1

2

Antechodynamics and Antechokinetics: Dynamics and Kinetics of Antibiotic Resistance Molecules

5

F. Baquero,[a,b] R. Cantón,[a,c] A.E. Pérez-Cobas,[a,c], T.M. Coque,[a,c] B. Levin,[d] J. Rodríguez-Beltrán,[a,c].

[a]Department of Microbiology, Ramón y Cajal University Hospital, Ramón y Cajal Institute for Health Research (IRYCIS), Madrid, Spain

[b]Network Center for Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain

[c]Network Center for Research in Infectious Diseases (CIBERINFEC), Madrid, Spain

[d]Department of Biology, Emory University, Atlanta, GE, USA.

13

Address correspondence to F. Baquero, baquero@bitmailer.net.

15
16
17
18
19
20

SUMMARY Pharmacology of antimicrobial drugs comprises pharmacodynamics (PD) and pharmacokinetics (PK). PD refers to studying drugs' mode of action at different concentrations on their molecular targets and the resulting effect(s). PK refers to studying the way(s) by which drugs enter and are distributed to reach their targets in different compartments (such as tissues in the body) and how the local drug concentrations are modified in time, as by metabolism or excretion. PD and PK constitute pivotal knowledge to establish breakpoints used to identify the appropriate antimicrobial agents in therapy of infections. Antibiotic resistance is the biological force opposing antimicrobial pharmacological effects. However, we do not have a term similar to pharmacology for microbial resistance reactions. Here, we propose the new scientific field of Antechology (from classic Geek *antechó,* resistance) studying the dynamics and kinetics of antibiotic resistance molecules, opposing the effect of antimicrobial drugs. Antechodynamics (AD) refers to the study of the molecular mechanisms by which antibiotic molecules are chemically modified or degraded by particular bacterial resistance enzymes (primary effectors), or drive the modification of the antibiotic target inhibition sites by molecules released by the antibiotic action on the microorganism (secondary effectors). Antechokinetics (AK) refers to the study of the processes leading to bacterial spatial cellular (subcellular, pericellular, extracellular) localizations of the molecules involved in antibiotic detoxifying mechanisms. Molecules' local concentrations change over time due to their production, degradation, and, eventually, excretion rates. The AD and AK for the different antimicrobial families, and the relation PD/PK and AD/AK is examined here.

INTRODUCTION

ANTIBIOTIC RESISTANCE DYNAMICS AND KINETICS, AN ACTION AND REACTION PROCESS

"Pharmacodynamics" and "pharmacokinetics" are well-accepted terms in the chemotherapeutic community. The first (PD) refers to the study of the mode of **action** of drugs at different concentrations on their molecular targets and the resulting effect(s). The second (PK) refers to the study of how drugs enter and are distributed to reach their targets in different compartments (such as tissues in the body) and how the local drug concentrations are modified in time, for instance, by metabolism or excretion. In antimicrobial chemotherapy, both PK and PD are considered when choosing a given drug to treat a particular infection, as PK influences PD and vice versa (1, 2). In fact, both PK and PD are considered by International Committees on Antibiotic Susceptibility Testing (as CLSI in the US or EUCAST in Europe) to determine breakpoints categorizing microorganisms as susceptible and resistant to agents approved for use in treating infectious diseases.

Antibiotic resistance is the opposite biological force, *reaction*, to antimicrobial pharmaceutical *action*. However, we do not have a term opposing "pharmacology". The question of how to designate the "science of resistance" was informally discussed in the 1970s by one of the authors of this work (FB) with the distinguished French microbiologist Yves A. Chabbert (1921-2018) – from the Pasteur Institute and one of the fathers of antibiotic susceptibility testing– and the distinguished Greek pharmacologist John Kosmidis (1936-2016), who immediately coined the word "Antechology". The verb "to resist" in classic Greek is ἀντέχω (antechó). In this review, we propose an antechological reaction (resistance) mediated by bacterial molecules acting as the primary effectors of drug-specific resistance in a way that explicitly opposes, by degradation,

122 extrusion or chemical modification, the pharmacological action of antimicrobial molecules on

123 primarily susceptible cells. We also consider here the secondary effectors. These are the bacterial

124 molecules, eventually resulting from the effect of antibiotics on the cell, that are specifically

125 triggering the synthesis of the primary effectors, or altering the antibiotic target (Fig 1). The

126 concept of *reaction* excludes from antechology the resistance mechanisms associated with the

127 intrinsic resistome, or the random, unspecific mutational alterations of the antibiotic targets, as,

128 even if involved in selective processes, they do not constitute any specific *reaction* to the antibiotic

129 effect.

130 As in drug pharmacology (pharmacodynamics), resistance mechanisms can be studied by

131 measuring their effects on their targets, the antimicrobial agents; this is **Antechodynamics**. The

132 molecular effectors of the "mechanisms of resistance" to most antimicrobials have been identified,

133 as have the involved genes. However, the details about how they exert their antibiotic deactivation

134 are not always well determined.

135 Similarly, we should also consider how the primary or secondary effectors of antibiotic resistance

136 are produced in different periods of cellular time (as growth phases), under or without induction,

137 how their concentrations vary in different intracellular and extracellular compartments depending

138 on carriers, and how they are affected by natural processes of degradation, including in the

139 environment. This is **Antechokinetics**. Certainly, antechodynamics and antechokinetics

140 parameters interact between them and with pharmacodynamics and pharmacokinetics, to provide

141 the complete frame of antimicrobial action. For example, PK/PD parameters (C_{max}, AUC,

142 t/MIC,..) correlate with the *in vivo* efficiency of antimicrobials. Conversely, AK/AD parameters

143 (K_{cat}, K_m, V_{max}…) correlate with a bacterium's resistance level (i.e., its MIC). The interplay

144 between PK/PD and AK/AD is ultimately responsible for the success or failure of the antimicrobial

145 treatment.

146 Antibiotic resistance mechanisms (note that in our view antechodynamics does not include

147 mutational mechanisms, only molecules acting on antibiotics) have been reviewed on many

148 occasions (3, 204). Strikingly, research in the field of the kinetics of antibiotic resistance

149 mechanisms has been disdained (4).

ANTECHODYNAMICS

151 Antechodynamics refers to the study of the molecular mechanisms by which antibiotic molecules

152 are chemically modified or degraded by particular bacterial resistance enzymes (primary

153 effectors), or drive the modification of the antibiotic target inhibition sites by molecules released

154 by the antibiotic action on the microorganism (secondary effectors). In both cases, the result is the

155 detoxification of the antibiotic agent. Efflux pumps, as multimolecular entities poorly specific in

156 molecular interaction/detoxification with particular antibiotics, are not directly counteracting the

157 mode of action of antibiotics and will be treated in more detail in the antechokinetics section. In

158 fact, many of these macromolecular complexes can specifically recognize antibiotic molecules and

159 interact chemically with them to proceed to the extrusion from the cell, in a process that could also

160 be considered from an antechodynamic perspective. Antechodynamics also deals with the

161 combined effect of resistance mechanisms in providing resistance phenotypes to particular drugs.

162

163

Primary effectors of antibiotic resistance: modifying and drug-degrading enzymes

Antibiotic resistance mechanisms are frequently based on drug-inactivation enzymes, hydrolyzing or modifying the antimicrobial agent (5). The **affinity** of the resistance enzyme for the antibiotic substrate (target) is classically measured by **the *Km* value**, determined by incubating the enzyme with varying substrate concentrations. This affinity expresses the intensity of **substrate recognition,** based on the functional **dynamics of ligand binding** (6). The **strength** of the link between the enzyme and the antibiotic depends on intermolecular interactions between these partners. This can be evaluated by **all-atom molecular dynamics** (MD) computational simulations (6). An alternative is **molecular docking**, able to model possible binding and provide scoring affinity functions by using a known tridimensional structure of a resistance enzyme, and the antibiotic substrate (7). Depending on the concentration of the antibiotic, a proportion of binding sites are filled with the substrate molecule; in fact, the *Km* refers to that proportion. Indeed, the direct functional part of an antibiotic-detoxifying enzyme is the **active site** within the folded protein, where the antibiotic enters a pocket or groove and is captured by temporary hydrogen bonds, forming an enzyme-antibiotic complex. The antibiotic should bind at this (or at the vicinity) specific region, which catalyzes the detoxifying chemical reaction. It is formed by the folding pattern of the protein and appears as a pocket or groove that is shaped to accommodate the antibiotic. The difference among members of a single antibiotic family (as beta-lactams, or aminoglycosides) to resist a particular detoxifying enzyme (beta-lactamases or aminoglycoside-modifying enzymes, respectively) essentially depends on the **degree of molecular adjustment** to the active site. Because of that, the evolutionary biology of antibiotic-inactivating enzymes consists of the acquisition of mutations altering the topology of the active site to accommodate

187 new (previously non-accepted) compounds. That explains that these "modified sites" are

188 frequently less effective in deactivating old antibiotics. For instance, acquiring resistance to third-

189 generation cephalosporins normally results in less enzymatic activity over aminopenicillins

190 (antagonistic pleiotropy or collateral susceptibility). However, the active site can still accept poorly

191 bound molecules of old drugs, so that these "modern" conformations can be selected by old drugs

192 (8).

193 It is to be noted that a high ligand binding does not necessarily correlate with a high enzymatic

194 activity, but is required for such a function.The number of substrate molecules transformed per

195 unit of time by an enzyme (its turnover rate) is traditionally expressed by the kcat value. Therefore,

196 enzymatic efficiency depends on both the affinity of the enzyme for its substrate (Km) and the

197 turnover rate of the enzyme (kcat). Traditionally, this has been expressed by the ratio kcat/Km. In

198 general, according to classic enzymology (9), what is expected for the catalytic reaction (covalent

199 bond making and bond breaking) of a large molecule (an enzyme) and a small molecule (as an

200 antibiotic) is to have a kcat/Km value ranging from 10^8 to 10^9 $M^{-1}s^{-1}$. Many antibiotic detoxifying

201 enzymes have reached an "antechological perfection", where they are no longer limited by bond

202 making and bond breaking, but by the diffusion of the substrate in and out of the active site.

203 Therefore, their catalytic efficiency might depend more on the possibilities of enzyme-antibiotic

204 encounters, and the diffusion hurdles might be critical in the process, as has been shown for beta-

205 lactamases (10). Moreover, the catalytic efficiency and diffusion could also depend on the

206 macromolecular crowding of the cells (11).

207

208

209   **Beta-lactams**.

210   In the case of beta-lactams, the detoxification mechanism occurs by the action of a protease, the

211   beta-lactamase, a globular protein composed of alpha-helices and beta-pleated sheets. In the case

212   of A, C or D beta-lactamases, detoxification is based on nucleophilic Serine residue in the enzyme

213   active site, which attacks the carbonyl moiety of the beta-lactam to form an acyl-enzyme

214   intermediate; other amino acids of the vicinity may contribute to substrate binding, facilitating

215   proton transfer, or orienting catalytic residues (12, 13). In class B beta-lactamases, the hydrolytic

216   reaction is facilitated by one or two essential zinc ions in the active site (14, 15). More than 2,300

217   potential beta-lactamases in 673 bacterial genera have been detected (16).

218   **Aminoglycosides.**

219   Aminoglycosides are deactivated by *N*-acetyltransferases (AAC), *O*-phosphoryltransferases

220   (APH), and nucleotydyltransferases (ANT, frequently known as adenyl transferases, AAD),

221   modifying the antibiotic molecule. Most acetyltransferases (AACs) belong to the GCN5

222   superfamily of acyltransferases and include slightly different ApmA enzymes (17). AACs transfer

223   an acetyl group to a free aminoglycoside amino group, APH transfers a phosphate group to a free

224   hydroxyl, and also ANT or nucleotidyl transferase transfers a nucleotide to a free hydroxyl. The

225   consequence is altering drug transport or the binding of the drug at the site of antibacterial action,

226   the 16S subunit at the tRNA acceptor site A in the 30S ribosomal unit (18–20). AAC(1) and

227   AAC(3) target the amino groups found at positions 1 and 3 of the 2-deoxystreptamine ring,

228   whereas AAC(2′) and AAC(6′) target amino groups found at the 2′ and 6′ positions of the 2,6-

229   dideoxy-2,6-diaminoglucose ring. Typically acetylation interferes with the binding of

230   aminoglycoside to 16S rRNA. O-Phosphorylation is exerted at aminoglycoside positions 3', 2",

231   3'off', 6, 9, 4 and 7" (21). The process involves a succession of ATP binding to the enzyme (acting

232 as monomers or dimers), followed by binding and phosphorylation of the aminoglycoside, release

233 of the modified inactivated drug, and rate-limiting dissociation of ADP (22). Adenylation follows

234 the formation of a complex with adenosine monophosphate (AMP) and the aminoglycoside, with

235 the involvement of pyrophosphate. A catalytic base is probably involved in a direct AMP transfer

236 mechanism from nucleotide to the aminoglycoside. The chemical modification occurs at positions

237 2, 3, 4, 6, and 9 of substrate aminoglycosides.

238 **Macrolides, lincosamides, streptogramins**

239 As a first example, macrolide 2´phosphotransferase is an enzyme that phosphorylates the

240 2´hydroxyl group of the C5-linked desoxamine or mycaminose moiety of macrolides and

241 ketolides. Phosphorylation involves the transfer of gamma-phosphate group of GTP to these

242 antibiotics. The C5 phosphorylation prevents the binding of the drug by specific hydrogen bond

243 interactions to the A2058 and A2059 of 23S rRNA, detoxifying the antibiotic action. There are at

244 least 15 types of macrolide **phosphotransferases** (MPHs) differing in the spectrum of macrolide-

245 ketolide inactivation (23, 24). Erythromycin can also be inactivated by the action of macrolide

246 **esterases**. Esterases act on the critical ester-bond involved in the construction of the macrocyclic

247 structure, linearizing and detoxifying the molecule, now unable to attach to the ribosomal binding

248 target site to produce the bacteriostatic effect (25). There are several macrolide esterases in a

249 variety of organisms (23). However, some macrolide-like compounds as ketolides, telithromycin,

250 or solithromycin exhibit moderate to strong cidality against several bacterial species; probably that

251 depends on the association/dissociation kinetics with the ribosome; long-term association leads to

252 a bactericidal effect (26). The structure of the rRNA binding site (long-distance base pair) might

253 also contribute to such association/dissociation kinetics (27). The more tightly associated

254 molecules are possibly less prone to being inactivated by detoxifying enzymes. Long-term

255   exposure to macrolides might produce bactericidal effects (28). However, the dissociation constant

256   (K_{diss}) is very low for macrolides and ketolides (10^{-8} to 10^{-9}) (29).

257   Lincosamides (lincomycin, clindamycin) are inactivated by **nucleotidyltransferases** (NTAses) in

258   the 3´-OH group of the drug, probably with the cooperation of chelation of magnesium cation. The

259   modified lincosamide can not bind to 23S rRNA in the 50S subunit of the ribosome and cannot

260   interfere with the peptidyltransferase reaction. In the microbial world, there are a big variety of

261   NTAses, probably over 120 potential enzymes (30).

262   Streptogramins (as streptogramin B, virginiamycin, pristinamycin, dalfopristin) are mostly

263   inactivated by **acetyltransferase** enzymes (31). In addition, **nucleotidyltransferases,** also

264   inactivating lincosamides, and **hydrolases** of streptogramins are inactivating enzymes (32).

265   **Phenicols**

266   **Phenicol acetyl-transferases** are among the predominant resistance mechanisms to

267   chloramphenicol and related drugs. These enzymes have amino acids with side chains involved in

268   catalysis (acetylation), which depends on the appropriate folding and packing of the polypeptide

269   chains, frequently forming heterotrimers. The process includes deprotonation of the primary (C-

270   3) alcohol of the antibiotic, and the resulting oxyanion attacks the carbonyl carbon of the acetyl

271   moiety of acetyl-CoA. The product is a tetrahedral intermediate sharing a hydrogen atom with the

272   side chain oxygen of a serine residue, resulting in a close approximation of two oxygen atoms. The

273   collapse of the tetrahedral intermediate yields the inactivated drug (33). The resulting chemical

274   alteration of the antibiotic prevents the exertion of ribosomal peptidyltransferase activity. **Fusidic**

275   **acid** can be inactivated by chloramphenicol acetyltransferases (34).

276

Tetracyclines.

Tetracycline molecules (including the modern compounds tigecycline, eravacycline, and omadacycline) can be degraded (destructed) by flavin-dependent **monooxygenases** (tetracycline destructases) originally discovered in *Bacteroides fragilis* (35–38). Tetracycline destruction prevents access and binding to the 30S subunit's helix 34 of the 16S rRNA, which overlaps the anticodon stem-loop of the A-site tRNA, interfering with ribosomal protein synthesis.

Fluoroquinolones.

A variant of the gene encoding aminoglycoside **acetyltransferase** AAC(6′)-Ib inactivates fluoroquinolones by *N*-acetylation at the amino nitrogen on its piperazinyl substituent (39)**.** In addition, *Labrys portucalensis* F11, an Alphaproteobacteria, specialized in degrading fluoro-organic compounds, uses a **monooxygenase** replacing fluorine with a hydroxyl group, inactivating fluoroquinolones, particularly in the presence of high acetate. A similar case occurs in *Rhodococcus* (40). Fortunately, these mechanisms have not spread into pathogenic bacteria.

Fosfomycin.

The activity of fosfomycin can be impaired by Mn^{++}-dependent **glutathione thiol-transferases,** also known **as metallo-glutatione transferases** (Fos enzymes) (41). FosA conjugate glutathione (GSH; L-γ-glutamyl-L-cysteinyl-glycine) or BSH/L-cysteine in the fosfomycin oxirane ring. Glutatione nucleophilic attack and degradation of fosfomycin is facilitated by the K^+ ion binding close to the active site, which increases the rate of reaction ~100-fold (42, 43). Conjugated fosfomycin is unable (or greatly reduced) to exert its mode of action on the active site cysteine residue of MurA (UDP-N-acetylglucosamine enolpyruvyl transferase) which is essential for bacterial cell wall synthesis.

299   **Rifampicin**.

300   Low-level rifampicin inactivation occurs by **glycosyl-transferases**, **nucleotidylyl-transferases**,

301   **phospho-transferases** and **monooxygenases**. Still, these enzymes have not been spreading in

302   most pathogens (44).

303   **Glycopeptides and lipopeptides**

304   To our knowledge**,** vancomycin-degrading enzymes have not been found in bacteria, but

305   microsomes from hepatic cells can fragment the aminoglycoside and the polypeptide parts of

306   vancomycin, probably involving mixed-function **oxidases or monooxygenases** (45). More

307   research is requested to find similar functions in bacterial organisms, eventually leading to

308   vancomycin resistance. However, a **deacylase** heterodimeric enzyme was found in *Actinoplanes*

309   species, which can detoxify members of teicoplanin family of glycopeptides, also acting on the

310   lipid tail and inactivating daptomycin, a lipopeptide antibiotic. In addition, daptomycin is

311   detoxified by a **serin protease with hydrolase activity** in actinomycetes (46).

312   **Polymyxins.**

313   Polymyxins are cyclic peptides resistant to degradation by the currently known proteases, probably

314   due to their cyclic structure, the presence of unusual amino acids, the attached lipid tail, and the

315   strong binding with the bacterial envelope.

316   **Sulphonamides**.

317   Little is known about bacterial sulphonamides enzymatic degradation. However, *Microbacterium*,

318   a genus belonging to Actinomycetota, can use sulphonamides as a single carbon source, using two

319 **flavin-dependent monooxygenases** possessing an acyl-CoA dehydrogenase domain and a **flavin**

320 **reductase** (47).

321 **Nitrofurantoin.**

322 Some environmental strains that are capable of using nitrofurantoin as a source of carbon and

323 energy; 1-aminohydantoin and semicarbazide were detected as nitrofurantoin biotransformation

324 products; however, inactivating enzymes have not been well characterized (48).

325

Secondary effector molecules triggering the expression of genes involved in antibiotic resistance.

328 Here, we consider the secondary effectors of specific antibiotic resistance counteracting antibiotic

329 action: those molecules that start the process(es) by which specific antibiotic detoxification occurs

330 by primary effectors. In some cases, these molecules are encoded in the genome of susceptible

331 organisms but either are not expressed or have a constitutive remarkably low expression,

332 insufficient to provide a significant resistance phenotype. However, they can be overexpressed

333 (de-repressed) in the presence of antimicrobials or by effector bacterial molecules resulting from

334 the early action of antimicrobials on bacterial cells. The processes more frequently involved are:

335 1) inducible hyperexpression of drug-degrading or modifying enzymes and 2) inducible

336 modification of the antibiotic target site. Such gene expression leads to an antibiotic-resistant

337 phenotype. The scarcely known field of molecules involved in the induction of genes involved in

338 antibiotic efflux pumps, including antibiotics but also many non-antibiotic unspecific inducers of

339 extrusion of a broad spectrum of chemical structures, will be mostly treated in the section of

340 Antechokinetics. In this section, we briefly mention the induction of efflux pumps when the

341   antibiotic is presumptively considered the main (more specific) inducer of pump-mediated

342   resistance, as in the case of antibiotic-triggered RNA-mediated regulation processes (49).

343   **Beta-Lactams**

344   The transcription of a group of beta-lactamase chromosomal enzymes, typically Class C serine

345   beta-lactamases (frequently known as cephalosporinases, as AmpC), is strongly repressed under

346   natural circumstances by the AmpR protein, a LysR-type transcriptional regulator. That occurs in

347   certain clinically relevant microorganisms such as *Enterobacter cloacae* complex, *Klebsiella*

348   *aerogenes, Citrobacter freundii, Morganella morganii, Serratia marcescens* complex, or

349   *Pseudomonas aeruginosa.* Their expression probably involves a high fitness cost in the absence

350   of beta-lactams. The presence of the antibiotic is detected by the early effects it produces on the

351   bacterial cell wall, releasing **"signaling" murein fragments** (**muropeptides)**, typically NAG-

352   NAM disaccharides attached to a peptide chain containing 2- to 5 amino acid residues (50, 51).

353   Such muropeptides are transported by AmpG symporter permease into the cytoplasm, and their

354   catabolites, as 1,6-anhydroMurNAc-peptides, bind the uridine diphosphate (UDP)-N-

355   acetylmuramic acid (52). Such complexes competitively displace UDP-MurNAC peptides that

356   maintain the AmpR repression, acting as a negative regulator of AmpR, a tetramer molecule that

357   recognizes D-ala-D-ala motif of the muropeptide, resulting in the activation of *ampC* transcription

358   and AmpC beta-lactamase hyperproduction resulting in β-lactam resistance (53–55). The reason

359   explaining the weak induction of AmpC in strains of *Serratia nevei* remains elusive at the time of

360   writing (56).

361   Resistance to beta-lactam agents in Gram positives can be also inducible by the presence of

362   antibiotics. In *Staphylococcus aureus* the activation of the synthesis of *blaZ,* the gene coding beta-

363   lactamase is regulated by **the transmembrane sensor/signal transducer proteins BlaR1 and**

364 **MecR1.** The extracellular part of BlaR1 interacts with the antibiotic, activating the intracellular

365 proteolytic activity of BlaR1 which cleaves the BlaI repressor and allows the synthesis of the beta-

366 lactamase blaZ. A similar mechanism of induction (involving *mecRI* and *mecI*) applies to the

367 synthesis of an alternative beta-lactam insensitive PBP2a encoded by *mecA* in MRSA (57, 58). In

368 *Streptococcus*, β-lactam antibiotics at low concentrations induce a decrease in the protein targets

369 of these antibiotics (penicillin-binding proteins, PBPs) using the response **regulator protein**

370 **CiaR**, which mediated **transcriptional increase of ccn-microRNAs** (ccn: central communication

371 networks proteins) **and PBP degradation of pbp-mRNAs** (59).

372 **Aminoglycosides**

373 The expression of aminoglycoside acetylases and adenylylases located in type 1 integrons was

374 proposed to be controlled by an aminoglycoside-sensing riboswitch RNA, influencing integron

375 internal recombination (60). However, further work did not confirm such a view and proposed that

376 the hyperexpression was due to the **increased translation rate of the integron cassettes** (61, 62).

377 . **16S rRNA methyltransferases** acting on the aminoacyl site of 16S rRNA, where the binding of

378 aminoglycosides occurs (A1408), confers high-level resistance to aminoglycosides. At least six

379 types of these enzymes have been detected: ArmA, RmtA, RmtB, RmtC, RmtD, NpmA and

380 NpmC (63–65). If in the current clinical resistance landscape ArmA has been frequently found in

381 mobile genetic elements, from plasmids to ISCR elements (66). Expression analysis has shown

382 that **aminoglycoside stress increases the expression of 16S rRNA methyltransferases**,

383 including RsmI (67). Proteins similar to the previously mentioned 16S rRNA methylases are found

384 in aminoglycoside-producing actinomycetes, suggesting that they might be inducible by low

385 aminoglycoside concentrations.

386   Any decrease in the aminoglycoside concentration inside the cell will reduce the antimicrobial

387   effect. Subinhibitory concentrations of kanamycin, probably disturbing the cell envelope, induce

388   the acriflavine resistance protein **AcrD**, a **multidrug efflux pump** extruding aminoglycosides

389   (also novobiocin, and fusidic acid), a member of the RND family of transporters energized by

390   proton motive force. Efflux of aminoglycosides by the transporter should produce coupled

391   transmembrane movement of H^+. Aminoglycosides are captured in a binding site located within

392   the ceiling of the central cavity of a AcrD trimer. Thus, it is likely that AcrD is capable of picking

393   up aminoglycosides via this central cavity (68–70).

394   **Macrolides, lincosamides and streptogramins (MLS)**

395   The antimicrobial effect of MLS antibiotics, mostly based on the dissociation of peptidyl-tRNAs

396   from the ribosome resulting in translational attenuation (reduced protein synthesis), has been

397   proposed to be the mechanism by which the genes involved in resistance (typically *erm(B)* gene)

398   is induced. Erm resistance proteins (about 50 orthologous genes have been reported) demethylate

399   a single adenine (A2058) in nascent 23S rRNA, a component of the large (50S) ribosomal subunit.

400   The effect of this **23S-methyl-transferase** is that the binding of MLS antibiotics to their target is

401   impaired. In the absence of antibiotics, the methyl-transferase gene is inactive (non-transcribed in

402   the normal folding structure of the mRNA of the *erm* gene) due to an attenuator upstream from the

403   structural gene. The presence of the MLS antibiotic leads to physical rearrangements of the mRNA

404   folding, exposing and stabilizing the 23S methyltransferase secondary sequence and allowing

405   ribosomes to process with the translation of the resistance enzyme (71). The MLS effect of

406   inducing resistance ultimately depends on ribosome stalling of the leader mRNA at Arg/Lys-X-

407   Arg/Lys motifs (72, 73). A putative-inducing signal may be the ribosomal release of short peptides

408   after the stalling event (74). In addition, it has been suggested that macrolides might allow passage

409   of some nascent peptides, contributing to a "selective translation" and peptide bond modulation

410   (75). A new mechanism of inducible erythromycin resistance based on ribosome recycling has

411   been observed in *L. monocytogenes*. The process is mediated by a **GTPase** named HflXr, a

412   **ribosome splitting factor** that is specifically produced in the presence of antibiotics targeting the

413   ribosome, such as macrolides and lincosamides (76).

414   **Phenicols**

415   Similar dynamics of inducible resistance occur with phenicols. In this case, the **acetyl-transferase**

416   and **CmlA efflux pump** genes are regulated by a translation attenuation process. In the absence

417   of antibiotics, the ribosome binding site sites are sequestered by the secondary structure of their

418   mRNA. Induction results when the ribosome becomes stalled at a specific site in the nine-codon

419   leader as a consequence of antibiotic action. The resulting alternative mRNA stem-loop structure

420   discloses the ribosome binding site allowing translation of chloramphenicol resistance genes (77).

421   In the case of CmlA efflux pump, the protein is localized in the inner membrane. It extrudes

422   chloramphenicol in a process driven by the proton motive force (78). **The Cfr rRNA**

423   **methyltransferase,** methylating 23S rRNA at position A2503, has a broad detoxification range

424   including chloramphenicol (79). Finally, the **ATP binding cassette proteins** PoxtA and OptrA,

425   are able to reduce the affinity of chloramphenicol (and linezolid) from the ribosome, resulting in

426   chloramphenicol resistance (see below, oxazolidinones-resistance) (80).

427   **Tetracyclines**

428   Tetracycline binds to the 30S ribosomal subunit preventing the access of charged tRNAs to the A-

429   site. A widespread mechanism of tetracycline resistance is the direct induction by tetracycline of

430   an specific efflux pump, TetA. In the absence of tetracycline, the transcriptional repressor TetR

431   constitutively binds the *tetA* promoter and inhibits the expression of the TetA resistance gene (81).

432   The **direct binding of tetracycline to the *tetR* repressor** leads to its dissociation from the DNA

433   and drives *tetA* expression, leading to antibiotic resistance. Another important mechanism of

434   tetracycline resistance is mediated by secondary effectors such as ribosome protection proteins

435   (82) induced by tetracycline exposure, and probably originated (for self-protection) in the original

436   tetracycline producer, *Streptomyces rimosus*. The proteins TetM and TetO are frequently found in

437   clinical strains, both Gram positive and Gram negative. These proteins are able to **displace**

438   **tetracyclines** (not glycylcyclines, as tigecycline) from their target, in a way resembling the binding

439   of elongation factor G to the ribosome, allowing the reassumption of protein synthesis. The

440   conformation of the tetracycline binding site is probably modified by TetM, preventing rebinding

441   of the drug (83). The process is favored by the GTPase hydrolysis.

442   **Fluoroquinolones**

443   Fluoroquinolones act by binding at the DNA-ligation active site required for topoisomerases

444   (topoisomerase IV and DNA gyrase) unwinding of the DNA, leading to DNA strand breaks and

445   aborting the replication process. **Qnr pentapeptide repeat protein** protects the topoisomerases-

446   DNA interface by binding to the topoisomerases units and the holoenzymes (84). Qnr proteins

447   occur both in the chromosome and in bacterial plasmids. Subinhibitory concentrations of

448   ciprofloxacin produce the induction of Qnr (*qnrS1*) by a mechanism independent of SOS response.

449   **Qnr induction requires** intact **integration host factors (LhfA and LhFB)**, specific DNA-

450   binding proteins involved in transcriptional control, and probably **DnaA** (initiating the process of

451   replication) influences the induction process. However, the possible natural Qnr inducers remain

452   elusive (85).

453   **Fosfomycin**

454   Fosfomycin resistance is controlled by the bacterial two-component **signal transduction system**

455   **CpxAR**. Fosfomycin, altering the construction of the cell wall, triggers this envelope stress

456   response system. CpxR directly represses the expression of two genes, *glpT* and *uhpT*, which

457   encode fosfomycin transporters into the *c*ell (86).

458   **Sulphonamides and Trimethoprim**

459   **The** antibacterial effect of sulfonamides depends on inhibiting the bacterial dihydropteroate

460   synthase (DHPS) through chemical mimicry of its co-substrate p-aminobenzoic acid (pABA).

461   Resistance is frequently mediated by the acquisition of *sul genes* (present in many mobile genetic

462   elements), which code for sulfa-insensitive, divergent DHPS enzymes. The reason for insensibility

463   is the sulphonamide binding in the DHPS pABA binding sites. Sul encodes an alternative **DHPS**

464   **synthase with an additional phenylalanine** residue lacking in sensitive DHPS, which results in

465   a conformational change, blocking the sulphonamide target. It can be suggested that the induction

466   of the expression of *sul* genes could be dependent on the sulfonamide effect decreasing the

467   **thymidine levels** (87). Similarly, trimethoprim resistance is typically achieved by acquiring

468   trimethoprim-insensitive dihydrofolate reductases (DHFR) encoded in dfr genes or by the

469   overexpression of the endogenous DHFR enzyme folA. Indeed, it has been shown that the **two-**

470   **component system PhoP/PhoQ** is involved in trimethoprim resistance under the regulation of

471   **MgrB**, such a way modulating the expression of FolA by influencing the synthesis of thymidine

472   (88).

473   **Glycopeptides and Lipopeptides**

474   Vancomycin resistance (particularly worrisome in *Enterococcus*) mostly depends on the

475   expression of the resistance gene *vanA*. VanA, a **d-Ala-d-lac ligase**, mediates the replacement of

476   an ester for an amide in the peptide target molecule, converting d-Ala-d-Ala in d-Ala-d-lac in the

477   terminal aminoacids in Lipid II, by forming five hydrogen bonds and multiple hydrophobic van

478   der Waal forces, such a way altering the vancomycin binding site and reducing by 1000-fold the

479   activity of the antibiotic (89). The induction of *vanA* (and the accompanying gene cluster) depends

480   on a canonical **two-component regulation system** composed of the transmembrane sensor

481   histidine kinase VanS and its cytoplasmic transcriptional regulator VanR, which allows *vanA*

482   transcription (90). The presence of vancomycin is detected by the membrane sensory kinase VanS,

483   which phosphorylates and activates VanR, a transcription regulator that drives the expression of

484   the vanH**AX** resistance operon. Induction by internal signals cannot be excluded, as cell wall

485   precursor accumulation (91). Interestingly, subinhibitory concentrations of beta-lactam agents

486   might induce heterogeneous vancomycin intermediary-resistance in *Staphylococcus aureus* (92).

487   Daptomycin resistance in *Enterococcus* is mediated by the LiaFSR system, a three-component

488   **regulatory system responsive to cell envelope stress** produced by the antibiotic. The membrane

489   stress response is controlled by **sensor histidine kinase-response** regulator pairs communicating

490   by signal transduction. LiaR regulates the expression of the gene LiaX, which can bind daptomycin

491   and regulate cell membrane remodeling, adapting the cell membrane to the DAP "attack" in the

492   words of Axell-House et al. (93).

493   **Polymyxins**

494   Polymyxins (polycation proteins such as colistin or polymyxin B) target the negatively charged

495   bacterial lipopolysaccharide (LPS). Physical disturbance of the LPS layer can be associated with

496   other effects, such as damaging the function of essential respiratory enzymes located in the

497   cytoplasmic membrane. Resistance results from chemical modifications of the LPS. Such

498   processes involve the activation (triggered by extracytoplasmic Mg^{++} and Ca^{++} concentrations)

499   of **two-component systems PhoP/PhoQ and PmrA/PmrB**, involving an inner membrane sensor

500   and a cytoplasmic regulator. In *Salmonella*, the result is the expression of PagL, a **deacetylase of**

501   **the lipid A** moiety of the LPS. In *E. coli*, the two-component systems activate EptA (PmrC) and

502   ArnT (PmrK), respectively **phosphoethanolamine and 4-amino-4-deoxy-L-arabinose lipid A**

503   **transferases**, which results in a reduced negative charge and thus less colistin binding, leading to

504   resistance and heteroresistance (94, 95). The widespread *mcr* plasmid genes determining colistin

505   resistance have probably originated from EtpA orthologs, encoding phosphoethanolamine

506   transferase which alters the structure of the binding site of colistin to lipid A in the bacterial

507   lipopolysaccharide layer membrane (96)*;* indeed, *mcr-9* is inducible by low antibiotic

508   concentrations of polymyxins (97).

509   **Oxazolidinones**.

510   Oxazolidinones (as linezolid) interact with the peptidyl transferase center of the bacterial

511   ribosome, inhibiting protein synthesis. Oxazolidinone resistance gene, *cfr*, mediates resistance not

512   only to linezolid, but also to phenicols, lincosamides, pleuromutilins, and streptogramin A type

513   antibiotics by encoding a **methyltransferase that modifies the 23S rRNA** at position A2503 (79).

514   Tedizolid is not affected by this resistance mechanism, as presents improved affinity not only

515   against wild-type 23S rRNA but also Cfr-methylated 23S rRNA (98). In addition, linezolid is

516   deactivated (together with chloramphenicol) by PoxtA and OptrA, apparently non-inducible **ATP**

517   **binding cassette (ABC) proteins** of the F subtype, which distorts the P-site tRNA in the ribosome

518   and contributes to reducing the affinity of the drugs for their binding site, in a sense "brushing"

519   the drug from the ribosome (99).

520

Fusidic acid

Fusidic acid prevents the release of elongation factor G (EF-G) from the ribosome due to changes in EF-G conformational flexibility. After each translocation event, the A ribosomal site should be vacant to allow incorporation of the next incoming aminoacyl-tRNA species. Fusidic acid deactivation is produced by the FusB protein family, which encodes an **EF-G-binding protein**, acting when EF-G is either unbound or bound to the ribosome (100). The origin of these target protection small proteins is unknown, but they certainly preceded the anthropogenic production of fusidic acid (101). FusB seems to be a fusidic acid-inducible protein. Induction probably involves (as in the case of methylase genes in macrolide resistance) a system of **translational attenuation,** involving fusidic-acid ribosomal stalling, resulting in the folding of the *fusB* leader mRNA; this folding releases the *fusB* Shine-Dalgarno sequestration, allowing transcription of the EF-G-binding protein that detoxify fusidic acid (102).

Nitrofurantoin

Nitrofurantoin, furazolidone, and nitrofurazone's antibiotic action depends on bacterial nitroreductases (mostly NfsA and NfsB), NAD(P)H-dependent flavoenzymes which activate the toxicity of the compounds. In fact, hyperexpression of these enzymes (for instance involving *cpxA/R* **two-component system** signaling) increases nitrofurantoin activity. Resistance to nitrofurans could result from a lower transcription of nitroreductases. Transcription/expression of *nfsA* is repressed by the oxidative stress **transcriptional regulator OxyR** and (postranscriptionally) by a small anti-sense RNA (*sdsN137*) in *E. coli,* and perhaps also the multidrug resistance **r**egulator *mprA* (103, 104). As OxyR is activated by oxidative and nitrosative stress*,* it should reduce nitroreductase transcription and might thus inactivate nitrofurantoin effect.

Combined effects of antibiotic resistance molecules

Pharmacodynamic drug-drug interactions (DDIs) occur when the pharmacological effect of one drug is altered by that of another drug in a combination regimen. DDIs often are classified as synergistic, additive, neutral or antagonistic (105). Antechological resistance mechanism-mechanism interactions (MMIs) can be expected when molecules involved in antibiotic resistance might have different combined effects influencing antibiotic detoxification. In the multiresistant organisms present in nosocomial infections, an apparent "functional redundancy" of beta-lactamases, for instance multiple different carbapenemases in the same strain is not an infrequent finding (106). In some cases, this might produce a kind of polyploidy, but other explanations cannot be excluded. The reactive production of efflux pumps reduces the accumulation of antibiotics inside of the bacterial cells and might facilitate the induction of primary or secondary resistance effectors before the drug causes irreversible cell damage (107). This important topic of interactions between antibiotic resistance mechanism has been recently reviewed (3).

Metabolic molecules influencing antibiotic detoxification

A recent field of research in antibiotic resistance is the effect of metabolism on antibiotic resistance. In a sense, metabolic molecules can act as "non-canonical", eventually poorly specific mechanisms of antibiotic detoxification, highly dependent on the nutritional and environmental conditions of the microorganism. Such an effect casts doubts about using the standard determination of minimal inhibitory concentrations in rich media as the only pharmacodynamic function used in susceptibility testing (108). For instance, rich media might contribute to a higher beta-lactamase concentration in the cell (109).Functional metabolomics studies have shown that

565 different metabolic states are related to antibiotic resistance phenotypes (110, 111). For instance,

566 core enzymes involved in metabolic regulation might prevent the antibiotic-mediated induction of

567 the tricarboxylic acid cycle functioning, reducing metabolic toxicity, basal respiration, and

568 consequently drug lethality (112, 113). A particularly interesting fact in this process is the

569 antibiotic induction of the "acetylome", an ensemble of multiple acetylating enzymes, resulting in

570 a decrease in antibiotic action (114). "Intrinsic" resistance to colistin in *Staphylococcus aureus*

571 entirely depends on a functional ATP synthase (115, 116). It is difficult to differentiate if these

572 effects due to metabolic functioning are consequences of the antibiotic action or adaptive cell

573 responses (reaction) to the drug exposure. In any case, antibiotics frequently "disorganize" the cell

574 metabolism, in some cases by altering the shape and subcellular structure of the microorganism

575 (117). Such effects can produce a heterogeneous response to antibiotic action in exposed

576 populations (118). Finally, some antibiotics, as sulfonamides or trimethoprim, are essentially

577 antimetabolic drugs. Sulphonamides and trimethoprim are structural analogs and competitive

578 antagonists of *p*-aminobenzoic acid (PABA)interfering with the dihydrofolate reductase (DHFR)

579 and dihydropteroate synthase (DHPS), respectively, which are sequentially involved in the

580 synthesis of folate for the production of nucleic acids. One of the very first mechanisms of

581 resistance that was elucidated was sulphonamide resistance resulting from hyperproduction of

582 para-aminobenzoic acid (119). Here we can see a stoichiometric example of metabolic resistance,

583 and an important gene-dosing effect has been shown for both sulphonamides and trimethoprim.

584

585

586

ANTECHOKINETICS

Antechokinetics refers to the study of the processes leading to bacterial spatial cellular (subcellular, pericellular, extracellular) localizations of the molecules involved in antibiotic detoxifying mechanisms. These molecules' local concentrations change over time due to their production, degradation, and, eventually, excretion rates. Variations in the kinetics of antibiotic resistance mechanisms should influence the rate of interaction and detoxification of the antibiotic agents. To show what we know (and particularly what we do not know) about the effects of antechokinetics in antibiotic resistance, we are obliged to recall here, in a succinct way, the intracellular kinetics of the different drugs.

In an extended meaning of the field of "antechokinetics," we could also consider the movement of antibiotic resistance genes across cells, species, and populations. This aspect will not be treated here; but reviews are widely available (120). This might also apply to the dissemination of resistance genes or resistance proteins in microvesicles, spherical nanoparticles composed of bacterial lipid membranes (121).

Three previous questions on antechodynamics

The question of efflux pumps

The field of efflux pumps, a homogeneous group of trans-envelope multimolecular complexes, is **hard to contextualize in the antechodynamics field**; as stated before, we consider that, in most cases, they do not directly influence the mechanisms of resistance by antibiotic detoxification nor the molecules involved in resistance by target modification. The induction of efflux pumps by repressor inactivation can be achieved by ligand binding including metabolites, antibiotics, biocides, pharmaceuticals, additives, plant extracts, or compounds released by oxidative stress

609   (122). The genes regulated by the antibiotic-responsive cis-acting RNA elements include several

610   different classes of multidrug antibiotics exporters and efflux pumps (123, 124). When the

611   antibiotic itself is the inducer or is specifically captured by the pump proteins, we can consider

612   these interactions in the antechodynamic field. As an example, in *E. coli*, tetracycline resistance

613   TetA pump is inducible by subinhibitory tetracycline concentrations, releasing the effect of the

614   repressor TetR (125).

615   In the antechokinetics perspective, the cellular density and perhaps the topology of efflux pumps

616   could influence the effectiveness of antibiotic degrading mechanisms, not only by modifying the

617   antibiotic concentration and thus the stoichiometry with these mechanisms, but eventually by

618   scarcely known spatial relations with them (co-localization, influencing stoichiometry in cellular

619   microspaces). In *Pseudomonas*, the maximal efflux efficiency occurs from the periplasm, being

620   two orders of magnitude faster than from the cytosol (126). TetA (see above) selectively transport

621   tetracycline from the cytosol to the periplasm in exchange of a proton (125). On the other hand,

622   the *action* of the antibiotics on the cell alters the cellular chemical structure and their metabolic

623   networks, and it can be suspected that certain molecules, Including non-antibiotics could serve as

624   inducers of the synthesis of efflux pumps (127). Antechokinetics could study the nature,

625   expression, location, and degradation of these presumed molecules, possibly related to those

626   involved in general stress responses.

627   **The question of the number of reduced affinity genes**

628   In our definition of antechology, and more particularly antechodynamics, we have discarded to

629   formally include antibiotic resistance due to mutated targets with low affinity for the antibiotic, as

630   they do not constitute any specific "reaction" against the "action" of the antibiotics. However, In

631   some cases, they could be considered from an antechokinetics perspective, for instance, when the

632 number of molecules resulting from the expression of these genes modify the antibiotic resistance

633 phenotype. For instance, where the beta-lactam resistance mechanism is not a beta-lactamase but

634 a modified target with reduced affinity for the antibiotic, as in the case of staphylococcal cassette

635 SCCmec element, the tandem amplification of this gene drives high-level methicillin resistance

636 (128). To our knowledge, nothing similar has been observed for penicillin low-affinity proteins

637 (about 5,000-20,000 per cell) in *Streptococcus pneumoniae*, as PBP2x; however, the number of

638 PBP2x molecules can be modulated in the activation of the HtrA serine protease that degrades

639 PBP2x (129). As a final "classic" *example*, a mutant resistant allele of *gyrA*, encoded in a

640 multicopy plasmid, was capable of producing a quinolone resistance phenotype when expressed

641 by a formerly susceptible strain (130). Such examples show how, to a certain extent, there is a

642 certain antechokinetic approach that can be applied to mutational events, but this perspective is

643 not treated in the current work.

644 **The question of intracellular topology in transcription-translation efficiency**

645 The interaction between antibiotic molecules, the antibiotic resistance molecules, and the bacterial

646 organelles and cellular structures where they meet occurs in defined (yet variable) spaces of the

647 cell. These encounters should depend on their relative density and their proximity in the space.

648 Very few have been done to clarify this antechokinetic problem. As an example, the number of

649 plasmid copies carrying antibiotic resistance genes is highly variable in an otherwise monoclonal

650 population (131), which results in a populational tuning of gene expression under different

651 exposure intensities to antibiotic agents. For instance, the spatial distribution in the cell of the

652 plasmids and frequent carriers of antibiotic resistance genes might influence their interaction with

653 the translating ribosomes by mRNAs. Apparently, during the growth cycle of bacilli, both large

654 plasmids with active segregation systems and small plasmids frequently colocalize with the

655 microspaces with higher ribosome density in the poles of the cell and close to the cellular

656 membrane, forming a transcription-translation microspatial factory (132, 133). The chromosomal

657 genes encoding antibiotic resistance effectors are relatively distant, as the nucleoid is located near

658 the cell center (134). However, the supercoiled DNA nucleoid (volume: 1 μm^3), with an average

659 pore diameter of $\sim$ 50 nm, allows the internal circulation of free ribosomes, with an average size

660 of $\sim$ 20 nm. On the contrary, polysomes, mRNAs with multiple bound ribosomes are much larger

661 and diffuse to the areas of higher ribosome density (135). A significant point in antechokinetics is

662 mRNA localization, meaning that mRNAs are directed to the subcellular microcompartments

663 where their protein products are targeting (for instance, to degrade an antibiotic or protect a vital

664 target) (134, 136). If a wealth of new knowledge is needed in this field, bacteria presumably have

665 an intracellular "road map" network system apparatus involving motor proteins and cytoskeleton-

666 like filaments, as those that have started to be known for plasmid partitioning (133).

667

Antechokinetics in resistance to different antibiotic classes.

Beta-lactam resistance

670 The access of beta-lactamases to bacterial cells occurs by transcription and translation of

671 chromosomal genes, but, at least in pathogenic species, much more frequently by the uptake and

672 expression (also in the progeny) of beta-lactamase genes acquired with mobile genetic elements,

673 as plasmids, transposons (eventually containing integrons), or by the capture of free extracellular

674 microvesicles containing the resistance proteins. In the case of gene capture, the biogenesis of the

675 active enzyme starts a complex physiological process attracted some attention in the 1980s but

676 was overshadowed by the genetics-bioinformatics obsession of recent research. The number of

677 beta-lactamase genes present in the cell, for instance, in relation to a plasmid (gene) copy number,

678 the number of active ribosomes, or the position of the beta-lactamase gene in integron strings (that

679 is, more or less distant to the promoter sequence), should influence the total concentration of beta-

680 lactamase in the cell, but very few is known of about these aspects. The protein genes should be

681 first transcribed, giving rise to pre-beta-lactamases, carrying an N-terminal signal leader sequence,

682 which interacts with the general Sec secretion system, or the twin-arginine (Tat) translocation

683 system. The Sec system involves a SecYEG integral membrane protein complex heterotrimer

684 probably acting as a single protein-conducting channel. This tetrameric arrangement of SecYEG

685 complexes and the highly dynamic peripherical bound ATPase SecA dimer together form a proton-

686 motive force- and ATP-driven molecular machine that drives the stepwise translocation of targeted

687 polypeptides across the cytoplasmic membrane (137). These secretion systems correlate with the

688 type of beta-lactamases; TEM-1, AmpC, CTX-M, or KPC enzymes use the Sec system; more

689 "chromosomal" beta-lactamases, such as L2, BlaC, or PenA (and also TEM-1!) can be exported

690 by both systems (138). The altered COOH-terminal part of the leader signal sequence is attached

691 to the outer face of the inner membrane. In some cases, the beta-lactamase, in its active form, can

692 be permanently bound to the membrane, without being excreted (139). Leader sequences can be

693 used to define beta-lactamase alleles (140). The leader sequence is proteolytically excised (by the

694 leader peptidase) when the beta-lactamase molecule crosses the cellular membrane and is

695 exported. Therefore, the export of the beta-lactamases localizes these proteins in the periplasmic

696 space in gram-negatives, or protruding in part outside the outer membrane, or reaching the extra-

697 membrane space, including the close exterior of the cell, mostly in the gram-positives. The signal

698 sequence and first nine N-terminal amino acids of Lpp, the major *Escherichia coli* lipoprotein, are

699 necessary for proper localization in the outer membrane (141). Possibly capsular material, mostly

700   polysaccharides, could retain beta-lactamases (142). There is a possibility of catalytically active

701   beta-lactamases inside the cytoplasm. Still, they are unable, (probably dependent on the degree of

702   excision of the leader peptide to be secreted (143). In some cases. Some enzymes (as TEM) cross

703   the cytoplasmic membrane immediately following translation. That is due to the spatial

704   connectivity between the cytoplasmic membrane and the dense "ribosome crown" below the

705   membrane (117). It has been suggested that cytoplasmic chaperones influence the beta-lactamase

706   protein oxidative folding, which results in membrane translocation (144). Then a rapid and

707   energetically favorable folding process allows the transported enzyme to adopt the lowest energy

708   conformation, ensuring that it will be soluble in the aqueous extra-cytoplasmic space (138). If beta-

709   lactamases are produced and secreted in high quantity (as under induction) in the periplasm, they

710   can form inclusion bodies with low catalytic efficiency (145); in fact, increasing the propensity of

711   beta-lactamases to aggregate might be a therapeutical strategy (146). Both in Gram positives and

712   Gram negatives, beta-lactamases can be transported into extracellular vesicles, occasionally

713   captured by other closely located bacteria, sometimes unable to produce beta-lactamases by

714   themselves (147, 148). The release of beta-lactamases during the bacterial lytic processes

715   (bacteriophages, bacterial predators, envelope-disrupting antimicrobials) and their stability in the

716   environment (as free molecules or granules) is a scarcely investigated field.

717   There is also meager information about the concentration of beta-lactamases in the different

718   cellular compartments concerning induction, growth cycle, and shape-alternative cellular

719   conformations. The volume versus surface of single cells and its consequences in the periplasm

720   total volume should modify these concentrations (117, 149). This question is critical to evaluate

721   the relationship between the quantity of beta-lactamase and resistance. In pharmacological terms,

722   the parameter V_{max} reflects the amount of beta-lactamase multiplied by the maximum number of

723   catalytic events each enzyme molecule can achieve per unit of time. Therefore, in principle,

724   increasing the amount of beta-lactamase should increase resistance to beta-lactams (142, 150, 151).

725   Probably shortly, fluorogenic-beta-lactam-based substrates could serve to measure beta-lactamase

726   concentration/activity (152).

727   The correlation between levels of inducibility of chromosomal AmpC beta-lactamase and

728   resistance level is a good example of the relation between the quantity of beta-lactamase and

729   antibiotic resistance. Even if the classically considered "inducible" genus *Serratia*, containing the

730   whole inducibility system AmpR-AmpC, contains low inducible species that are susceptible to

731   cephalosporins (56). However, the relationship between the quantity of beta-lactamase and

732   hydrolytic efficiency is not necessarily linear; the effect of efflux pumps, transcriptional regulators,

733   and porins can influence the final phenotypic outcome (153). On the other hand, a critical but

734   hitherto poorly explored point is the **speed of induction;** the canonical bacterial response could

735   be eventually delayed to localize enough beta-lactamase in the periplasm to avoid cellular

736   destruction. To overcome such a "death-before-induction," some strategies have been suggested.

737   A "rapid mechanism" based on an alternative signaling system in which a membrane-associated

738   histidine kinase directly binds β-lactams, triggering the expression of a β-lactamase before

739   muropeptide disturbance has been suggested (154). In the case of AmpC induction resulting from

740   the lack of AmpR repression of the AmpC promoter, we can consider that AmpR is a LysR family

741   master regulator whose deletion influences the expression of hundreds of genes (155). That might

742   suggest that AmpR-mediated derepression of AmpC could be considered a side effect triggered by

743   other bacterial stresses, not necessarily the antibiotic exposure. This probably includes "envelope

744   stress", as AmpC might contribute to the recovery of the damages in the outer membrane-

745   peptidoglycan architecture (156).

746 Another process leading to variable concentration levels of beta-lactamases is gene amplification

747 leading to an **increased number of copies** of a particular gene (polyploidy), which results in more

748 resistant phenotypes. One of the first examples was the effect of multiple copies of the beta-

749 lactamase TEM-1 (by cloning the enzyme in a multicopy plasmid) in the emergence of resistance

750 to beta-lactam/clavulanate, a beta-lactamase inhibitor (157, 158). This is a general phenomenon in

751 many species (159). But beta-lactamase polyploidy occurs more frequently by gene amplification

752 (gene duplication in its simplest version); the steady-state frequencies of gene duplication are

753 extremely high, typically ranging between 10^{-5} and 10^{-2} per cell per gene (160). Polyploidy is

754 expected to occur under bacterial stress driving filamentation; however, it remains to be

755 ascertained if the collective protective effect of an increased number of beta-lactamase molecules

756 per elongated multinuclear cell is diluted by the increase in total cell volume.

757 Surprisingly, the degradation kinetics of beta-lactamases in the bacterial cell, the host (body,

758 microbiota) or external environments under natural conditions has been scarcely examined in

759 recent years. Body proteases (as trypsin) or microbial proteases (as ClpXP) seem to be inactive in

760 degrading beta-lactamases and eventually might increase antibiotic resistance (161). Early

761 observations with TEM-1 suggest that molecular folding exerts a critical role and that the disulfide

762 bond can be essential in the process (162, 163). Outside the cell, AmpC beta-lactamase from *E.*

763 *coli* is reversibly denatured by temperature in a two-state manner with a temperature of melting of

764 54.6 ° (164).

765 **Aminoglycosides resistance**

766 Aminoglycosides (polycationic compounds) can bind the outer membrane lipopolysaccharide,

767 followed by a displacement of magnesium ions (self-promoted uptake) and increase cytoplasmic

768 membrane permeability, which might result in passive rapid uptake and eventually membrane

769   disruption (165); they do not enter through hydrophilic porins (18, 166). The first stage of uptake

770   is followed by a slow, energy-dependent, electron-transport-mediated process. Aminoglycosides

771   should immediately reach their ribosomal target, in the vicinity of the cytoplasmic membrane.

772   Even if the information is very limited, aminoglycoside resistance enzymes were classically

773   considered cytoplasmically located. However, the efficiency of interaction and detoxification of

774   aminoglycoside molecules acting on the ribosomes might suggest a condensation in the

775   cytoplasmic sub-inner membrane "ribosome crown space". Another possibility is the

776   detoxification of the antibiotic before entering the cytoplasm. The efforts to locate

777   aminoglycoside-modifying enzymes in the periplasm of gram-negatives have provided

778   controversial results. Osmotic shock technology has been used to release the periplasmic

779   molecules, but the possibility of contamination with cytoplasmic molecules cannot be discarded.

780   The examination of putative signal sequences involved in putative periplasmic transport has been

781   addressed. Aminoglycoside acetyl-transferases have signal-like sequences integrating a long

782   hydrophobic stretch of amino acids, but they might also have a stabilizing function. These

783   sequences have not been found in aminoglycoside phosphotransferases. Experiments have been

784   done by fusing beta-lactamases (TEM type) leader peptides in the acetylase (6´)-Ib. The cells with

785   this hybrid protein, now periplasmically located, significantly increased aminoglycoside

786   resistance. These results suggested that the cellular location of the modifying enzyme may be

787   important in determining resistance levels (167). Later works, on the contrary, indicate that even

788   if the TEM leader peptide is present, it is not processed (removed), so it becomes part of a mature

789   AAC (6´)-Ib. The conclusion was that the protein is probably located in the cytoplasm and is

790   evenly distributed in this compartment (168). In addition, *in vivo* imaging of this protein confirms

791   that it diffuses freely within the cytoplasm of the cell, but it tends to form inclusion bodies at higher

792   concentrations in rich culture media (169).

793   The cellular concentration of aminoglycoside-modifying enzymes has effects on the bacterial

794   resistance phenotype, as it is shown by gene amplification. Phosphotransferase *aphA1* results in

795   clinical resistance to tobramycin (170). Also, bleomycin acts as a transcriptional inducer of the

796   neo-ble-str operon contained in Tn5, and the increase of the phosphorylase *aph3´II* results in

797   amikacin resistance (171, 172). In a much more recent study, the level of resistance to amikacin

798   increases linearly with a higher concentration of AAC(6′)-Ib until it reaches a plateau at a specific

799   protein concentration (173).

800   **Macrolides, lincosamides and streptogramins (MLS) resistance**

801   Macrolides are hydrophobic molecules, their self-promoted uptake entry in the cell being favored

802   by the hydrophobic nature of lipid A in the outer membrane LPS. The macrolides bind to the

803   nascent peptide exit tunnel in the ribosome (74). The number of 50S ribosomal units where MLS

804   drugs bind, inhibiting protein synthesis, is about 20,000/cell, but this number is variable with the

805   growth phase and the bacterial species. The number of genes involved in the most frequent

806   mechanism of macrolide resistance, 23S rRNA methylation, is comparatively low; as these genes

807   are usually harbored by plasmids, only one gene is present per plasmid, and a generally few copies

808   of the plasmids are harbored in the bacterial cell. If a single 23S rRNA methylase is sufficient for

809   the methylation-deactivation process, resistance depends on the transcription rate under conditions

810   of induction. To our knowledge, the number of intracellular macrolide molecules needed for an

811   efficient induction of 23S rRNA methylase remains undetermined. We should also consider the

812   ribosomal rescue and recycling rate after the prematurely terminated translation events (74, 75,

813   174). On the other hand, independently of ribosome stalling, macrolides might exert a protective

814   role on mRNA decay, favoring ErmB hyperproduction (175).

Tetracyclines resistance

816   Tetracycline enters the bacterial cell by passive diffusion through the hydrophilic β-barrel protein

817   bacterial porins (OmpC, OmpF), crossing the outer membrane and thus connecting the periplasmic

818   space of Gram-negatives with pericellular space. The diffusion is facilitated by positive cation-

819   tetracycline complexes, which dissociate in the periplasm to make a more lipophilic molecule able

820   to cross the cytoplasmic membrane, an energy-dependent process involving proton motive force

821   (176). There is a dense "ribosome crown" below the cytoplasmic membrane where most ribosomes

822   are located. Certainly, the effect of tetracyclines should depend on the number of available

823   ribosomal targets which depends on growth rate, and the bacterial species. The number of

824   tetracycline molecules inside the cell is highly variable (1-100 micromolar, so that the number can

825   reach 10^9 molecules). As stated in a previous section, the TetR promoter binds tetracycline,

826   allowing induction of the TetA efflux pump, also, tetracycline can be displaced from his by 30S

827   ribosomal target by TetM or TetO. This free tetracycline might then serve to induce TetA (if

828   present). The spatial location of these mechanisms depends on the location of the mobile genetic

829   elements that host the corresponding genes; it might be conceived that their resistance efficiency

830   depends on their chances of meeting translating ribosomes, but this is a poorly investigated field.

Fluoroquinolones resistance

832   Uptake of hydrophilic fluoroquinolones occurs by passive diffusion using bacterial porins. The

833   translocation across the bilayered cytoplasmic membrane seems to occur by permeation of the

834   neutral form of ciprofloxacin, so that the zwitterionic ciprofloxacin, approaching the membrane in

835   stacks, diffuse through the membrane as neutral monomers (177). Depending on the external

836   concentration, and the natural efflux systems (mostly AcrAB), calculations based on

837   spectrofluorimetry and mass spectrometry yield a bias of a number of 263 ciprofloxacin

838   molecules/cell (178). The average number of GyrA topoisomerase target molecules per cell has

839   been estimated to be 2,200, which outnumbers that of ciprofloxacin molecules almost by a factor

840   of 10 (179). This indicates that, assuming that all ciprofloxacin molecules are bound to a

841   topoisomerase complex, roughly 90% of cellular topoisomerases are not inhibited by the antibiotic

842   and should be able to unwind DNA and facilitate replication. The binding kinetics to

843   topoisomerases is probably biphasic, with adhesion first and then cross-linking (180). *However,*

844   *topoisomerase-ciprofloxacin complexes are poisonous to the cell as they produce replication-*

845   *assisted double-strand breaks which are the ultimate cause of quinolone-mediated cell death.*

846   *Therefore,* the number of cleaved complexes containing ciprofloxacin, topoisomerase, and DNA

847   should determine the antibacterial action. In fact, the stoichiometry of fluoroquinolone

848   action/resistance was suspected long time ago, as when mutated *gyrA* was cloned in a multicopy

849   plasmid, that resulted in an increase in quinolone resistance (130). More recently, it has shown that

850   ploidy facilitates fluoroquinolone persister survival (181)**.**

851   **Trimethoprim resistance**

852   Trimethoprim can be detoxified by pumping out the molecule; efflux pumps can be inducible, as

853   in the case of *Acinetobacter baumannii*. The efflux pump SxtP, a member of major facilitator

854   superfamily, is activated by a **LysR-type transcriptional regulator**, SxtR (182)*.*

855

856

Glycopeptide and lipopeptide resistance

Vancomycin molecules freely diffuse through the layers of Gram-positive peptidoglycan that enclose a Gram-positive bacterial cell to reach the peptide target (183). We have previously mentioned LiaX as a molecule determining daptomycin resistance. In *E. faecalis*, its N-terminal domain is released to the extracellular medium, where it binds daptomycin; the complex is probably recognized on the cell surface, maintaining the cell membrane stress adaptive response. The level of daptomycin resistance is probably related to an increase in LiaX molecules (93).

CROSSROADS BETWEEN ANTECHOLOGY (AD/AK) AND PHARMACOLOGY (PD/PK)

In the former sections, the reader can appreciate the operative interactions linking Antechodynamics and Antechokinetics of molecules involved in bacterial resistance against antimicrobial agents. The most evident example is the effect of changing concentrations of antibiotic resistance effectors (Antechokinetics), exerting different resistance antibiotic detoxification activities (Antechodynamics), as a result of the induction of expression of resistance genes. This relation parallels what occurs with antibiotic molecules in pharmacokinetics and pharmacodynamics. In fact, PD/PK, and AD/AK studies should be combined to provide data of potential therapeutical interest.

For a given antimicrobial agent, how many antimicrobial resistance molecules are needed to detoxify the antibiotic molecules present in the bacterial cell? In other words, how important is the determination of the stoichiometry of antibiotic and resistance molecules? The stoichiometric

878   values will probably be variable in different environments and cellular growth phases, and most

879   importantly the presence of multimolecular mechanisms of resistance (as efflux pumps).

880   The starting point for these (scarcely developed) studies is the determination of intra-bacterial

881   **antibiotic molecular concentrations per cell** considering different external concentrations of the

882   antibiotic. In recent years, progress has been made by applying spectrofluorimetry (including

883   microspectrofluorimetry), and mass spectrometry to achieve this goal. These techniques can be

884   complemented with time-lapse imaging methods able to evaluate the antibiotic transport kinetics

885   and the subcellular localization of antibiotics in individual cells, revealing the pharmacokinetic

886   kinetic heterogeneity in bacterial populations. An important driver of the intracellular

887   concentration of antibiotics in the cell (for a given external concentration) is the rate of antibiotic

888   influx and efflux. Antibiotic structure-to-intracellular-accumulation (SICAR) studies,

889   comprehending the rate of influx across the bacterial envelope (SICAR^{IN}), the antibiotic efflux

890   rate by particular mechanisms (SICAR^{EF}) provide insights on antibiotic accumulation inside

891   bacteria (184). However, these studies do not provide general de quantitation in terms of the

892   number of molecules.

893   Second, the determination of the **number of target molecules of particular antibiotics**, and the

894   number of molecules needed to inactivate a target molecule.

895   Third, the **number of antibiotic resistance molecules present in the cell** should be known. That

896   is work for future research, as our currently available data concerning these parameters are

897   incomplete. These calculations should consider the growth phase and metabolic conditions of the

898   cell and the variability in antibiotic-resistance molecules (number of gene copy numbers, inductive

899   processes). Gene copy numbers are dependent on tandem amplification and an increased number

900   of mobile genetic elements carrying the resistance gene, eventually resulting from the insertion of

901   the gene in cryptic high-copy plasmids (185). The kinetics of the number of antibiotic resistance

902   is work for future research, as our currently available data concerning these parameters are really

903   incomplete.

904   As an example, using bacterial lysates after exposure to different **ciprofloxacin** concentrations,

905   the intracellular concentrations in *E. coli* are about 30 times lower than the external ones; for

906   instance, 0.08 μg/mL in the lysate when the external concentration is 2,5-3 μg/mL. That should

907   correspond to about 200-500 ciprofloxacin molecules per cell (178), a close number to the

908   estimated number of 300 gyrase molecules stably bound to the *E. coli* chromosome at any time,

909   among the total number of DNA gyrase molecules determined by epifluorescence in the whole

910   cell (186) distributed randomly throughout the cytoplasm (187). The number of Qnr ciprofloxacin

911   inactivating molecules could be estimated to range from a few hundred to a few thousand

912   molecules per cell (188). Now, the protection Antechodynamics of Qnr, in particular, the

913   ciprofloxacin inhibitory interactions with DNA gyrase, should be also known (189).

914   Beta lactams inhibit different PBPs (mostly transpeptidases); these targets construct the

915   peptidoglycan and, thus, should be mostly spatially linked to this sacculus. In Gram-negatives, the

916   peptidoglycan is a 2.5-thick structure located in a 15 nm wide periplasm, occupying from 20 to

917   40% of the total cell volume (190, 191). As stated above, beta-lactamases are mostly located in

918   the periplasm, protecting against beta-lactam inhibition of PBPs. However, different beta-lactams

919   target different PBPs, which are not homogeneously located in the cell. PBP2, involved in bacterial

920   elongation, is located in a spot at the lateral wall and also at the cell division site. PBP3, involved

921   in cell division, is located in the space corresponding to the division septum (192). This target's

922   compartmentalization is probably assured by the fibrillar actin-like structures of the protein MreB

923   (193). The local stoichiometry of PBPs and beta-lactamases should certainly be better known to

924 understand the effect of different concentrations of beta-lactam agents. The number of PBP

925 molecules in *Staphylococcus aureus* has been estimated (more than 20 years ago) from

926 approximately 150 to 825 PBPs/cell (194). In spite of having thinner peptidoglycan, early

927 calculations for *E. coli* yielded about 2,000 PBPs/cell, but many of them are carboxypeptidases

928 (195). The number of beta-lactamase molecules per cell in resistant organisms is highly variable,

929 probably ranging from 10^3 to 10^6 molecules per cell under different conditions. The whole plot

930 should also consider the number of beta-lactam molecules in the cell, but against expectations, the

931 information is also scarce, more focused on changes in indirect markers, such as fluorescence,

932 immunoblotting of the resistance beta-lactamase, or mRNA transcription of the resistance gene,

933 rather than in the intracellular molecular concentrations (196, 197).

934 In general, it is difficult to find this type of data for most antibiotics and their inactivating

935 molecules (198). To add complexity, the three main parameters that are needed (the number of

936 antibiotic molecules in the cell, the number of target molecules, and the number of antibiotic

937 resistance molecules) should probably be considered in the different subcellular locations,

938 including membrane microdomains (199).

939 Something to consider as an extension of the scope of the AD/AK are the presumable future fields

940 are antechotoxicodynamics (ATD) and antechotoxicokinetics (ATK), mimicking what occurs with

941 antimicrobial drugs (200). Similarly, as drugs may produce toxic effects in the hosts, including

942 their normal microbiota, bacterial resistance mechanisms could be toxic for the resistant bacterial

943 organisms, the microbiota, or directly the human or animal hosts. Such perspective has been

944 extensively treated in the case of mutational "fitness costs" of resistance, or those associated with

945 the presence of mobile genetic elements carrying resistance genes, which is critical to envisage

946 possible biorestoration strategies (201–203). In conclusion, and in spite of our apparent extensive

knowledge of the processes and mechanisms associated with bacterial antibiotic resistance, the study of such mechanisms of resistance should be "continuous, resilient, and steady" (204). We hope that the antechological approach that we are proposing might offer novel research challenges leading to a complete understanding and eventually to the control of antibiotic resistance.

Acknowledgments

REFERENCES

1. Jacobs MR. 2001. Optimisation of antimicrobial therapy using pharmacokinetic and pharmacodynamic parameters. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 7:589–596.

2. Mouton JW, Dudley MN, Cars O, Derendorf H, Drusano GL. 2005. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. J Antimicrob Chemother 55:601–607.

3. Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA, Blair JMA. 2023. Molecular mechanisms of antibiotic resistance revisited. Nat Rev Microbiol 21:280–295.

4. Luz CF, van Niekerk JM, Keizer J, Beerlage-de Jong N, Braakman-Jansen LMA, Stein A, Sinha B, van Gemert-Pijnen JEWC, Glasner C. 2022. Mapping twenty years of antimicrobial resistance research trends. Artif Intell Med 123:102216.

5. Schaenzer AJ, Wright GD. 2020. Antibiotic Resistance by Enzymatic Modification of Antibiotic Targets. Trends Mol Med 26:768–782.

6. Avery C, Baker L, Jacobs DJ. 2022. Functional Dynamics of Substrate Recognition in TEM Beta-Lactamase. Entropy Basel Switz 24:729.

7. Pantsar T, Poso A. 2018. Binding Affinity via Docking: Fact and Fiction. Mol Basel Switz 23:1899.

8. Baquero F, Martínez JL, Novais Â, Rodríguez-Beltrán J, Martínez-García L, Coque TM, Galán JC. 2021. Allogenous Selection of Mutational Collateral Resistance: Old Drugs Select for New Resistance Within Antibiotic Families. Front Microbiol 12:757833.

9. Albery WJ, Knowles JR. 1976. Evolution of enzyme function and the development of catalytic efficiency. Biochemistry 15:5631–5640.

991 10. Bulychev A, Mobashery S. 1999. Class C β-Lactamases Operate at the Diffusion Limit for

992   Turnover of Their Preferred Cephalosporin Substrates. Antimicrob Agents Chemother

993   43:1743–1746.

994 11. Monterroso B, Margolin W, Boersma AJ, Rivas G, Poolman B, Zorrilla S. 2024.

995   Macromolecular Crowding, Phase Separation, and Homeostasis in the Orchestration of

996   Bacterial Cellular Functions. Chem Rev 124:1899–1949.

997 12. Di Cera E. 2009. Serine proteases. IUBMB Life 61:510–515.

998 13. Hedstrom L. 2002. Serine protease mechanism and specificity. Chem Rev 102:4501–4524.

999 14. Bush K. 1989. Characterization of beta-lactamases. Antimicrob Agents Chemother 33:259–

1000   263.

1001 15. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y,

1002   Spencer J. 2019. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. J Mol Biol

1003   431:3472–3500.

1004 16. Zdarska V, Kolar M, Mlynarcik P. 2024. Occurrence of beta-lactamases in bacteria. Infect

1005   Genet Evol J Mol Epidemiol Evol Genet Infect Dis 122:105610.

1006 17. Bordeleau E, Stogios PJ, Evdokimova E, Koteva K, Savchenko A, Wright GD. 2024.

1007   Mechanistic plasticity in ApmA enables aminoglycoside promiscuity for resistance. Nat

1008   Chem Biol 20:234–242.

1009 18. Krause KM, Serio AW, Kane TR, Connolly LE. 2016. Aminoglycosides: An Overview.

1010   Cold Spring Harb Perspect Med 6:a027029.

1011 19. Smith CA, Baker EN. 2002. Aminoglycoside antibiotic resistance by enzymatic deactivation.

1012   Curr Drug Targets Infect Disord 2:143–160.

1013 20. Revillo Imbernon J, Weibel J-M, Ennifar E, Prévost G, Kellenberger E. 2024. Structural

analysis of neomycin B and kanamycin A binding Aminoglycosides Modifying Enzymes

(AME) and bacterial ribosomal RNA. Mol Inform 43:e202300339.

21. Wright GD, Thompson PR. 1999. Aminoglycoside phosphotransferases: proteins, structure, and mechanism. Front Biosci J Virtual Libr 4:D9-21.

22. McKay GA, Wright GD. 1995. Kinetic mechanism of aminoglycoside phosphotransferase type IIIa. Evidence for a Theorell-Chance mechanism. J Biol Chem 270:24686–24692.

23. Golkar T, Zieliński M, Berghuis AM. 2018. Look and Outlook on Enzyme-Mediated Macrolide Resistance. Front Microbiol 9:1942.

24. Pawlowski AC, Stogios PJ, Koteva K, Skarina T, Evdokimova E, Savchenko A, Wright GD. 2018. The evolution of substrate discrimination in macrolide antibiotic resistance enzymes. Nat Commun 9:112.

25. Barthélémy P, Autissier D, Gerbaud G, Courvalin P. 1984. Enzymic hydrolysis of erythromycin by a strain of Escherichia coli. A new mechanism of resistance. J Antibiot (Tokyo) 37:1692–1696.

26. Kinetics of drug-ribosome interactions defines the cidality of macrolide antibiotics - PubMed. https://pubmed.ncbi.nlm.nih.gov/29229833/. Retrieved 27 November 2024.

27. Svetlov MS, Cohen S, Alsuhebany N, Vázquez-Laslop N, Mankin AS. 2020. A long-distance rRNA base pair impacts the ability of macrolide antibiotics to kill bacteria. Proc Natl Acad Sci U S A 117:1971–1975.

28. Gil-Gil T, Berryhill BA, Manuel JA, Smith AP, McCall IC, Baquero F, Levin BR. 2024. The evolution of heteroresistance via small colony variants in Escherichia coli following long term exposure to bacteriostatic antibiotics. Nat Commun 15:7936.

29. Douthwaite S, Champney WS. 2001. Structures of ketolides and macrolides determine their

1037   mode of interaction with the ribosomal target site. J Antimicrob Chemother 48 Suppl T1:1–

1038   8.

1039 30. Stogios PJ, Evdokimova E, Morar M, Koteva K, Wright GD, Courvalin P, Savchenko A.

1040   2015. Structural and functional plasticity of antibiotic resistance nucleotidylyltransferases

1041   revealed by molecular characterization of lincosamide nucleotidylyltransferases lnu(A) and

1042   lnu(D). J Mol Biol 427:2229–2243.

1043 31. Stogios PJ, Kuhn ML, Evdokimova E, Courvalin P, Anderson WF, Savchenko A. 2014.

1044   Potential for Reduction of Streptogramin A Resistance Revealed by Structural Analysis of

1045   Acetyltransferase VatA. Antimicrob Agents Chemother 58:7083–7092.

1046 32. Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. 1999. Nomenclature

1047   for macrolide and macrolide-lincosamide-streptogramin B resistance determinants.

1048   Antimicrob Agents Chemother 43:2823–2830.

1049 33. Murray IA, Shaw WV. 1997. O-Acetyltransferases for chloramphenicol and other natural

1050   products. Antimicrob Agents Chemother 41:1–6.

1051 34. Farrell DJ, Castanheira M, Chopra I. 2011. Characterization of global patterns and the

1052   genetics of fusidic acid resistance. Clin Infect Dis Off Publ Infect Dis Soc Am 52 Suppl

1053   7:S487-492.

1054 35. Gasparrini AJ, Markley JL, Kumar H, Wang B, Fang L, Irum S, Symister CT, Wallace M,

1055   Burnham C-AD, Andleeb S, Tolia NH, Wencewicz TA, Dantas G. 2020. Tetracycline-

1056   inactivating enzymes from environmental, human commensal, and pathogenic bacteria cause

1057   broad-spectrum tetracycline resistance. Commun Biol 3:241.

1058 36. Forsberg KJ, Patel S, Wencewicz TA, Dantas G. 2015. The Tetracycline Destructases: A

1059   Novel Family of Tetracycline-Inactivating Enzymes. Chem Biol 22:888–897.

1060   37. Cheng Q, Cheung Y, Liu C, Chan EWC, Wong KY, Zhang R, Chen S. 2022. Functional and

1061   phylogenetic analysis of TetX variants to design a new classification system. Commun Biol

1062   5:522.

1063   38. Park J, Gasparrini AJ, Reck MR, Symister CT, Elliott JL, Vogel JP, Wencewicz TA, Dantas

1064   G, Tolia NH. 2017. Plasticity, dynamics, and inhibition of emerging tetracycline resistance

1065   enzymes. Nat Chem Biol 13:730–736.

1066   39. Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, Bush K, Hooper

1067   DC. 2006. Fluoroquinolone-modifying enzyme: a new adaptation of a common

1068   aminoglycoside acetyltransferase. Nat Med 12:83–88.

1069   40. Maia AS, Tiritan ME, Castro PML. 2018. Enantioselective degradation of ofloxacin and

1070   levofloxacin by the bacterial strains Labrys portucalensis F11 and Rhodococcus sp. FP1.

1071   Ecotoxicol Environ Saf 155:144–151.

1072   41. Bernat BA, Laughlin LT, Armstrong RN. 1997. Fosfomycin resistance protein (FosA) is a

1073   manganese metalloglutathione transferase related to glyoxalase I and the extradiol

1074   dioxygenases. Biochemistry 36:3050–3055.

1075   42. Klontz EH, Tomich AD, Günther S, Lemkul JA, Deredge D, Silverstein Z, Shaw JF,

1076   McElheny C, Doi Y, Wintrode PL, MacKerell AD, Sluis-Cremer N, Sundberg EJ. 2017.

1077   Structure and Dynamics of FosA-Mediated Fosfomycin Resistance in Klebsiella pneumoniae

1078   and Escherichia coli. Antimicrob Agents Chemother 61:e01572-17.

1079   43. Tossounian M-A, Zhao Y, Yu BYK, Markey SA, Malanchuk O, Zhu Y, Cain A, Gout I.

1080   2024. Low-molecular-weight thiol transferases in redox regulation and antioxidant defence.

1081   Redox Biol 71:103094.

1082   44. Goldstein BP. 2014. Resistance to rifampicin: a review. J Antibiot (Tokyo) 67:625–630.

1083   45. Cao M, Feng Y, Zhang Y, Kang W, Lian K, Ai L. 2018. Studies on the metabolism and

1084   degradation of vancomycin in simulated in vitro and aquatic environment by UHPLC-Triple-

1085   TOF-MS/MS. Sci Rep 8:15471.

1086   46. D'Costa VM, Mukhtar TA, Patel T, Koteva K, Waglechner N, Hughes DW, Wright GD,

1087   Pascale GD. 2012. Inactivation of the Lipopeptide Antibiotic Daptomycin by Hydrolytic

1088   Mechanisms. Antimicrob Agents Chemother 56:757.

1089   47. Paris L, Devers-Lamrani M, Joly M, Viala D, De Antonio M, Pereira B, Rouard N, Besse-

1090   Hoggan P, Hébraud M, Topp E, Martin-Laurent F, Batisson I. 2023. Effect of subtherapeutic

1091   and therapeutic sulfamethazine concentrations on transcribed genes and translated proteins

1092   involved in Microbacterium sp. C448 resistance and degradation. FEMS Microbiol Ecol

1093   99:fiad064.

1094   48. Pacholak A, Juzwa W, Zgoła-Grześkowiak A, Kaczorek E. 2023. Multi-faceted analysis of

1095   bacterial transformation of nitrofurantoin. Sci Total Environ 874:162422.

1096   49. Dar D, Sorek R. 2017. Regulation of antibiotic-resistance by non-coding RNAs in bacteria.

1097   Curr Opin Microbiol 36:111–117.

1098   50. Zeng X, Lin J. 2013. Beta-lactamase induction and cell wall metabolism in Gram-negative

1099   bacteria. Front Microbiol 4:128.

1100   51. Irazoki O, Hernandez SB, Cava F. 2019. Peptidoglycan Muropeptides: Release, Perception,

1101   and Functions as Signaling Molecules. Front Microbiol 10:500.

1102   52. Sverak HE, Yaeger LN, Worrall LJ, Vacariu CM, Glenwright AJ, Vuckovic M, Al Azawi Z-

1103   D, Lamers RP, Marko VA, Skorupski C, Soni AS, Tanner ME, Burrows LL, Strynadka NC.

1104   2024. Cryo-EM characterization of the anydromuropeptide permease AmpG central to

1105   bacterial fitness and β-lactam antibiotic resistance. Nat Commun 15:9936.

1106   53. Vadlamani G, Thomas MD, Patel TR, Donald LJ, Reeve TM, Stetefeld J, Standing KG,

1107   Vocadlo DJ, Mark BL. 2015. The β-lactamase gene regulator AmpR is a tetramer that

1108   recognizes and binds the D-Ala-D-Ala motif of its repressor UDP-N-acetylmuramic acid

1109   (MurNAc)-pentapeptide. J Biol Chem 290:2630–2643.

1110   54. Mallik D, Jain D, Bhakta S, Ghosh AS. 2022. Role of AmpC-Inducing Genes in Modulating

1111   Other Serine Beta-Lactamases in Escherichia coli. Antibiot Basel Switz 11:67.

1112   55. Tamma PD, Doi Y, Bonomo RA, Johnson JK, Simner PJ, Antibacterial Resistance

1113   Leadership Group. 2019. A Primer on AmpC β-Lactamases: Necessary Knowledge for an

1114   Increasingly Multidrug-resistant World. Clin Infect Dis Off Publ Infect Dis Soc Am

1115   69:1446–1455.

1116   56. Aracil-Gisbert S, Fernández-De-Bobadilla MD, Guerra-Pinto N, Serrano-Calleja S, Pérez-

1117   Cobas AE, Soriano C, de Pablo R, Lanza VF, Pérez-Viso B, Reuters S, Hasman H, Cantón

1118   R, Baquero F, Coque TM. The ICU environment contributes to the endemicity of the

1119   "Serratia marcescens complex" in the hospital setting. mBio 15:e03054-23.

1120   57. Belluzo BS, Abriata LA, Giannini E, Mihovilcevic D, Dal Peraro M, Llarrull LI. 2019. An

1121   experiment-informed signal transduction model for the role of the Staphylococcus aureus

1122   MecR1 protein in β-lactam resistance. Sci Rep 9:19558.

1123   58. Clarke SR, Dyke KGH. 2001. The signal transducer (BlaRI) and the repressor (BlaI) of the

1124   Staphylococcus aureus beta-lactamase operon are inducible. Microbiol Read Engl 147:803–

1125   810.

1126   59. Yang M-J, Li M-J, Huang L-D, Zhang X-W, Huang Y-Y, Gou X-Y, Chen S-N, Yan J, Du P,

1127   Sun A-H. 2024. Response regulator protein CiaR regulates the transcription of ccn-

1128   microRNAs and β-lactam antibiotic resistance conversion of Streptococcus pneumoniae. Int

1129   J Antimicrob Agents 107387.

1130   60. Jia X, Zhang J, Sun W, He W, Jiang H, Chen D, Murchie AIH. 2013. Riboswitch regulation

1131   of aminoglycoside resistance acetyl and adenyl transferases. Cell 153:1419–1420.

1132   61. Hipólito A, García-Pastor L, Blanco P, Trigo da Roza F, Kieffer N, Vergara E, Jové T,

1133   Álvarez J, Escudero JA. 2022. The expression of aminoglycoside resistance genes in

1134   integron cassettes is not controlled by riboswitches. Nucleic Acids Res 50:8566–8579.

1135   62. Carvalho A, Hipólito A, Trigo da Roza F, García-Pastor L, Vergara E, Buendía A, García-

1136   Seco T, Escudero JA. 2024. The expression of integron arrays is shaped by the translation

1137   rate of cassettes. Nat Commun 15:9232.

1138   63. González-Zorn B, Teshager T, Casas M, Porrero MC, Moreno MA, Courvalin P, Domínguez

1139   L. 2005. armA and aminoglycoside resistance in Escherichia coli. Emerg Infect Dis 11:954–

1140   956.

1141   64. Fritsche TR, Castanheira M, Miller GH, Jones RN, Armstrong ES. 2008. Detection of

1142   Methyltransferases Conferring High-Level Resistance to Aminoglycosides in

1143   Enterobacteriaceae from Europe, North America, and Latin America. Antimicrob Agents

1144   Chemother 52:1843–1845.

1145   65. Matamoros BR, Serna C, Wedel E, Montero N, Kirpekar F, Gonzalez-Zorn B. 2024. NpmC,

1146   a novel A1408 16S rRNA methyltransferase in the gut of humans and animals. Int J

1147   Antimicrob Agents 107382.

1148   66. Yuan M, Nie L, Huang Z, Xu S, Qiu X, Han L, Kang Y, Li F, Yao J, Li Q, Li H, Li D, Zhu

1149   X, Li Z. 2024. Capture of armA by a novel ISCR element, ISCR28. Int J Antimicrob Agents

1150   64:107250.

1151   67. Wangkheimayum J, Paul D, Chanda DD, Melson Singha K, Bhattacharjee A. 2022. Elevated

expression of rsmI can act as a reporter of aminoglycoside resistance in Escherichia coli using kanamycin as signal molecule. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis 98:105229.

68. Rosenberg EY, Ma D, Nikaido H. 2000. AcrD of Escherichia coli is an aminoglycoside efflux pump. J Bacteriol 182:1754–1756.

69. Sidhu K, Talbot M. 2012. Treatment with Sub-inhibitory Kanamycin Induces Adaptive Resistance to Aminoglycoside Antibiotics via the AcrD Multidrug Efflux Pump in Escherichia coli K-12 16.

70. Zhang Z, Morgan CE, Cui M, Yu EW. 2023. Cryo-EM Structures of AcrD Illuminate a Mechanism for Capturing Aminoglycosides from Its Central Cavity. mBio 14:e0338322.

71. Leclercq R. 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis Off Publ Infect Dis Soc Am 34:482–492.

72. Min Y-H, Kwon A-R, Yoon E-J, Shim M-J, Choi E-C. 2008. Translational attenuation and mRNA stabilization as mechanisms of erm(B) induction by erythromycin. Antimicrob Agents Chemother 52:1782–1789.

73. Sothiselvam S, Neuner S, Rigger L, Klepacki D, Micura R, Vázquez-Laslop N, Mankin AS. 2016. Binding of Macrolide Antibiotics Leads to Ribosomal Selection against Specific Substrates Based on Their Charge and Size. Cell Rep 16:1789–1799.

74. Seefeldt AC, Aguirre Rivera J, Johansson M. 2021. Direct Measurements of Erythromycin's Effect on Protein Synthesis Kinetics in Living Bacterial Cells. J Mol Biol 433:166942.

75. Vázquez-Laslop N, Mankin AS. 2018. How Macrolide Antibiotics Work. Trends Biochem Sci 43:668–684.

1175   76. Duval M, Dar D, Carvalho F, Rocha EPC, Sorek R, Cossart P. 2018. HflXr, a homolog of a

1176   ribosome-splitting factor, mediates antibiotic resistance. Proc Natl Acad Sci U S A

1177   115:13359–13364.

1178   77. Lovett PS. 1996. Translation attenuation regulation of chloramphenicol resistance in

1179   bacteria--a review. Gene 179:157–162.

1180   78. George AM, Hall RM. 2002. Efflux of chloramphenicol by the CmlA1 protein. FEMS

1181   Microbiol Lett 209:209–213.

1182   79. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B. 2006. The Cfr rRNA

1183   methyltransferase confers resistance to Phenicols, Lincosamides, Oxazolidinones,

1184   Pleuromutilins, and Streptogramin A antibiotics. Antimicrob Agents Chemother 50:2500–

1185   2505.

1186   80. Structural basis for PoxtA-mediated resistance to phenicol and oxazolidinone antibiotics -

1187   PubMed. https://pubmed.ncbi.nlm.nih.gov/35387982/. Retrieved 27 November 2024.

1188   81. Hinrichs W, Kisker C, Düvel M, Müller A, Tovar K, Hillen W, Saenger W. 1994. Structure

1189   of the Tet repressor-tetracycline complex and regulation of antibiotic resistance. Science

1190   264:418–420.

1191   82. Starosta AL, Lassak J, Jung K, Wilson DN. 2014. The bacterial translation stress response.

1192   FEMS Microbiol Rev 38:1172–1201.

1193   83. Dönhöfer A, Franckenberg S, Wickles S, Berninghausen O, Beckmann R, Wilson DN. 2012.

1194   Structural basis for TetM-mediated tetracycline resistance. Proc Natl Acad Sci U S A

1195   109:16900–16905.

1196   84. Aldred KJ, Kerns RJ, Osheroff N. 2014. Mechanism of quinolone action and resistance.

1197   Biochemistry 53:1565–1574.

1198   85. Monárrez R, Wang Y, Fu Y, Liao C-H, Okumura R, Braun MR, Jacoby GA, Hooper DC.

1199   2018. Genes and Proteins Involved in qnrS1 Induction. Antimicrob Agents Chemother

1200   62:e00806-18.

1201   86. Kurabayashi K, Hirakawa Y, Tanimoto K, Tomita H, Hirakawa H. 2014. Role of the CpxAR

1202   Two-Component Signal Transduction System in Control of Fosfomycin Resistance and

1203   Carbon Substrate Uptake. J Bacteriol 196:248–256.

1204   87. Venkatesan M, Fruci M, Verellen LA, Skarina T, Mesa N, Flick R, Pham C, Mahadevan R,

1205   Stogios PJ, Savchenko A. 2023. Molecular mechanism of plasmid-borne resistance to

1206   sulfonamide antibiotics. Nat Commun 14:4031.

1207   88. Shi H, Li T, Xu J, Yu J, Yang S, Zhang X-E, Tao S, Gu J, Deng J-Y. 2021. MgrB

1208   Inactivation Confers Trimethoprim Resistance in Escherichia coli. Front Microbiol

1209   12:682205.

1210   89. Guberman-Pfeffer M. Resistance: How VanA Strains Get Away With It.

1211   90. Stogios PJ, Savchenko A. 2020. Molecular mechanisms of vancomycin resistance. Protein

1212   Sci Publ Protein Soc 29:654–669.

1213   91. Grissom-Arnold J, Alborn WE, Nicas TI, Jaskunas SR. 1997. Induction of VanA

1214   vancomycin resistance genes in Enterococcus faecalis: use of a promoter fusion to evaluate

1215   glycopeptide and nonglycopeptide induction signals. Microb Drug Resist Larchmt N 3:53–

1216   64.

1217   92. Roch M, Clair P, Renzoni A, Reverdy M-E, Dauwalder O, Bes M, Martra A, Freydière A-M,

1218   Laurent F, Reix P, Dumitrescu O, Vandenesch F. 2014. Exposure of Staphylococcus aureus

1219   to subinhibitory concentrations of β-lactam antibiotics induces heterogeneous vancomycin-

1220   intermediate Staphylococcus aureus. Antimicrob Agents Chemother 58:5306–5314.

1221   93. Axell-House DB, Simar SR, Panesso D, Rincon S, Miller WR, Khan A, Pemberton OA,

1222   Valdez L, Nguyen AH, Hood KS, Rydell K, DeTranaltes AM, Jones MN, Atterstrom R,

1223   Reyes J, Sahasrabhojane PV, Suleyman G, Zervos M, Shelburne SA, Singh KV, Shamoo Y,

1224   Hanson BM, Tran TT, Arias CA. 2024. LiaX is a surrogate marker for cell envelope stress

1225   and daptomycin non-susceptibility in Enterococcus faecium. Antimicrob Agents Chemother

1226   68:e0106923.

1227   94. Olaitan AO, Morand S, Rolain J-M. 2014. Mechanisms of polymyxin resistance: acquired

1228   and intrinsic resistance in bacteria. Front Microbiol 5:643.

1229   95. Gallardo A, Iglesias M-R, Ugarte-Ruiz M, Hernández M, Miguela-Villoldo P, Gutiérrez G,

1230   Rodríguez-Lázaro D, Domínguez L, Quesada A. 2023. Plasmid-mediated Kluyvera-like

1231   arnBCADTEF operon confers colistin (hetero)resistance to Escherichia coli. Antimicrob

1232   Agents Chemother 65:e00091-21, AAC.00091-21.

1233   96. Zhang H, Srinivas S, Xu Y, Wei W, Feng Y. 2019. Genetic and Biochemical Mechanisms

1234   for Bacterial Lipid A Modifiers Associated with Polymyxin Resistance. Trends Biochem Sci

1235   44:973–988.

1236   97. Kieffer N, Royer G, Decousser J-W, Bourrel A-S, Palmieri M, Ortiz De La Rosa J-M,

1237   Jacquier H, Denamur E, Nordmann P, Poirel L. 2019. mcr-9, an Inducible Gene Encoding an

1238   Acquired Phosphoethanolamine Transferase in Escherichia coli, and Its Origin. Antimicrob

1239   Agents Chemother 63:e00965-19.

1240   98. Locke JB, Finn J, Hilgers M, Morales G, Rahawi S, G C K, Picazo JJ, Im W, Shaw KJ, Stein

1241   JL. 2010. Structure-activity relationships of diverse oxazolidinones for linezolid-resistant

1242   Staphylococcus aureus strains possessing the cfr methyltransferase gene or ribosomal

1243   mutations. Antimicrob Agents Chemother 54:5337–5343.

1244 99. Crowe-McAuliffe C, Murina V, Turnbull KJ, Huch S, Kasari M, Takada H, Nersisyan L,

1245   Sundsfjord A, Hegstad K, Atkinson GC, Pelechano V, Wilson DN, Hauryliuk V. 2022.

1246   Structural basis for PoxtA-mediated resistance to phenicol and oxazolidinone antibiotics. Nat

1247   Commun 13:1860.

1248 100.   Tomlinson JH, Thompson GS, Kalverda AP, Zhuravleva A, O'Neill AJ. 2016. A target-

1249   protection mechanism of antibiotic resistance at atomic resolution: insights into FusB-type

1250   fusidic acid resistance. Sci Rep 6:19524.

1251 101.   O'Neill AJ, McLaws F, Kahlmeter G, Henriksen AS, Chopra I. 2007. Genetic basis of

1252   resistance to fusidic acid in staphylococci. Antimicrob Agents Chemother 51:1737–1740.

1253 102.   O'Neill AJ, Chopra I. 2006. Molecular basis of fusB-mediated resistance to fusidic acid

1254   in Staphylococcus aureus. Mol Microbiol 59:664–676.

1255 103.   Roemhild R, Linkevicius M, Andersson DI. 2020. Molecular mechanisms of collateral

1256   sensitivity to the antibiotic nitrofurantoin. PLoS Biol 18:e3000612.

1257 104.   Hausladen A, Privalle CT, Keng T, DeAngelo J, Stamler JS. 1996. Nitrosative stress:

1258   activation of the transcription factor OxyR. Cell 86:719–729.

1259 105.   Niu J, Straubinger RM, Mager DE. 2019. Pharmacodynamic Drug-Drug Interactions.

1260   Clin Pharmacol Ther 105:1395–1406.

1261 106.   Cabello M, Hernández-García M, Maruri-Aransolo A, Michelena M, Pérez-Viso B,

1262   Ponce-Alonso M, Cantón R, Ruiz-Garbajosa P. 2024. Occurrence of multi-carbapenemase-

1263   producing Enterobacterales in a tertiary hospital in Madrid (Spain): A new epidemiologic

1264   scenario. J Glob Antimicrob Resist 38:281–291.

1265 107.   Li X-Z, Nikaido H. 2009. Efflux-Mediated Drug Resistance in Bacteria: an Update.

1266   Drugs 69:1555–1623.

1267   108.   Berryhill BA, Gil-Gil T, Manuel JA, Smith AP, Margollis E, Baquero F, Levin BR. 2023.

1268   What's the Matter with MICs: Bacterial Nutrition, Limiting Resources, and Antibiotic

1269   Pharmacodynamics. Microbiol Spectr 11:e0409122.

1270   109.   Wang Y, Ran M, Wang J, Ouyang Q, Luo C. 2015. Studies of Antibiotic Resistance of

1271   Beta-Lactamase Bacteria under Different Nutrition Limitations at the Single-Cell Level.

1272   PLoS ONE 10:e0127115.

1273   110.   Peng B, Su Y-B, Li H, Han Y, Guo C, Tian Y-M, Peng X-X. 2015. Exogenous alanine

1274   and/or glucose plus kanamycin kills antibiotic-resistant bacteria. Cell Metab 21:249–262.

1275   111.   Peng B, Li H, Peng X-X. 2015. Functional metabolomics: from biomarker discovery to

1276   metabolome reprogramming. Protein Cell 6:628–637.

1277   112.   Lopatkin AJ, Bening SC, Manson AL, Stokes JM, Kohanski MA, Badran AH, Earl AM,

1278   Cheney NJ, Yang JH, Collins JJ. 2021. Clinically relevant mutations in core metabolic genes

1279   confer antibiotic resistance. Science 371:eaba0862.

1280   113.   Jiang M, Su Y-B, Ye J-Z, Li H, Kuang S-F, Wu J-H, Li S-H, Peng X-X, Peng B. 2023.

1281   Ampicillin-controlled glucose metabolism manipulates the transition from tolerance to

1282   resistance in bacteria. Sci Adv 9:eade8582.

1283   114.   Yi X, Feng M, He F, Xiao Z, Wang Y, Wang S, Yao H. 2024. Multi-omics analysis

1284   explores the impact of ofloxacin pressure on the metabolic state in Escherichia coli. J Glob

1285   Antimicrob Resist 39:59–68.

1286   115.   Vestergaard M, Nøhr-Meldgaard K, Bojer MS, Krogsgård Nielsen C, Meyer RL,

1287   Slavetinsky C, Peschel A, Ingmer H. 2017. Inhibition of the ATP Synthase Eliminates the

1288   Intrinsic Resistance of Staphylococcus aureus towards Polymyxins. mBio 8:e01114-17.

1289   116.   Baquero F, Martínez J-L. 2017. Interventions on Metabolism: Making Antibiotic-

1290   Susceptible Bacteria. mBio 8:e01950-17.

117.   Baquero F, Martínez J-L, Sánchez A, Fernández-de-Bobadilla MD, San-Millán A,

Rodríguez-Beltrán J. 2023. Bacterial Subcellular Architecture, Structural Epistasis, and

Antibiotic Resistance. Biology 12:640.

118.   Stevanovic M, Teuber Carvalho JP, Bittihn P, Schultz D. 2024. Dynamical model of

antibiotic responses linking expression of resistance genes to metabolism explains

emergence of heterogeneity during drug exposures. Phys Biol 21:036002.

119.   Landy M, Larkum NW, Oswald EJ, Streightoff F. 1943. INCREASED SYNTHESIS OF

p-AMINOBENZOIC ACID ASSOCIATED WITH THE DEVELOPMENT OF

SULFONAMIDE RESISTANCE IN STAPHYLOCOCCUS AUREUS. Science 97:265–267.

120.   Baquero F, Coque TM, Martínez J-L, Aracil-Gisbert S, Lanza VF. 2019. Gene

Transmission in the One Health Microbiosphere and the Channels of Antimicrobial

Resistance. Front Microbiol 10:2892.

121.   MacNair CR, Tan M-W. 2023. The role of bacterial membrane vesicles in antibiotic

resistance. Ann N Y Acad Sci 1519:63–73.

122.   Novelli M, Bolla J-M. 2024. RND Efflux Pump Induction: A Crucial Network Unveiling

Adaptive Antibiotic Resistance Mechanisms of Gram-Negative Bacteria. Antibiot Basel

Switz 13:501.

123.   Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, Luisi BF.

2018. Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol

16:523–539.

124.   Dar D, Shamir M, Mellin JR, Koutero M, Stern-Ginossar N, Cossart P, Sorek R. 2016.

Term-seq reveals abundant ribo-regulation of antibiotics resistance in bacteria. Science

1313   352:aad9822.

125.   Møller TSB, Overgaard M, Nielsen SS, Bortolaia V, Sommer MOA, Guardabassi L,

1315   Olsen JE. 2016. Relation between tetR and tetA expression in tetracycline resistant

1316   Escherichia coli. BMC Microbiol 16:39.

126.   Quantifying bacterial efflux within subcellular domains of Pseudomonas aeruginosa -

1318   PubMed. https://pubmed.ncbi.nlm.nih.gov/39475289/. Retrieved 27 November 2024.

127.   Costafrolaz J, Panis G, Casu B, Ardissone S, Degeorges L, Pilhofer M, Viollier PH.

1320   2023. Adaptive β-lactam resistance from an inducible efflux pump that is post-translationally

1321   regulated by the DjlA co-chaperone. PLoS Biol 21:e3002040.

128.   Gallagher LA, Coughlan S, Black NS, Lalor P, Waters EM, Wee B, Watson M, Downing

1323   T, Fitzgerald JR, Fleming GTA, O'Gara JP. 2017. Tandem Amplification of the

1324   Staphylococcal Cassette Chromosome mec Element Can Drive High-Level Methicillin

1325   Resistance in Methicillin-Resistant Staphylococcus aureus. Antimicrob Agents Chemother

1326   61:e00869-17.

129.   Peters K, Schweizer I, Hakenbeck R, Denapaite D. 2021. New Insights into Beta-Lactam

1328   Resistance of Streptococcus pneumoniae: Serine Protease HtrA Degrades Altered Penicillin-

1329   Binding Protein 2x. Microorganisms 9:1685.

130.   Martínez JL, Alonso A, Gómez-Gómez JM, Baquero F. 1998. Quinolone resistance by

1331   mutations in chromosomal gyrase genes. Just the tip of the iceberg? J Antimicrob Chemother

1332   42:683–688.

131.   Hernandez-Beltran JCR, Rodríguez-Beltrán J, Aguilar-Luviano OB, Velez-Santiago J,

1334   Mondragón-Palomino O, MacLean RC, Fuentes-Hernández A, San Millán A, Peña-Miller R.

1335   2024. Plasmid-mediated phenotypic noise leads to transient antibiotic resistance in bacteria.

1336   Nat Commun 15:2610.

132.   Wang Y, Penkul P, Milstein JN. 2016. Quantitative Localization Microscopy Reveals a

        Novel Organization of a High-Copy Number Plasmid. Biophys J 111:467–479.

133.   Niki H, Hiraga S. 1997. Subcellular distribution of actively partitioning F plasmid during

        the cell division cycle in E. coli. Cell 90:951–957.

134.   Kannaiah S, Amster-Choder O. 2014. Protein targeting via mRNA in bacteria. Biochim

        Biophys Acta 1843:1457–1465.

135.   Castellana M, Hsin-Jung Li S, Wingreen NS. 2016. Spatial organization of bacterial

        transcription and translation. Proc Natl Acad Sci U S A 113:9286–9291.

136.   Rudner DZ, Losick R. 2010. Protein Subcellular Localization in Bacteria. Cold Spring

        Harb Perspect Biol 2:a000307.

137.   Manting EH, Driessen AJ. 2000. Escherichia coli translocase: the unravelling of a

        molecular machine. Mol Microbiol 37:226–238.

138.   Kaderabkova N, Bharathwaj M, Furniss RCD, Gonzalez D, Palmer T, Mavridou DAI.

        2022. The biogenesis of β-lactamase enzymes. Microbiol Read Engl 168:001217.

139.   Plückthun A, Pfitzinger I. 1988. Membrane-bound beta-lactamase forms in Escherichia

        coli. J Biol Chem 263:14315–14322.

140.   Bradford PA, Bonomo RA, Bush K, Carattoli A, Feldgarden M, Haft DH, Ishii Y, Jacoby

        GA, Klimke W, Palzkill T, Poirel L, Rossolini GM, Tamma PD, Arias CA. 2022. Consensus

        on β-Lactamase Nomenclature. Antimicrob Agents Chemother 66:e0033322.

141.   Francisco JA, Earhart CF, Georgiou G. 1992. Transport and anchoring of beta-lactamase

        to the external surface of Escherichia coli. Proc Natl Acad Sci U S A 89:2713–2717.

142.   Livermore DM. 1997. beta-Lactamases: quantity and resistance. Clin Microbiol Infect

1359   Off Publ Eur Soc Clin Microbiol Infect Dis 3 Suppl 4:S10–S19.

1360   143.   Kadonaga JT, Gautier AE, Straus DR, Charles AD, Edge MD, Knowles JR. 1984. The

1361   role of the beta-lactamase signal sequence in the secretion of proteins by Escherichia coli. J

1362   Biol Chem 259:2149–2154.

1363   144.   Balchin D, Hayer-Hartl M, Hartl FU. 2020. Recent advances in understanding catalysis

1364   of protein folding by molecular chaperones. FEBS Lett 594:2770–2781.

1365   145.   Bowden GA, Paredes AM, Georgiou G. 1991. Structure and morphology of protein

1366   inclusion bodies in Escherichia coli. Biotechnol Nat Publ Co 9:725–730.

1367   146.   Khodaparast L, Khodaparast L, Wu G, Michiels E, Gallardo R, Houben B, Garcia T, De

1368   Vleeschouwer M, Ramakers M, Wilkinson H, Duran-Romaña R, Van Eldere J, Rousseau F,

1369   Schymkowitz J. 2023. Exploiting the aggregation propensity of beta-lactamases to design

1370   inhibitors that induce enzyme misfolding. Nat Commun 14:5571.

1371   147.   Kim SW, Seo J-S, Park SB, Lee AR, Lee JS, Jung JW, Chun JH, Lazarte JMS, Kim J,

1372   Kim J-H, Song J-W, Franco C, Zhang W, Ha MW, Paek S-M, Jung M, Jung TS. 2020.

1373   Significant increase in the secretion of extracellular vesicles and antibiotics resistance from

1374   methicillin-resistant Staphylococcus aureus induced by ampicillin stress. Sci Rep 10:21066.

1375   148.   Jiang B, Lai Y, Xiao W, Zhong T, Liu F, Gong J, Huang J. 2024. Microbial extracellular

1376   vesicles contribute to antimicrobial resistance. PLoS Pathog 20:e1012143.

1377   149.   Herencias C, Álvaro-Llorente L, Ramiro-Martínez P, Fernández-Calvet A, Muñoz-

1378   Cazalla A, DelaFuente J, Graf FE, Jaraba-Soto L, Castillo-Polo JA, Cantón R, San Millán Á,

1379   Rodríguez-Beltrán J. 2024. β-lactamase expression induces collateral sensitivity in

1380   Escherichia coli. Nat Commun 15:4731.

1381   150.   Reguera JA, Baquero F, Perez-Diaz JC, Martinez JL. 1988. Synergistic effect of dosage

1382   and bacterial inoculum in TEM-1 mediated antibiotic resistance. Eur J Clin Microbiol Infect

1383   Dis Off Publ Eur Soc Clin Microbiol 7:778–779.

1384   151.   San Millan A, Escudero JA, Gifford DR, Mazel D, MacLean RC. 2016. Multicopy

1385   plasmids potentiate the evolution of antibiotic resistance in bacteria. Nat Ecol Evol 1:10.

1386   152.   Canabal R, González-Bello C. 2024. Chemical sensors for the early diagnosis of bacterial

1387   resistance to β-lactam antibiotics. Bioorganic Chem 150:107528.

1388   153.   Gross R, Yelin I, Lázár V, Datta MS, Kishony R. 2024. Beta-lactamase dependent and

1389   independent evolutionary paths to high-level ampicillin resistance. Nat Commun 15:5383.

1390   154.   Li L, Wang Q, Zhang H, Yang M, Khan MI, Zhou X. 2016. Sensor histidine kinase is a

1391   β-lactam receptor and induces resistance to β-lactam antibiotics. Proc Natl Acad Sci U S A

1392   113:1648–1653.

1393   155.   Balasubramanian D, Schneper L, Merighi M, Smith R, Narasimhan G, Lory S, Mathee K.

1394   2012. The regulatory repertoire of Pseudomonas aeruginosa AmpC ß-lactamase regulator

1395   AmpR includes virulence genes. PloS One 7:e34067.

1396   156.   Morè N, Martorana AM, Biboy J, Otten C, Winkle M, Serrano CKG, Montón Silva A,

1397   Atkinson L, Yau H, Breukink E, den Blaauwen T, Vollmer W, Polissi A. 2019.

1398   Peptidoglycan Remodeling Enables Escherichia coli To Survive Severe Outer Membrane

1399   Assembly Defect. mBio 10:e02729-18.

1400   157.   Martinez JL, Vicente MF, Delgado-Iribarren A, Perez-Diaz JC, Baquero F. 1989. Small

1401   plasmids are involved in amoxicillin-clavulanate resistance in Escherichia coli. Antimicrob

1402   Agents Chemother 33:595.

1403   158.   Martinez JL, Cercenado E, Rodriguez-Creixems M, Vincente-Perez MF, Delgado-

1404   Iribarren A, Baquero F. 1987. Resistance to beta-lactam/clavulanate. Lancet Lond Engl

1405   2:1473.

159.   San Millan A, Santos-Lopez A, Ortega-Huedo R, Bernabe-Balas C, Kennedy SP,
        Gonzalez-Zorn B. 2015. Small-plasmid-mediated antibiotic resistance is enhanced by
        increases in plasmid copy number and bacterial fitness. Antimicrob Agents Chemother
        59:3335–3341.

160.   Sandegren L, Andersson DI. 2009. Bacterial gene amplification: implications for the
        evolution of antibiotic resistance. Nat Rev Microbiol 7:578–588.

161.   Culp E, Wright GD. 2017. Bacterial proteases, untapped antimicrobial drug targets. J
        Antibiot (Tokyo) 70:366–377.

162.   Schultz SC, Dalbadie-McFarland G, Neitzel JJ, Richards JH. 1987. Stability of wild-type
        and mutant RTEM-1 beta-lactamases: effect of the disulfide bond. Proteins 2:290–297.

163.   Shimizu-Ibuka A, Matsuzawa H, Sakai H. 2006. Effect of disulfide-bond introduction on
        the activity and stability of the extended-spectrum class A beta-lactamase Toho-1. Biochim
        Biophys Acta 1764:1349–1355.

164.   Beadle BM, McGovern SL, Patera A, Shoichet BK. 1999. Functional analyses of AmpC
        beta-lactamase through differential stability. Protein Sci Publ Protein Soc 8:1816–1824.

165.   Baquero F, Levin BR. 2021. Proximate and ultimate causes of the bactericidal action of
        antibiotics. Nat Rev Microbiol 19:123–132.

166.   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.

167.   Shaw KJ, Rather PN, Hare RS, Miller GH. 1993. Molecular genetics of aminoglycoside
        resistance genes and familial relationships of the aminoglycoside-modifying enzymes.

1428   Microbiol Rev 57:138–163.

168.   Dery KJ, Søballe B, Witherspoon MSL, Bui D, Koch R, Sherratt DJ, Tolmasky ME.
        2003. The Aminoglycoside 6′-N-Acetyltransferase Type Ib Encoded by Tn1331 Is Evenly
        Distributed within the Cell's Cytoplasm. Antimicrob Agents Chemother 47:2897–2902.

169.   d'Acoz O d'Udekem, Hue F, Ye T, Wang L, Leroux M, Rajngewerc L, Tran T, Phan K,
        Ramirez MS, Reisner W, Tolmasky ME, Reyes-Lamothe R. 2023. Dynamics and
        quantitative contribution of the aminoglycoside 6'-N-acetyltransferase type Ib [AAC(6')-Ib]
        to amikacin resistance. BioRxiv Prepr Serv Biol 2023.09.05.556435.

170.   McGann P, Courvalin P, Snesrud E, Clifford RJ, Yoon E-J, Onmus-Leone F, Ong AC,
        Kwak YI, Grillot-Courvalin C, Lesho E, Waterman PE. 2014. Amplification of
        aminoglycoside resistance gene aphA1 in Acinetobacter baumannii results in tobramycin
        therapy failure. mBio 5:e00915.

171.   Martinez JL, Blazquez J, Vicente MF, Martinez-Ferrer M, Reguera JA, Culebras E,
        Baquero F, Martinez JL, Blazquez J, Vicente MF, Martinez-Ferrer M, Reguera JA, Culebras
        E, Baquero F. 1989. Influence of gene dosing on antibiotic resistance mediated by
        inactivating enzymes. 4 Suppl. J Chemother Florence Italy 1:265–266.

172.   Blazquez J, Martinez JL, Baquero F. 1993. Bleomycin increases amikacin and
        streptomycin resistance in Escherichia coli harboring transposon Tn5. Antimicrob Agents
        Chemother 37:1982–1985.

173.   d'Udekem d'Acoz O, Hue F, Ye T, Wang L, Leroux M, Rajngewerc L, Tran T, Phan K,
        Ramirez MS, Reisner W, Tolmasky ME, Reyes-Lamothe R. 2024. Dynamics and
        quantitative contribution of the aminoglycoside 6'-N-acetyltransferase type Ib to amikacin
        resistance. mSphere 9:e0078923.

1451   174.   Vázquez-Laslop N, Mankin AS. 2018. Context-Specific Action of Ribosomal

1452   Antibiotics. Annu Rev Microbiol 72:185–207.

1453   175.   Dzyubak E, Yap MN. 2016. The Expression of Antibiotic Resistance Methyltransferase

1454   Correlates with mRNA Stability Independently of Ribosome Stalling. Antimicrob Agents

1455   Chemother 60:7178–7188.

1456   176.   Chopra I, Roberts M. 2001. Tetracycline Antibiotics: Mode of Action, Applications,

1457   Molecular Biology, and Epidemiology of Bacterial Resistance. Microbiol Mol Biol Rev

1458   65:232–260.

1459   177.   Cramariuc O, Rog T, Javanainen M, Monticelli L, Polishchuk AV, Vattulainen I. 2012.

1460   Mechanism for translocation of fluoroquinolones across lipid membranes. Biochim Biophys

1461   Acta 1818:2563–2571.

1462   178.   Dumont E, Vergalli J, Conraux L, Taillier C, Vassort A, Pajovic J, Réfrégiers M, Mourez

1463   M, Pagès J-M. 2019. Antibiotics and efflux: combined spectrofluorimetry and mass

1464   spectrometry to evaluate the involvement of concentration and efflux activity in antibiotic

1465   intracellular accumulation. J Antimicrob Chemother 74:58–65.

1466   179.   García-López M, Megias D, Ferrándiz M-J, de la Campa AG. 2022. The balance between

1467   gyrase and topoisomerase I activities determines levels of supercoiling, nucleoid compaction,

1468   and viability in bacteria. Front Microbiol 13:1094692.

1469   180.   Mustaev A, Malik M, Zhao X, Kurepina N, Luan G, Oppegard LM, Hiasa H, Marks KR,

1470   Kerns RJ, Berger JM, Drlica K. 2014. Fluoroquinolone-gyrase-DNA complexes: two modes

1471   of drug binding. J Biol Chem 289:12300–12312.

1472   181.   Murawski AM, Brynildsen MP. 2021. Ploidy is an important determinant of

1473   fluoroquinolone persister survival. Curr Biol CB 31:2039-2050.e7.

1474   182.   Gaona M, Corral J, Campoy S, Barbé J, Pérez Varela M, Aranda J. 2024. The novel MFS

1475   efflux pump SxtP, regulated by the LysR-type transcriptional activator SxtR, is involved in

1476   the susceptibility to sulfamethoxazole/trimethoprim (SXT) and the pathogenesis of

1477   Acinetobacter baumannii. Antimicrob Agents Chemother 68:e0071224.

1478   183.   Nicolaou KC, Boddy CN, Bräse S, Winssinger N. 1999. Chemistry, Biology, and

1479   Medicine of the Glycopeptide Antibiotics. Angew Chem Int Ed Engl 38:2096–2152.

1480   184.   Masi M, Réfregiers M, Pos KM, Pagès J-M. 2017. Mechanisms of envelope permeability

1481   and antibiotic influx and efflux in Gram-negative bacteria. Nat Microbiol 2:17001.

1482   185.   Nicoloff H, Hjort K, Andersson DI, Wang H. 2024. Three concurrent mechanisms

1483   generate gene copy number variation and transient antibiotic heteroresistance. Nat Commun

1484   15:3981.

1485   186.   Stracy M, Wollman AJM, Kaja E, Gapinski J, Lee J-E, Leek VA, McKie SJ, Mitchenall

1486   LA, Maxwell A, Sherratt DJ, Leake MC, Zawadzki P. 2019. Single-molecule imaging of

1487   DNA gyrase activity in living Escherichia coli. Nucleic Acids Res 47:210–220.

1488   187.   Thornton M, Armitage M, Maxwell A, Dosanjh B, Howells AJ, Norris V, Sigee DC.

1489   1994. Immunogold localization of GyrA and GyrB proteins in Escherichia coli. Microbiol

1490   Read Engl 140 (Pt 9):2371–2382.

1491   188.   Garoff L, Yadav K, Hughes D. 2018. Increased expression of Qnr is sufficient to confer

1492   clinical resistance to ciprofloxacin in Escherichia coli. J Antimicrob Chemother 73:348–352.

1493   189.   Xiong X, Bromley EHC, Oelschlaeger P, Woolfson DN, Spencer J. 2011. Structural

1494   insights into quinolone antibiotic resistance mediated by pentapeptide repeat proteins:

1495   conserved surface loops direct the activity of a Qnr protein from a gram-negative bacterium.

1496   Nucleic Acids Res 39:3917–3927.

1497 190. Stock JB, Rauch B, Roseman S. 1977. Periplasmic space in Salmonella typhimurium and

1498 Escherichia coli. J Biol Chem 252:7850–7861.

1499 191. Garde S, Chodisetti PK, Reddy M. 2021. Peptidoglycan: Structure, Synthesis, and

1500 Regulation. EcoSal Plus 9:eESP-0010-2020.

1501 192. Scheffers D-J, Pinho MG. 2005. Bacterial cell wall synthesis: new insights from

1502 localization studies. Microbiol Mol Biol Rev MMBR 69:585–607.

1503 193. Bailey BWA, Stansfeld PJ. 2024. The bacterial cytoskeleton alters the cell membrane in

1504 molecular dynamics simulations. Biophys J 123:374a.

1505 194. Pucci MJ, Dougherty TJ. 2002. Direct quantitation of the numbers of individual

1506 penicillin-binding proteins per cell in Staphylococcus aureus. J Bacteriol 184:588–591.

1507 195. Dougherty TJ, Kennedy K, Kessler RE, Pucci MJ. 1996. Direct quantitation of the

1508 number of individual penicillin-binding proteins per cell in Escherichia coli. J Bacteriol

1509 178:6110–6115.

1510 196. Allam A, Maigre L, Vergalli J, Dumont E, Cinquin B, Alves de Sousa R, Pajovic J, Pinet

1511 E, Smith N, Herbeuval J-P, Réfrégiers M, Artaud I, Pagès J-M. 2017.

1512 Microspectrofluorimetry to dissect the permeation of ceftazidime in Gram-negative bacteria.

1513 Sci Rep 7:986.

1514 197. Geyer CN, Fowler RC, Johnson JR, Johnston B, Weissman SJ, Hawkey P, Hanson ND.

1515 2016. Evaluation of CTX-M steady-state mRNA, mRNA half-life and protein production in

1516 various STs of Escherichia coli. J Antimicrob Chemother 71:607–616.

1517 198. Vergalli J, Dumont E, Pajović J, Cinquin B, Maigre L, Masi M, Réfrégiers M, Pagés J-M.

1518 2018. Spectrofluorimetric quantification of antibiotic drug concentration in bacterial cells for

1519 the characterization of translocation across bacterial membranes. Nat Protoc 13:1348–1361.

1520   199.   Ukleja M, Kricks L, Torrens G, Peschiera I, Rodrigues-Lopes I, Krupka M, García-

1521   Fernández J, Melero R, Del Campo R, Eulalio A, Mateus A, López-Bravo M, Rico AI, Cava

1522   F, Lopez D. 2024. Flotillin-mediated stabilization of unfolded proteins in bacterial

1523   membrane microdomains. Nat Commun 15:5583.

1524   200.   Welling PG. 1995. Differences between pharmacokinetics and toxicokinetics. Toxicol

1525   Pathol 23:143–147.

1526   201.   Andersson DI, Hughes D. 2010. Antibiotic resistance and its cost: is it possible to reverse

1527   resistance? Nat Rev Microbiol 8:260–271.

1528   202.   San Millan A, MacLean RC. 2017. Fitness Costs of Plasmids: a Limit to Plasmid

1529   Transmission. Microbiol Spectr 5.

1530   203.   Baquero F, Rodríguez-Beltrán J, Coque TM, Del Campo R. 2024. Boosting Fitness Costs

1531   Associated with Antibiotic Resistance in the Gut: On the Way to Biorestoration of

1532   Susceptible Populations. Biomolecules 14:76.

1533   204.   Munita JM, Arias CA. 2016. Mechanisms of antibiotic resistance. Microbiol Spectr

1534   4:10.1128.

1535

1536

1537

1538 **Author Bios**

1539 To be submitted after the acceptation of the manuscript.

1540

1541

1542

1543

1544

1545

1546

Table I: Antechodynamics: primary detoxifying effector molecules causing direct effect on antimicrobial agents

Antibiotics	Primary detoxifying effector molecules
Beta-lactams	Beta-lactamases (proteases-hydrolases)
Aminoglycosides	Acetyl-transferases, Phospho-transferases, Nucleotydyl-transferases
Macrolides, Lincosamides, Streptogramins	Phospho-transferases, Esterases, Nucleotydyl-transferases, Acetyl-transferases, Hydrolases.
Phenicols	Acetyltransferases
Tetracyclines	Monooxygenases
Fluoroquinolones	Acetyl-transferases, Monooxygenases
Fosfomycin	Metallo-glutathione-transferases
Rifampicin	Glycosyl-transferases, Nucleotydyl-transferases, Phospho-transferases, Monooxygenases
Glyco-Lipopeptides	Monooxygenases (?), Deacylases, Serin-protease-hydrolases
Sulphonamides	Flavin-Monooxygenases, Flavin-Reductases

1549

1550

1551

1552

1553

1554

1555

1556

1557

1558

1559

1560

1561

1562

1563

Table II: Antechodynamics: secondary effector molecules triggering the
expression of genes involved in antibiotic resistance.

Antibiotics	Secondary effector molecules triggering antibiotic detoxification	Detoxification mechanism
Beta-lactams	Muropeptides (murein fragments), Transmembrane sensor transducers MicroRNAs transcriptases	Induction beta-lactamases Induction beta-lactamases PBP degradation
Aminoglycosides	AttC-site integron recombinases 16SrRNA methyl-transferases AcrD efflux pump synthases	Increase acetyl-transferases Increase nucleotydyl-transferases Reduced ribosome binding Efflux pump AcrD
Macrolides Lincosamides, Streptogramins	23S-rRNA methyl-transferase	Reduced ribosome binding
Phenicols	23S-rRNA methyltransferase ATP binding cassette proteins	Reduced ribosome binding
Tetracyclines	*tetR* repressor-tetracycline complex TetM and TetO proteins	Expression efflux pump TetA Tetracycline target displacement
Fluoroquinolones	Qnr pentapeptide repeat protein, requiring integration host factors	DNA target protection
Fosfomycin	Two-component signal transduction	Decreased uptake
Sulphonamides	Two-component signal transduction activated by reduced thymidine levels	Increase in thymidine levels
Glyco-Lipopeptides	Two-component signal transduction	d-Ala-d-lac ligase, modifying the target in the cell wall
Polymyxins	Two-component signal transduction	Induction of lipid A acetylase, phosphoethanolamine, or 4-amino-4-deoxy-L-arabinose transferases: target modification
Oxazolidinones	23S-rRNA methyltransferase ATP-binding cassette	Reduced ribosome binding Target modification
Fusidic acid	Elongation Factor-G-binding protein	Target protection
Nitrofurantoin	Two-component signal transduction	Lower transcription of nitroreductases with reduced nitrofurantoin effect.
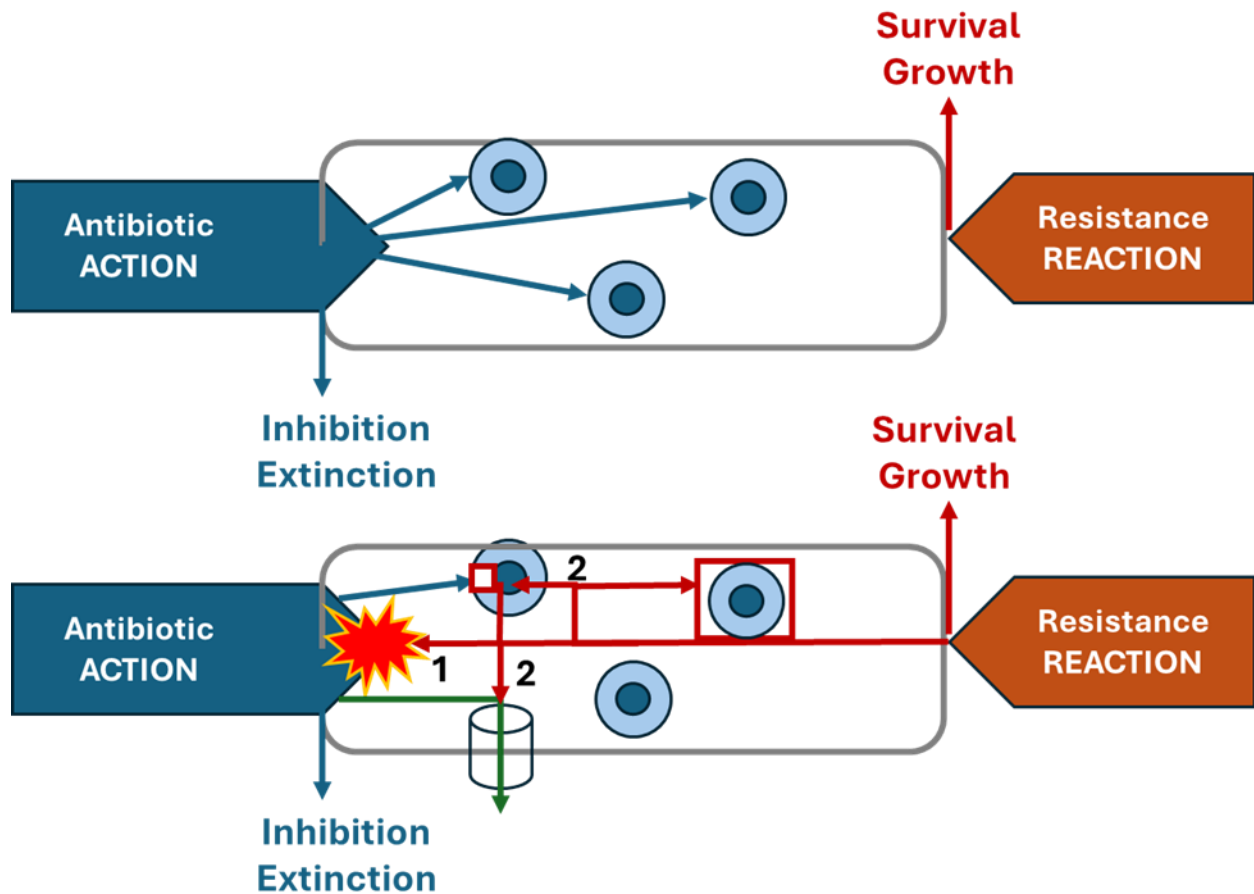
1569

1570

1571   Fig 1. Antibiotics action and Resistance reaction. Blue circles, antibiotic targets; when disturbed (dark green

1572   arrows), the result is bacterial extinction or growth inhibition. Molecules involved in resistance counteract

1573   antibiotics' action (red arrows), destroying or altering the antibiotic (blast) by antechodynamic primary (1)

1574   effectors, or secondary (2) effectors, acting by triggering primary effectors, preventing antibiotic-target

1575   binding (red squares), or pumping out the antibiotic (cylinder), as a result of the antibiotic action on targets.

1576   The result is bacterial cell survival or growth. Antibiotic pharmacology predicts antibiotic effectiveness;

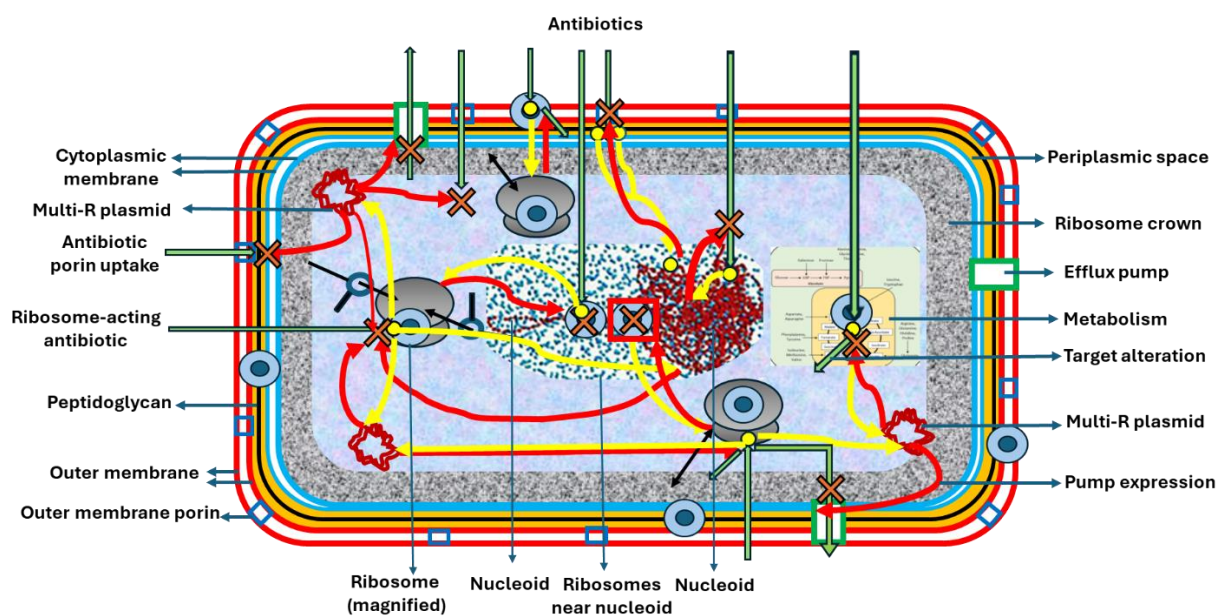1577   antechology predicts antibiotic resistance.

1578

1579

1580

1581

1582

1583

Fig 2. A schema of antechodynamic and antechokinetic processes in a bacterial cell. Blue double circles represent antibiotic targets. The dotted frame represents the bacterial ribosomes, mainly located in the region below the cytoplasmic membrane; the double grey ovals are a magnification of the ribosomes (see magnifying glasses). Green arrows represent antibiotics entering and eventually being detoxified, either destroyed, structurally modified to prevent binding to the target, or pumped out (red-bladed-crosses). Antechodynamic primary effector molecules (red lines) are directly targeting (often destroying or modifying) the antibiotic. Antechodynamic secondary effectors (yellow lines) are molecules resulting from antibiotic action that activate primary effectors or modify the antibiotic target preventing drug binding. The intracellular spatial trajectories of the detoxifying molecules (red and yellow lines), as their relative abundance in relation to the target density and their stability in the cell, are much less known aspects; this is the field of Antechokinetics. See the text for more detailed information.

1595