associated with mitochondrial DNA mutations. *Egypt J Med Hum Genet* 17:287–293.
10. Serio AW, Keepers T, Andrews L, Krause KM. 2018. Aminoglycoside Revival: Review of a Historically Important Class of Antimicrobials Undergoing Rejuvenation. *EcoSal Plus* 8.
11. World Health Organization. 2018. Critically important antimicrobials for human medicine : 6th revision. <https://www.who.int/publications-detail-redirect/9789241515528>. Retrieved 1 August 2022.
12. Eljaaly K, Alharbi A, Alshehri S, Ortwine JK, Pogue JM. 2019. Plazomicin: A Novel Aminoglycoside for the Treatment of Resistant Gram-Negative Bacterial Infections. *Drugs* 79:243–269.
13. Krause KM, Serio AW, Kane TR, Connolly LE. 2016. Aminoglycosides: An Overview. *Cold Spring Harb Perspect Med* 6:a027029.
14. Magnet S, Blanchard JS. 2005. Molecular Insights into Aminoglycoside Action and Resistance. *Chem Rev* 105:477–498.
15. Fourmy D, Recht MI, Blanchard SC, Puglisi JD. 1996. Structure of the A Site of *Escherichia coli* 16S Ribosomal RNA Complexed with an Aminoglycoside Antibiotic. *Science* 274:1367–1371.
16. Moazed D, Noller HF. 1987. Interaction of antibiotics with functional sites in 16S ribosomal RNA. *Nature* 327:389–394.
17. Borovinskaya MA, Pai RD, Zhang W, Schuwirth BS, Holton JM, Hirokawa G, Kaji H, Kaji A, Cate JHD. 2007. Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nat Struct Mol Biol* 14:727–732.
18. Mehta R, Champney WS. 2002. 30S Ribosomal Subunit Assembly Is a Target for Inhibition by Aminoglycosides in *Escherichia coli*. *Antimicrob Agents Chemother* 46:1546–1549.
19. Wang L, Pulk A, Wasserman MR, Feldman MB, Altman RB, Cate JHD, Blanchard SC. 2012. Allosteric control of the ribosome by small-molecule antibiotics. 9. *Nat Struct Mol Biol* 19:957–963.
20. Ying L, Zhu H, Shoji S, Fredrick K. 2019. Roles of specific aminoglycoside-ribosome interactions in the inhibition of translation. *RNA N Y N* 25:247–254.
21. Aguirre Rivera J, Larsson J, Volkov IL, Seefeldt AC, Sanyal S, Johansson M. 2021. Real-time measurements of aminoglycoside effects on protein synthesis in live cells. *Proc Natl Acad Sci* 118:e2013315118.
22. Poehlsgaard J, Douthwaite S. 2005. The bacterial ribosome as a target for antibiotics. *Nat Rev Microbiol* 3:870–881.
23. Chowdhury HM, Siddiqui MA, Kanneganti S, Sharmin N, Chowdhury MW, Nasim MT. 2018. Aminoglycoside-mediated promotion of translation readthrough occurs through a non-stochastic mechanism that competes with translation termination. *Hum Mol Genet* 27:373–384.
24. Davies J, Gilbert W, Gorini L. 1964. Streptomycin, suppression, and the code. *Proc Natl Acad Sci U S A* 51:883–890.
25. Demirci H, Murphy F, Murphy E, Gregory ST, Dahlberg AE, Jogle G. 2013. A structural basis for streptomycin-induced misreading of the genetic code. *Nat Commun* 4:1355.
26. Hewitt J, Kogut M. 1977. An investigation of mistranslation in vivo induced by streptomycin by an examination of the susceptibility of abnormal proteins to degradation. *Eur J Biochem* 74:285–

292.

27. Wohlgenuth I, Garofalo R, Samatova E, Günenç AN, Lenz C, Urlaub H, Rodnina MV. 2021. Translation error clusters induced by aminoglycoside antibiotics. *Nat Commun* 12:1830.

28. Webster CM, Shepherd M. 2022. A mini-review: environmental and metabolic factors affecting aminoglycoside efficacy. *World J Microbiol Biotechnol* 39:7.

29. Davis BD, Chen LL, Tai PC. 1986. Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. *Proc Natl Acad Sci U S A* 83:6164–6168.

30. Kohanski MA, Dwyer DJ, Wierzbowski J, Cottarel G, Collins JJ. 2008. Mistranslation of Membrane Proteins and Two-Component System Activation Trigger Antibiotic-Mediated Cell Death. *Cell* 135:679–690.

31. Ezraty B, Vergnes A, Banzhaf M, Duverger Y, Huguenot A, Brochado AR, Su S-Y, Espinosa L, Loiseau L, Py B, Typas A, Barras F. 2013. Fe-S Cluster Biosynthesis Controls Uptake of Aminoglycosides in a ROS-Less Death Pathway. *Science* 340:1583–1587.

32. Wessel AK, Yoshii Y, Reder A, Boudjemaa R, Szczesna M, Betton J-M, Bernal-Bayard J, Beloin C, Lopez D, Völker U, Ghigo J-M. 2023. Escherichia coli SPFH Membrane Microdomain Proteins HflKC Contribute to Aminoglycoside and Oxidative Stress Tolerance. *Microbiol Spectr* 11:e0176723.

33. Mingeot-Leclercq M-P, Glupczynski Y, Tulkens PM. 1999. Aminoglycosides: Activity and Resistance. *Antimicrob Agents Chemother* 43:727–737.

34. 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* 15:5–14.

35. Price KE, Kresel PA, Farchione LA, Siskin SB, Karpow SA. 1981. Epidemiological studies of aminoglycoside resistance in the U.S.A. *J Antimicrob Chemother* 8 Suppl A:89–105.

36. Dalhoff A. 1983. Transport of aminoglycosides in Escherichia coli. *Zentralblatt Bakteriologie Mikrobiologie Hygiene 1 Abt Originale Mikrobiologie Infektions- und Parasitenhygiene Int J Microbiol Hyg Med Microbiol Infect* 254:379–387.

37. Taber H, Halfenger GM. 1976. Multiple-Aminoglycoside-Resistant Mutants of Bacillus subtilis Deficient in Accumulation of Kanamycin. *Antimicrob Agents Chemother* 9:251–259.

38. Stead DA, Richards RM. 1997. Sensitive high-performance liquid chromatographic assay for aminoglycosides in biological matrices enables the direct estimation of bacterial drug uptake. *J Chromatogr B Biomed Sci App* 693:415–421.

39. Stead DA, Richards RME. 1996. Sensitive fluorimetric determination of gentamicin sulfate in biological matrices using solid-phase extraction, pre-column derivatization with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci App* 675:295–302.

40. Clarindo Lopes L, Lima D, Hayat M, Li Y, Kumar A, Kuss S. 2022. Electrochemical Quantification of Tobramycin Retention in Pseudomonas aeruginosa as Antimicrobial Susceptibility Indicator. *Anal Chem* 94:12553–12558.

41. Bryant RE, Fox K, Oh G, Morthland VH. 1992.  $\beta$ -Lactam Enhancement of Aminoglycoside Activity under Conditions of Reduced pH and Oxygen Tension That May Exist in Infected Tissues. *J Infect Dis* 165:676–682.

42. Aslangul E, Massias L, Meulemans A, Chau F, Andremont A, Courvalin P, Fantin B, Ruimy R. 2006. Acquired gentamicin resistance by permeability impairment in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 50:3615–3621.
43. Deguchi T, Okumura S, Ishii A, Tanaka M. 1977. Synthesis of carbon-14 and tritium labeled sagamicin. *J Antibiot (Tokyo)* 30:993–998.
44. Lee BK, Condon RG, Munayyer H, Weinstein MJ. 1978. Uptake of (methyl-14C)-sisomicin and (methyl-14C)-gentamicin into bacterial cells. *J Antibiot (Tokyo)* 31:141–146.
45. El-Kawy OA, Abdelaziz G, Abdel-Razek AS. 2022. Radiolabeling, characterization, and preclinical evaluation of plazomicin: A potential tracer for bacterial infection. *Chem Biol Drug Des* 99:688–702.
46. Jolley ME, Stroupe SD, Wang CH, Panas HN, Keegan CL, Schmidt RL, Schwenzer KS. 1981. Fluorescence polarization immunoassay. I. Monitoring aminoglycoside antibiotics in serum and plasma. *Clin Chem* 27:1190–1197.
47. Henry-Stanley MJ, Hess DJ, Wells CL. 2014. Aminoglycoside inhibition of *Staphylococcus aureus* biofilm formation is nutrient dependent. *J Med Microbiol* 63:861–869.
48. Allison KR, Brynildsen MP, Collins JJ. 2011. Metabolite-enabled eradication of bacterial persisters by aminoglycosides. *Nature* 473:216–220.
49. Shan Y, Lazinski D, Rowe S, Camilli A, Lewis K. 2015. Genetic basis of persister tolerance to aminoglycosides in *Escherichia coli*. *mBio* 6:e00078-15.
50. Shin B, Park W. 2015. Synergistic Effect of Oleanolic Acid on Aminoglycoside Antibiotics against *Acinetobacter baumannii*. *PloS One* 10:e0137751.
51. Short FL, Lee V, Mamun R, Malmberg R, Li L, Espinosa MI, Venkatesan K, Paulsen IT. 2021. Benzalkonium chloride antagonises aminoglycoside antibiotics and promotes evolution of resistance. *EBioMedicine* 73:103653.
52. Chen H, Gong X, Fan Z, Xia Y, Jin Y, Bai F, Cheng Z, Pan X, Wu W. 2023. *Pseudomonas aeruginosa* Citrate Synthase GltA Influences Antibiotic Tolerance and the Type III Secretion System through the Stringent Response. *Microbiol Spectr* 11:e03239-22.
53. Meylan S, Porter CBM, Yang JH, Belenky P, Gutierrez A, Lobritz MA, Park J, Kim SH, Moskowitz SM, Collins JJ. 2017. Carbon Sources Tune Antibiotic Susceptibility in *Pseudomonas aeruginosa* via Tricarboxylic Acid Cycle Control. *Cell Chem Biol* 24:195–206.
54. Wang Y, Tian T, Zhang J, Jin X, Yue H, Zhang X-H, Du L, Bai F. 2019. Indole Reverses Intrinsic Antibiotic Resistance by Activating a Novel Dual-Function Importer. *mBio* 10:e00676-19.
55. Thomae AV, Wunderli-Allenspach H, Krämer SD. 2005. Permeation of Aromatic Carboxylic Acids across Lipid Bilayers: The pH-Partition Hypothesis Revisited. *Biophys J* 89:1802–1811.
56. Sabeti Azad M, Okuda M, Cyrenne M, Bourge M, Heck M-P, Yoshizawa S, Fourmy D. 2020. Fluorescent Aminoglycoside Antibiotics and Methods for Accurately Monitoring Uptake by Bacteria. *ACS Infect Dis* 6:1008–1017.
57. Carvalho A, Mazel D, Baharoglu Z. 2021. Deficiency in cytosine DNA methylation leads to high chaperonin expression and tolerance to aminoglycosides in *Vibrio cholerae*. *PLOS Genet* 17:e1009748.
58. Lang M, Krin E, Korlowski C, Sismeiro O, Varet H, Coppée J-Y, Mazel D, Baharoglu Z. 2021.

Sleeping ribosomes: Bacterial signaling triggers RaiA mediated persistence to aminoglycosides. *iScience* 24:103128.

59. Pierlé SA, Lang M, López-Igual R, Krin E, Fourmy D, Kennedy SP, Val M-E, Baharoglu Z, Mazel D. 2023. Identification of the active mechanism of aminoglycoside entry in *V. cholerae* through characterization of sRNA *ctrR*, regulating carbohydrate utilization and transport. *bioRxiv* <https://doi.org/10.1101/2023.07.19.549712>.
60. Lang M, Renard S, El-Meouche I, Amoura A, Denamur E, Brosschot T, Ingersoll M, Bacqué E, Mazel D, Baharoglu Z. 2023. Uridine as a potentiator of aminoglycosides through activation of carbohydrate transporters. *bioRxiv* <https://doi.org/10.1101/2023.07.31.551273>.
61. Delcour AH. 2009. Outer membrane permeability and antibiotic resistance. *Biochim Biophys Acta* 1794:808–816.
62. Masi M, Winterhalter M, Pagès J-M. 2019. Outer Membrane Porins. *Subcell Biochem* 92:79–123.
63. Stokes JM, French S, Ovchinnikova OG, Bouwman C, Whitfield C, Brown ED. 2016. Cold Stress Makes *Escherichia coli* Susceptible to Glycopeptide Antibiotics by Altering Outer Membrane Integrity. *Cell Chem Biol* 23:267–277.
64. Anand N, Davis BD, Armitage AK. 1960. Effect of Streptomycin on *Escherichia coli*: Uptake of Streptomycin by *Escherichia coli*. *Nature* 185:23–24.
65. Taber HW, Mueller JP, Miller PF, Arrow AS. 1987. Bacterial uptake of aminoglycoside antibiotics. *Microbiol Rev* 51:439–457.
66. Hurwitz C, Rosano CL. 1962. Accumulation of label from C14-streptomycin by *Escherichia coli*. *J Bacteriol* 83:1193–1201.
67. Martin NL, Beveridge TJ. 1986. Gentamicin interaction with *Pseudomonas aeruginosa* cell envelope. *Antimicrob Agents Chemother* 29:1079–1087.
68. Nichols WW, Young SN. 1985. Respiration-dependent uptake of dihydrostreptomycin by *Escherichia coli*. Its irreversible nature and lack of evidence for a uniport process. *Biochem J* 228:505–512.
69. Xie M, Wang H, Chen Y. 2009. Insight into the interaction of *E. coli* with gentamycin by ultrafast electrophoresis achieved within the microscopic visual field. *Electrophoresis* 30:3884–3890.
70. Engelberg H, Artman M. 1961. Studies on streptomycin-dependent bacteria: uptake of 14C streptomycin by a streptomycin-dependent mutant of *Escherichia coli*. *Biochim Biophys Acta* 54:533–542.
71. Newton BA. 1954. Site of action of polymyxin on *Pseudomonas aeruginosa*: antagonism by cations. *J Gen Microbiol* 10:491–499.
72. Peterson AA, Fesik SW, McGroarty EJ. 1987. Decreased binding of antibiotics to lipopolysaccharides from polymyxin-resistant strains of *Escherichia coli* and *Salmonella typhimurium*. *Antimicrob Agents Chemother* 31:230–237.
73. Moore RA, Hancock RE. 1986. Involvement of outer membrane of *Pseudomonas cepacia* in aminoglycoside and polymyxin resistance. *Antimicrob Agents Chemother* 30:923–926.
74. Abdel-Sayed S, González M, Eagon RG. 1982. The role of the outer membrane of *Pseudomonas aeruginosa* in the uptake of aminoglycoside antibiotics. *J Antimicrob Chemother* 10:173–183.

75. Raulston JE, Montie TC. 1989. Early cell envelope alterations by tobramycin associated with its lethal action on *Pseudomonas aeruginosa*. *J Gen Microbiol* 135:3023–3034.
76. Leviton IM, Fraimow HS, Carrasco N, Dougherty TJ, Miller MH. 1995. Tobramycin uptake in *Escherichia coli* membrane vesicles. *Antimicrob Agents Chemother* 39:467–475.
77. Miller MH, Edberg SC, Mandel LJ, Behar CF, Steigbigel NH. 1980. Gentamicin uptake in wild-type and aminoglycoside-resistant small-colony mutants of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 18:722–729.
78. Jackson GG, Lolans VT, Daikos GL. 1990. The inductive role of ionic binding in the bactericidal and postexposure effects of aminoglycoside antibiotics with implications for dosing. *J Infect Dis* 162:408–413.
79. John T, Thomas T, Abel B, Wood BR, Chalmers DK, Martin LL. 2017. How kanamycin A interacts with bacterial and mammalian mimetic membranes. *Biochim Biophys Acta BBA - Biomembr* 1859:2242–2252.
80. Saika T, Hasegawa M, Kobayashi I, Nishida M. 1998. Ionic binding of 3H-gentamicin and short-term bactericidal activity of gentamicin against *Pseudomonas aeruginosa* isolates differing in lipopolysaccharide structure. *Kansenshogaku Zasshi* 72:97–104.
81. Hasegawa M, Kobayashi I, Saika T, Nishida M. 1997. Gentamicin-induced alteration in drug susceptibility and lipopolysaccharide-composition of *Pseudomonas aeruginosa* isolates. *Kansenshogaku Zasshi* 71:199–206.
82. Bryan LE, O'Hara K, Wong S. 1984. Lipopolysaccharide changes in impermeability-type aminoglycoside resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 26:250–255.
83. Hancock RE, Farmer SW, Li ZS, Poole K. 1991. Interaction of aminoglycosides with the outer membranes and purified lipopolysaccharide and OmpF porin of *Escherichia coli*. *Antimicrob Agents Chemother* 35:1309–1314.
84. Raetz CRH, Reynolds CM, Trent MS, Bishop RE. 2007. Lipid A modification systems in gram-negative bacteria. *Annu Rev Biochem* 76:295–329.
85. Edrington TC, Kintz E, Goldberg JB, Tamm LK. 2011. Structural basis for the interaction of lipopolysaccharide with outer membrane protein H (OprH) from *Pseudomonas aeruginosa*. *J Biol Chem* 286:39211–39223.
86. Bell A, Hancock RE. 1989. Outer membrane protein H1 of *Pseudomonas aeruginosa*: purification of the protein and cloning and nucleotide sequence of the gene. *J Bacteriol* 171:3211–3217.
87. Hancock RE, Raffle VJ, Nicas TI. 1981. Involvement of the outer membrane in gentamicin and streptomycin uptake and killing in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 19:777–785.
88. Nicas TI, Hancock RE. 1983. Alteration of susceptibility to EDTA, polymyxin B and gentamicin in *Pseudomonas aeruginosa* by divalent cation regulation of outer membrane protein H1. *J Gen Microbiol* 129:509–517.
89. Macfarlane EL, Kwasnicka A, Ochs MM, Hancock RE. 1999. PhoP-PhoQ homologues in *Pseudomonas aeruginosa* regulate expression of the outer-membrane protein OprH and polymyxin B resistance. *Mol Microbiol* 34:305–316.
90. Loh B, Grant C, Hancock RE. 1984. Use of the fluorescent probe 1-N-phenylnaphthylamine to study the interactions of aminoglycoside antibiotics with the outer membrane of *Pseudomonas*

aeruginosa. *Antimicrob Agents Chemother* 26:546–551.

91. Joshi T, Voo ZX, Graham B, Spiccia L, Martin LL. 2015. Real-time examination of aminoglycoside activity towards bacterial mimetic membranes using Quartz Crystal Microbalance with Dissipation monitoring (QCM-D). *Biochim Biophys Acta* 1848:385–391.
92. Khondker A, Bider R-C, Passos-Gastaldo I, Wright GD, Rheinstädter MC. 2021. Membrane interactions of non-membrane targeting antibiotics: The case of aminoglycosides, macrolides, and fluoroquinolones. *Biochim Biophys Acta Biomembr* 1863:183448.
93. Nakae R, Nakae T. 1982. Diffusion of aminoglycoside antibiotics across the outer membrane of *Escherichia coli*. *Antimicrob Agents Chemother* 22:554–559.
94. Yoshimura F, Nikaido H. 1985. Diffusion of beta-lactam antibiotics through the porin channels of *Escherichia coli* K-12. *Antimicrob Agents Chemother* 27:84–92.
95. Charbit A. 2003. Maltodextrin transport through LamB. *Front Biosci* 8:s265-274.
96. Schmid K, Ebner R, Jahreis K, Lengeler JW, Titgemeyer F. 1991. A sugar-specific porin, ScrY, is involved in sucrose uptake in enteric bacteria. *Mol Microbiol* 5:941–950.
97. Iscla I, Wray R, Wei S, Posner B, Blount P. 2014. Streptomycin potency is dependent on MscL channel expression. *Nat Commun* 5:4891.
98. Wray R, Iscla I, Gao Y, Li H, Wang J, Blount P. 2016. Dihydrostreptomycin Directly Binds to, Modulates, and Passes through the MscL Channel Pore. *PLoS Biol* 14:e1002473.
99. Hancock RE, Farmer SW, Li ZS, Poole K. 1991. Interaction of aminoglycosides with the outer membranes and purified lipopolysaccharide and OmpF porin of *Escherichia coli*. *Antimicrob Agents Chemother* 35:1309–1314.
100. Kobayashi Y, Nakae T. 1985. The mechanism of ion selectivity of OmpF-porin pores of *Escherichia coli*. *Eur J Biochem* 151:231–236.
101. Bafna JA, Sans-Serramitjana E, Acosta-Gutiérrez S, Bodrenko IV, Hörömpöli D, Berscheid A, Brötz-Oesterhelt H, Winterhalter M, Ceccarelli M. 2020. Kanamycin Uptake into *Escherichia coli* Is Facilitated by OmpF and OmpC Porin Channels Located in the Outer Membrane. *ACS Infect Dis* 6:1855–1865.
102. Paul E, Ghai I, Hörömpöli D, Brötz-Oesterhelt H, Winterhalter M, Bafna JA. 2022. Uptake of aminoglycosides through outer membrane porins in *Escherichia coli*. *bioRxiv* <https://doi.org/10.1101/2022.09.05.506620>.
103. Foulds J, Chai TJ. 1978. New major outer membrane proteins found in an *Escherichia coli* tolF mutant resistant to bacteriophage Tu1b. *J Bacteriol* 133:1478–1483.
104. Chai TJ, Foulds J. 1977. *Escherichia coli* K-12 tolF mutants: alterations in protein composition of the outer membrane. *J Bacteriol* 130:781–786.
105. Rahaman SkO, Mukherjee J, Chakrabarti A, Pal S. 1998. Decreased membrane permeability in a polymyxin B-resistant *Escherichia coli* mutant exhibiting multiple resistance to  $\beta$ -lactams as well as aminoglycosides. *FEMS Microbiol Lett* 161:249–254.
106. Ude J, Tripathi V, Buyck JM, Söderholm S, Cunrath O, Fanous J, Claudi B, Egli A, Schleberger C, Hiller S, Bumann D. 2021. Outer membrane permeability: Antimicrobials and diverse nutrients bypass porins in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci* 118:e2107644118.
107. Pratt LA, Hsing W, Gibson KE, Silhavy TJ. 1996. From acids to osmZ: multiple factors



- influence synthesis of the OmpF and OmpC porins in *Escherichia coli*. *Mol Microbiol* 20:911–917.
108. Sugawara E, Nestorovich EM, Bezrukov SM, Nikaido H. 2006. *Pseudomonas aeruginosa* Porin OprF Exists in Two Different Conformations. *J Biol Chem* 281:16220–16229.
109. Click EM, McDonald GA, Schnaitman CA. 1988. Translational control of exported proteins that results from OmpC porin overexpression. *J Bacteriol* 170:2005–2011.
110. Bruni GN, Kralj JM. 2020. Membrane voltage dysregulation driven by metabolic dysfunction underlies bactericidal activity of aminoglycosides. *eLife* 9:e58706.
111. Bryan LE, Kwan S. 1983. Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. *Antimicrob Agents Chemother* 23:835–845.
112. Campbell BD, Kadner RJ. 1980. Relation of aerobiosis and ionic strength to the uptake of dihydrostreptomycin in *Escherichia coli*. *Biochim Biophys Acta* 593:1–10.
113. Damper PD, Epstein W. 1981. Role of the membrane potential in bacterial resistance to aminoglycoside antibiotics. *Antimicrob Agents Chemother* 20:803–808.
114. Setty OH, Hendler RW, Shrager RI. 1983. Simultaneous measurements of proton motive force, delta pH, membrane potential, and H<sup>+</sup>/O ratios in intact *Escherichia coli*. *Biophys J* 43:371–381.
115. Bryan LE, Van DenElzen HM. 1976. Streptomycin Accumulation in Susceptible and Resistant Strains of *Escherichia coli* and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 9:928–938.
116. Tran QH, Uden G. 1998. Changes in the proton potential and the cellular energetics of *Escherichia coli* during growth by aerobic and anaerobic respiration or by fermentation. *Eur J Biochem* 251:538–543.
117. Bot CT, Prodan C. 2010. Quantifying the membrane potential during *E. coli* growth stages. *Biophys Chem* 146:133–137.
118. Kashket ER. 1981. Effects of aerobiosis and nitrogen source on the proton motive force in growing *Escherichia coli* and *Klebsiella pneumoniae* cells. *J Bacteriol* 146:377–384.
119. Becker K, Cao S, Nilsson A, Erlandsson M, Hotop S-K, Kuka J, Hansen J, Haldimann K, Grinberga S, Berruga-Fernández T, Huseby DL, Shariatgorji R, Lindmark E, Platzack B, Böttger EC, Crich D, Friberg LE, Lundberg CV, Hughes D, Brönstrup M, Andrén PE, Liepinsh E, Hobbie SN. 2021. Antibacterial activity of apramycin at acidic pH warrants wide therapeutic window in the treatment of complicated urinary tract infections and acute pyelonephritis. *eBioMedicine* 73.
120. Schlessinger D. 1988. Failure of aminoglycoside antibiotics to kill anaerobic, low-pH, and resistant cultures. *Clin Microbiol Rev* 1:54–59.
121. Bryan LE, Kowand SK, Van Den Elzen HM. 1979. Mechanism of Aminoglycoside Antibiotic Resistance in Anaerobic Bacteria: *Clostridium perfringens* and *Bacteroides fragilis*. *Antimicrob Agents Chemother* 15:7–13.
122. Chareyre S, Barras F, Mandin P. 2019. A small RNA controls bacterial sensitivity to gentamicin during iron starvation. *PLoS Genet* 15:e1008078.
123. Lobritz MA, Belenky P, Porter CBM, Gutierrez A, Yang JH, Schwarz EG, Dwyer DJ, Khalil AS, Collins JJ. 2015. Antibiotic efficacy is linked to bacterial cellular respiration. *Proc Natl Acad Sci U S A* 112:8173–8180.
124. Krin E, Carvalho A, Lang M, Babosan A, Mazel D, Baharoglu Z. 2023. RavA-ViaA links *Vibrio*

cholerae Cpx- and Zra2- envelope stress to antibiotic response. bioRxiv <https://doi.org/10.1101/2023.04.24.538083>.

125. Bryan LE, Kwan S. 1981. Mechanisms of aminoglycoside resistance of anaerobic bacteria and facultative bacteria grown anaerobically. *J Antimicrob Chemother* 8:1–8.
126. Muir ME, Hanwell DR, Wallace BJ. 1981. Characterization of a respiratory mutant of *Escherichia coli* with reduced uptake of aminoglycoside antibiotics. *Biochim Biophys Acta* 638:234–241.
127. Bryan LE, Van Den Elzen HM. 1977. Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. *Antimicrob Agents Chemother* 12:163–177.
128. Bryan LE, Van Den Elzen HM. 1975. Gentamicin accumulation by sensitive strains of *E. coli* and *P. aeruginosa*. *J Antibiot (Tokyo)* 28:696–703.
129. Holtje J-V. 1978. Streptomycin Uptake via an Inducible Polyamine Transport System in *Escherichia coli*. *Eur J Biochem* 86:345–351.
130. McCollister BD, Hoffman M, Husain M, Vázquez-Torres A. 2011. Nitric oxide protects bacteria from aminoglycosides by blocking the energy-dependent phases of drug uptake. *Antimicrob Agents Chemother* 55:2189–2196.
131. Zemke AC, Gladwin MT, Bomberger JM. 2015. Sodium nitrite blocks the activity of aminoglycosides against *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother* 59:3329–3334.
132. Zhang Y, Guo K, Meng Q, Gao H. 2020. Nitrite modulates aminoglycoside tolerance by inhibiting cytochrome heme-copper oxidase in bacteria. *Commun Biol* 3:1–10.
133. Loughman K, Hall J, Knowlton S, Sindeldecker D, Gilson T, Schmitt DM, Birch JW-M, Gajtka T, Kobe BN, Florjanczyk A, Ingram J, Bakshi CS, Horzempa J. 2016. Temperature-Dependent Gentamicin Resistance of *Francisella tularensis* is Mediated by Uptake Modulation. *Front Microbiol* 7.
134. Fraimow HS, Greenman JB, Leviton IM, Dougherty TJ, Miller MH. 1991. Tobramycin uptake in *Escherichia coli* is driven by either electrical potential or ATP. *J Bacteriol* 173:2800–2808.
135. Muir ME, Wallace BJ. 1979. Isolation of mutants of *Escherichia coli* uncoupled in oxidative phosphorylation using hypersensitivity to streptomycin. *Biochim Biophys Acta* 547:218–229.
136. Goss SR, Spicer AB, Nichols WW. 1988. Bioenergetics of dihydrostreptomycin transport by *Escherichia coli*. *FEBS Lett* 228:245–248.
137. Muir ME, Ballesteros M, Wallace BJ. 1985. Respiration rate, growth rate and the accumulation of streptomycin in *Escherichia coli*. *J Gen Microbiol* 131:2573–2579.
138. Nielsen PL. 1978. The inhibitory effect of salt, cyanide and chloramphenicol on the uptake of streptomycin by *Escherichia coli* K 12. *Acta Pathol Microbiol Scand [B]* 86B:321–326.
139. Baharoglu Z, Krin E, Mazel D. 2013. RpoS Plays a Central Role in the SOS Induction by Sub-Lethal Aminoglycoside Concentrations in *Vibrio cholerae*. *PLOS Genet* 9:e1003421.
140. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. 2007. A Common Mechanism of Cellular Death Induced by Bactericidal Antibiotics. *Cell* 130:797–810.
141. Ling J, Söll D. 2010. Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. *Proc Natl Acad Sci* 107:4028–4033.

142. Davis BB. 1988. The lethal action of aminoglycosides. *J Antimicrob Chemother* 22:1–3.
143. Davis BD. 1987. Mechanism of bactericidal action of aminoglycosides. *Microbiol Rev* 51:341–350.
144. Busse HJ, Wöstmann C, Bakker EP. 1992. The bactericidal action of streptomycin: membrane permeabilization caused by the insertion of mistranslated proteins into the cytoplasmic membrane of *Escherichia coli* and subsequent caging of the antibiotic inside the cells due to degradation of these proteins. *J Gen Microbiol* 138:551–561.
145. Hurwitz C, Braun CB, Rosano CL. 1981. Role of ribosome recycling in uptake of dihydrostreptomycin by sensitive and resistant *Escherichia coli*. *Biochim Biophys Acta BBA - Nucleic Acids Protein Synth* 652:168–176.
146. Chang FN, Flaks JG. 1972. Binding of dihydrostreptomycin to *Escherichia coli* ribosomes: characteristics and equilibrium of the reaction. *Antimicrob Agents Chemother* 2:294–307.
147. Hancock RE. 1981. Aminoglycoside uptake and mode of action--with special reference to streptomycin and gentamicin. I. Antagonists and mutants. *J Antimicrob Chemother* 8:249–276.
148. D'Alessandri RM, McNeely DJ, Kluge RM. 1976. Antibiotic synergy and antagonism against clinical isolates of *Klebsiella* species. *Antimicrob Agents Chemother* 10:889–892.
149. Muir ME, Van Heeswyck RS, Wallace BJ. 1984. Effect of Growth Rate on Streptomycin Accumulation by *Escherichia coli* and *Bacillus megaterium*. *Microbiology* 130:2015–2022.
150. 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* 26:1355-1364.e4.
151. Höltje JV. 1979. Regulation of polyamine and streptomycin transport during stringent and relaxed control in *Escherichia coli*. *J Bacteriol* 137:661–663.
152. Babosan A, Fruchard L, Krin E, Carvalho A, Mazel D, Baharoglu Z. 2022. Nonessential tRNA and rRNA modifications impact the bacterial response to sub-MIC antibiotic stress. *microLife* 3:18.
153. Moukaddem M, Tangy F, Capmau ML, Le Goffic F. 1986. Effects of cations, polyamines and other aminoglycosides on gentamicin C2. Binding to ribosomes from sensitive and resistant *Escherichia coli* strains. *J Antibiot (Tokyo)* 39:136–140.
154. Kwon DH, Lu C-D. 2006. Polyamines Induce Resistance to Cationic Peptide, Aminoglycoside, and Quinolone Antibiotics in *Pseudomonas aeruginosa* PAO1. *Antimicrob Agents Chemother* 50:1615–1622.
155. Garcia-Riestra C, Perlin MH, Lerner SA. 1985. Lack of accumulation of exogenous adenylyl dihydrostreptomycin by whole cells or spheroplasts of *Escherichia coli*. *Antimicrob Agents Chemother* 27:114–119.
156. Muto A, Goto S, Kurita D, Ushida C, Himeno H. 2021. Involvement of GcvB small RNA in intrinsic resistance to multiple aminoglycoside antibiotics in *Escherichia coli*. *J Biochem (Tokyo)* 169:485–489.
157. Monod J. 1942. *Recherches sur la croissance des cultures bactériennes*. Institut Pasteur, Paris.
158. 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* 9:1599.
159. Usui M, Yoshii Y, Thiriet-Rupert S, Ghigo J-M, Beloin C. 2023. Intermittent antibiotic

treatment of bacterial biofilms favors the rapid evolution of resistance. *Commun Biol* 6:275.

160. Van den Bergh B, Michiels JE, Wenseleers T, Windels EM, Boer PV, Kestemont D, De Meester L, Verstrepen KJ, Verstraeten N, Fauvart M, Michiels J. 2016. Frequency of antibiotic application drives rapid evolutionary adaptation of *Escherichia coli* persistence. *Nat Microbiol* 1:16020.

161. Van den Bergh B, Schramke H, Michiels JE, Kimkes TEP, Radzikowski JL, Schimpf J, Vedelaar SR, Burschel S, Dewachter L, Lončar N, Schmidt A, Meijer T, Fauvart M, Friedrich T, Michiels J, Heinemann M. 2022. Mutations in respiratory complex I promote antibiotic persistence through alterations in intracellular acidity and protein synthesis. *Nat Commun* 13:546.

162. Girgis HS, Hottes AK, Tavazoie S. 2009. Genetic Architecture of Intrinsic Antibiotic Susceptibility. *PLoS ONE* 4:e5629.

163. Luo X, Esberard M, Bouloc P, Jacq A. 2021. A Small Regulatory RNA Generated from the *malK* 5' Untranslated Region Targets Gluconeogenesis in *Vibrio* Species. *mSphere* e0013421.

164. Beisel CL, Storz G. 2011. The base-pairing RNA spot 42 participates in a multioutput feedforward loop to help enact catabolite repression in *Escherichia coli*. *Mol Cell* 41:286–297.

165. Papenfort K, Vogel J. 2011. Sweet business: Spot42 RNA networks with CRP to modulate catabolite repression. *Mol Cell* 41:245–246.

166. Boos W, Shuman H. 1998. Maltose/Maltodextrin System of *Escherichia coli*: Transport, Metabolism, and Regulation. *Microbiol Mol Biol Rev* 62:204–229.

167. Dannelly HK, Roseman S. 1992. NAD<sup>+</sup> and NADH regulate an ATP-dependent kinase that phosphorylates enzyme I of the *Escherichia coli* phosphotransferase system. *Proc Natl Acad Sci U S A* 89:11274–11276.

168. Peterkofsky A, Gazdar C. 1979. *Escherichia coli* adenylate cyclase complex: regulation by the proton electrochemical gradient. *Proc Natl Acad Sci U S A* 76:1099–1103.

169. Andersson M, Bondar A-N, Freitas JA, Tobias DJ, Kaback HR, White SH. 2012. Proton-coupled dynamics in lactose permease. *Struct Lond Engl* 1993 20:1893–1904.

170. Foster DL, Garcia ML, Newman MJ, Patel L, Kaback HR. 1982. Lactose-proton symport by purified lac carrier protein. *Biochemistry* 21:5634–5638.

171. Andersson DI, Hughes D. 2014. Microbiological effects of sublethal levels of antibiotics. 7. *Nat Rev Microbiol* 12:465–478.

172. Baharoglu Z, Krin E, Mazel D. 2013. RpoS Plays a Central Role in the SOS Induction by Sub-Lethal Aminoglycoside Concentrations in *Vibrio cholerae*. *PLoS Genet* 9:e1003421.

173. Baharoglu Z, Mazel D. 2011. *Vibrio cholerae* Triggers SOS and Mutagenesis in Response to a Wide Range of Antibiotics: a Route towards Multiresistance. *Antimicrob Agents Chemother* 55:2438–2441.

174. Cruz-Loya M, Kang TM, Lozano NA, Watanabe R, Tekin E, Damoiseaux R, Savage VM, Yeh PJ. 2019. Stressor interaction networks suggest antibiotic resistance co-opted from stress responses to temperature. 1. *ISME J* 13:12–23.

175. Gutierrez A, Laureti L, Crussard S, Abida H, Rodríguez-Rojas A, Blázquez J, Baharoglu Z, Mazel D, Darfeuille F, Vogel J, Matic I. 2013.  $\beta$ -lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity. 1. *Nat Commun* 4:1610.

176. Kindrachuk KN, Fernández L, Bains M, Hancock REW. 2011. Involvement of an ATP-

- Dependent Protease, PA0779/AsrA, in Inducing Heat Shock in Response to Tobramycin in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 55:1874–1882.
177. Roemhild R, Bollenbach T, Andersson DI. 2022. The physiology and genetics of bacterial responses to antibiotic combinations. 8. *Nat Rev Microbiol* 20:478–490.
178. Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V. 2000. Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. 6802. *Nature* 407:340–348.
179. Mohler K, Ibba M. 2017. Translational fidelity and mistranslation in the cellular response to stress. 9. *Nat Microbiol* 2:1–9.
180. Dale T, Fahlman RP, Olejniczak M, Uhlenbeck OC. 2009. Specificity of the ribosomal A site for aminoacyl-tRNAs. *Nucleic Acids Res* 37:1202–1210.
181. Fluitt A, Pienaar E, Viljoen H. 2007. Ribosome kinetics and aa-tRNA competition determine rate and fidelity of peptide synthesis. *Comput Biol Chem* 31:335–346.
182. Vicens Q, Westhof E. 2002. Crystal Structure of a Complex between the Aminoglycoside Tobramycin and an Oligonucleotide Containing the Ribosomal Decoding A Site. *Chem Biol* 9:747–755.
183. Gromadski KB, Rodnina MV. 2004. Streptomycin interferes with conformational coupling between codon recognition and GTPase activation on the ribosome. 4. *Nat Struct Mol Biol* 11:316–322.
184. Richter K, Haslbeck M, Buchner J. 2010. The heat shock response: life on the verge of death. *Mol Cell* 40:253–266.
185. Goltermann L, Good L, Bentin T. 2013. Chaperonins fight aminoglycoside-induced protein misfolding and promote short-term tolerance in *Escherichia coli*. *J Biol Chem* 288:10483–10489.
186. Peng S, Chu Z, Lu J, Li D, Wang Y, Yang S, Zhang Y. 2017. Heterologous Expression of Chaperones from Hyperthermophilic Archaea Inhibits Aminoglycoside-Induced Protein Misfolding in *Escherichia coli*. *Biochem Biokhimiia* 82:1169–1175.
187. Hinz A, Lee S, Jacoby K, Manoil C. 2011. Membrane Proteases and Aminoglycoside Antibiotic Resistance  $\nu$ . *J Bacteriol* 193:4790–4797.
188. Ling J, Cho C, Guo L-T, Aerni H, Rinehart J, Söll D. 2012. Protein aggregation caused by aminoglycoside action is prevented by a hydrogen peroxide scavenger. *Mol Cell* 48:713–722.
189. Carvalho A, Mazel D, Baharoglu Z. 2021. Deficiency in cytosine DNA methylation leads to high chaperonin expression and tolerance to aminoglycosides in *Vibrio cholerae*. *PLOS Genet* 17:e1009748.
190. Lin JT, Connelly MB, Amolo C, Otani S, Yaver DS. 2005. Global Transcriptional Response of *Bacillus subtilis* to Treatment with Subinhibitory Concentrations of Antibiotics That Inhibit Protein Synthesis. *Antimicrob Agents Chemother* 49:1915–1926.
191. Wüllner D, Gesper M, Haupt A, Liang X, Zhou P, Dietze P, Narberhaus F, Bandow JE. 2022. Adaptive Responses of *Pseudomonas aeruginosa* to Treatment with Antibiotics. *Antimicrob Agents Chemother* 66:e00878-21.
192. Cardoso K, Gandra RF, Wisniewski ES, Osaku CA, Kadowaki MK, Felipach-Neto V, Haus LFA-Á, Simão R de CG. 2010. DnaK and GroEL are induced in response to antibiotic and heat shock in *Acinetobacter baumannii*. *J Med Microbiol* 59:1061–1068.
193. Carvalho A, Krin E, Korlowski C, Mazel D, Baharoglu Z. 2021. Interplay between Sublethal

Aminoglycosides and Quorum Sensing: Consequences on Survival in *V. cholerae*. *Cells* 10:3227.

194. Fruchard L, Babosan A, Carvalho A, Lang M, Li B, Duchateau M, Giai-Gianetto Q, Matondo M, Bonhomme F, Fabret C, Namy O, de Crécy-Lagard V, Mazel D, Baharoglu Z. 2022. Queuosine modification of tRNA-Tyrosine elicits translational reprogramming and enhances growth of *Vibrio cholerae* with aminoglycosides. preprint. bioRxiv <https://doi.org/10.1101/2022.09.26.509455>.

195. Kohanski MA, Dwyer DJ, Wierzbowski J, Cottarel G, Collins JJ. 2008. Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. *Cell* 135:679–690.

196. Bryan LE, Kwan S. 1983. Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. *Antimicrob Agents Chemother* 23:835–845.

197. Lv B, Huang X, Lijia C, Ma Y, Bian M, Li Z, Duan J, Zhou F, Yang B, Qie X, Song Y, Wood TK, Fu X. 2023. Heat shock potentiates aminoglycosides against gram-negative bacteria by enhancing antibiotic uptake, protein aggregation, and ROS. *Proc Natl Acad Sci* 120:e2217254120.

198. Grabowicz M, Silhavy TJ. 2017. Envelope Stress Responses: An Interconnected Safety Net. *Trends Biochem Sci* 42:232–242.

199. Mitchell AM, Silhavy TJ. 2019. Envelope stress responses: balancing damage repair and toxicity. *Nat Rev Microbiol* 17:417–428.

200. Raivio TL, Leblanc SKD, Price NL. 2013. The *Escherichia coli* Cpx Envelope Stress Response Regulates Genes of Diverse Function That Impact Antibiotic Resistance and Membrane Integrity. *J Bacteriol* 195:2755–2767.

201. Thorbjarnardóttir SH, Magnúsdóttir RA, Eggertsson G. 1978. Mutations determining generalized resistance to aminoglycoside antibiotics in *Escherichia coli*. *Mol Gen Genet MGG* 161:89–98.

202. Guest RL, Wang J, Wong JL, Raivio TL. 2017. A Bacterial Stress Response Regulates Respiratory Protein Complexes To Control Envelope Stress Adaptation. *J Bacteriol* 199:e00153-17.

203. Jing W, Liu J, Wu S, Li X, Liu Y. 2021. Role of *cpxA* Mutations in the Resistance to Aminoglycosides and  $\beta$ -Lactams in *Salmonella enterica* serovar Typhimurium. *Front Microbiol* 12.

204. Kashyap DR, Kuzma M, Kowalczyk DA, Gupta D, Dziarski R. 2017. Bactericidal peptidoglycan recognition protein induces oxidative stress in *Escherichia coli* through a block in respiratory chain and increase in central carbon catabolism. *Mol Microbiol* 105:755–776.

205. El Khoury JY, Zamarreño Beas J, Huguenot A, Py B, Barras F. 2023. Bioenergetic State of *Escherichia coli* Controls Aminoglycoside Susceptibility. *mBio* 14:e0330222.

206. Snider J, Gutsche I, Lin M, Baby S, Cox B, Butland G, Greenblatt J, Emili A, Houry WA. 2006. Formation of a distinctive complex between the inducible bacterial lysine decarboxylase and a novel AAA+ ATPase. *J Biol Chem* 281:1532–1546.

207. Wong KS, Bhandari V, Janga SC, Houry WA. 2017. The RavA-ViaA Chaperone-Like System Interacts with and Modulates the Activity of the Fumarate Reductase Respiratory Complex. *J Mol Biol* 429:324–344.

208. Tsviklist V, Guest RL, Raivio TL. 2022. The Cpx Stress Response Regulates Turnover of Respiratory Chain Proteins at the Inner Membrane of *Escherichia coli*. *Front Microbiol* 12.

209. Lee S, Hinz A, Bauerle E, Angermeyer A, Juhaszova K, Kaneko Y, Singh PK, Manoil C. 2009. Targeting a bacterial stress response to enhance antibiotic action. *Proc Natl Acad Sci* 106:14570–14575.
210. Rome K, Borde C, Taher R, Cayron J, Lesterlin C, Gueguen E, De Rosny E, Rodrigue A. 2018. The Two-Component System ZraPSR Is a Novel ESR that Contributes to Intrinsic Antibiotic Tolerance in *Escherichia coli*. *J Mol Biol* 430:4971–4985.
211. Imlay JA. 2013. The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. 7. *Nat Rev Microbiol* 11:443–454.
212. Chiang SM, Schellhorn HE. 2012. Regulators of oxidative stress response genes in *Escherichia coli* and their functional conservation in bacteria. *Arch Biochem Biophys* 525:161–169.
213. Dukan S, Farewell A, Ballesteros M, Taddei F, Radman M, Nyström T. 2000. Protein oxidation in response to increased transcriptional or translational errors. *Proc Natl Acad Sci U S A* 97:5746–5749.
214. Dahl J-U, Gray MJ, Jakob U. 2015. Protein quality control under oxidative stress conditions. *J Mol Biol* 427:1549–1563.
215. Schramm FD, Schroeder K, Jonas K. 2020. Protein aggregation in bacteria. *FEMS Microbiol Rev* 44:54–72.
216. Lin DM, Koskella B, Lin HC. 2017. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World J Gastrointest Pharmacol Ther* 8:162–173.
217. López-Igual R, Bernal-Bayard J, Rodríguez-Patón A, Ghigo J-M, Mazel D. 2019. Engineered toxin-intein antimicrobials can selectively target and kill antibiotic-resistant bacteria in mixed populations. *Nat Biotechnol* <https://doi.org/10.1038/s41587-019-0105-3>.
218. Palacios Araya D, Palmer KL, Duerkop BA. 2021. CRISPR-based antimicrobials to obstruct antibiotic-resistant and pathogenic bacteria. *PLoS Pathog* 17:e1009672.
219. Chawla M, Verma J, Gupta R, Das B. 2022. Antibiotic Potentiators Against Multidrug-Resistant Bacteria: Discovery, Development, and Clinical Relevance. *Front Microbiol* 13:887251.
220. Wright GD. 2016. Antibiotic Adjuvants: Rescuing Antibiotics from Resistance. *Trends Microbiol* 24:862–871.
221. Avent ML, Rogers BA, Cheng AC, Paterson DL. 2011. Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity: Aminoglycosides: review and monitoring. *Intern Med J* 41:441–449.
222. Gonzalez 3rd LS, Spencer JP. 1998. Aminoglycosides: A Practical Review. *Am Fam Physician* 58:1811–1820.
223. Isaksson B, Nilsson L, Maller R, Sörén L. 1988. Postantibiotic effect of aminoglycosides on gram-negative bacteria evaluated by a new method. *J Antimicrob Chemother* 22:23–33.
224. Labovitz E, Levison ME, Kaye D. 1974. Single-Dose Daily Gentamicin Therapy in Urinary Tract Infection. *Antimicrob Agents Chemother* 6:465–470.
225. Roberts JA, Kruger P, Paterson DL, Lipman J. 2008. Antibiotic resistance—What’s dosing got to do with it? *Crit Care Med* 36:2433–2440.
226. Moore RD, Lietman PS, Smith CR. 1987. Clinical Response to Aminoglycoside Therapy: Importance of the Ratio of Peak Concentration to Minimal Inhibitory Concentration. *J Infect Dis* 155:93–99.

227. Rybak MJ, Abate BJ, Kang SL, Ruffing MJ, Lerner SA, Drusano GL. 1999. Prospective evaluation of the effect of an aminoglycoside dosing regimen on rates of observed nephrotoxicity and ototoxicity. *Antimicrob Agents Chemother* 43:1549–1555.
228. Drlica K, Zhao X. 2007. Mutant Selection Window Hypothesis Updated. *Clin Infect Dis* 44:681–688.
229. Kashuba ADM, Bertino JS, Nafziger AN. 1998. Dosing of Aminoglycosides To Rapidly Attain Pharmacodynamic Goals and Hasten Therapeutic Response by Using Individualized Pharmacokinetic Monitoring of Patients with Pneumonia Caused by Gram-Negative Organisms. *Antimicrob Agents Chemother* 42:1842–1844.
230. Deng W, Fu T, Zhang Z, Jiang X, Xie J, Sun H, Hu P, Ren H, Zhou P, Liu Q, Long Q. 2020. L-lysine potentiates aminoglycosides against *Acinetobacter baumannii* via regulation of proton motive force and antibiotics uptake. *Emerg Microbes Infect* 9:639–650.
231. Lebeaux D, Chauhan A, Létoffé S, Fischer F, de Reuse H, Beloin C, Ghigo J-M. 2014. pH-mediated potentiation of aminoglycosides kills bacterial persisters and eradicates in vivo biofilms. *J Infect Dis* 210:1357–1366.
232. Crabbé A, Ostyn L, Staelens S, Rigauts C, Risseuw M, Dhaenens M, Daled S, Van Acker H, Deforce D, Van Calenbergh S, Coenye T. 2019. Host metabolites stimulate the bacterial proton motive force to enhance the activity of aminoglycoside antibiotics. *PLOS Pathog* 15:e1007697.
233. Pan X, Dong Y, Fan Z, Liu C, Xia B, Shi J, Bai F, Jin Y, Cheng Z, Jin S, Wu W. 2017. In vivo Host Environment Alters *Pseudomonas aeruginosa* Susceptibility to Aminoglycoside Antibiotics. *Front Cell Infect Microbiol* 7.
234. Hall CW, Farkas E, Zhang L, Mah T-F. 2019. Potentiation of Aminoglycoside Lethality by C4-Dicarboxylates Requires RpoN in Antibiotic-Tolerant *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 63:e01313-19.
235. Chen Y-T, Ma Y-M, Peng X-X, Li H. 2022. Glutamine potentiates gentamicin to kill lab-evolved gentamicin-resistant and clinically isolated multidrug-resistant *Escherichia coli*. *Front Microbiol* 13:1071278.
236. Zhao X-L, Chen Z-G, Yang T-C, Jiang M, Wang J, Cheng Z-X, Yang M-J, Zhu J-X, Zhang T-T, Li H, Peng B, Peng X-X. 2021. Glutamine promotes antibiotic uptake to kill multidrug-resistant uropathogenic bacteria. *Sci Transl Med* 13:eabj0716.
237. Kitzenberg DA, Lee JS, Mills KB, Kim J-S, Liu L, Vázquez-Torres A, Colgan SP, Kao DJ. 2022. Adenosine Awakens Metabolism to Enhance Growth-Independent Killing of Tolerant and Persister Bacteria across Multiple Classes of Antibiotics. *mBio* 13:e00480-22.
238. Saura P, Kaila VRI. 2019. Energetics and Dynamics of Proton-Coupled Electron Transfer in the NADH/FMN Site of Respiratory Complex I. *J Am Chem Soc* 141:5710–5719.
239. Peng B, Su Y, Li H, Han Y, Guo C, Tian Y, Peng X. 2015. Exogenous Alanine and/or Glucose plus Kanamycin Kills Antibiotic-Resistant Bacteria. *Cell Metab* 21:249–262.
240. Yong Y, Zhou Y, Liu K, Liu G, Wu L, Fang B. 2021. Exogenous Citrulline and Glutamine Contribute to Reverse the Resistance of *Salmonella* to Apramycin. *Front Microbiol* 12:759170.
241. Su Y, Peng B, Li H, Cheng Z, Zhang T, Zhu J, Li D, Li M, Ye J, Du C, Zhang S, Zhao X, Yang M, Peng X. 2018. Pyruvate cycle increases aminoglycoside efficacy and provides respiratory energy in bacteria. *Proc Natl Acad Sci* 115:E1578–E1587.



242. Li Y, Liu B, Guo J, Cong H, He S, Zhou H, Zhu F, Wang Q, Zhang L. 2019. L-Tryptophan represses persister formation via inhibiting bacterial motility and promoting antibiotics absorption. *Future Microbiol* 14:757–771.
243. Ye J-Z, Su Y-B, Lin X-M, Lai S-S, Li W-X, Ali F, Zheng J, Peng B. 2018. Alanine Enhances Aminoglycosides-Induced ROS Production as Revealed by Proteomic Analysis. *Front Microbiol* 9:29.
244. Zhou Y, Yong Y, Zhu C, Yang H, Fang B. 2022. Exogenous D-ribose promotes gentamicin treatment of several drug-resistant *Salmonella*. *Front Microbiol* 13:1053330.
245. Su Y, Peng B, Han Y, Li H, Peng X. 2015. Fructose restores susceptibility of multidrug-resistant *Edwardsiella tarda* to kanamycin. *J Proteome Res* 14:1612–1620.
246. Tang X-K, Su Y-B, Ye H-Q, Dai Z-Y, Yi H, Yang K-X, Zhang T-T, Chen Z-G. 2021. Glucose-Potentiated Amikacin Killing of Cefoperazone/Sulbactam Resistant *Pseudomonas aeruginosa*. *Front Microbiol* 12:800442.
247. Jiang M, Li X, Xie C-L, Chen P, Luo W, Lin C, Wang Q, Shu D-M, Luo C, Qu H, Ji J. 2023. Fructose-enabled killing of antibiotic-resistant *Salmonella enteritidis* by gentamicin: Insight from reprogramming metabolomics. *Int J Antimicrob Agents* 62:106907.
248. Barraud N, Buson A, Jarolimek W, Rice SA. 2013. Mannitol Enhances Antibiotic Sensitivity of Persister Bacteria in *Pseudomonas aeruginosa* Biofilms. *PLOS ONE* 8:e84220.
249. Rosenberg CR, Fang X, Allison KR. 2020. Potentiating aminoglycoside antibiotics to reduce their toxic side effects. *PLoS ONE* 15:e0237948.
250. Lv B, Bian M, Huang X, Sun F, Gao Y, Wang Y, Fu Y, Yang B, Fu X. 2022. n-Butanol Potentiates Subinhibitory Aminoglycosides against Bacterial Persisters and Multidrug-Resistant MRSA by Rapidly Enhancing Antibiotic Uptake. *ACS Infect Dis* 8:373–386.
251. Brochado AR, Telzerow A, Bobonis J, Banzhaf M, Mateus A, Selkrig J, Huth E, Bassler S, Zamarreño Beas J, Zietek M, Ng N, Foerster S, Ezraty B, Py B, Barras F, Savitski MM, Bork P, Göttig S, Typas A. 2018. Species-specific activity of antibacterial drug combinations. *Nature* 559:259–263.
252. Ma X, Fu S, Wang Y, Zhao L, Yu W, He Y, Ni W, Gao Z. 2022. Proteomics Study of the Synergistic Killing of Tigecycline in Combination With Aminoglycosides Against Carbapenem-Resistant *Klebsiella pneumoniae*. *Front Cell Infect Microbiol* 12:920761.
253. Donkor GY, Anderson GM, Stadler M, Tawiah PO, Orellano CD, Edwards KA, Dahl J-U. 2023. The Novel Silver-Containing Antimicrobial Potentiates Aminoglycoside Activity Against *Pseudomonas aeruginosa*. *bioRxiv* <https://doi.org/10.1101/2023.03.15.532855>.
254. Luo M-L, Huang W, Zhu H-P, Peng C, Zhao Q, Han B. 2022. Advances in indole-containing alkaloids as potential anticancer agents by regulating autophagy. *Biomed Pharmacother* 149:112827.
255. Dou Q, Zhu Y, Li C, Bian Z, Song H, Zhang R, Wang Y, Zhang X, Wang Y. 2023. 4F-Indole Enhances the Susceptibility of *Pseudomonas aeruginosa* to Aminoglycoside Antibiotics. *Microbiol Spectr* e0451922.
256. Yarlagadda V, Medina R, Wright GD. 2020. Venturicidin A, A Membrane-active Natural Product Inhibitor of ATP synthase Potentiates Aminoglycoside Antibiotics. *Sci Rep* 10:8134.
257. Bryant RE, Fox K, Oh G, Morthland VH. 1992. Beta-lactam enhancement of aminoglycoside activity under conditions of reduced pH and oxygen tension that may exist in infected tissues. *J Infect Dis* 165:676–682.

258. Moellering RC, Weinberg AN. 1971. Studies on antibiotic synerism against enterococci. II. Effect of various antibiotics on the uptake of 14 C-labeled streptomycin by enterococci. *J Clin Invest* 50:2580–2584.
259. Yee Y, Farber B, Mates S. 1986. Mechanism of penicillin-streptomycin synergy for clinical isolates of viridans streptococci. *J Infect Dis* 154:531–534.
260. Winstanley TG, Hastings JG. 1990. Synergy between penicillin and gentamicin against enterococci. *J Antimicrob Chemother* 25:551–560.
261. Jiafeng L, Fu X, Chang Z. 2015. Hypoionic shock treatment enables aminoglycosides antibiotics to eradicate bacterial persisters. *Sci Rep* 5:14247.
262. Zhao Y, Lv B, Sun F, Liu J, Wang Y, Gao Y, Qi F, Chang Z, Fu X. 2020. Rapid Freezing Enables Aminoglycosides To Eradicate Bacterial Persisters via Enhancing Mechanosensitive Channel MscL-Mediated Antibiotic Uptake. *mBio* 11:e03239-19, /mbio/11/1/mBio.03239-19.atom.
263. Poole K, Gilmour C, Farha MA, Mullen E, Lau CH-F, Brown ED. 2016. Potentiation of Aminoglycoside Activity in *Pseudomonas aeruginosa* by Targeting the AmgRS Envelope Stress-Responsive Two-Component System. *Antimicrob Agents Chemother* 60:3509–3518.
264. Laws M, Shaaban A, Rahman KM. 2019. Antibiotic resistance breakers: current approaches and future directions. *FEMS Microbiol Rev* 43:490–516.
265. Poole K, Gilmour C, Farha MA, Parkins MD, Klinoski R, Brown ED. 2018. Meropenem potentiation of aminoglycoside activity against *Pseudomonas aeruginosa*: involvement of the MexXY-OprM multidrug efflux system. *J Antimicrob Chemother* 73:1247–1255.
266. Herisse M, Duverger Y, Martin-Verstraete I, Barras F, Ezraty B. 2017. Silver potentiates aminoglycoside toxicity by enhancing their uptake. *Mol Microbiol* 105:115–126.
267. Morones-Ramirez JR, Winkler JA, Spina CS, Collins JJ. 2013. Silver Enhances Antibiotic Activity Against Gram-negative Bacteria. *Sci Transl Med* 5:190ra81.
268. Dove AS, Dzurny DI, Dees WR, Qin N, Nunez Rodriguez CC, Alt LA, Ellward GL, Best JA, Rudawski NG, Fujii K, Czyż DM. 2023. Silver nanoparticles enhance the efficacy of aminoglycosides against antibiotic-resistant bacteria. *Front Microbiol* 13:1064095.
269. Prestinaci F, Pezzotti P, Pantosti A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health* 109:309–318.
270. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ. 2020. Antimicrobial Resistance in ESKAPE Pathogens. *Clin Microbiol Rev* 33:e00181-19.
271. McKenna M. 2020. The antibiotic paradox: why companies can't afford to create life-saving drugs. *Nature* 584:338–341.
272. Walesch S, Birkelbach J, Jézéquel G, Haeckl FPJ, Hegemann JD, Hesterkamp T, Hirsch AKH, Hammann P, Müller R. 2023. Fighting antibiotic resistance—strategies and (pre)clinical developments to find new antibacterials. *EMBO Rep* 24:e56033.
273. Narendrakumar L, Chakraborty M, Kumari S, Paul D, Das B. 2023.  $\beta$ -Lactam potentiators to re-sensitize resistant pathogens: Discovery, development, clinical use and the way forward. *Front Microbiol* 13:1092556.
274. Theuretzbacher U, Outtersson K, Engel A, Karlén A. 2020. The global preclinical antibacterial pipeline. 5. *Nat Rev Microbiol* 18:275–285.

275. Mallalieu NL, Winter E, Fettner S, Patel K, Zwanziger E, Attley G, Rodriguez I, Kano A, Salama SM, Bentley D, Geretti AM. 2020. Safety and Pharmacokinetic Characterization of Nacubactam, a Novel  $\beta$ -Lactamase Inhibitor, Alone and in Combination with Meropenem, in Healthy Volunteers. *Antimicrob Agents Chemother* 64:e02229-19.
276. Eckburg PB, Lister T, Walpole S, Keutzer T, Utley L, Tomayko J, Kopp E, Farinola N, Coleman S. 2019. Safety, Tolerability, Pharmacokinetics, and Drug Interaction Potential of SPR741, an Intravenous Potentiator, after Single and Multiple Ascending Doses and When Combined with  $\beta$ -Lactam Antibiotics in Healthy Subjects. *Antimicrob Agents Chemother* 63:e00892-19.
277. Vaara M, Siikanen O, Apajalahti J, Fox J, Frimodt-Møller N, He H, Poudyal A, Li J, Nation RL, Vaara T. 2010. A Novel Polymyxin Derivative That Lacks the Fatty Acid Tail and Carries Only Three Positive Charges Has Strong Synergism with Agents Excluded by the Intact Outer Membrane. *Antimicrob Agents Chemother* 54:3341–3346.
278. Krol E, Werel L, Essen LO, Becker A. 2023. Structural and functional diversity of bacterial cyclic nucleotide perception by CRP proteins. *microLife* 4:uqad024.
279. Park H, McGill SL, Arnold AD, Carlson RP. 2020. Pseudomonad reverse carbon catabolite repression, interspecies metabolite exchange, and consortial division of labor. *Cell Mol Life Sci CMLS* 77:395–413.
280. Emmer M, deCrombrughe B, Pastan I, Perlman R. 1970. Cyclic AMP Receptor Protein of *E. coli*: Its Role in the Synthesis of Inducible Enzymes. *Proc Natl Acad Sci* 66:480–487.
281. Perlman RL, De Crombrughe B, Pastan I. 1969. Cyclic AMP regulates Catabolite and Transient Repression in *E. coli*. *Nature* 223:810–812.
282. Phillips AT, Mulfinger LM. 1981. Cyclic adenosine 3',5'-monophosphate levels in *Pseudomonas putida* and *Pseudomonas aeruginosa* during induction and carbon catabolite repression of histidase synthesis. *J Bacteriol* 145:1286–1292.
283. Collier DN, Hager PW, Phibbs PV. 1996. Catabolite repression control in the *Pseudomonads*. *Res Microbiol* 147:551–561.
284. West SE, Sample AK, Runyen-Janecky LJ. 1994. The *vfr* gene product, required for *Pseudomonas aeruginosa* exotoxin A and protease production, belongs to the cyclic AMP receptor protein family. *J Bacteriol* 176:7532–7542.
285. Suh S-J, Runyen-Janecky LJ, Maleniak TC, Hager P, MacGregor CH, Zielinski-Mozny NA, Phibbs PV, West SEH. 2002. Effect of *vfr* mutation on global gene expression and catabolite repression control of *Pseudomonas aeruginosa*. *Microbiology* 148:1561–1569.
286. Rojo F. 2010. Carbon catabolite repression in *Pseudomonas*: optimizing metabolic versatility and interactions with the environment. *FEMS Microbiol Rev* 34:658–684.
287. Sonnleitner E, Wulf A, Campagne S, Pei X-Y, Wolfinger MT, Forlani G, Prindl K, Abdou L, Resch A, Allain FH-T, Luisi BF, Urlaub H, Bläsi U. 2018. Interplay between the catabolite repression control protein Crc, Hfq and RNA in Hfq-dependent translational regulation in *Pseudomonas aeruginosa*. *Nucleic Acids Res* 46:1470–1485.
288. Moreno R, Martínez-Gomariz M, Yuste L, Gil C, Rojo F. 2009. The *Pseudomonas putida* Crc global regulator controls the hierarchical assimilation of amino acids in a complete medium: evidence from proteomic and genomic analyses. *Proteomics* 9:2910–2928.
289. MacGregor CH, Wolff JA, Arora SK, Phibbs PV. 1991. Cloning of a catabolite repression control (*crc*) gene from *Pseudomonas aeruginosa*, expression of the gene in *Escherichia coli*, and

- identification of the gene product in *Pseudomonas aeruginosa*. *J Bacteriol* 173:7204–7212.
290. Filiatrault MJ, Stodghill PV, Wilson J, Butcher BG, Chen H, Myers CR, Cartinhour SW. 2013. CrcZ and CrcX regulate carbon source utilization in *Pseudomonas syringae* pathovar tomato strain DC3000. *RNA Biol* 10:245–255.
291. Sonnleitner E, Bläsi U. 2014. Regulation of Hfq by the RNA CrcZ in *Pseudomonas aeruginosa* carbon catabolite repression. *PLoS Genet* 10:e1004440.
292. Sonnleitner E, Abdou L, Haas D. 2009. Small RNA as global regulator of carbon catabolite repression in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 106:21866–21871.
293. Moreno R, Fonseca P, Rojo F. 2012. Two small RNAs, CrcY and CrcZ, act in concert to sequester the Crc global regulator in *Pseudomonas putida*, modulating catabolite repression. *Mol Microbiol* 83:24–40.
294. Shawkat H, Westwood M-M, Mortimer A. 2012. Mannitol: a review of its clinical uses. *Contin Educ Anaesth Crit Care Pain* 12:82–85.
295. Albert Leyva, C J Van Groeningen, I Kraal, H Gall, G J Peters, J Lankelma, H M Pinedo. 1984. Phase I and Pharmacokinetic Studies of High-Dose Uridine Intended for Rescue from 5-Fluorouracil Toxicity. *Cancer Res* 44:5928–5933.
296. Van Groeningen GJ, Peters GJ, Nadal JC, Laurensse E, Pinedo HM. 1991. Clinical and Pharmacologic Study of Orally Administrated Uridine. *JNCI J Natl Cancer Inst* 83:437–442.
297. Thompson J, Neutel J, Homer K, Tempero K, Shah A, Khankari R. 2014. Evaluation of D-ribose pharmacokinetics, dose proportionality, food effect, and pharmacodynamics after oral solution administration in healthy male and female subjects. *J Clin Pharmacol* 54:546–554.
298. Pizzorno A, Padey B, Julien T, Trouillet-Assant S, Traversier A, Errazuriz-Cerda E, Fouret J, Dubois J, Gaymard A, Lescure F-X, Dulière V, Brun P, Constant S, Poissy J, Lina B, Yazdanpanah Y, Terrier O, Rosa-Calatrava M. 2020. Characterization and Treatment of SARS-CoV-2 in Nasal and Bronchial Human Airway Epithelia. *Cell Rep Med* 1:100059.
299. Martínez-Salgado C, López-Hernández FJ, López-Novoa JM. 2007. Glomerular nephrotoxicity of aminoglycosides. *Toxicol Appl Pharmacol* 223:86–98.
300. Selimoglu E. 2007. Aminoglycoside-induced ototoxicity. *Curr Pharm Des* 13:119–126.