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CHAPTER ONE

Past, present, and future of insect-borne diseases

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Insect-Borne Diseases in the 21st Century
https://doi.org/10.1016/B978-0-12-818706-7.00001-2

Listening to the silence

We start with two books, which contain seeds of the further arguments. The first one is *The Silent Spring* by Rachel Carson (Carlson, 1962). The second one is *The Origin of Species by Means of Natural Selection* by Charles R. Darwin (Darwin, 2003). Both had deep cultural and scientific influences, for different reasons, and nowadays they must be considered as the starting points of theories about habitats needing careful consideration.

In 1962, Rachel Carson published *The Silent Spring*, intending to document the effects on the environment of the indiscriminate use of synthetic pesticides, starting from the absence of the usual songs of birds in spring. The book explicitly identifies DDT and other pesticides as responsible for enormous damage to the environment, causing in general threat to wildlife and a series of negative effects like the increase of cancer cases in humans. Carson directly evidences the responsibility of the chemical industry not only in its excessive use of chemicals, but also by covering its activity through spreading misleading information about the consequences, in collaboration with public officials. Chemical companies reacted by masking and justifying their actions, but the book had a great impact on the American public, acting as a seminal event for the environmental movement and generating a strong debate about habitat care. The policy on the utilization of chemical insecticides was in part affected and another consequence was the creation of national agencies in defense of environmental equilibria. The argument is still totally open, since the effects of neonicotinoids on honeybees and birds are nowadays under consideration. However, the most interesting point is arguably that until *The Silent Spring* was published, all the above issues were considered acceptable and even normal.

Probably, no ideas were subjected more to misleading and manipulation than the results presented by Charles Darwin about animal social behaviors. A clear example of a diverted utilization of scientific theories by people, who never had read one word of his books. Some interpretations of Darwinism gave the impression of a giant omnipresent struggle among organisms for surviving and winning the competition for resources and reproduction, obtaining sexual advantages. However, we know that there are innumerable examples of cooperation, mutual help, positive coexistence, and symbiosis between very different organisms. However, there are also example of slavery by an organism for another to satisfy personal needs, and examples of clear sloth (as reported also by Darwin). This is probably the key to

understand several factors affecting the future of the environment and humankind, from the gut microbiome to the Angiosperms reproduction.

In several examples, selecting those related to insect-borne diseases, the integration and incorporation between very different organisms—i.e., bacteria, insects, plants, and fungi—gives rise to a complete network, working as a natural mechanism, wherein each organism has a precise and defined role.

We are in the midst of epochal planetary changes, whose consequences are becoming more and more evident in the developing scenarios. The powerful weapons utilized by humans to control insect-borne diseases, consisting of chemical-made antibiotics and insecticides, are becoming useless. For a long time, the pathogen organisms were easily killed and controlled. Insects and microorganisms, thanks also to other allied organisms, after a long period of passivity are finally reacting properly to the lethal continuous attacks by humans, whose minimum goal was the complete extermination of these insects and microorganisms. So far, the counteractions by microorganism and insects have been mainly defensive and limited, but they are ready to become brutal and offensive, and perhaps decisive.

The counteraction by target organisms is the most obvious in any war: nullify the enemy's weapons by resistance, and fight back. This resistance is already being worked on, and is ready to become very effective on a large scale. The incoming front of the resistance's phenomenon is based also on more efficient methods of diffusion, including organisms so far latent and in a revision of strategies to survive and diffuse. Insect-borne diseases are evolving in this scenario and therefore they are ready to play again their central role in the never-ending fight for survival (Mehlhorn, 2015a,b; Mahmud et al., 2017).

Entomology will need to play an important role. However, it is necessary to reconsider its goals, which have too often focused on taxonomic problems, and consider the needs for new and original approaches able to face novel challenges. It is time to revise several dominating axioms on the light of the occurrence of a series of important phenomenons, which are acting as current motors of radical changes. In this book, we will introduce the key concepts of superorganisms, system biology, and bionetwork, and present some examples to verify these approaches. Examples must consider the several aspects involved, including target organisms or selectivity effects. It is necessary to understand what is going on and the role played by each organism. Several examples will be presented and their related solutions, based on current or recent episodes of public concern, including health and production of food. Therefore, the philosophy and the strategies reported will generally find their evidence and concreteness in selected cases.

The main goal of the book consists in encouraging readers to consider the possibility of thinking in another way, without accepting the dominant paradigms. The book will also ask each reader to contribute where possible to another possible style of living, considering surrounding organisms, such as insects or bacteria, to be not annoyances to be removed, but potential allies to play a daily and fascinating scenario with alternative costs and new hopes.

In this chapter, the two main actors of an insect-borne disease, the microparasite and the vector, will be examined, considering in particular their current evolution and consequent effects on the occurrence of diseases. Let us start with the parasite, in consideration of its key responsibility in the disease.

Several signals of changes are converging to create a new environmental scenario. The 21st century announced its advent with radical planetary events disclosing enormous impacts. Among the main influencing factors, the enormous advances obtained by technology are changing any aspect of our life. The instantaneous planetary connection is opening the door to a planet globalization of the information, but not only the news are travelling everywhere. Therefore, continuous innovations in ordinary life are fueled by a progressive dependence on the artificial intelligence, allowing the possibility to exchange everything can be moved, like ideas and materials of any kind, including pathogens and parasited. Changes are rapidly affecting quality of life and health, including deep evolvements in social organization, evident in the crisis of the tribal and family models. Continuous and rapid challenges of dominant paradigms inside the global network are actively changing the planet, but with different effects in each part. Most people consider these changes to be simple collateral effects of scientific advancements, whereas everything is still moved by the usual eternal motivations: the possibility of surviving and growing in the best environmental conditions, the research of habitat sources to be utilized efficiently in the best way, in total indifference of the consequences necessary to achieve the expected goal. Now, as ever, climate changes generate migrations of humans and animals, moved by their usual needs. In some cases, organisms move to conquer territories previously closed to them, disrupting previous equilibria. Migration is a natural phenomenon, and always has consequences (Bezirtzoglou et al., 2011; Lamb, 1995; Cook, 1992; Wigley et al., 1981).

Environmental changes can offer new possibilities, not only damages. Survival needs, or simply homeostasis rules and imperatives, push organisms to find better territories or more favorable living conditions. It is a thermodynamic contest, like water moving freely from two containers or the

equilibration of the temperatures between two adjoining rooms. Continuously, the brave vanguard of any organism try out the boundary of their territory in search of opportunities. When movements are successful, they become massive and overflow, finding resistance from previous native inhabitants, but defensive damage is largely counterbalanced by the absence of the usual natural enemies remaining in the old territory. The fight for natural sources is open. Therefore, the alien species enlarges its distribution as soon as possible, whereas autochthonous species experience difficulties and the whole environment is highly affected.

It is important to focus on the mechanism of the migration. During the first steps of the migration, some epigenetic changes can occur, generating more aggressive populations, and these are more motivated to move. Several genotypes of the alien species can move in sequence and the strongest one takes supremacy during the starting step. When the rooting is completed and the migrant alien population is integrated and favorable, the second step, consisting of diffusion, can start with increasing efficiency, causing the dramatic diffusion, like an expansion of the oil stain can start with increasing efficiency, causing the dramatic diffusion, like an expansion of the oil stain. Therefore, the epidemic stage, so large, abundant, and evident at the height of its manifestation, is the result of the action of a super-selected vanguard. The success of the initial step is crucial, and explains why for a long time the invasion was not possible. The route of the invading species in the whole phenomenon, from the starting initial territory to the final one, can be visualized in the form of an hourglass. Over time, the sand in the upper glass tube (the starting population of the species in its territory) will decrease in favor of the other one (the new habitat), but it is necessary to satisfy the initial condition of flowing.

The environmental changes influence our ordinary life, including the possible advent of great threats, which will affect previous situations already ripening over a short or long time. The changes are just the development of previous situations already ripening over a short or long time. Several signals are announcing the incoming future, but correct interpretations and, in particular, necessary counteractions are largely lacking. Scientists are modern haruspices, like Cassandras dedicated to the interpretation of signals from the habitat, and as in ancient times they may not be listened to by powerful people, who are more concerned with maintaining their power. Therefore, the only hope is in the ordinary people. The rise of a general and capillary consciousness is the necessary key to face the new challenges, influencing the behaviors of everyone and forcing solutions that will benefit all mankind.

Let us recall the aforementioned concepts, using the key words: signals, interpretation, and counteractions. Lack of knowledge of this sequence increases the chances of something unexpected happening, such as a sudden catastrophe or real emergency. The natural consequences of the planet's movements and migrations of its inhabitants are considered unexpected and unusual. It is a return to times of ignorance. Once the hubris of gods was considered as responsible of outbreaks, and now we live in times when that ignorance is simply substituted by other kinds of fear, counterbalanced by frantic manifestation of man omnipotence. There are many examples of this aptitude. For these reasons, the themes of this book are fundamental and crucial to the pathway toward our secure future. A general consciousness of the peril of the current pathway is vital. Some causes of the changes can be attributed to the planet, others to human influences, but in any case the lack of counteractions in the right direction will be our fault. Selected cases will be exposed to evidence the ongoing trends and speculate on the coming years.

Possible scenarios are in conflict

Current changes in planet climate are going to be fundamental as never before for the success of every human activity and enterprise, from agriculture to trade. Temperature rise and desertification are generating massive migrations from rural areas to urban ones, and from the global south to the global north, remodeling animal and human distribution (Reiter, 2001; Mouchet and Carnevale, 1997; Córdoba-Aguilar, 2018). Technology is a key actor in this changing scenario. Resources availability and food production are highly dependent on access to high technology, causing a new form of colonization and continuous migration toward advanced countries for humans and constant consequent movement of the myriad creatures associated with human activities. Several factors are remodeling everyone's concept of life and welfare. Current changes are likely to be connected mainly with the increase in life expectancy, including the emerging of new pathologies and health disorders, and with the revolution in the nutritional environment, due to radical changes in food. If we want to imagine our near future, considering the effects of this "evolution sap" derived from these concomitant factors, we must consider the alternative utilizations of natural resources, facing the challenge between sustainability and overconsumption. In this moment, mankind are not alone, deciding their destiny.

In every moment of human history, we have had to face direct or indirect discrete formidable attacks from invisible enemies, able to threaten our lives. The COVID-19 outbreak is only a further example of the potentiality of parasite of changing economy and lifestyle. Although in decline, pathogen microorganisms and parasites remain among the main causes of deaths globally, and their virulence is far from being dominated. Each episode of this eternal fight is different and needs careful interpretation. In the last period, much attention was focused in production and use of insecticides useful against vector-borne diseases, with the aim of eradicating their presence and therefore save as many lives as possible. Epidemic emergencies can occur everywhere. Insects are vectors of important diseases involving non-human targets, causing important effects on plants and animals. Recently, some of these diseases rapidly increased in profile and generated great alarm about the potential consequences of their diffusion. The economic negative effects are enormous and damage to the local living system is dramatic. The global incidence of insect-borne diseases is relevant considering the population at risk and the number of reported cases, but the percentage of death is around 0.2% compared to 15% for tuberculosis and 0.3% for the similar disease influenza. These data indicate an endemic presence of these diseases, whose effects must be mainly considered from social and production points of view (Reiter, 2001; Mouchet and Carnevale, 1997).

Besides the insect-borne diseases concerning human beings, recent cases of widespread insect-borne diseases not directly endangering human health will also be reported. In these cases, no successful strategies or pesticides are available, but many new proposals have been presented (Lounibos, 2002; Wright and Sutherland, 2007; Khater et al., 2017; Benelli, 2015, 2019, Nicoletti et al., 2016; Rogers and Randolph, 2000; Tanwar et al., 2014; Benelli and Mehlhorn, 2018; Willcox et al., 2005).

Super agents, supervectors, and superbugs

Microorganisms are dominant in the planet's biomass and affect any organic equilibrium. One of the main roles of pathogen microorganisms in habitats is the turnover of organic matter. Nobody knows how, but they are able to feel the absence of life, and, as soon as possible, immediately after the death of an organism, a plethora of "wreckers," mainly virus, bacteria, and microfungi, assault the body to obtain short and available substances, useful as their food. A side effect is the cleaning and scavenger action on

the habitat. Otherwise, we should be covered by residues of organic matter, as happens with plastic. The main reason is that molecules must be exchanged, and rapidly, to ensure new organisms can replace the old ones. This can be achieved by acceleration of the catabolism up to the point of death of the attacked target. Infection is the first step, often consisting of a small vanguard, which are usually destroyed by the natural defenses of the target, but in some cases not totally, causing a rapid increase of the infecting population. The start is the crucial step and needs first the introduction of the pathogen in the body of the target organism, but there is a preliminary act. Initially, the microorganism must be present near to the target—near enough to be able to obtain the inoculation. As a result, microorganisms need to solve the problem of their dimensions. The world around them is at least 10^6 bigger, and any movement is therefore virtually irrelevant. The difficulties of the microorganism reaching the target are usually insurmountable, preventing it from performing its mission. Furthermore, the utilization of abiotic agents, like wind or rain, have low probabilities of success. The transfer must be efficient and performed to keep the pathogen alive and efficient.

This problem is related to the next step and is focused on the diffusion of the infection, meaning reproduction of the agent and consequent propagation of the infected organisms. We have already established the key role of the vanguard, which is to test possibilities. In an epidemic scenario there is a starting point—an insignificant place on the map—wherein a more potent and efficient population unexpectedly appears, as a consequence of a genome change or a migration caused by environmental situations. The starting epidemic area is called the "plague focus'" or "plague reservoir." The Ebola epidemic started in one small village in Guinea and was able to generate a rapid spread in Central Africa. This minuscule key starting point is normal in biology. According to genetic data, the 7 billion *Homo sapiens* currently on the planet can be phylogenetically related to the population of a village in some part of Eastern Africa, later widespread in every part of the world. In the epidemic model, the diffusion initially develops slowly, but at a certain point it increases dramatically to reach the exponential curve, until it reaches a plateau due to the shortage of nutritional input in comparison with the quantity of the population (Fig. 1.1). However, the shape of the resulting curve, meaning the time to wait until the end of the epidemic, cannot be easily predicted, as is evident in many cases. It is often the same when we have a fever, being sure that in the next two days everything will be solved; we should probably be more patient, avoiding the

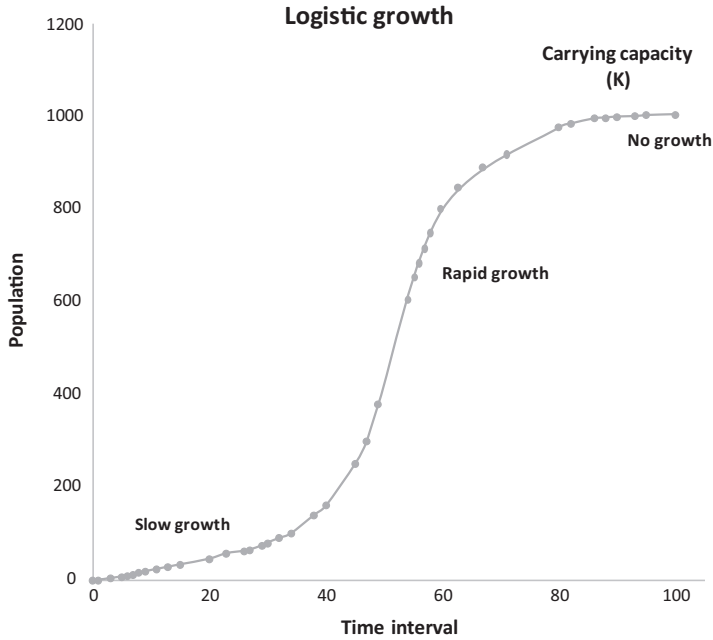


Fig. 1.1 The classical curve of diffusion of an organism's population.

use of drugs including antibiotics. Another complication is that the virulence of the insect-borne disease can differ in accordance with the agent, perhaps a virus or a bacterium or a protozoan, but the initial symptoms are very similar, and not very different from ordinary infections. Furthermore, medical doctors often do not have the necessary experience to make a diagnosis that relates to tropical diseases.

In other words, the detection of the origin of the disease and its nature are fundamental to act positively in the first steps, and also when choosing the therapy. In other words, more the patient is able to live, more are the possibilities to survive. After vaccination, giving the immunity system time to react is the most efficient therapy to fight and defeat the agent of the infection. However, vaccination is not always available, in particular for emerging diseases. A new insect-borne disease can contain several elements of novelty. Even the re-emergence of an already known disease will contain new aspects, and the approach of control must be reconsidered. This is evident in particular for viremias, which are predominant in insect-borne diseases.

Usually, the propagation is obtained by utilizing the subjects already attacked, like in influenza and pneumonia, not necessarily being the main target. Another efficient and smart method, essential in the first steps, consists of transportation, a sort of lift, by an efficient and rapid agent. In our cases, the adjutant is called a vector and is a flying insect or another arthropod. The choice of an insect, in particular a biting one, has excellent advantages. In this way, the disease can be diffused rapidly and the infection of a large territory achieved without any loss of efficiency or energy by the microorganism, which is hosted and protected. The insect is also in charge of the introduction of the microorganism inside the target. However, the main target is an organism very different from the vector, requiring important morphological changes by the pathogen. The result is an astonishing protean performing capacity, which is completely absent in organisms considered much more advanced and specialized, like us. Imagine waking up in a completely different form, like in Kafka's *The Metamorphosis*.

In Greek mythology, Proteus was a prophetic old sea god, subject to Neptune. Proteus knew all things—past, present, and future—but he disliked divulging what he knew. To consult him, you first had to surprise and bind him during his noonday slumber. However, even when caught, he would try to escape by assuming all sorts of shapes, from an elephant to a mouse, but this was possible only for a short period. If his captor held him fast and for long enough (like Heracles did), the god at last returned to his proper shape, gave the wished-for answer, and then plunged into the sea. The word *protean*, one meaning of which is "changeable in shape or form," is derived from Proteus. Proteus was able to assume whatever shape he pleased, and therefore, he can be regarded as a symbol of the original matter (Gea) from which the world was created. In our contest, it could be considered the metaphor of the ancestral bacterium, the first type of Life, still able to convert easily itself, thanks to an astonishing changing capacity, whereas we are forced to be coherent to our unique form. However, it is noteworthy that insect vectors, although in a limited way, are also able to perform metamorphosis.

However, I believe that the legend of Proteus can be considered as a metaphor of the research in biology. The researcher's aim is to understand what is going on in an organism under examination, but meanwhile the biological continuum is in action, able to change the results and the data already obtained, under the influence of a pressure due to a series of variables, acting

together and generating confusion during the experiment and the interpretation of data. The researcher must be absolutely patient and consistent throughout their experiments, repeating everything many times, until the correct explanation discloses the shadow of the inherent complexity of the phenomenon, to obtain the coherent explanation.

Once inside its target, a microorganism is subjected to a series of changes to survive in its new environment and to multiply efficiently. In many cases, the consequence is a disease or even the death of the target. This appears to be an incongruence. Why should the etiological agent attack the host, causing debilitation and damage to its health? This is an energetic problem, i.e., transfer of negative entropy: the microorganism is a parasite subtracting the energy of the host (the transient habitat) for its homeostasis, i.e., to survive and reproduce.

Living systems, like any type of organic organisms, work by subtracting negative entropy from the environment. A similar phenomenon can be observed in other systems, like crystals, but life is based on a specialized order, constantly fighting against the environmental chaos, which is asking back its subtracted energy. Thus, insect-borne diseases, as well as resistance, are only consequences of these energy transfers, occurring to ensure survival and maintain the adequate favorable state. Once they have utilized the negative entropy of other living systems, they react in opposite manner, defending themselves and counteracting. The environment seems to have gained back its energy and the organism will desperately fight to survive until death, when its energy and its molecules will be recycled. Biologists are attempting to understand and reveal the mechanisms through which the negative entropy's transfer is obtained.

However, why should the microorganism kill the host, which is necessary for its reproduction and survival? After all the procedures to infect the host and the necessary metamorphoses? The reason for this apparent suicidal behavior is in the interest of the species versus that of the individual. The best way to propagate an infection is a dead target body: the cadaver is a perfect medium for the reservoir of the pathogen and its subsequent expansion, without the opposition of the immunity system. This is why corpses were often burned in ancient times, even though nothing was known about the strategies of the infective agents. Nowadays, we have a lot of information and science, but it is necessary to remember that if this is the general scenario, every infection is different and the strategy must be tailored carefully for any single case.

First act: The attacked microorganism reacts

We must consider the entity of the subject in question. Fear, terror, dear, and desperation are ancestral words associated with major pathogen diseases. Microorganism pathogens were from early on in history the most dangerous enemy to mankind. As already reported, in principle they are appointed with the important mission to clean habitats of degenerated organic matter and to accelerate the turnover of molecules by causing the death and decomposition of living organisms. The work of micropathogens is therefore continuous and necessary, but unpleasant when they cause diseases. In terms of the latter, micropathogens are able to cause death on a large scale. The history of humanity contains plenty of epidemic episodes that can be attributed to infections of various types. Let us consider, for instance, the ten plagues in the Bible and the situation at the opening of the *Iliad*, based on a medical emergency due to an epidemic causing the death of Achaean warriors and their animals.

The greatest catastrophe in the history was not a war, but a bubonic epidemic plague, known as the Black Death or Great Plague. The Black Death epidemic killed 30%–50% of the entire population of Europe, affecting between 75 million and 200 million people within a few years. The disease started in 1348 when the plague reached the harbor of London, where the city was extremely dirty and overcrowded, but no one knew what caused this dreadful pestilence. Only a few years ago from now, scientists confirmed by DNA analysis that it was caused by the bacterium *Yersinia pestis* and first appeared in wild rodents in places where they lived in great numbers and density. The plague reached humans when the black rats—rodents very common in human habitations—became infected.

The pandemic moved fast, spreading everywhere with terrifying speed and staggering mortality. Whole villages died within a few weeks, and fear spread even faster than the infectious agent. Some towns barricaded themselves in the futile hope of saving themselves via isolation. Mothers abandoned husbands and children—and vice versa—for fear of catching the contagion. Otherwise, fighting the contagion by fire was considered the final, unique solution. Houses and villages were burned to the ground—with the inhabitants inside, if they were known to be ill. Disease can spread easily, causing a new supply of victims, and every time the efforts to contain such pandemics become far more difficult. Ordinary parish burial grounds

were insufficient to hold the massive numbers of dead, and new plague cemeteries were opened, often consisting of a mass grave roughly dug. The social and economic havoc created by a plague is almost beyond imagining, and the impacts of pandemics are still stamped in the minds of humans, such as when a new epidemic jeopardizes humanity or an old one reappears, like recent Ebola or Zika epidemics. It is noteworthy that the mass graves were the best source of information for recent studies based on molecular biology, which were able to rewrite all the story of human plagues.

Until recent times, the causes were totally unknown, but the reports are sufficiently clear. At that time, the physical responses were simple and linear: burning of the cadavers and performing any tentative to limit the area of diffusion of the outbreak by separation of the bodies. The spiritual sphere was involved with prayers and offers to divinities, the plague being considered by some a punishment for some unknown sin. For a long time such diseases remained a mystery, until a fundamental episode, consisting of the discovery of the secret world of microorganisms through the invention of the microscope, became evident in the classic case of ergotism. However, this was not a solution to the diseases.

The situation changed radically in our favor with the introduction of antibiotics. In 1929, penicillin was reported as an antibacterial agent by Alexander Fleming, produced by the mold *Penicillium*. In 1938, Howard Florey, Professor of Pathology at Oxford University, began research on the use of penicillin as a medical drug. Doctor Florey started his treatment due to the potential consequences of the war with Germany and the possible invasion of Britain. Therefore, he focused on cultivating the most productive mold and purifying penicillin. Microorganisms live in a very difficult situation, where most competitors are other microorganisms, like bacteria and fungi, searching for exactly the same opportunities to feed and grow. This microwar is not physical, but based on production of secondary metabolites, synthetized to damage the proliferation of competitors and called antibiotics by us.

In 1941, a police constable called Albert Alexander was the first patient to be clinically treated with penicillin as an antibacterial drug. Constable Alexander was a human volunteer, being in a terminal condition due to an infection accidentally achieved from a rose scratch 2 months earlier. Twenty-four hours after intravenous infusion of 160 mg of penicillin, the infection had begun to heal. After 4 days of treatment, Alexander was well on the way to recovery, but the stock of penicillin ran out. He died a month

later. Therefore, the treatment was switched to sick children, who required smaller quantities of the drug, demonstrating the efficacy of this "miracle drug." In 1944, penicillin was followed by streptomycin, chloramphenicol (1947), cephalosporin (1948), etc., and selection of abnormal strains able to produce enormous quantity of antibiotics. The Age of Antibiotics was born.

Since then, antibiotics have been used successfully to cure a series of infections, such as septicemia, meningitis, pneumonia, and infections of sinuses, joints, and bone, with effects absolutely never experienced by human populations, but also with social consequences.

In advanced countries, life expectancies rose over the centuries in accordance with the increase of civilization and availability of food. During the Egyptians' age, life expectancy was about until 20 years. At the times of Jesus Christ and Alexander the Great, the life expectancy was 33 years, exactly the length of their lives, until an arrow or a pestilence or an accident or simply a deficiency of food or water might bring life to an end. Life expectancy then increased slowly until the advent of antibiotics, and within a few decades the estimated lifetime advanced from 50 to 65 years, at least in advanced countries. The introduction of antimicrobial drugs was fundamental to save lives from simple infections and insect-borne diseases. Nowadays, life expectancy in advanced countries is 77–78 for men and 81–82 for women. This difference occurs because during a woman's fertile years, she is protected by the production of special hormones. After this time, Nature's special care ends and the speed of aging is exactly the same for both sexes. Considering a future when cancer and cardiovascular diseases may be defeated, and aging may be slowed down due to better living conditions, life expectancy in advanced countries is considered to reach an average of 112 years. The rest of the world is still fighting an ongoing war against hunger, famine, and diseases, including old and new vector-borne diseases.

The use of antibiotics was extended to any kind of animal of interest and even in agriculture. The confidence in the value and efficacy of antibiotics was without any shadow. As a consequence, widespread abuse of antibiotics occurred. However, microorganisms are trained to react to environmental changes. In the exact moment that antibiotics were first used, some bacteria resulted that were resistant to these drugs. In other words, when a population of sensitive bacteria are exposed to antibiotics, they will mainly die except the few resistant bacteria, which are already present in the population or created by mutation. These bacteria can continue to grow due to the absence of competitors. Continuing the use of the antibiotics, they will be favored until they are the only dominant ones, causing the inefficiency of the old antibiotic.

Insecticide resistance

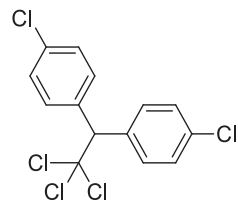
In 1946, a year after the use of penicillin became widespread, some Staphylococcus aureus strains had already become resistant to it. During the next decades, the cases of resistance raised exponentially, including strains of the most common bacteria, starting the phenomenon of the multidrug resistance. Resistance means that in a population of organisms, some of them develop the capacity to render harmful the substances or drugs currently used (Semmler et al., 2009; Mehlhorn, 2015a,b; Gale et al., 1981; Natham and Cars, 2014; Dondorp et al., 2009; Trdan, 2016; Karaagac, 2011; Naqqash et al., 2016). Multidrug resistance is the result of appearance of bacterial strains that could survive exposure to several different classes of antibiotics, and on the other side allied insect vectors became themselves resistant to insecticides. The eternal fight against our most dangerous enemies could be lost in the near future, or at least we may be defeated in the current battle. Several studies are predicting the end of the Antibiotic Era, when most antibacterial drugs will have no effect against microorganism attacks. This situation was already clear to some scientists, but it became an emergency when very important institutions alerted their populations about the incoming problems. Measures adopted so far are too late or insufficient. Currently, use of antibiotics is banned in agriculture and should be for farm animals only, in cases of real necessity, but the real issue concerns inefficacy due to increasing multi-resistance. Once used, the antibiotic remains in the processed food and is accumulated by the consumer. Considering the difficulty of producing new active molecules in the pharmaceutical industry using the established model, it is time to explore new solutions, like the use of natural substances, novel mechanisms of action, and multi-component drugs.

The eternal invisible and devious enemy is coming back. Nowadays in advanced countries the principal causes of death these days are cancer, cardiovascular diseases, and diabetes. In contrast, in the global south, the situation is practically unchanged, with malaria and other insect-borne diseases still dominant. The Antibiotic Age is now in decline. It is possible a more democratic equalized future, at least in terms of causes of death.

Timeline of DDT (dichlorodiphenyltrichloroethane)'s rise and fall (U.S. EPA, 1975)

1900–1935: Most insecticides are constituted of inorganic ingredients, and a few organic compounds, such as nicotine, pyrethrin, and rotenone.

1914: First records on resistance to inorganic insecticides.
1939: DDT's insecticidal action was discovered by the Swiss chemist Paul Hermann Müller (Fig. 1.2).
1940: DDT was introduced as an insecticide, becoming rapidly the principal actor of the period known as the "pesticide revolution," responsible of the wide utilization of pesticides everywhere. In this year the use of DDT became dominant in pest control. DDT was mainly employed with the aim of eliminating or controlling the density of undesired insect populations, but it also affects other insects. DDT was used latterly in World War II to control malaria and typhus among civilians and troops.
1945: In October, DDT was made available for public sale in the United States. Its use was promoted by the government and industry as a safe and efficient agricultural and household pesticide. Once DDT became available, it played a key role in the eradication of malaria in Europe and North America.
1947: Report on occurrence of DDT resistance in houseflies, followed by many other reports in next years. Among others, Dr. Bradbury Robinson, a physician and nutritionist practicing in St. Louis, USA, warned of the negative effects of DDT in agriculture.
1948: Müller was awarded the Nobel Prize in Physiology or Medicine "for his discovery of the high efficiency of DDT as a contact poison against several arthropods."
1955–1965: Relying largely on DDT utilization for mosquito control, the World Health Organization (WHO) started a worldwide program to eradicate malaria in countries with low to moderate transmission rates. The program was able to eliminate the disease in North America, Europe, and the former Soviet Union, and to reduce mortality in several countries. Therefore, it seemed possible to eradicate malaria forever; however, in practice the program was only really effective in areas with



Structure of DDT (dichlorophenyltrichloroethane)

Fig. 1.2 The structure of DDT.

"high socio-economic status, well-organized healthcare system and relatively less intensive or seasonal malaria transmission," as later reported by the same WHO. On the other hand, the failure to sustain the program everywhere resulted in an increasing mosquito tolerance to DDT and a parallel parasite tolerance, leading to a progressive resurgence of the disease. In many areas, early successes were partially or completely reversed, and in some cases rates of transmission increased.

1962: Rachel Carson published the book *The Silent Spring*, focusing on the concerns about massive use of DDT from the beginning of its utilization and denouncing the negative effects on habitats, including birds. The book had an increasing impact on public concern and generated a large public outcry about the environmental damage from widespread use of DDT and other pesticides, in particular in terms of harm to beneficial insects.

1972: Spraying programs (especially using DDT) were curtailed due to concerns over safety and environmental effects (accumulation of insecticide in the soil and in beneficial organisms), as well as problems regarding administrative, managerial, and financial implementation. Utilization of DDT was reduced and its agricultural use was finally banned in the United States.

2001–2004: A worldwide ban on DDT's agricultural use was formalized under the Stockholm Convention on Persistent Organic Pollutants, but its limited and still-controversial use in disease vector control continued in several parts of the world. Attempts at eradication were abandoned and attention was instead focused on controlling and treating the disease. Efforts shifted from spraying to the use of bednets impregnated with insecticides and other interventions.

2014: At least 590 species of insects were reported as resistant to insecticides as registered to one or more insects.

Second act: The post-antibiotic era

Meanwhile, although micropathogens were considered defeated, they were preparing a great return. Resistance can be extended to the entire repertoire of available therapeutic agents. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer (or even sometimes no) effective antimicrobial agents available for infections caused by these bacteria. Gram-positive

and Gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance. The problem of increasing antimicrobial resistance is even more threatening when considering the very limited number of new antimicrobial agents that are in development.

The economic costs of antimicrobial resistance are dramatic. For example, the yearly cost to the US health system alone has been estimated at US $21–34 billion dollars, accompanied by more than 8 million additional days in hospital. The European Centre for Disease Prevention and Control estimated that 25,000 deaths per year were caused by antimicrobial resistant organisms and a cost of approximately 1.5 billion euros per year and 2.5 million additional days in hospital, as reported by the WHO in 2014. In 1970, at least 440,000 cases of multi-drug resistance tuberculosis were detected in 69 countries, resulting in around 150,000 deaths. In 2011, around 25,000 deaths a year in the EU were caused by multidrug resistant infections, with the paradox that two-thirds of these were caught by hospital in-patients. Resistant bacteria from hospitals are also causing more "community-acquired" infections. Difficulty in treating infections with effective antibiotics has increased, because some resistant bacteria have also acquired toxins that make them more virulent, like leukocitin, which causes necrotic lesions that can kill patients in 72 h.

So far, most antibiotics have been used not for therapeutic purposes, but for prevention of infectious diseases in livestock and to promote animal growth in intensive livestock production, amplifying their diffusion behind the abuse of medicinal drugs. Therefore, European farmers are moving to alternative measures such as improved husbandry, increased biosecurity, and nutrition, as well as selective vaccination programs. As a consequence, more than 80% of Organisation for Economic Co-operation and Development (OECD) countries have banned the use of antibiotics for growth promotion, but across developed and developing countries they are widely used to prevent disease, and often when one animal becomes sick the whole herd is treated.

After a long period of prevalence thanks to antibiotics, bacteria are getting out of control. What will happen when there are no more antibiotics left to treat infections? This possibility is not so distant as we might think.

In 2011, Margaret Chan, General Director of the WHO, choosing the theme "Combat Drug Resistance," reported:

"We are now on the brink of losing this precious arsenal of medicines. The use and misuse of antimicrobials in human medicine and animal husbandry over the past 70 years have increased the number and types of micro-organisms resistant to these medicines, causing deaths, greater suffering and

disability. If this phenomenon continues unchecked, many infection diseases risk becoming uncontrollable. In the absence of urgent corrective and protective action, the world is heading towards a post-antibiotic era, in which many common infections will no longer have a cure."

Her words evidence a change in the antibiotic story, when the inadequacy of medicine enters into the problem. The infinite trust in the power of therapy is cracked.

Let's reconsider the resistance phenomenon and the possibility of avoiding its insurgence. To be effective, an antibiotic or an insecticide should be lethal to the great majority of individuals in a normal population. The treatment can lose its efficacy if many populations, or many individuals in a population, develop resistance to the toxic effects. Let us focus on this key point, considering that it is only a further example of the consequences of the human tendency to overexploit natural resources, in order to obtain the maximum effects and not considering the consequences. The problem is inherent: resistance is related to a massive and persistent use of chemicals. Many species may have numerous resistant populations, which can resist one or many treatments. As a result of the chemical treatment, some individuals in a among the population become resistant. Individual genomic differences are inherent to biology. Sensitive microorganisms exposed to the chemical will die, except for the few resistant ones, which can continue to develop and proliferate. Continuing in this way, they will be favored. More use of chemicals fuels the dominance of the resistant part of a population. The consequence of the mechanism is that sooner or later, medicines or insecticides that were once effective are not sufficient to control microorganisms or insects, respectively. However, recalcitrance is not solely caused by resistance but also implies peculiar cells, named persistents, which are drug-tolerant. Tolerance of persistents is not genetically manifested, since they are as susceptible as their parent strains. Stress responses, as in the case of antimicrobial use, may act as general activators of persistents formation.

Nowadays, the situation concerning the future efficacy of antibiotics is wondering, but the key argument is that current resistance could be only the tip of the iceberg. Resistance could be associated with changes at the genome level, giving rise to more virulent organisms. In this case, the scenario changes radically and dangers arise. Bacteria can resist the action of antimicrobial agents by several mechanisms: target modification, target over-expression (e.g., folate inhibitors pathway), antibiotic inactivation (e.g., beta-lactams and aminoglycosides), and modifications of the outer membrane permeability by reducing the expression of outer membrane-proteins (OMPs) or by increasing the expression of multidrug transporters.

Resistance to chemotherapeutic agents can be the consequence of horizontal or vertical transfer of resistance genes and/or intrinsic resistance arising by adaptive response to drugs exposure.

In nature, i.e., in normal situations, the mechanism of multi-resistance is possible, but very unlikely, and challenging to develop. A massive introduction of human-derived products in the environment enables exceptional effects to occur, like speed genomic changes, outside of the Darwinian natural selection laws. Newer organisms are forced to spread out as a consequence of this unnatural treatment. Therefore, resistance could be considered a natural phenomenon, which can be simply considered as a retort of the micropopulation to abnormal environmental conditions. The meaning of the world abnormal, in this case must be identified with massive utilization of antibiotics or insecticides.

Antibiotics nowadays in use, just as is the case for insecticides, all act with three or four mechanisms, and in a short time multi-resistance is a reality. Multi-resistance means that all the substances in use lose any effect, independently by the structure and the action mechanism. Multi-resistance is an important phenomenon first studied in medicinal drugs, like those used against cancer. It has generated several novel concepts in treated complex pathologies.

Third act: The antibiotic emergency

More than 150 antibiotics belonging to at least 17 different classes are now potentially available. They are used mainly for medical treatments and for farm animals and pets. Each antibiotic operates on a specific target or site within the bacterial cell. On the other side, the microorganism has a defense to counteract the effect of the drug.

The range of antibiotics' mechanisms of action is large. Very common antibiotics attack the cell wall, inhibiting the wall synthesis. This class includes the beta-lactams, i.e., glycopeptides, cephalosporins, carbapenems, monobactams, and glycopeptides and cyclic lipopetides, including daptomycin. The response of the bacterium is the enzymatic cleavage of the beta-lactame ring. Other antibiotics act on cell membranes (like polymyxins) or at the metabolic level inhibiting synthesis of proteins (e.g., aminoglycosides, chloramphenical and tetracycline), nucleic acids (e.g., fluoroquinones, rifamycins), or target particular biochemical pathways (e.g., methotrexate, sulfonamides) or cross-link to cysteines on enzymes (e.g., metronidazole). The response of antibiotics includes alteration of

the target site, bypassing an inhibited reaction by alteration of the metabolic pathway, reduced drug accumulation by decreasing the drug permeability, expulsion of the drug, and dilution of the drug's concentration inside the target cell.

Among the issues at the beginning of the 21st century, we must consider a sort of rapid swinging in political tendencies. Any speculation about the future is complicated by this and other factors causing rapid and dramatic changes of counteractions. Further steps in the struggle against multi-resistance are not clear. It is necessary that any counteraction should be based on the knowledge of the peculiarity of the phenomenon and its effects, but other influences can interact and confuse the context. The same debate is taking place about consequences of climate changes, even if a real change is going on. Although scientists are almost in total accordance about the situation and its strong impact, as well the need of rapid responses, the related decisions are out of their hands. What is going on in the US administration is a clear example, with the radical changes after the election of Donald Trump, whose administration stopped former important acts in the right direction. The administration of former US President Barack Obama stepped up its efforts to combat the rising problem of antibiotic resistance. In 2014, Obama's policy started a series of acts to face the multi-resistance phenomenon. First, he signed an executive order establishing a new inter-agency task force charged with developing a national strategy to combat antibiotic-resistant bacteria.

Dr. John Holdren, Director of the White House Office of Science and Technology Policy and Assistant to the President, said the problem is a serious challenge to public health and national security: "We are clearly in a fight against … bacteria where no permanent treatment is possible." The order also established a Presidential Advisory Council made up of non-governmental experts, who would provide advice and recommendations to strengthen surveillance of infections, research new treatments, and develop alternatives to antibiotics for use in agriculture. The administration released the "National Strategy on Combating Antibiotic-Resistant Bacteria," a 5-year plan to prevent and contain outbreaks and develop the next generation of tests, antibiotics, and vaccines. The President's Council of Advisers on Science and Technology (PCAST) reported on future scenarios and also released their opinions on combating antibiotic resistance. There are three main components to the report: (a) improve surveillance of antibiotic-resistant bacteria and stop outbreaks; (b) increase the shelf-life of current antibiotics and develop new ones, as well as promote research accelerating

clinical trials; and (c) finally increase economic incentives to develop new antibiotics. A $20 million prize was set up to be given to spur development of tests that health care professionals can use to identify highly resistant bacterial infections. The task force, responsible for counteractions, was to be co-chaired by the secretaries of Health & Human Services, the Department of Defense, and the Department of Agriculture. The task force submitted its national action plan to the President by February 15, 2015. As a consequence, in March 2015 President Obama declared the fight against multi-resistance and the decision to ban definitely every use of antibiotics for farm animals.

Obama's resolution is the result of an incredible situation. Most antibiotics used in the US and UK are given to animals, and not for therapeutic purposes. In 2001, the Union of Concerned Scientists estimated that more that 70% of the antibiotics used in the US were given to animals reared for food (chickens, pigs, and cattle) also in the absence of diseases. The idea is that antibiotics at low doses not only furnish a shield against microorganisms, but also promote health and therefore growth in farm animals, although there is no scientific evidence of these effects. The situation in the EU is not very different despite more restrictive regulations. The situation is similar for human use. In most countries, antibiotics are available from pharmacies without medical prescription, or this restriction can be easily overcome. This means that anyone who feels sick with an upset stomach or diarrhea or who feels any symptom of influenza can directly buy whatever antibiotic treatment is available, whether it is appropriate or not. Treated or untreated fecal matter is a source of contamination for water supplies. The potential for rapid spread of antibiotics in the environment appears to be greater in emerging countries, as well as in Europe and North America, but we must consider also the greater availability and the major possibility of expanding the sanitary service and reacting in case of emergency, epidemics, or pandemia, in richer countries. Obama's resolution was a crucial sign, but it was subjected to political influences and economic interest. It ended a long strike with farm industries that previously had been successfully blocked by the food and pharmaceutical industries.

Is it time to face the possible end of Antibiotics Era? Considering the consequences of the absence of these products and the necessity of their presence, we have only one answer: declare the end of the first period of antibiotics, and continue directly with the second period. We must hurry up, since we have only 30 years to solve the problem. The way to solve the problem is based on the exploration in other directions with research and helping

mankind to progress the lifestyle against any dystopia. Once again, if this is the scenario proposed by scientists and confirmed by common experience, the consequent decisions can be incoherent. Furthermore, in these arguments there is always a sort of tunnel vision, focusing on human health and not considering side environmental effects.

Of course, there are already signs of reactions. The WHO called for "push" incentives to encourage certain classes of antibiotics. The US Senate introduced the Generating Antibiotic Incentives Now Act in 2011, to "spur development of new antibiotics to combat the spread of antibiotic resistant bacteria." However, President Trump is now denying any global environmental changes and effects of antibiotics, just as previous president Ronald Reagan did, who focused his electoral campaign on this argument.

Nowadays, the Trump administration is resisting the WHO's effort to limit sharply use of antibiotics in farm animals, a move intended to preserve these drugs' effectiveness. Instead, the US is helping to draft an alternative approach that appears more favorable to agribusiness, since antibiotics are mainly used as growth promoters, although this use should be not allowed. Therefore, on name of the independence of any country in matter of developing, the U.S. Agriculture Department termed the "WHO position as shoddy science," causing the leave of WHO representatives out of the agreement to avoid potential conflicts. This is a clear example of a conservative position. Even if one considers the scientific considerations about resistance to be exaggerated, research on this field should be reinforced, just in case.

In recent years, the spread of antibiotic resistance among bacteria has reached worldwide proportions. In ordinary consideration, antibiotics have realized a utopian role as a panacea against all kinds of invisible attacks to health. All the policies and measures intended to contain or slow the development of antibiotic resistance have become inadequate and immediate actions are vital to understand the phenomenon of "antibiotic resistance pollution." The use of antibiotics in a wide range of applications, from health care to agriculture, is key to the evolution of antibiotic-resistant organisms following the phenomenon defined as "use it and lose it." This wide utilization of antibiotics means that the limit of use in medical drugs is only part of the problem, as a consequence of the food production chain. However, lacking of other solutions and drugs, in this moment we cannot renounce to antibiotics.

So far, as matter of fact, the pendulum between outbreak and control of pathogens resulted in a stand by and, as for the environmental changes, no

real solutions or changes came from the rituality of global conferences and consequent declarations. Therefore, the key word is "new." To face new challenges, we need new solutions. To obtain new substances we must explore new possibilities, and to do it we must be brave and intelligent. Most pharmaceutical companies are not able to be free in this exploration.

We must learn from our enemies. In the struggle, they are able to adapt quickly and even sacrifice their precious past. These organisms, so simple and microscopic, have ruled Earth from the beginning and now, again, they try to claim absolute dominion.

To face an epidemic alert, attention is usually focused on the present, in search of efficient responses to halt the disease as soon as possible. The expectation is that usually the disease disappears, but this could be only for a while, and then the disease may return, stronger and more virulent. Activities should thus be concentrated also on the reasons and the events related to the origin of the disease. It is worth focusing on point zero at time zero—the start of the disease, when the pathogen changes and becomes a powerful danger. In the past, the organism was subjected to control by antibiotics or insecticide. Resistance modified the scenario radically. What are the origins of resistance? How does the resistant strain rise in the population? What factors activate and influence this event? What was the deflagrating factor, which was able to cause a significant change inside a species that suddenly became so destructive after a long history of normal disease? Let us reconsider the nature of the pathogen in the light of actual knowledge and some recent important inputs.

In clinical practice, an organism is resistant to a therapeutic agent if treatment with that agent results in clinical failure at the in vivo concentration achieved. As we have seen, resistance is the key phenomenon to understand the present and imagine the future of the therapeutic treatment. Resistance involves both protagonists, insect vector and microorganisms, in the first place, and is a consequence of human activity. Most resistance factors can be intrinsic or acquired. The acquisition of antibiotic resistance genes is possible through the acquisition of genetic mobile elements such as plasmids, transposons, and gene cassettes. In bacteria, horizontal gene transfer (HGT) plays an important role in the spread of antibiotic resistance genes and virulence factors also among phylogenetically unrelated organisms.

Bacteria can resist the action of each antimicrobial agent type by several mechanisms:

- target modification (e.g., fluoroquinolones);
- target over-expression (e.g., folate inhibitors pathway);

- antibiotic inactivation (e.g., beta-lactams and aminoglycosides);
- modifications of the outer membrane permeability by reducing the expression of outer membrane-proteins (OMPs); and
- increasing the expression of multidrug transporters.

Genetic lessons

An evolutionary change should consist of a genetic modification, resulting in the acquisition of characters in better accordance with the habitat changes (Barlow and Hall, 2002; Andersson and Levin, 1999; Miller Jr, 2013). The correlation between environmental factors and related genome changes is not easy to verify. A character may be the result of the co-occurrence of several genes and casual breeding. A gene can consist of thousands of bases, and the change of a single base in a key position can generate the production of an amino acid or something else, but this does not result automatically in a phenotypic change, as is evident in genetic studies. Most attempts at correlation between the presence of a certain allele and the phenotypic character of a human population were wasting time and misunderstanding, although in several cases the occurrence of an interested utilization of data can be usually used to denigrate or morally criticize certain targeted groups. Like the phoenix rising from the ashes, eugenics reappears with its promises of improving genetic quality by excluding. Neanderthals and *Homo sapiens* were considered a priori incompatible, until the DNA sequences evidenced exactly the contrary. However, science needs models and formulas, based on the study of simplified situations.

The lesson of genetic starts with Mendel's laws, although they are not really useful to clarify our genetic origins, and all the results reported by the agencies to reveal the pedigrees of confident citizens are arguably a smart method to accumulate money, flying the flag of genome sequencing reliability. The reality is that in very few cases the human characters were demonstrated as a consequence of an adaptation response to the environmental pressure in defined regions, although everybody knows the example of melanin production. Interestingly, another successful example is related to insect-borne diseases. Among some populations subjected to malaria caused by *Plasmodium falciparum* and *Plasmodium vivax*, owing to a mutation of gene of hemoglobin beta, the shape and structure of red cells are altered. The erythrocytes changes their usual classic discoidal round form into a stretched shape, and therefore acquire partial protection against malaria. In sickle cell

anemia, the red blood cells become rigid and sticky and are shaped like sickles or crescent moons. However, there are two variants of the mutation. People with only a single changed copy of the gene will have a sufficient number of regular blood cells and few symptoms of the disease. People with two modified genes will develop pains, infection, and ictus, because irregularly shaped cells can get stuck in small blood vessels, which can slow or block blood flow and oxygen to parts of the body. However, they will also develop a resistance against malaria as a collateral effect, since the *Plasmodium* is used to living inside normal blood cells. The shift in blood composition toward the prevalence of modified red cells is shown in Fig. 1.3.

The development of antibiotic resistance by bacteria is considered by evolutionists to be a demonstration of evolutionary change. Bacteria can be used as an appropriate model for studying evolution steps in a homogenous population selecting the environmental factors and their influences. Therefore, the so-called "evolution in a Petri dish" is based on some aspects very useful in the study: rapid rate of replication, easy of analysis and detection of changes, wide range of conditions generated in the laboratory, and recently molecular analysis of the bacterial genome, in such a way that the responses of various strains can be compared. The bacterial genome is a powerful tool to understand trends, since it is possible to use it to write the events of thousands of years of evolution.

The development of antibiotic resistance was referred to by Miller as the consequence of evolution's "creative force" and by Barlow and Hall as "the unique opportunity to observe evolutionary process over the course of a few decades of the several millennia that are generally required for these processes to occur." In this way, an evolutionary change is the result of a so-called adaptation, which is a consequence of "beneficial" changes in the genotype. The consequent descent with modification from the prototype should require "evolutionary" acquisition of characters obtained by a vertical sequence of generations, which means a consequence of mutations capable of the adequate genetic changes.

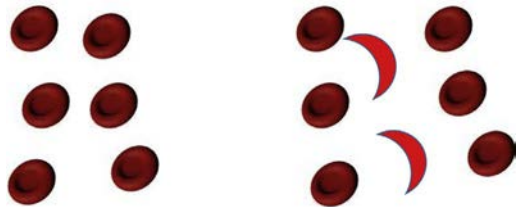


Fig. 1.3 The comparison between normal blood cells and sickle cell anemia.

The study of resistance mechanisms, although forced by the necessity to find a therapeutic solution, has afforded important information about the biology of microorganisms, including real discoveries. Molecular biology studies on bacteria have shown that these organisms, so simple and microscopic, are able to become resistant by different and unexpected mechanisms, some already discovered and others under examination.

This is a classic interpretation of the genomic response in case of the resistance phenomenon. We can imagine the situation as a tensor acting in vertical and determining the normal sequence of the generations in the species and another tensor operating as a disturbance able to generate changes in the above sequence.

Resistance to chemotherapeutic agents can be the consequence of horizontal or vertical transfer of resistance genes and/or intrinsic resistance arising by adaptive response to antimicrobial exposure. In Gram-negative bacteria, many of these genes are associated with mobile genetic elements such as plasmids, transposons, and gene cassettes with their integrons.

In fact, this is an efficient mechanism quite common in resistant bacterial strains, but it can account only for the rapid spread since resistance genes must be present already in the bacterial world. The only origin of real evolutionary changes must be in mutations that must be regarded as beneficial when they increase the survival chances of bacteria in the presence of antibiotics, by a homeostatic mechanism. However, resistance can occur in the reverse direction to the ordinary sense, i.e., subtracting and not adding. This mechanism is often underestimated or even considered absent in multicellular organisms, but it has been clearly evidenced in unicellular populations. This approach could be a key explanation of many evolutionary changes for many organisms, including mammalians. The general interpretation of an evolutionary step consists of an advance of the complexity and possibilities of an organism, but this is not correct. In these cases, modifications consist in reduction or loss of cell functions previously occurring, such as lack of membrane selective transport by proteins or porins and protein binding affinities, a decrease or block of enzyme activity, and modification of proton motive force and other regulatory control systems. In such cases, introduction of a cell drug can be efficiently limited or the drug easily extracted, disarming the drug by limiting its concentration inside the cell.

Another hypothesis of mechanisms concerning resistance is based on genetic mobile elements, which are part of a huge "intrinsic resistome" in bacteria. The resistome is composed of genes of varied phylogenetic origin that act as resistance genes only in the presence of the ultimate drug, as a form of survival by the target organism. The success of resistant organisms

contributes to the constant accumulation of a genetic platform and vehicles able to recruit and spread novel resistance genes efficiently. This phenomenon is called "genetic capitalism."

Antibiotics and synthetic insecticides produce effects that should be analyzed under the light of the multilevel selection theory. All biological-genetic elements at any level of hierarchy should become targets for intervention against the antibiotic-resistant phenomenon. Such a perspective indicates the need and possibility of drugs acting not necessarily to cure the individual, but to cure specific environments from resistance and to prevent or weaken the evolutionary possibilities of the biological elements involved in this phenomenon. This approach is referred to as the "ecological and evolutionary" approach.

Classes of antibiotics

Antibiotics belong to several classes that are classified by their mode of action (Table 1.1). As consequence, the organism target can generate several ways to counteract the pathogen. The general idea is that in the next 20–30 years, bacteria could develop resistance to any kind of antibacterial drug, independently of the mode of action. This situation is called multi-resistance and means that it is necessary to investigate several areas to understand the what, where, and when of the insurgence and success of the resistance. Let us start from the current situation.

It is important to stress again the importance of the resistance phenomenon in microbial pathogens, always considering that in all this argumentation, most of the considerations with some obvious differences can be translated to insecticides. However, microorganisms could be considered a better model to observe, study, and understand the phenomenon.

Research in the field of antimicrobial agents has reached a dead end. The production of antibiotics was and still is totally in private hands. Large pharmaceutical companies, for economic reasons, are no more interested in developing new antibiotics. The costs of research and the risk of antibiotic failure caused by the almost simultaneous appearance of resistance are unsustainable. Based on clinical outcomes, the costs of new antibiotics would too high to be comparable with the older ones. This is only one aspect, probably the most searing of the general problem in producing new medicinal drugs because of the enormous cost of the clinical trials required. Research centers, mainly within universities, are working to explore new solutions, but the shortage of public grants is a great limit.

Table 1.1 Summary of antibacterial classes and mode of action.

Antibacterial classes	Mode of action
Penicillins, cephalosporins, carbapenems, monobactems, glycopeptides, polypeptides	Cell-wall construction inhibitors of peptidoglycan synthesis or cross-linking functions resulting in osmotic lysis
Lipopeptides, polypeptides	Cell-membrane disruption altering the structure and function of the cell membrane, thus causing cellular leakage
Aminoglycosides, tetracyclines	Protein synthesis inhibitors binding to the 30S ribosomal subunit, thus preventing translation initiation and tRNA binding
Macrolides, oxazolidinones, streptogramins, phenicols	Protein synthesis inhibitors binding to the 50S ribosomal subunit, thus disrupting translocation and peptidyl transferase activity
Rifampin	RNA synthesis inhibitors preventing the synthesis of mRNA by binding to DNA-directed RNA polymerase
Quinolones	DNA synthesis inhibitors prevent DNA replication by binding to topoisomerase IV or DNA gyrase
Trimethoprim	Folic acid metabolism inhibitors preventing the synthesis of nucleotide bases by blocking the synthesis of tetrahydrafolate
Sulfonamides	Folic acid metabolism inhibitors inhibiting nucleic acid synthesis by preventing the synthesis of folate

Toward a new antibiotic age, or the end of a fundamental health tool?

The beginning or the end? Among the new approaches, we must consider the increasing consideration assigned to the whole human microbiome, in relation to homeostasis and performances of the human body. Remember that, until the human genome changes in terms of acquiring resistance to bacteria, we are dependent on antibiotics. The possible speed of change is about 0.1% in 10,000 years, although we can see that something has already moved under the disease pressure. Use of antibiotics in the last 70 years has transformed human health. We can survive bacterial infections that routinely killed our ancestors, but without new antibiotics, we may soon be exposed once more to terrible epidemics. In any case, we need a solution, urgently.

Recent studies on our microbiota are changing radically our point of view about bacteria. Usually, people are concerned about microorganisms only in the case of infection. Attention is focused on effects of the presence of pathogens and people are only interested in killing the bad bacteria as soon as possible to reach the previous status of health again. The problem is that the consequence of antibiotics treatments are not only beneficial. We have more bacterial cells (around 10^{14}, accounting for 1–2.5 kg of our body weight) than eukaryotic cells (around 10^{13}). The bacterial biodiversity is far more abundant, considering that in our gut, more than 500 different species have been found, albeit 10 are predominant, and in our mouth we have hundreds of other species different from those of the gut; the human skin carries several hundred more. Introducing huge numbers of fast-growing and virulent bacteria, any antibiotic resistant genes that appear in response to selection pressure could become established and continue to spread. However, the most important cause for alarm concerns not their misuse as medical drugs, but the environmental aspect.

The widespread antibiotic resistance among pathogenic bacteria affects not only the treatment of infectious diseases, but also many other medical practices such as surgery and immunosuppression in transplants. The spread of antibiotic-resistant bacteria in hospitals "means that commonplace medical procedures once previously taken for granted could be conceivably consigned to medical limbo. The repercussions are almost unimaginable" (WHO "Overcoming antibiotic resistance report," 2009). Hospitals, instead of being helpful, constitute one of the main reservoirs of antibiotic-resistant microorganisms. Patients with resistant bacterial infections are in close proximity with other patients whose vulnerable states make them susceptible to acquire such nosocomial infections.

The studies of our microbiota have completely changed the paradigm of the role of the invisible enemies. Currently, we know that without this microscopic symbiotic help, we could not be able to live and that even our feelings are probably influenced by our microbiota. Therefore, we know that there are good and bad bacteria. In addition, a good bacterium can change and become aggressive and dangerous. The microbe world is continuously subjected to change according to its environment.

Furthermore, it is clear that our microbiota are different and change in parts or organs of our body. Therefore, probiotic and prebiotic products must be tailored on this consideration. Products must be also tailored for ambient situation. For instance, a computer keyboard is usually a preferred area for some aggressive bacteria, and several bacteria may be transferred by

the use of the same computer by different persons. Therefore, in particular epidemic situations, some places must be monitored and cleaned. The cleaner must consider the type of bacteria usually present in this case. A place can appear clean, but might not be disinfected. Nowadays there are very simple and low-cost kits to detect the presence of dangerous bacteria, but few people know them and very few are using these cell sensors. An alternative solution is the use of gloves or/and other protection, but workers do not usually like this solution, especially for long periods of time. These aspects were not usually considered as part of people's experience until an emergence generates a general need to face the attack of a new pathogen, more dangerous than those already known. It was so far strange, until the Coronavirus pandemia, for Europeans to see Asiatic tourists wearing face masks in public places, but this is familiar in other countries, and several epidemics have had their origins in the Orient. In other words, the need for familiar products to maintain hygiene and prevent diffusion of microscopic parasites, from viruses to microorganisms, is fundamental, being the first frontline for control of insect-borne diseases, as well as for other outbreaks.

The microbial communities of humans are characteristic and complex mixtures of microorganisms that have co-evolved with their human hosts, for better and for worse. Humans and their bacteria share the same evolutionary fate in which mutualistic interactions are essential for human health. For instance, several diseases are the result of perturbation of this equilibrium caused by changes occurring in the ecology or genetic of the bacterial world. Taking ecology and evolution into account might provide new strategies for restoring and maintaining human health. However, the dominant aspect of the economic cost must always be considered. The "win-win" approach, based on sustainability and respect for the environment joined with economic benefit, is now probably a utopia, but it is a new perspective for the future, probably the unique able to work to maintain the perspective of a sustainable state.

A current definition of resistance

This section is dedicated to a partial revision of the arguments already exposed, in consideration of the current debate about the resistance and the consequent counteractions. As a consequence of the multiple attacks, bacteria have evolved a large range of protection to deactivate, remove, or otherwise circumvent the toxicity of antibacterial compounds, thereby leading to today's

multidrug-resistant organisms. As a result of this evolutionary ping-pong between attack and defenses, a bacterium is able to decrease the concentration of the drug in its cell, enable the effects, and/or interfere in the mechanism of action. In such cases, the medical response is to change the drug to another considered more active or able to surprise the pathogen. The final consequence is an incredible sequel of antibiotic drugs, exploring all the possible chemical derivatives of the leading molecules, which evidenced some kind of efficacy (Table 1.2). Although the possibilities of chemical variations are practically infinite, several routes have already been exhausted.

A table similar to Table 1.2 can be obtained also in case of insecticides resistance (Table 1.3). The sequence of emergence of resistance for the antibiotic vancomycin is reported in Fig. 1.4. However, the scenario evidenced in Table 1.3 does not totally explain the resistance to antibiotics. In many cases, such as cephalosporins and tetracyclines, several generations of related drugs have been developed and introduced in therapy with the hope of obtaining a better result. This strategy is still being used, but so far the problem has not been overcome. As can be deduced from the data reported in Table 1.3, it is possible that the time for the appearance of resistance is going to be progressively reduced, in accordance with the multidrug phenomenon being the result of a wider utilization of antibiotics in any field. The use of antibiotics in a wide range of applications, from health care to agriculture, is

Table 1.2 Timelines of the introduction of the antibacterial drug in chemotherapy and first appearance of resistance for most common antibiotics.

Antibiotic	Introduction of antibiotic drug in therapy	First resistance report
Sulfonamides	1935	1945
Penicillin	1940	1945
Chloramphenicol	1945	1957
Tetracycline	1948	1953
Streptomycin	1952	1986
Erythromycin	1957	1985
Vancomycin	1960	1987
Methicillin	1962	1964
Ampicillin	1963	1973
Linezolid	2000	2004
Daptomycin	2004	2005
Tigecycline	2005	–

Table 1.3 Examples of utilized insecticides and insurgence of resistance.

Type of insecticide	Year of introduction to the market	Year of insurgence of resistance
Modulator of sodium channels		
▪ DDT ▪ Pyrethroids	1940 1960	1947 (banned in 1950s and 1960s) 2018
Acetylcholinesterases (AChE)		
▪ Organophosphates (parathion and malathion)	1938	1961 (banned in 1970s)
Chlorine channel antagonist regulated by GABA		
▪ Synthetic phenylpyrazolones	1930	2010
Juvenile hormones		
▪ Pheromones	1960	1989
Chitin synthesis inhibitors		
▪ Diflubenzuron	1970	1978

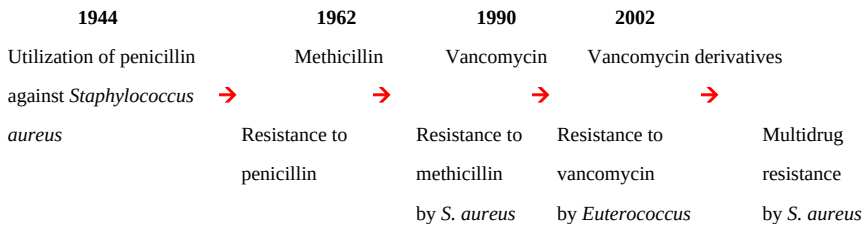


Fig. 1.4 The sequence of emergence of resistance for vancomycin.

the key of the evolution of antibiotic-resistant organisms following the phenomenon defined as "use it and lose it." Accordingly to the WHO definition, "antimicrobial resistance (AMR) is the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others." Consulting other current definitions, resistance can be defined as "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control

when used according to the label recommendation for that pest species," as reported by IRAC (Insecticide Resistance Action Committee) of the University of Nebraska. Here, we will consider also a further approach, wherein resistance will be considered as "the inherited ability of an organism to become tolerant and/or resistant to a dosage of the chemical that would be lethal to a definite species." In fact, resistance, being related to genome, appears to be a no-turn phenomenon, meaning that we have to face its consequences in mankind's future.

Antibiotics are the natural result of the usual war between microorganisms to dominate a common territory. In addition, infected hosts are involved in the production of such compounds. The consequence is that when an antibiotic drug is no longer effective, the usual response involves a shift to a new drug. This measure is always wasting time, or at least delaying, since in a short time the new drug will become ineffective. The manifestation of the antibiotic resistance is very rapid, as is well evidenced by the sequence in Fig. 1.4. This is in accordance with the presence of resistant strains already inside the targeted population of microorganisms. How is this possible? Most antibiotics are obtained or derived from natural products, whose structures are already in the memory of the attacked microorganism. After a short gap for adaptation, the resistance resurges.

However, it is possible that the natural reservoir of biocides natural products is not totally explored, in particular in plants, considering the lower use of antibiotics in agriculture and the important case of pyrethroids. Pyrethroids are a group of a synthetic pesticides, whose structures are deduced from the natural pesticide substances, pyrethrins and related terpenes, contained in the flowers of the perennial plant pyrethrum (*Chrysanthemum cynerariifolium*, Compositae) (Fig. 1.5). The use of seeds and flowers as insecticide dates from thousands of years, since in China and in Iran, chrysanthemums were crushed and used as insecticide powder as early as 1000 BCE. This so-called Persian Powder was widely used for centuries, as an insecticide in household use and as a repellent for mosquitos, fleas, and body lice, such as by French soldiers in the Napoleonic Wars. Flower of this plant are

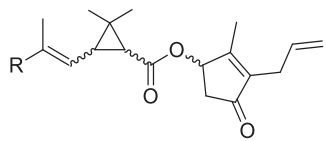


Fig. 1.5 The general structure of pyrethroids.

similar to those of the common daisy, but they are bigger and all yellow. Although more than 1000 pyrethroids have been made, only a few have been selected for this use, mainly as a domestic insecticide. The strange structure of these compounds is characterized by the presence of one rare cyclopropane unit, essential for the insecticide activity a pentacyclic lactone alpha, beta unsaturated and several chiral centers. In particular, pyrethrum is the extract from the chrysanthemum plant, containing pyrethrins. Therefore, pyrethroids are the man-made version of the natural pyrethrins, but while pyrethrum extract is composed of six esters which are insecticidal, a synthetic pyrethroid is usually composed of only one chemically active compound, in accordance with the kind of activity typical of natural products, consisting of the effects of a mixture of constituents.

Resistance is an emerging phenomenon, demonstrated in interesting several cases of the interaction of humans with the environment, including microorganisms' resistance to antibiotics and that of insects to insecticides. Resistance is an increasing problem, whose solution may be crucial for any future scenario for mankind, dramatically involving not only directly human health, but also future feed and food. Furthermore, it is necessary to consider that resistance is a widespread phenomenon. Antibiotics are everywhere, and bacteria that are resistant to chemically modified and synthetized antibiotics are present in any environment.

Multidrug resistance

Multidrug resistance (MDR) is among the most important causes of infections in nosocomial and community settings. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer (or even sometimes no) effective antimicrobial agents available for infections caused by these parasites.

The same definition of MDR is actually inadequate to describe the phenomenon. To date, the adjectives "extensively drug resistant" (XDR) and "pandrug resistant" (PDR) have been introduced to describe the degree of resistance to a determined number of different classes of antibiotics. For instance, PDR organisms show resistance to all available antimicrobials. The idea is describe the different levels of the phenomenon, and this distinction is useful to identify its mechanism.

As already reported, to be effective, an antibiotic should be lethal to the great majority of the individuals in a normal population. However, a

population is a mix of individuals, each having similar but different metabolisms, and therefore having personalized responses to the antibiotic attack. The antibiotic can lose its efficacy if many populations, or many individuals in a population, develop resistance to the toxic effects. Let us focus on this key point, considering that is only a further example of the consequences of the human tendency to overexploit natural resources, in order to obtain the maximum effects and not considering the consequences. The problem is inherent: resistance is related to a massive and persistent use of chemicals. More use of chemicals fuels the dominance of the resistant part of a population. The consequence of the mechanism is that chemicals that were once effective become insufficient to control insects. However, antibiotic recalcitrance is not solely caused by resistance, but also implies peculiar cells, named persistents, which are drug-tolerant. Antibiotic tolerance of persistents is not genetically manifested, since persistents are as susceptible as their parent strains. Stress responses, as in the case of antimicrobial use, may act as general activators of persistents formation.

The key argument is that the current resistance occurrence could be only the tip of the iceberg. This resistance could be associated with genome changes, giving rise to more virulent organisms. In this case, the scenario changes radically and dangers arise. The dominance of resilient organisms have consequences not limited to the target population, but all the environment is engaged and affected.

Multi-resistance is the final boomerang step, related to an intense and massive use of insecticides, exactly like antibiotics in microorganisms: many species have numerous resistant populations, as the normal range of possible reactions to the habitat changes. In nature, i.e., in normal situations, the mechanism is possible, but very unlikely and challenging to develop, and subjected to randomness. Newer organisms are forced to spread out as a consequence of this unnatural treatment. However, the environmental conditions are very important and even decisive, as we already have seen, for the emergence and spread of the epidemic phases.

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