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Antimicrobial Resistance: Factors to Findings

Omics and Systems Biology Approaches

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Preface

The evolutionary arms race is a constant battle for life and death. The monarch butterfly, for example, warns predators of its poisonous nature via its bright orange color. This method protects the monarchs only until their natural predators develop a tolerance or resistance to the poison. With the poison no longer a deterrent, the monarchs are free game yet again. Pathogens are the same way. Bacteria, viruses, fungi, and parasitic worms all aim to find a comfortable home within you so that they can grow and proliferate to their (proverbial) heart's content. Host animals have evolved with these infectious agents to overcome their attacks. As pathogens evolve to overcome host strategies, humans use their mighty prefrontal cortices to develop alternative methods to protect ourselves from these pathogens. We wash our hands multiple times a day, apply a generous layer of Neosporin on open wounds, and receive vaccinations against life-threatening pathogens. We've developed a wide array of antibiotics through careful study. However, the incorrect use of antibiotics has been consistently reducing their efficacy as bacteria evolve to subvert their effects. Bacteria are beginning to one-up us.

This book, "Antimicrobial Resistance: Factors to Findings—Omics and Systems Biology Approaches," describes the story of antimicrobial resistance from its origins to what scientists are doing to combat it today. Each chapter highlights different aspects of this story, with meaningful analyses of the costs and benefits of certain actions and recommendations for the next step forward written by leading researchers in the field. Chapter 1 begins with an introduction to antimicrobial resistance (AMR), outlines the emergence of drug resistance, and reports the consequences of this resistance on life as we know it. It delves into the mechanisms of the evolution of AMR and the diagnostic methods used to detect AMR in clinical practice. In continuity, Chap. 2 gives an overview of genetic tools like plasmid overexpression and targeted chromosome mutagenesis used to study AMR, using *Mycobacteria* to illustrate their uses. We move on to learning more about the evolutionary arms race in Chap. 3, as well as what scientists are doing to identify novel drug targets to gain a leg-up on evolving pathogens. The authors expand on the heterogeneity in bacterial gene expression that contributes to AMR, in addition to other factors. Next, moving toward the protein side of the story, Chap. 4 provides an introduction to

proteomics and its methods in the context of AMR, including methods that have revealed new classes of virulence factors in recent years. Since proteins are the last step of the central dogma and responsible for the biochemical processes, Chap. 5 introduces metabolomics with a focus on bacterial metabolism, drug metabolism, and the use of metabolomics in diagnostic applications of AMR. Further, Chap. 6 addresses the topic of the microbiome and its role in AMR, including the impact of antibiotic use on the microbiome, microbiome-based therapies for AMR, and the limitations of microbiome-based approaches to AMR. The progression of AMR within natural ecosystems and the impact of human activities, such as waste management and industrial practices, facilitate the evolution of AMR. Hence, Chap. 7 highlights the importance of genomic epidemiology in public health and the latest genomic technologies that assist in epidemiological investigations. Further, Chap. 8 discusses the integration of multiomics in AMR research and communicates the computational and statistical tools to combine different types of omics data. Subsequently, Chap. 9 emphasizes the need to look at the bigger picture by advocating a system biology based approach towards AMR. Specifically, the chapter delves into modeling heterogeneity in bacterial populations as well as modeling the evolutionary landscapes that contribute to AMR. Besides bacterial factors, host also plays a central role in the bacterial adaptation against antibiotic challenges. Thus, Chap. 10 depicts AMR from the host's perspective, describing the effects of bacterial infection and subsequent drug treatments on the host. The chapter emphasizes the importance of a host-direct approach toward AMR and treatment research. Toward advanced developments of data analytics in AMR-system biology, Chap. 11 examines the controversial role of artificial intelligence (AI) and machine learning (ML) in omics biology, and how they can be used to develop novel therapeutics against AMR and its diagnosis. It also expands on the limitations and ethical considerations in the use of AI and ML in the biological sciences. Consequently, Chap. 12 relates information about the latest innovative technologies used in drug discovery against AMR and the use of omics knowledge in identifying drugs that can be effectively repurposed for AMR. Finally, Chap. 13 concludes the book with an overview of omics-systems biology in the study of AMR, the various pipelines that can be utilized to fast-track drug discovery, and recommendations and future directions for the field overall.

Altogether, in this book, we intend to convey the story of AMR as it stands today such that readers can utilize the given information in their lives and at work. AMR is a pressing global issue and requires all the attention we can give it. The evolution of drug-resistant bacteria and their spread to geographical areas where antibiotics are already in scarce supply is a recipe for disaster. We aim to provide researchers, both in academia and industry, with a compiled and up-to-date reference that they can use to investigate AMR and develop novel therapeutics against it.

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This book serves as a testament to the collaborative efforts of experts in the field who have dedicated their research endeavors to unraveling the complexities of AMR. This book project was not possible alone. We express our deep gratitude to many contributors, researchers, and mentors for their guidance, support, and inspiration throughout this project.

First and foremost, we would like to thank all the contributors and authors who agreed to take on the challenge of writing chapters. Their dedication to advancing the understanding of antimicrobial resistance through Omics and Systems Biology approaches has enriched this book. Further, their consent efforts, generosity, patience, time, and enthusiasm made this project possible. Additional thanks to those exceptional authors who agreed to write at the last moment, despite time constrain, and successfully made it on time.

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A heartfelt thank you to our family for their unwavering support, encouragement, and patience. Their love and belief in us have been the driving force behind our work, and we could not have completed this project without them. We are also indebted to our friends, colleagues, and mentors who have been a constant source of motivation and inspiration. Their constructive feedback and insightful comments have helped us to refine the structure of the book.

We would like to express our acknowledgment to the broader system biology research community for their ongoing contributions to this dynamic field. Lastly, we would like to thank our readers for their interest in this book. It is an honor to share our ideas with you, and we hope that this book will enrich your understanding and inspire you to explore new horizons in the field of AMR system biology.

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Chapter 1
Antimicrobial Resistance and Factors: An Introduction

Vijay Soni, Andréa Beltrami Doltrario, Eric H. Rosenn, Sohini Das, Biplab Singha, Rahul Singh Rawat, and Saurabh Mishra

Abbreviations

AMR	Anti-Microbial Resistance
MDR	Multi-Drug Resistant
GLASS	Global Antimicrobial Resistance and Use Surveillance System
NGS	next-generation sequencing
WGS	Whole Genome Sequencing
GEMs	Genome-Scale Metabolic Models
QSP	Quantitative Systems Pharmacology
FQ	Fluoroquinolone
XDR-TB	Extensively drug resistant tuberculosis
PPE	personal protective equipment
ARG	Antibiotic resistant genes
AMPs	Antimicrobial peptides

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ASP	Antimicrobial Stewardship Programs
MIC	minimum inhibitory concentration
SDA	strand displacement amplification
TMA	transcription-mediated amplification
NASBA	nucleic acid sequence-based amplification
RCA	rolling circle amplification
RPA	recombinase polymerase amplification
LAMP	loop-mediated isothermal amplification
HDA	helicase-dependent amplification
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry
NMR	nuclear magnetic resonance spectroscopy

1.1 Introduction

> If we use antibiotics when not needed, we may not have them when they are most needed. – Tom Frieden, former director of the Centers for Disease Control and Prevention (CDC)

According to the World Health Organization (WHO), *"antimicrobial resistance is a natural phenomenon that occurs when microorganisms no longer respond to antibiotics to which they were previously susceptible and that were previously active in treating infections caused by these microorganisms."* The WHO estimates that, if unchecked, by 2050 more people will die from infections with multi-drug-resistant (MDR) bacteria than from both automobile accidents and all incidences of cancer combined [2] (Fig. 1.1). In 2019, the WHO called attention to the problem by naming anti-microbial resistance (AMR) as one of the top ten threats to global health [3]. At the turn of the century, Louis Pasteur and his contemporaries theorized that germs release substances to kill other bacteria. However, it was not till the 1930s that a systematic search for microbes producing these chemicals and their classification began; leading to the "golden age" of antibiotic discovery [4] (Fig. 1.2). The abundance of natural molecules serving as lead compounds seemed endless, but by the 1970s, new drug development had stalled, and science was confronted with the ensuing growth of microbial resistance. Realizing the diminishing potential for discoveries, pharmaceutical companies divested antimicrobial research departments, which compounded the problem [5]. Even so, antibiotics continued to be prescribed liberally, not only for treating human pathologies but also for inoculating livestock and treating processed foods. As a result of misuse in hospitals, nosocomial infections emerged [6], but a greater factor in MDR development was the release of these compounds into the environment through agricultural use and improper waste management [7]. The effects of this on shifting pressures and genetic influences on

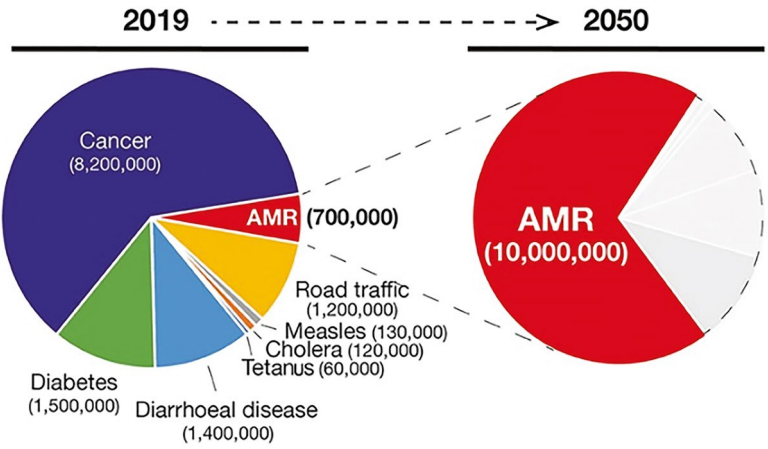

Fig. 1.1 Number of deaths and the main causes (left) in 2019 and the projection of the number of deaths due to AMR infections in 2050 (in red in the right). Gray areas represent other causes of deaths. (The figure and caption are reprinted from [1])

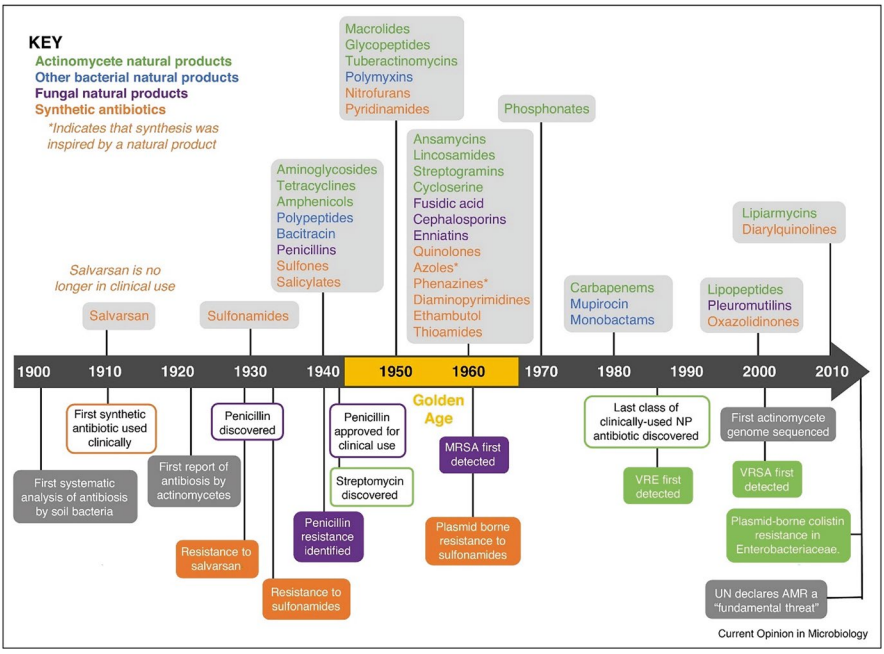

Fig. 1.2 Timeline showing the decade new classes of antibiotics reached the clinic. The antibiotics are colored per their source: green = actinomycetes, blue = other bacteria, purple = fungi, and orange = synthetic. At the bottom of the timeline are key dates relating to antibiotic discovery and antimicrobial resistance, including the first reports of drug-resistant strains such as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), vancomycin-resistant *S. aureus* (VRSA), and plasmid-borne colistin resistance in Enterobacteriaceae. (The figure and caption are reprinted from [4])

interacting bacteria within ecological systems and biota across the world are unquantifiable.

The WHO's global action plan on AMR, launched in 2015, aims to regulate antimicrobial agent use, encourage investment in research for new agents, and ensure accessibility for low-resource areas. The plan emphasizes a "one health" approach, involving various sectors like human and veterinary medicine, agriculture, and the environment [8]. The WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) standardizes AMR surveillance data collection and sharing worldwide. The first GLASS report in 2018 included data from 22 countries, detailing over 500,000 infections and the system has since spread to 109 countries [9].

Antimicrobial therapies are still an effective avenue when applied strategically and there is renewed interest in combination drugs [10]. In order to rationally design new drugs and properly apply new therapies, we need a better understanding of bacterial biology [11]. Studying mechanisms of pathogenesis can allow us to predict if AMR is likely to develop, identifying at-risk populations, potential avenues for spread, and likely reservoirs [12]. Epidemiological surveys strive to track the "movement" of MDR strains across the globe as well as analyze pathogen populations for mutations to predict the emergence of new strains [13]. This allows for preparations such as prophylactic vaccinations or research on proper antibiotic therapy protocols to be developed in advance, establishing procedures to stifle the spread of a new strain [14].

> There is probably no chemotherapeutic drug to which, in suitable circumstances, the bacteria cannot react by in some way acquiring fastness. – Alexander Flemming

As antimicrobial compounds occur in nature and are produced by microbes to defend against other microbes, microbial resistance is something that can be acquired through natural evolution [2, 15]. When administered in pharmaceutical doses, resistance is acquired through the same mechanisms, but under greater selective pressure, the process speeds up [16]. In this way, natural resistance can occur intrinsically or be induced by the activation of certain ubiquitous genes [17]. Acquired resistance can occur through an adaptive mutation that is passed vertically to progeny or by horizontal gene transfer that introduces new genetic material [6]. Activation of dormant genes or activating mutations confers the ability to resist drug molecules by the following typical strategies [19]:

(i) The pathogen may produce enzymes or toxins that inactivate a drug by hydrolysis, inducing structural modification (e.g., aminoglycoside-modifying enzymes), or directly destroying the antibiotic (e.g., β-lactamases).
(ii) By decreasing membrane permeability or overexpressing efflux pumps, a pathogen can hinder a drug from entering or reduce its residence time within the bacteria, reducing the drug's action or/or preventing it from reaching a target site.

(iii) The pathogen can change the antibiotic target (changing the amino acid sequence at a reactive site on an enzyme), such as to make it inaccessible or non-reactive, by inducing genetic changes altering the primary transcript, or via post-translational modifications.

Transient phenotypic and fundamental genetic changes may both confer resistance, and furthermore, the occurrence of one adaptation may lead to the stable mutagenesis of the other [18]. Not only do internal genetic factors need to be considered but also inter-organismal interactions such as the formation of biofilms, another method of resistance. Any of these resistance mechanisms may allow for prolonged survival that may act as a "stepping stone" for genetic AMR development [19].

> Systems biology—has exactly the same goals and purposes as general biology—with a whole new arsenal of tools.
> – Eberhard Voit

Almost 20 years ago, Dr. Trey Ideker defined systems biology as *"the use of systematic genomic, proteomic, and metabolomic technologies to acquire data for developing models of complex biologic systems and pathologies"* [20]. Systems biology thus integrates many fields, amalgamating the studies of biophysical chemistry, data science, and engineering, among others, resulting in a distinct ethos. Understanding the multilevel effects involved in a biologic pathway and its interrelation with other processes allows for comprehensive modeling of the system [21]. This holistic perspective yields information about the most basic components of the system, such as cellular mechanisms, which in turn leads to more accurate inferences about how small changes, like inducing a slight pH shift, can result in large effects on a whole organism. This approach has many implications for solving specific problems, like predicting pharmacokinetics, as well as providing solutions for broader human health and environmental concerns. Integrative omics approaches have been essential in the development of the systems biology approach [22] (Fig. 1.3). It is important to understand how techniques in genomics, transcriptomics, proteomics, and metabolomics and the integration of these fields in modeling the interactome contribute to fighting MDR.

The advent of next-generation sequencing (NGS), via high-throughput, parallel sequencing of DNA fragments, has allowed pathogen genomes to be determined rapidly and at comparatively low cost [23]. Genomics looks at whole genome data and encompasses techniques most commonly used in the experimental and clinical identification of bacterial species, as well as in establishing and analyzing bacterial phylogenetic relationships [24]. Comparative phylogenetic analysis can be exploited to quantify the degree of relatedness, which, overlaid with epidemiological data, can elucidate the temporospatial dynamics of AMR and transmission. Whole Genome Sequencing (WGS) is being increasingly employed to address the public health challenge of AMR, supporting surveillance, outbreak investigation, and contributing to improved diagnostics.

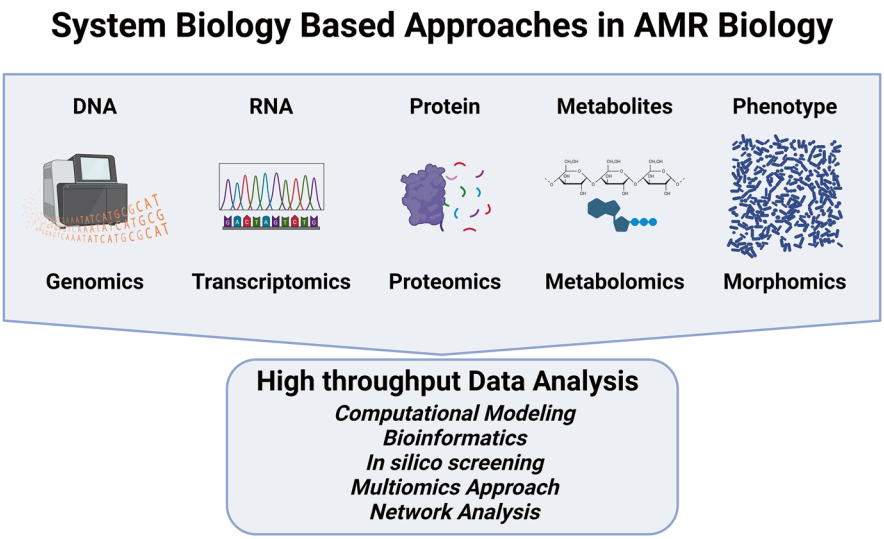

Fig. 1.3 Overview of system biology and its role to understand the microbiology of AMR (Created using Biorender.com)

Proteomics information such as proteome constitution or the relative abundance of isoforms can yield information on pathogenesis as well as aid in identifying strains [25]. Analysis of protein pathways in MDR bacteria has led to predictions of proteins in the same pathway that are likely to undergo additional mutagenesis. Furthermore, the analysis of a mutated protein can inform the probability of AMR development in homologs [26].

Metabolomics views the metabolome as the complete set of small molecules involved in cellular biochemical processes [27]. The metabolome closely corresponds with the phenotype and gives a snapshot of intracellular changes in response to antibiotics and adaptations that can confer resistance [18]. Shifts in metabolism underlie stages of pathogenesis and mechanisms of immune invasion that may confer resistance. For instance, many bacteria can switch from a high-energy state utilizing aerobic respiration to an anaerobic state when resources are scarce or depleted. This can also serve as a strategy for evasion of host defenses and confer resistance to aminoglycosides. If bacteria revert to a "dormant" state, antimicrobials that utilize active cellular processes for uptake to reach their targets are less effective. Likewise, antibiotics such as β-lactams show increased effects in conjunction with higher bacterial respiration [28].

Increases in metabolism may also confer resistance, as bacteria can prompt energetically costly changes in their own cell envelopes [22]. Regulating permeability makes antibiotic access difficult and bacteria can induce specific alterations to membrane structures targeted by antibiotics. For instance, alterations in lipid biosynthesis in Gram-negative bacteria can result in a modified Lipid A [29], the

regular binding site for polymyxins [30]. Another active process is the upregulation and production of efflux pumps. In either case of increased or decreased metabolism that confers resistance, there is a fitness trade-off that occurs. Precise modeling of the new metabolic equilibrium caused by these adaptations could identify state-specific homeostatic foci as novel targets in MDR bacteria [31].

As inhibition or excitation of metabolism can both act as methods conferring MDR, a complex balance must be maintained to effectively resist drugs. Understanding this balance may be the key to understanding a method to disrupt the bacterial lifecycle, making them more susceptible to pharmaceuticals. Tracking spatiotemporal shifts in phenotype by proteomics and metabolite measurements with genome-scale metabolic models (GEMs) can help predict MDR emergence [22]. Further analysis of the fitness tradeoff involved in acquiring resistance can determine the method and rate of population stabilization, allowing for threat assessment and proactive action against dissemination [32].

There are many factors to consider that contribute to AMR—a complex system of interactions. From subcellular changes within an organism to the commensal effects of bacterial communities to ecological and whole biome considerations that affect the spatiotemporal dispersion of bacterial lineages and mutagenic influences, a vast amount of data must be considered [33]. Forming a more comprehensive model of these multilevel interactions is key to revealing how specific MDR mechanisms have developed and predicting the emergence of new AMR capabilities [34]. A system-level understanding allows us to address the problem of MDR from multiple avenues. First, with increasingly accurate modeling, a quantitative systems pharmacology (QSP) approach can be used which may potentially lead to the discovery of many new drugs or combination therapies [35]. Second, a better understanding of the environmental factors involved in MDR may highlight ways humans may be influencing the evolution of MDR strains without even realizing; and conversely, how to manipulate this evolution [36]. Lastly, understanding the development of the most resistant pathogens and their interactions with their human hosts over an evolutionary timescale may yield insights into bacterial adaptability and aid us in predicting their future behavior [37].

The objective of this chapter is to explore the general scope of AMR and the factors behind it. We have included a comprehensive discussion on bacterial evolution in the context of AMR, interpreting its connections with diverse clinical factors. Furthermore, we highlighted the implications of basic knowledge to understand the global consequences of AMR, encompassing both the disease aspect and policy considerations. The chapter further extends to different diagnostic methods for AMR, followed by an assessment of its impact on healthcare. We have also reviewed the pivotal role of systems biology in the battle against AMR. We envision this chapter as a simple guide for all readers of this book, serving as a resource to develop a deeper understanding of the fundamental biology of AMR and the imminent challenges that demand timely attention.

1.2 Evolution of AMR: Environmental and Biological Factors

> A band of bacterial brothers, swigging ATP with some others. In a jocular fit, they laughed til' they split, now they're all microbial mothers. – Richard Cowen

In the introduction section, we indicated some pivotal points in the history of AMR development. In recent years, the rapid spread of AMR bacteria has given us a clear view of its devastating trajectory. We have also mentioned the implications for progressive therapeutic innovation necessary to combat AMR, stressing the need for a deeper understanding of AMR mechanisms as well as continued research into new antimicrobial treatments. When we consider the misuse of antibiotics and other environmental impacts of humans that have caused the more rapid emergence of AMR, we mainly focus on the last 100 or so years. This is quite a short period on an evolutionary timeline. While the direct human impact on the spread of AMR is evident, we must also realize that millennia of evolution have taken place that primed these bacteria to be able to mutate so rapidly in response to stress.

From the beginning of mankind, there has been a co-evolution of pathogenic bacteria [38]. In fact, it is posited that many modern diseases arose after agricultural revolution, as large and concentrated human populations were required to maintain the pathogens' minimal viable populations [39]. Subsequentially, the immune system of a modern human is quite distinct from that of our ancestors, even a few thousand years ago. Likewise, new bacterial strains emerged in response to these new defense mechanisms [40]. The intertwined nature of human and bacterial coevolution has resulted in myriad complicated relationships; this can be seen in modern bacterial species that range from symbiotic gut microbes to pathogens so hostile that mere contact is a death sentence.

Yersinia pestis, for instance, which has caused three major pandemics throughout history, including the bubonic plague, has been isolated from corpses dated 3800 years old and remains endemic to some regions today [41]. It was not until the 1930s through the analysis of epidemiologic data that it was understood that rats not only could carry the disease but were also acting as reservoirs, allowing it to persist and cause sporadic outbreaks. Such reservoirs present another important aspect influencing the evolution of AMR. Revelations like these have underlined the importance of historical microbiology and research into past bioevents [42].

We can see over shorter time spans how pathogens exclusive to animal hosts can mutate and gain the ability to be transmitted to humans, as was determined to be the case regarding the COVID-19 pandemic. Additionally, the phylogenic relationship between the animal host and humans has been shown to be a possible factor affecting the transmissibility and even infectivity of a pathogen [43]. By comparing differences in immune responses between other host species and humans, the immune evasive mechanisms of the pathogen can be better understood, in addition to its mode of transmission. Practically, this could reveal a novel inducible immune factor that might aid in resistance to AMR bacteria, as well as expose new strategies for disrupting transmission [44].

The integration of environmental and genetic influences on and between humans and bacteria reveals the emergent properties of a system that can explain processes like the response of bacteria to selective pressures or give insight into the evolution of natural resistance mechanisms such as gene transfer between bacterial species. Moreover, there is importance in identifying the locus of evolutionarily conserved genetic elements and other genetic features such as points of rapid mutation that seem to incorporate many active genes without being overly detrimental to the organism [45]. Having identified these possibly significant genes, a network-based approach can be applied using omics techniques to elucidate genetic interaction pathways [46]. If any of these pathways are related to drug-resistance genes, then the other pathways related to the locus can be considered high-profile leads for further understanding the molecular basis of AMR. Changes in bacteria through the process of gaining AMR present a number of fitness trade-offs that can be exploited in therapeutic development [47]. For instance, the gain of AMR function is usually accompanied by morphologic and metabolic changes that may present new modalities for drug action [48].

It is also important to note the intrinsic resistome of many bacteria, which is an independent general defense mechanism that has evolved through natural evolution and also functions in AMR. *P. aeruginosa,* for instance, has developed such adaptability that it is nearly ubiquitous in every environment and species [49]. Moreover, populations cultured under various conditions do not represent distinct strains, but rather fluid physiological and metabolic changes driven by highly adaptable expression systems [50]. The mutational resistome also displays intrinsic versatility in that a large number of loci represent functional phenotypic shifts that contribute to acquired immunity. Under stress from any particular antimicrobial, a vast number of single mutations are possible that can confer resistance [51]. Many other pathogens display similar adaptability in that established intrinsic defenses are highly effective in interfering with antibiotics, despite this being a novel functional role. It is posited that these intrinsic mechanisms are simply far too adaptable for any one antimicrobial formulation to be an effective therapeutic solution. Subsequently, current research is examining the metabolic changes that occur in response to regimens of multiple antibiotics and how these changes might build off each other to expose a novel target or "weak point." [52] In addition to this evolutionary approach, by studying historic changes in metabolism under environmental pressure, we might predict how changing nutrient availability in disease states might affect susceptibility to antimicrobials and other pharmaceuticals [53].

1.2.1 Scope of Influences Driving AMR

> Working Upward multicellular organisms such as you and I are social groups of Eukaryotic cells. Working Downward bacteria are social groups of genes. —David Sloan Wilson

Under the theory of multi-level selection, natural selection may occur not only between individuals in a population but at various levels of organization [54]. In a simple example of "group selection," a population with greater genetic diversity is

more "fit" than a homogenous population and is more likely to have surviving members when confronted with a disease. While many scientists have critiqued the theory, we will use its definition in a limited scope here to discuss the phenomenon of emergent traits of levels of organization within bacteria and consider distinct "selective" pressures at these levels that have influenced the development of antibiotic resistance genes.

As we have described previously, bacteria are highly adaptable due to plasticity at many levels. At the genetic level, we see a high mutation rate and a fluid genome with transmissible elements [55]. At higher levels of organization, we see promiscuous proteins, asymmetric phenotypic expression, tradeoffs between variation and privileged environmental access, regional strain variants displaying unique characteristics, and emergent strains with significant functional distinctions. Each of these represents an emergent and variable trait of a hierarchical organization, with each level experiencing its own selective pressures. An important facet of multilevel selection is that we consider selective pressures at the level of organization where they are most useful in a particular context. Take our population example above; a more infectious but homogenous strain populating an organ may contain more fit, rapidly reproducing, individuals but can be more easily killed with an antibacterial than a more temperate strain that grows slower while generating greater variation.

There are so many processes producing adaptive variability that seem redundant throughout the hierarchical structure of bacterial species; however, there is an order to the madness. In examining the number of naturally occurring resistance genes as well as those that can be recruited through other transfer mechanisms, there is a question posed about why an even higher number of AMR traits are not observed [56]. In what is referred to in some works as a bottleneck, there is high competition between AMR elements, which are selected by bacterial genetic recruitment mechanisms based on stringent criteria. One of the major factors is the ability of the new genetic material to be easily integrated into bacterial epigenetic mechanisms, which is the basis for many higher-level functions.

Bacteria multiply and spread rapidly through various environments, resulting in population growth under many different influences [57]. There will thus be divergent evolution between populations, resulting in greater genetic variation within the species or strain. A larger population will be more stable as it will encompass greater variation as well as be more adaptable as there is increased competition within the group. However, an opposing situation may occur when smaller subpopulations are formed, which may result in more radical adaptations. Consider the case of small amounts of bacteria being passed from one host to another or dispersed throughout a host body. In sufficiently small, isolated subpopulations, genetic drift may occur, resulting in the incubation of a particular phenotype [58]. A weak AMR gene with a neutral fitness effect may become highly resistant, and subsequent exposure may introduce this novel resistance to the greater bacterial population.

Another way in which AMR can develop in a non-Darwinian fashion is through phenotypic mutations. Intrapopulation phenotypic heterogeneity occurring from noise in expression mechanisms can result in greater adaptive variation as well as novel AMR resistance traits. If these are concurrent with some antimicrobial

pressure, the mutation may be passed on via epigenetic inheritance. Furthermore, through gene amplification, small genotype variances can have a disproportionate but reversible phenotypic response and result in heteroresistance to moderate antibiotic pressure or a high probability of adaptive mutation under strong antibiotic stress. This epigenetic-based phase variation underlies *Neisseria meningitidis* susceptibility to ceftazidime and ciprofloxacin [59].

At the various levels of organizational hierarchy, we see cases where adaptive variation has facilitated exaptations that have ultimately evolved into AMR mechanisms. We can tell transposases' primary function is in environmental adaptation as only a small percent carry AMR element; however, it does so highly efficiently, resulting in a large effect on AMR [60]. Promiscuous proteins in ancient bacteria were also able to bind and disrupt natural antimicrobials, an initial exaptation forming a portion of intrinsic resistance, and later gained broader functions. In fact, the ability of most portions of the intrinsic resistome to function in clinical antibiotic resistance represents many exaltations.

Looking at how many ways AMR elements can result from various mechanisms of adaptive variation, it seems bacteria might be able to shift any survival tool at their disposal toward the development of AMR under sufficient pressure. Examining AMR from an evolutionary perspective has given us great insight into a highly complex organism. In going forward in developing new therapies to circumvent AMR, we should be extremely considerate of these adaptive mechanisms as well as sources of random mutation that could present dangerous obstacles.

1.2.2 Evolutionary Development-Informed Strategies for AMR Treatment and Prevention

> Imagining a world without vaccines, anesthetics, contraception, and anti-infectives reveals how medicines revolutionized humanity. How to manipulate conditions that favor such discoveries is worth consideration. —John Warren

Understanding the basis of AMR development is important for the rational and effective development of all therapeutics to treat bacterial infections. As discussed, understanding the past evolution of bacteria and AMR informs us how certain stressors might affect a pathogen, thus allowing us to incorporate artificial selection into the rational design of a therapeutic mechanism [61]. In addition to manipulating a pathogen in this manner, we may also take advantage of fitness tradeoffs and important genomic expression systems we have uncovered.

Cross-sensitivity is a fitness trade-off effect observed when a new adaptive trait causes the weakening or reversal of a previously acquired adaptive trait. Collateral sensitivity (previously induced sensitivity) specifically describes increased sensitivity to one antibiotic when a bacterium gains resistance against another antibiotic [62]. In 1952, Szybalski and Bryson first observed that *E. coli* cultures could not develop increased resistance to both polymyxin B and chloromycetin. They later established that increased resistance to one agent caused an obligate increased sensitivity to the other [62].

The use of collateral sensitivity has since been shown to be a viable strategy for eliminating AMR strains. In particular, when a disproportionate collateral sensitivity relationship is observed, a treatment involving altering exposure between the antimicrobials involved can result in hypersensitivity to one of the agents that effectively clears the pathogen. In one experiment, for instance, MDR *P. aeruginosa* strains were treated with an alternating tobramycin/ceftazidime regimen [47]. Tobramycin administration first kills any bacteria in the population that have heterogeneous ceftazidime resistance. Ceftazidime treatment then causes hypersensitivity to tobramycin and a subsequent tobramycin administration then kills off the bacteria entirely.

Research is being conducted to identify conserved evolutionary networks involving the collateral resistance phenotype and inform the rational design of drug pairs with a predictable range of target strains [63]. However, the information generated has significant gaps due to processes like epistasis, polymorphisms, and allelic expression that confound predictions based on genetic similarity. Another problem is the potential heterogeneous emergence of collateral sensitivity, leading to intrapopulation variation in the robustness of induced sensitivity [64]. Convergent collateral sensitivity is a strategy employing a regimen comprising "steps" of controlled exposure to additional antibiotic compounds [65]. This takes advantage of the process of phenotypic convergence, in which a diverse population, or populations, subject to the same stress will adopt a similar phenotype. Applying a sequence of antibiotic stressors causes a significant shift toward a similar phenotype, making collateral sensitivity a more feasible approach for the treatment of diverse clinical strains as well as heterogeneous systematic infections.

Another evolutionary strategy examining metabolic changes in response to antimicrobial challenges might reveal combinations of drugs causing metabolic shifts that can be exploited [66, 67]. For instance, even an antimicrobial to which a particular bacterium has developed complete resistance might still induce some metabolic change that makes the bacteria more susceptible to another antibiotic. An eco-evolutionary angle is also being applied by considering antimicrobial resistance elements' original function, such as how drug efflux pumps may have functioned in bacterial metabolism before [68]. Understanding this state can help identify adjuvants, such as nutrient compounds that affect the element's function, that could be co-administered with an antibiotic and result in a greater effect. In addition to antibiotics and adjuvants, another way in which artificial selection for more susceptible bacteria might be accomplished is through alteration of the environment itself by affecting the host microbiome.

1.3 Clinical Pressures in the Development of Drug Resistance Pathogens

Clinical pressures are a crucial issue behind the development of drug-resistant pathogens. Factors such as antibiotic misuse, inadequate dosage, and improper prescribing practices contribute to the selective advantage of AMR. Hospital

environments, global travel, and the lack of new antibiotic development further amplify this challenge. These pressures underline the pressing need for wide-ranging approaches to alleviate antibiotic resistance, emphasizing judicious use, enhanced surveillance, and the development of novel treatment methods to ensure the sustained effectiveness of antimicrobial therapies.

1.3.1 Key Factors

Antibiotic Misuse and Wrong Clinical Practices

The use of antibiotics results in selective microbial pressure that promotes the growth of antibiotic-resistant organisms [69]. Negative consequences outweigh the benefits when antibiotics are used despite no clinical indication. Antibiotics are frequently used for the treatment of respiratory infections, even if they are not indicated. Shafrin et al. found that ciprofloxacin was prescribed to 15% of women with UTIs in the United States [70]. Apart from AMR, inappropriate antibiotics lead to poor patient outcomes and an increase in healthcare costs [70, 71]. Inappropriate antibiotic use includes the wrong antibiotic, dose, and duration.

The key question is, "If inappropriate antibiotic therapy has adverse effects and no benefits, why are antibiotics prescribed in these scenarios?" It is found that time pressure, diagnostic uncertainty, patient demand/satisfaction, concern for secondary bacterial infections, and high patient volumes lead to inappropriate antibiotic prescriptions [72, 73]. Physician-related factors associated with a higher likelihood of antibiotics include a long duration of practice (more than 10 years), medical liability, and the perception of severe illness. Often, taking a few minutes to talk to the patient is enough to address their concerns about the need for antibiotics [74]. Patients with a history of corticosteroid use, fever, and multiple comorbidities are likely to be treated with antibiotics. More than 85% of antibiotics are prescribed in clinics, of which 41% are prescribed in primary care [75–79]. Self-medication is common in countries where antibiotics are available over-the-counter. Measures to curb inappropriate use of antibiotics include the sale of antibiotics as prescription only, enabling prescribers to provide the exact dose and duration, patient education about appropriate antibiotic use by providers and pharmacists, antibiotic use and resistance surveillance systems, and public education campaigns [80].

Food Animals

Antibiotic use in food animals (poultry, swine, cattle, and sheep) is a significant contributor to AMR. Antibiotics are used for metaphylaxis, prophylaxis, treatment, and as growth promoters. Metaphylaxis is the mass medication of healthy animals when an infection is present in one or more individuals in the herd. Prophylaxis is antimicrobial administration in healthy animals to avert disease. Growth promoters are antibiotics (often at subtherapeutic levels) used to increase the feed-to-muscle conversion rate [81, 82]. Selective pressure leads to the predominance of resistant bacteria, which are transmitted to humans through food or the environment.

Fluoroquinolone (FQ) resistance rates in *E. coli* are less than 5% in the United States, where its use is prohibited in food animals. However, more than 40% of *E. coli* are FQ-resistant in Brazil, Europe, and China, which use FQ in food animals [83].

Overcrowding and Poor Sanitation

Overcrowding, poor sanitation, a lack of handwashing, and the drainage of contaminated water into rivers and lakes increase the spread of AMR. This is especially relevant in urban slums in LMICs and refugee camps. Overcrowding is associated with increased respiratory and MRSA skin and soft tissue infections (SSTI) [84, 85]. Emphasis on good hygiene practices is an important measure to prevent the spread of AMR.

1.3.2 Impact of Clinical Pressures on the Emergence of AMR

The rapid rate of emergence of AMR has outpaced the development of new antibiotics. As we have discussed in Sect. 1.2, this creates the possibility of a frightening situation like a hundred years ago, prior to the availability of antibiotics, when common infections like pneumonia, urinary tract infections, and meningitis had no effective treatment. The World Health Organization (WHO) declared AMR a top-ten global public health crisis in 2019 [86]. Overuse and misuse of antibiotics, poor infection prevention and control measures, poor sanitation and handwashing practices, limited access to vaccines, and effective antimicrobials hasten the development and spread of AMR [87].

During the initial phase of the COVID-19 pandemic, healthcare facilities were overwhelmed by critically ill patients requiring mechanical ventilation, central venous lines, and urinary catheters. Rates of carbapenem-resistant Enterobacterales, extended-spectrum β-lactamases (ESBLs) Enterobacterales, and MDR *P. aeruginosa* increased by 35%, 32%, and 32%, respectively. It is alarming that 47% of ESBL Enterobacterales infections were community-acquired. Vancomycin-resistant Enterococcus (VRE) and methicillin-resistant *S. aureus* (MRSA) increased by 14% and 13%, respectively.

This was attributed to [88]:

- Broad-spectrum use of antibiotics for sick patients before the availability of evidence-based COVID-19 pneumonia therapies
- Repurposing of AMR resources to tracking and testing for COVID-19
- Limited appointments in clinics, which delayed treatment for common infections

Tuberculosis poses another challenge in clinics. MDR tuberculosis (MDR-TB) is defined as resistance to both rifampicin and isoniazid. Extensively drug-resistant tuberculosis (XDR-TB) is defined as resistance to rifampicin, isoniazid, fluoroquinolones, and one second-line injectable drug. Compared to drug-susceptible tuberculosis, MDR-TB and XDR-TB are difficult to treat, with longer treatment courses,

severe adverse effects of second-line antituberculosis drugs, and higher morbidity and mortality. Healthcare workers and family members who take care of/live with these patients are at risk of acquiring the disease. After years of decline, there was an increase in MDR-TB cases from 2020 to 2021, from 437,000 to 451,000. This was attributed to the COVID-19-associated disruption of tuberculosis detection and treatment services [89]. In a South African study that included 273 patients with XDR TB, 74% had poor treatment outcomes that comprised treatment failure, default, relapse, or death on treatment. More than half (63%) were discharged into the community from the hospital. The median time to death after home discharge was 10 months. 20% of discharged patients had positive sputum smear results at 3 weeks, indicating that they were a potential source of the spread of infection [90].

1.3.3 Strategies to Address Clinical Pressures Contributing to Drug Resistance

Appropriate Use of Antibiotics Appropriate antibiotic prescribing is the cornerstone of slowing down AMR. These include:

- The initial antibiotic should be chosen keeping in mind the common etiologic organism(s).
- Use local antibiograms to select empiric antibiotics before culture results are available. An antibiogram is an aggregate of sensitivity reports collected from individual isolate data. Antibiograms can be created for each hospital as well as at a regional, national, and global level. They provide valuable information about antimicrobial susceptibility in a specific region and trends in AMR at a national and international level [91].
- Obtain a microbiological diagnosis with cultures. After culture reports are available, de-escalate antibiotics from a broad spectrum to a narrow spectrum.
- Use the appropriate dose and duration. Higher than recommended doses increase the risk of adverse effects. Suboptimal doses run the risk of therapeutic failure and can induce resistant pathogens. Treatment duration varies by site of infection [92, 93].
- Always aim for source control. Antibiotics without source control expose patients to the adverse effects of antibiotics, with the risk of persistent infection.
- Keep in mind antibiotic penetration in specific tissues while treating infections.
- Assess the need for central venous catheters, arterial lines, urinary catheters, and other devices daily. Remove catheters and lines when no longer required.

Antibiotic Stewardship The CDC defines antimicrobial stewardship as measures to ensure the appropriate use of antibiotics for providing the best possible care to the patient as well as to keep in check the threat of antimicrobial resistance [94]. Antibiotic stewardship applies to healthcare settings including hospitals, outpatient

clinics, dental and urgent care, dialysis centers, long-term care facilities, and veterinary medicine in food, farm, and companion animals.

Infection Prevention and Control Measures Standard precautions must be followed in all patient-care settings. These are measures to prevent exposure to blood, body secretions, mucosa, and non-intact skin. Two essential components of standard precautions are hand hygiene and the use of personal protective equipment (PPE). Other components of standard precautions include cough etiquette principles, safe disposal of sharps, handling textiles and laundry carefully, cleaning patient care equipment, and safe injection practices [95]. Transmission-based precautions have three categories: airborne, contact, and droplet precautions. These are additional precautions taken over and above standard precautions; a risk assessment at hospital admission must be done to assess if a patient requires transmission-based precautions [96].

Water, Sanitation, and Hygiene Measures (WaSH) As compared to developed countries, low- and middle-income countries (LMICs) treat less than 10% of their wastewater [97]. Suboptimal waste disposal leads to contamination of surface and groundwater with human and animal excreta and exposes the population to antibiotics and AMR bacteria. Factors that promote the spread of AMR include open defecation, septic tanks, pit latrines, a lack of isolation of animal excreta, and antibiotics that may get incorporated into plant produce when wastewater reaches crops [98]. Developing basic sanitation facilities like toilets, drainage systems, and proper waste disposal systems will help reduce the spread of AMR. Local community engagement, toilet ownership by households, and investment in infrastructure helped promote better WaSH behaviors [99]. Further resources dedicated to inspecting food preparation, food products, restaurants, and eating places will improve food hygiene [100].

1.4 Global Impact and Consequences of AMR in Disease Control and Treatment

During the COVID-19 pandemic, resources allocated to AMR control were redirected to the pandemic. Significant AMR testing backlogs occurred, and many samples remained untested [101]. The impact of AMR on the global health system is profound and multifaceted. AMR presents a serious threat to the efficiency of antibiotics and other antimicrobial agents, threatening our ability to control bacterial, viral, parasitic, and fungal infections. The increasing incidence of drug-resistant strains undermines standard treatment protocols, leading to prolonged illnesses, increased mortality rates, and heightened healthcare costs. Moreover, AMR sabotages the success of disease prevention measures, as regular health practices such as surgeries, chemotherapy, and organ transplants become riskier due to the potential