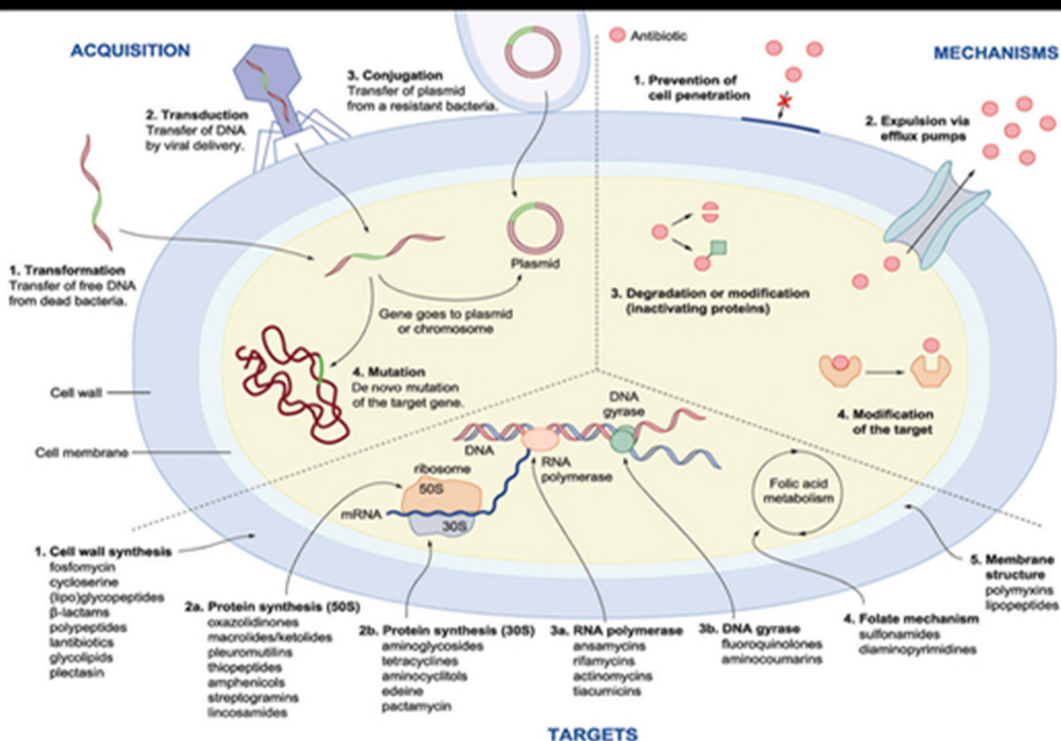


EDITED BY
JOSÉ-LUIS CAPELO-MARTÍNEZ | GILBERTO IGREJAS

ANTIBIOTIC DRUG RESISTANCE



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Antibiotic Drug Resistance

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Preface

In the fight to survive, bacteria have been able to find their own path to succeed despite what may be considered their worst-case evolutionary scenario, the advent of the antibiotic era. Bacterial resistance to antibiotics has reached levels of success unexpectedly 20 years ago. Some bacteria can resist, literally, everything human kind has invented to fight them. The situation is worsening as the bacteria causing pneumonia, tuberculosis, gonorrhea, and salmonellosis are becoming multiresistant to all known antibiotics. For humans, this problem is having a tremendous effect on our society, regardless of gender, age, or country, because the cost of medical care is increasingly high due to longer hospital stays and the need of sophisticated and expensive new drugs. There is an additional threat for immunodepressed patients who cannot survive multiresistant bacteria. Though the causes of multiresistance are complex, it seems the misuse of antibiotics in human medicine and more conspicuously in animal medicine and husbandry is one of the primary causes. To complicate things further, the spread of multiresistance is a pressing issue, as poor hygiene, unsafe sexual relationships, and poor food preservation are key factors that help to magnify the problem (World Health Organization 2019). Biocides and other antimicrobials could co-select for bacteria resistant to clinically relevant antibiotics, and therefore the use of these chemicals must be revisited (Oniciuc et al. 2019).

There is collateral damage in the use of antibiotics. The large amount of antibiotics produced every year (c. 100 K tons) has environmental implications of great concern. Antibiotics are now ubiquitous in the environment. For example, they have been detected in freshwater and in fish at sublethal concentrations that can contribute to spreading bacterial resistance and changing the composition of single-celled communities (Danner et al. 2019). The way antibiotic resistance is acquired is still under debate, but it is certainly multifactorial and complex.

Some of today's problems with antibiotic resistance are clearly growing so much and so rapidly that they seem intractable. Therefore, now more than ever, a holistic view of the problem is necessary. This book is intended to fill

this gap. The first part of the book addresses the mechanisms of action of the antibiotics most used nowadays, namely, aminoglycosides, quinolones, beta-lactams, glycopeptides, and macrolides. The mechanisms by which bacteria develop antibiotic resistance, including mutations and gene transfer, are also explained. As an issue that negatively affects the living conditions of many people, the socioeconomic impact of antibiotic resistance on public health is also discussed, with special emphasis in public policies aimed at reducing or eliminating pathogens in the environment. Special attention is given to strategies devoted to overcoming antibiotic resistance, with focus on (i) new strategies to design drugs, (ii) antibiotics from natural sources, (iii) strategies based on antimicrobials and bacteriophages, (iv) sensitizing agents to restore antibiotic activity, (v) nontraditional medicines, and (vi) therapeutic options to treat infections caused by pathogenic biofilms.

We believe this book offers a unique global perspective of the problem of antibiotic resistance, as it integrates current knowledge in all related areas from new antibiotics to the reuse of old ones, from new strategies to fight bacteria based on natural products or bacteriophages to new synthetic drugs, and from the strategies to prevent the spread of antibiotic resistance to public policies to reduce the impact of the problem.

We are in debt to everyone involved in bringing this project to fruition, especially to Professor Ramaiah who kindly proposed the idea of the book and to the Wiley Editorial Team who generously embraced the idea. We thank all the authors who generously gave their time and expertise and Gonalo Martins for helping us to compile the contributions.

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References

- Danner, M.-C., Robertson, A., Behrends, V., and Reiss, J. (2019). Antibiotic pollution in surface fresh waters: occurrence and effects (review). *Sci. Total Environ.* 664: 793–804.
- Oniciuc, E.-A., Likotrafiti, E., Alvarez-Molina, A. et al. (2019). Food processing as a risk factor for antimicrobial resistance spread along the food chain (review). *Curr. Opin. Food Sci.* 30: 21–26.
- WHO (2019). Antimicrobial resistance. <https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance> (accessed 26 February 2019).

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José-Luis Capelo-Martínez obtained his PhD in Analytical Chemistry from the University of Vigo in 2002 with Prof. Carlos Bendicho and his postdoc in IST in Lisbon with Prof. Ana Mota (2002–2005). He was appointed as researcher at REQUIMTE (FCT/UNL, 2005–2009) and returned to the University of Vigo to become a principal investigator for the Isidro Parga Pondal program and a researcher–lecturer (2009–2012). He was an assistant professor in FCT/UNL (2012–2018), and in 2018 he was appointed associate professor in the Department of Chemistry of the Faculty of Science and Technology in the same institution. In 2007 he obtained his Spanish habilitation in analytical chemistry and in 2017 the Portuguese habilitation in biochemistry (analytical proteomics). Dr. Capelo is a fellow of the Royal Society of Chemistry and member of the Portuguese Chemistry Society. He co-leads the BIOSCOPE Research Group (www.bioscopegroup.org). He is the co-CEO of the PROTEOMASS Scientific Society and founder/co-CEO of the Chemicals start-up Nan@rts. Dr. Capelo



has been researching on the following topics: (i) quantification of metal and metal species in environmental and food samples, (ii) new methods to speed protein identification using mass spectrometry-based workflows, (iii) accurate bottom-up protein quantification, (iv) bacterial identification through mass spectrometry, (v) fast determination of steroids in human samples, (v) biomarker discovery, (vi) application of sensors and chemosensor to the detection/quantification of metals, and (vii) nanoproteomics and nanomedicine.

He is an author or co-author of more than 200 manuscripts, 2 patents, 12 book chapters, and 4 books. He has mentored 12 PhD theses and currently he is mentoring 6.



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