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**JHARSUGUDA**

 **NATIONAL CHILDREN SCIENCE CONGRESS**

**THEME –HEALTH AND THE DEVELOPMENT**

**TIITLE-PREVENTION OF ANTIBIOTIC RESISITANT BY AQUACULTURE**

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IT IS TO CERTIFY THAT MASTER DEEPAK KU PATEL OF CLASS 9TH HAD DONE THE REASERCH AND EXPERIMENT FOR THE PROJECT TITLED “PREVENTION OF ANTIBIOTIC RESISTANT BYAQUACULTURE”WITH HIS TEAM AND COMPLETE THE SAME DURING THE ACADEMIC SESSION 2017-18 FOR NATIONAL CHILDREN SCIENCE CONGRESS

SIGN OF TEACHER SIGN OF PRINCIPAL

**WE PAY OUR SINCERE THANKS TO OUR PRICIPAL MAM SMT D.J MISHRA WHO ALLOW US TO DO THIS PROJECT ALSO PAY THANKS TO OUR GUIDE TEACHER WHO GIVE US IDEAS ABOUT THIS PROJECT ALSO SPECIAL THANKS TO ALL .THANKS TO ALL THE CLASSMATES AND FRIENDS WHO HELP US AT LASTST THANKS TO ALL THE TEACHERS AND THE PEOPLE WHO ENCOURAGE US AND COPERATE WITH US TO DO THE PROJECT**

**THANKS TO ALL**

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*Title- PREVENTION OF ANTIBIOTIC RESISTANT BY AQUACULTURE*

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***ABSTRACT***

*Antibiotics are medicines used to prevent and treat bacterial infections. Antibiotic resistance occurs when bacteria change in response to the use of these medicines.*

*Bacteria, not humans or animals, become antibiotic-resistant. These bacteria may infect humans and animals, and the infections they cause are harder to treat than those caused by non-resistant bacteria.*

*Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality.*

*The world urgently needs to change the way it prescribes and uses antibiotics. Even if new medicines are developed, without behaviour change, antibiotic resistance will remain a major threat. Behaviour changes must also include actions to reduce the spread of infections through vaccination, hand washing, practising safer sex, and good food hygiene.*

Background

 *Everyone has a role in helping to prevent antibiotic resistance. Canadians and healthcare professionals must work together to reduce its impacts on our health and healthcare system.*

*Practicing good hygiene, like washing your hands properly, helps keep you from getting sick. It also helps prevent antibiotic resistance. You are also helping when you and your family use antibiotics responsibly and properly from aquaculture* *Antibiotic resistance is rising to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. A growing list of infections – such as pneumonia, tuberculosis, blood poisoning and gonorrhoea – are becoming harder, and sometimes impossible, to treat as antibiotics become less effective.*

*Where antibiotics can be bought for human or animal use without a prescription, the emergence and spread of resistance is made worse. Similarly, in countries without standard treatment guidelines, antibiotics are often over-prescribed by health workers and veterinarians and over-used by the public.*

*Without urgent action, we are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill.*

***Objective***

***Antibiotic****allergic****reactions****. Around 1 in 15 people have an allergic****reaction****to****antibiotics****, especially penicillin and cephalosporins. In most cases, the allergic****reaction****is mild to moderate and can take the form of: a raised, itchy skin rash (urticaria, or hives)*

*We found that some disese are deadly so ipe*

*There are many unconvincing claims in the lawsuit, but that the lethal effects of the drug were not adequately communicated to physicians is not one of them.*

*“The pain is almost unbearable in both legs and left shoulder,” a user named our a neighbourhood tell that “I am now walking with a cane.” Ruptured tendons, which our neighbours was diagnosed with, are a rare but serious side effect of Levaquin, as are a host of other physical and mental issues.*

*“I can barely walk,” another named Linda wrote one month ago. “Can’t sit or lay without discomfort.”*

**Methology and workplan**

*The overall aim of the project is to improve antimicrobial prescribing in primary health centre and in the community by obtaining up-to-date, clinically relevant data on variation in clinical management and antimicrobial consumption and resistance rates and then to feed this back via a number of educational initiatives to paediatricians in-training and in clinical practice across village*

*Evaluation of the specific antibiotics and doses used to treat common infections in partner primary health centre*

*Collection and comparison of specific primary care and hospital antibiotic prescribing guidelines for the most common childhood infections*

*Development of a web-based educational training programme for paediatricians on the principles of prudent antibiotic prescribing containing country-specific prescribing and resistance data*

*Identification of the specific antibiotics used to treat common childhood infections in primary care*

*result in widely applicable methods for the surveillance of antibiotic use and antimicrobial resistance in neonates and children*

*Primary Care Antibiotic Prescribing for common childhood infections*

*Point Prevalence Survey of Paediatric Hospital Antimicrobial Consumption*

*Variation in paediatric antimicrobial prescribing guidelines*

*Existing primary care data sets will be used to determine the variation in specific antibiotic prescribing for common childhood infections*

*Antimicrobial resistance threatens the very core of modern medicine and the sustainability of an effective, global public health response to the enduring threat from infectious diseases. Effective antimicrobial drugs are prerequisites for both preventive and curative measures, protecting patients from potentially fatal diseases and ensuring that complex procedures, such as surgery and chemotherapy, can be provided at low risk. Yet systematic misuse and overuse of these drugs in human medicine and food production have put every nation at risk. Few replacement products are in the pipeline. Without harmonized and immediate action on a global scale, the world is heading towards a post-antibiotic era in which common infections could once again kill.*

*` to improve awareness and understanding of antimicrobial resistance through effective communication, education and training; ` to strengthen the knowledge and evidence base through surveillance and research; ` to reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures; ` to optimize the use of antimicrobial medicines in human and animal health; ` to develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions.*

***Result(conclusion) and obvservation***

* ***We do experiment and wqent different area of the village the practised different mode of problem and investigated***Antibiotic resistance is one of the biggest threats to global health, food security, and development today.
* Antibiotic resistance can affect anyone, of any age, in any country.
* Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process.
* A growing number of infections – such as pneumonia, tuberculosis, and gonorrhoea – are becoming harder to treat as the antibiotics used to treat them become less effective.
* Antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality.

ome bacteria have developed resistance to antibiotics that were once commonly used to treat them. For example, Staphylococcus aureus (‘golden staph’ or MRSA) and Neisseria gonorrhoeae (the cause of gonorrhoea) are now almost always resistant to benzyl penicillin. In the past, these infections were usually controlled by penicillin.

The most serious concern with antibiotic resistance is that some bacteria have become resistant to almost all of the easily available antibiotics. These bacteria are able to cause serious disease and this is a major public health problem. Important examples are:

* methicillin-resistant Staphylococcus aureus (MRSA)
* vancomycin-resistant Enterococcus (VRE)
* multi-drug-resistant Mycobacterium tuberculosis (MDR-TB)
* carbapenem-resistant Enterobacteriaceae (CRE) gut bacteria

By undergoing a simple mating process called "conjugation," bacteria can transfer **genetic** material, including **genes** encoding **resistance** to **antibiotics**(found on plasmids and transposons) from one bacterium to another. Viruses are another mechanism for passing **resistance** traits between bacteria

The successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed. This is true for agents used in the treatment of bacterial, fungal, parasitic, and viral infections and for treatment of chronic diseases such as cancer and diabetes; it applies to ailments caused or suffered by any living organisms, including humans, animals, fish, plants, insects, etc. A wide range of biochemical and physiological mechanisms may be responsible for resistance. In the specific case of antimicrobial agents, the complexity of the processes that contribute to emergence and dissemination of resistance cannot be overemphasized, and the lack of basic knowledge on these topics is one of the primary reasons that there has been so little significant achievement in the effective prevention and control of resistance development. Most international, national, and local agencies recognize this serious problem. Many resolutions and recommendations have been propounded, and numerous reports have been written, but to no avail: the development of antibiotic resistance is relentless.

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**TITLE OF THE PROJECT:**

*PREVENTION ANTIBIOTIC RESISTANT BY AQUACULTURE*

*INTRODUCTION*

*The successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed. This is true for agents used in the treatment of bacterial, fungal, parasitic, and viral infections and for treatment of chronic diseases such as cancer and diabetes; it applies to ailments caused or suffered by any living organisms, including humans, animals, fish, plants, insects, etc. A wide range of biochemical and physiological mechanisms may be responsible for resistance. In the specific case of antimicrobial agents, the complexity of the processes that contribute to emergence and dissemination of resistance cannot be overemphasized, and the lack of basic knowledge on these topics is one of the primary reasons that there has been so little significant achievement in the effective prevention and control of resistance development. Most international, national, and local agencies recognize this serious problem. Many resolutions and recommendations have been propounded, and numerous reports have been written, but to no avail: the development of antibiotic resistance is relentless.*

*The most striking examples, and probably the most costly in terms of morbidity and mortality, concern bacteria. The discovery of these infectious agents in the late 19th century stimulated the search for appropriate preventative and therapeutic regimens; however, successful treatment came only with the discovery and introduction of antibiotics half a century later. Antibiotics have revolutionized medicine in many respects, and countless lives have been saved; their discovery was a turning point in human history. Regrettably, the use of these wonder drugs has been accompanied by the rapid appearance of resistant strains. Medical pundits are now warning of a return to the preantibiotic era; a recent database lists the existence of more than 20,000 potential resistance genes (r genes) of nearly 400 different types, predicted in the main from available bacterial genome sequences (*[*85*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r85)*). Fortunately, the number existing as functional resistance determinants in pathogens is much smaller.*

*Many excellent reviews describing the genetics and biochemistry of the origins, evolution, and mechanisms of antibiotic resistance have appeared over the last 60 years. Two of note in recent times are those of Levy and Marshall (*[*82*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r82)*) and White et al. (*[*149*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r149)*). The goal of this short article is not to summarize such a wealth of information but to review the situation as we see it now (most particularly with respect to the origins and evolution of resistance genes) and to provide some personal views on the future of antibiotic therapy of infectious diseases.*

*Antibiotic discovery, modes of action, and mechanisms of resistance have been productive research topics in academia (*[*27*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r27)*) and, until recently, in the pharmaceutical industry. As natural products, they provide challenging intellectual exercises and surprises with respect to their chemical nature, biosynthetic pathways, evolution, and biochemical mode of action (*[*26*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r26)*,*[*134*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r134)*). The total synthesis of such natural products in the laboratory is difficult, since these small molecules are often extremely complex in functionality and chirality (*[*98*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r98)*). The antibiotic penicillin was discovered in 1928, but the complete structure of this relatively simple molecule was not revealed until 1949, by the X-ray crystallographic studies of Dorothy Crowfoot Hodgkin (*[*73*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r73)*), and was confirmed by total synthesis in 1959 (*[*125*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r125)*). Studies of modes of action have provided biochemical information on ligands and targets throughout antibiotic history (*[*59*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r59)*,*[*147*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r147)*), and the use of antibiotics as “phenotypic mutants” has been a valuable approach in cell physiology studies (*[*142*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r142)*). The field of chemical biology/genetics grew from studies of those interactions. We have a meager understanding of how antibiotics work, and in only a few instances can the intimate interactions of the small molecule and its macromolecular receptor be interpreted in terms of defined phenotypes. More surprisingly, there is a paucity of knowledge of the natural biological functions of antibiotics, and the evolutionary and ecological aspects of their chemical and biological reactions remain topics of considerable interest and value (*[*3*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r3)*,*[*8*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r8)*).*

*To begin, the definition of “antibiotic,” as first proposed by Selman Waksman, the discoverer of streptomycin and a pioneer in screening of soils for the presence of biologicals, has been seriously overinterpreted; it is simply a description of a use, a laboratory effect, or an activity of a chemical compound (*[*146*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r146)*). It does not define a class of compound or its natural function, only its application. At the risk of attack from purist colleagues, the generic term “antibiotic” is used here to denote any class of organic molecule that inhibits or kills microbes by specific interactions with bacterial targets, without any consideration of the source of the particular compound or class. Thus, purely synthetic therapeutics are considered antibiotics; after all, they interact with receptors and provoke specific cell responses and biochemical mechanisms of cross-resistance in pathogens. The fluoroquinolones (FQs), sulfonamides, and trimethoprim are good examples.*

*As in any field of biological study, antibiotic history is replete with misconceptions, misinterpretations, erroneous predictions, and other mistakes that have occasionally led to the truth. This account aspires to focus on the truth. The discovery of antibiotics is rightly considered one of the most significant health-related events of modern times, and not only for its impact on the treatment of infectious diseases. Studies with these compounds have often shown unexpected nonantibiotic effects that indicate a variety of other biological activities; the result has been a significant number of additional therapeutic applications of “antibiotics” as antiviral, antitumor, or anticancer agents. In some cases, the alternative applications have surpassed those of antibiotic activity in importance, such as in the treatment of cardiovascular disease or use as immunosuppressive agents (*[*45*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2937522/#r45)*).*

*Unfortunately, the colossal need for these valuable drugs has had a significant environmental downside. In the 60 years since their introduction, millions of metric tons of antibiotics have been produced and employed for a wide variety of purposes. Improvements in production have provided increasingly less expensive compounds that encourage nonprescription and off-label uses. The cost of the oldest and most frequently used antibiotics is (probably) mainly in the packaging. The planet is saturated with these toxic agents, which has of course contributed significantly to the selection of resistant strains. The development of generations of antibiotic-resistant microbes and their distribution in microbial populations throughout the biosphere are the results of many years of unremitting selection pressure from human applications of antibiotics, via underuse, overuse, and misuse. This is not a natural process, but a man-made situation superimposed on nature; there is perhaps no better example of the Darwinian notions of selection and survival*

*Antibiotics represent one of the most successful forms of therapy in medicine. But the efficiency of antibiotics is compromised by the growing number of antibiotic-resistant pathogens. Antibiotic resistance, which is implicated in elevated morbidity and mortality rates as well as in the increased treatment costs, is considered to be one of the major global public health threats*

*Frontiers in Microbiology, section of Antimicrobials, Resistance, and Chemotherapy. The articles in the eBook update the reader on various aspects and mechanisms of antibiotic resistance. A better understanding of these mechanisms should facilitate the development of means to potentiate the efficacy and increase the lifespan of antibiotics while minimizing the emergence of antibiotic resistance among pathogens.*

*. Antibiotic-resistant bacterial strains remain a major global threat, despite the prevention, diagnosis and antibiotherapy, which have improved considerably. In this thematic issue, the scientists present their results of accomplished studies, in order to provide an updated overview of scientific information and also, to exchange views on new strategies for interventions in antibiotic-resistant bacterial strains cases and outbreaks. As a consequence, the recently developed techniques in this field will contribute to a considerable progress in medical research*.

***AIM OF THE OBJECTIVES***

The scientific and technological objectives of the project are to use a multidisciplinary approach involving clinical, pharmacological, genetic, bacteriological, molecular biological and epidemiological expertise to study the impact of different antibiotics in selecting resistance among pathogenic and commensal members of the indigenous microbiota of humans. This overall objective will be achieved by the following sub-objectives:

**Objective #1:** to identify and quantify those cultivable antibiotic-resistant bacteria that emerge during the administration of four different antibiotics to healthy volunteers

**Objective #2:** to investigate, using 454 pyrosequencing, the full complement of resistance determinants (the resistome) in the cultivable and not-yet-cultivable microbiota and the effect on this of antibiotic administration.

**Objective #3:** to ascertain the dynamics of resistance development and the persistence of antibiotic-resistant strains.

**Objective #4:** to compare the pattern of antibiotic resistance development induced by different classes of antibiotics.

**Objective #5:** to investigate the ecological impact of antibiotic administration on the cultivable indigenous microbiota.

**Objective #6:** to investigate, using 454 pyrosequencing, the ecological impact of antibiotic administration on the cultivable and not-yet-cultivable indigenous microbiota.

**Objective #7:** to characterise, using state-of-the-art microarrays, the antibiotic resistance determinants in the isolates obtained from the clinical studies.

**Objective #8:**to determine the mobility of the resistance genes detected in the isolates obtained from the clinical studies.

**Objective #9:** to ascertain the biological cost of antibiotic resistance in a number of clinically-important organisms isolated during the clinical studies.

**Objective #10:**To disseminate the project findings to the clinical and scientific communities and to the general public and to ensure access to the ANTIRESDEV databases to enable future studies by other groups.

**Objective #11:**To use the ANTIRESDEV findings to inform health care decision makers of some of the factors influencing the emergence and persistence of antibiotic-resistant bacteria following the administration of particular antibiotics and thereby provide opportunities for them to implement appropriate policies concerning antibiotic use.

 Improve awareness and understanding of antimicrobial resistance through effective communication, education and training

* to develop an understanding of the structure of bacteria, viruses, protozoa and worms and how these interact with our immune response and on prevention and treatment of infection.
* to understand how the structural differences between bacterial and human cells provide targets for antibiotic action.
* to understand the conditions under which bacteria develop antibiotic resistance and how that resistance may be transferred; similarly how resistance has occurred to chloroquine used as a prophylactic against malaria.
* to understand the differences in structure between Gram positive and Gram negative bacteria and how this affects pathogenesis, immunity and antibiotic antibiotic action.
* to understand how parasites have evolved into a parasitic way of life from free living ancestors.
* to develop an understanding of how infectious units may be transmitted between individuals and colonise the body
* to understand the similarities and differences in how the immune defence system deals with infections;
* to understand molecular mechanisms by which pathogens may evade host defences and produce symptomatic disease;
* to understand how normal immune responses may be augmented for vaccine development and prevention of infection.

 *Methology*

*The occurrence of antibiotic-resistant bacteria is an increasingly serious problem world-wide. In addition, to phenotypically resistant bacteria, a threat may also be posed by isolates with silent, but intact, antibiotic resistance genes. Such isolates, which have recently been described, possess wild-type genes that are not expressed, but may convert to resistance by activating expression of the silent genes. They may therefore compromise the efficacy of antimicrobial treatment, particularly if their presence has not been diagnosed. This chapter describes the detection of silent resistance genes by PCR and DNA sequencing. A method to detect five potentially silent acquired resistance genes; aadA, bla (OXA-2), strAB, sul1, and tet(A) is described. First, the susceptibility of the isolates to the relevant antibiotics is determined by an appropriate susceptibility testing method, such as E-test. Then the presence of the genes is investigated by PCR followed by agarose gel electrophoresis of the amplification products. If a resistance gene is detected in a susceptible isolate, the entire open-reading frame and promoter sequence of the gene is amplified by PCR and their DNA sequences obtained. The DNA sequences are then compared to those of known resistant isolates, to detect mutations that may account for susceptibility. If no mutations are detected the expression of the gene is investigated by RT-PCR following RNA extraction. The methods described here can be applied to all acquired resistance genes for which sequence and normal expression data are available*.

*There are several antimicrobial susceptibility testing methods available today, and each one has their respective advantages and disadvantages. They all have one and the same goal, which is to provide a reliable prediction of whether an infection caused by  a bacterial isolate will respond therapeutically to a particular antibiotict reatment. This data may be utilized as guidelines for chemotherapy, or at the population level as indicators of emergence and spread of resistance based on passive or active surveillance.  Some examples of antibiotic sensitivity tesing methods are:*

* *Dilution method (broth and agar dilution method)*
* *Disk-diffusion method*
* *E-test*
* *Automated methods*
* *Mechanism-specific tests such as beta-lactamase detection test and chromogenic cephalosporin test*
* *Genotypic methods such as PCR and DNA hybridization methods*

 *Selection of  the appropriate method will depend on the intended degree of accuracy, convenience, urgency, availability of resources, availability of technical expertise and cost..    Interpretation should be based on veterinary standards whenever possible, rather than on human medical standards, which may not always be applicable.   Among these available tests, the two most commonly used methods in veterinary laboratories are the agar disk-diffusion method and the broth microdilution method.*

*At a time of rising concern about drug resistance and falling output of new antibacterial compounds, antibiotic research has once again returned to the forefront of medical science. In Antibiotic Resistance: Methods and Protocols, Stephen Gillespie and a panel of leading clinical and diagnostic microbiologists describe a series of detailed molecular and physical methods designed to study the growing problem of antibiotic resistance, as well as facilitate new antibiotic research programs for its effective redress. The techniques range widely from those that provide rapid diagnosis via DNA amplification and phage display, to those for plotting the transmission of resistant organisms and investigating their epidemiology. The methods are readily adaptable to a wide range of resistant bacterial organisms. In order to ensure successful results, each method is described in minute detail and includes tips on avoiding pitfalls.
Practical and wide-ranging, Antibiotic Resistance: Methods and Protocols provides a collection of indispensable techniques not only for illuminating the basic biology of antimicrobial resistance, but also for developing and implementing new diagnostic and epidemiological tools.*nfections need to be treated quickly to keep them from spreading, especially in critical cases such as blood infections. But bacteria are constantly mutating and achieving resistance to antibiotics, making it harder for medical personnel to respond in time with the right drugs.

*Empirical therapy is used when there isn’t enough time to analyse a specific bacteria for its susceptibility to antibiotics. This means probable antibiotics resistance is assessed on the basis of the population the patient belongs to – for instance a country's general public.*

*But there is always a risk that doctors are betting on the wrong horse. The bacteria behind an infection turns out to be resistant to the drugs. The next tactic is to treat the patient with broad spectrum antibiotics. But these are less effective than targeted antibiotics and their use can contribute to antibiotic resistance.*

***Resistance analysis within four hours***

*A group of Swedish researchers working at Uppsala University, the SciLifeLab in Stockholm and Uppsala University Hospital have discovered a way to quickly identify a bacterial species and its susceptibility profile to antibiotics.*

*“We’ve developed a new method which enables us to determine the species and the resistance pattern of bacteria causing urinary tract infections in less than four hours. By comparison, the present method takes one to two days,” states Professor Dan I. Andersson of Uppsala University in a press release.*

***Bacteria growth indicates susceptibility***

*The method utilises the fact that bacteria signal whether they are resistant when they are in the vicinity antibiotics. If they are resistant to a given type of antibiotics they continue to grow normally. If they are vulnerable they stop growing. Prospective growth is seen by an increase in a number of copies of a special DNA sequence.*

*The method has been shown to identify the correct bacteria species and its resistance pattern in all the tests that were analysed.*

*“We used the method to determine the antibiotic susceptibility profile of E. coli bacteria for two medications, with 100 percent precision and within just three-and-a-half hours,” write the researchers in the study.*

*Instruments are on the market which can test bacterial vulnerability to antibiotics in less than five hours. But the technique used by these is only 82.3 percent accurate, the Swedish scientists point out.*

***Can be used on all bacteria types***

*“The new method is general and in principle should be usable for all types of bacteria and antibiotics,” says Anja Mezger at SciLifeLab.*

*However, some modifications will be needed to make the method effective for a wider array of bacteria and infection types, conclude the researchers. For instance it needs to be adapted to a longer incubation period for slow-growing bacteria and longer cultivation times for slow-working antibiotics.*

*The scientists hope the method can be made automatic in the future for clinical use in hospitals and emergency wards.* the development of a rapid susceptibility test for Staphylococcus aureus,*a bacterium that causes some 60 percent of hospital-acquired infections and which has spread in communities, causing pneumonia and a variety of skin and tissue infections in both healthy and immune-compromised individuals.*

*The development is important, say biomedical scientists, because of the critical need for physicians to rapidly discriminate between drug resistant strains (commonly termed MRSA for methicillin-resistant* S. aureus*) and drug sensitive strains, since these infections can progress rapidly, especially MRSA strains with additional resistance to newer antibiotics designed to treat pathogens that are now appearing in hospitals.*

*According to the Centers for Disease Control and Prevention, antibiotic resistance causes two million illnesses and 23,000 deaths annually, costing the U.S. economy approximately $20-billion a year in direct health care costs and nearly eight million extra days in the hospital. Indeed, bacteria are evolving resistance to antibiotics much more quickly than global biomedical research efforts are delivering new drugs to market, leading to the appearance of infections caused by bacteria that are now resistant to every therapy.*

*Rapid antimicrobial susceptibility testing allows doctors to discriminate between infections caused by drug sensitive bacteria, which can be treated with safe and effective antibiotics developed in what scientists call the golden age of drug discovery (the mid-20th century) such as penicillin, and those caused by drug resistant bacteria, which might require newer antibiotics, such as daptomycin or cubicin. This approach will decrease the emergence of resistance by reserving the newest drugs for those infections where they are most needed.*

*The interdisciplinary team at UC San Diego was comprised of two infectious-disease specialists in the School of Medicine, Victor Nizet, MD, and George Sakoulas, MD; two biologists in the Division of Biological Sciences, Kit and Joe Pogliano; and Diana Quach, a bioengineering graduate student. The scientists applied a method previously developed in the Pogliano laboratories for drug discovery to antibiotic susceptibility testing.*

*“Previously we developed a microscopy-based method that performs an autopsy on bacterial cells that allows us to determine how each cell died, and we have shown that this method can identify new antibiotics and help understand how these antibiotics work,” said Kit Pogliano, a professor of biology at UC San Diego who headed the research team. “We tested to see if this method could be applied to antibiotic susceptibility testing. Surprisingly, we not only found that our method was able to accurately differentiate sensitive* S. aureus *strains from resistant MRSA strains, but that we were able to identify two subgroups of MRSA strains, one of which is susceptible to combinations of antibiotics that could be used in the hospital. We are excited by the accuracy and speed of this test, as well as by its unanticipated ability to identify these two types of MRSA infections, which would have been missed by other tests.”*

*Examining single cells has two key advantages over other testing methods, say the researchers. First, it is rapid, cutting days off the time for typical culture-based assays. It often takes days for a doctor to receive information on resistance, and this means that patients with life-threatening infections are often treated with the assumption that the infection is drug-resistant. Second, this method does not rely on having any detailed understanding of the bacterium causing the infection, or of the genes that convey resistance. This is particularly important in this case, since resistance to the drugs used to treat MRSA infections arise by several evolutionary pathways via different combinations of mutations, and it could also provide rapid treatment information for newly emerging bacterial pathogens, such as that which caused the infections transmitted by endoscopes.*

*“Regardless of the type of bacterium, a healthy and growing bacterium looks different from a dead bacterium, so whenever we detect a difference in how the cells look, we know that the bacterium is sensitive to the antibiotic we have applied. When we combine careful culture conditions, cutting edge imaging methods and a detailed quantitative analysis, we can turn this simple approach into a reliable test,” said Joe Pogliano, a professor of biology.*

*“Rapid and precise identification of antibiotic sensitivity patterns allows the most potent and effective drug to be administered,” said Nizet, a professor of pediatrics and pharmacy. "Equally important, more specific antibiotic therapy can help preserve the normal bacteria living in our gut microbiome that play an important role in our health and immune system function.”*

*The UC San Diego biologists say their new method has the potential to be applied to many different types of bacteria. “Our new method worked surprising well at rapidly detecting antibiotic resistant strains of*S. aureus*,” said Diana Quach, a graduate student and lead author on the study. “We are now optimizing it to provide a more accurate test for other types of antibiotic resistant bacteria, such as*Pseudomonas aeruginosa. ***Bacterial mechanisms of antibiotic resistance*** *Several mechanisms have evolved in bacteria which confer them with antibiotic resistance. These mechanisms can either chemically modify the antibiotic,  render it inactive through physical removal from the cell, or modify target site so that it is not recognized by the antibiotic.

The most common mode is enzymatic inactivation of the antibiotic. An existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganism. An alternative strategy utilized by many bacteria is the alteration of the antibiotic target site. These and other mechanisms are shown in the the figure and accompanying table below.*

*Three mechanisms of antibiotic resistance in bacteria. Most, but not all, resistance mechanisms are encoded by plasmids, which are potentially transmissible to other bacteria. Clockwise. 12 o'clock: Efflux pumps are high-affinity reverse transport systems located in the membrane that transport the antibiotic out of the cell. This is the mechanism of resistance to tetracycline. 4 o'clock: A specific enzyme modifies the antibiotic in a way that it loses its activity. In the case of streptomycin, the antibiotic is chemically modified so that it will no longer bind to the ribosome to block protein synthesis. 9 o'clock: An enzyme is produced that degrades the antibiotic, thereby inactivating it. For example, the penicillinases are a group of beta-lactamase enzymes that cleave the beta lactam ring of the penicillin molecule.*

|  |  |  |
| --- | --- | --- |
| **Antibiotic** | **Method of resistance** |  |
|  |  |  |
|  | Chloramphenicol | reduced uptake into cell |  |
|  | Tetracycline | active efflux from the cell |  |
|  | β-lactams, Erythromycin, Lincomycin | eliminates or reduces binding of antibiotic to cell target |  |
|  | β-lactams, Aminoglycosides, Chloramphenicol | enzymatic cleavage or modification to inactivate antibiotic molecule |  |
|  | Sulfonamides, Trimethoprim | metabolic bypass of inhibited reaction |  |
|  | Sulfonamides, Trimethoprim | overproduction of antibiotic target (titration) |  |

**The acquisition and spread of antibiotic resistance in bacteria**

The development of resistance is inevitable following the introduction of a new antibiotic. Initial rates of resistance to new drugs are normally on the order of 1%. However, modern uses of antibiotics have caused a huge increase in the number of resistant bacteria. In fact, within 8-12 years after wide-spread use, strains resistant to multiple drugs become widespread. Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment.

Antibiotic resistance in bacteria may be an inherent trait of the organism (e.g. a particular type of cell wall structure) that renders it **naturally resistant**, or it may be **acquired** by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source.

**Inherent (natural) resistance**. Bacteria may be inherently resistant to an antibiotic. For example, an organism lacks a transport system for an antibiotic; or an organism lacks the target of the antibiotic molecule; or, as in the case of Gram-negative bacteria, the cell wall is covered with an outer membrane that establishes a permeability barrier against the antibiotic.

**Acquired resistance**. Several mechanisms are developed by bacteria in order to acquire resistance to antibiotics. All require either the modification of existing genetic material or the acquisition of new genetic material from another source.

**Vertical gene transfer**
The spontaneous mutation frequency for antibiotic resistance is on the order of about 10-8- 10-9. This means that one in every 108- 109  bacteria in an infection will develop resistance through the process of mutation. In *E. coli*, it has been estimated that streptomycin resistance is acquired at a rate of approximately 10-9 when exposed to high concentrations of streptomycin. Although mutation is a very rare event, the very fast growth rate of bacteria and the absolute number of cells attained means that it doesn't take long before resistance is developed in a population.

Once the resistance genes have developed, they are transferred directly to all the bacteria's progeny during DNA replication. This is known as **vertical gene transfer**or **vertical evolution**. The process is strictly a matter of Darwinian evolution driven by principles of natural selection: a spontaneous mutation in the bacterial chromosome imparts resistance to a member of the bacterial population. In the selective environment of the antibiotic, the wild type (non mutants) are killed and the resistant mutant is allowed to grow and flourish

**Horizontal gene transfer**
Another mechanism beyond spontaneous mutation is responsible for the acquisition of antibiotic resistance. Lateral or **horizontal gene transfer** (HGT) is a process whereby genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species.

There are at least three possible mechanisms of HGT, equivalent to the three processes of genetic exchange in bacteria. These are transduction, transformation or conjugation.

Conjugation occurs when there is direct cell-cell contact between two bacteria (which need not be closely related) and transfer of small pieces of DNA called plasmids takes place. This is thought to be the main mechanism of HGT.

Transformation is a process where parts of DNA are taken up by the bacteria from the external environment. This DNA is normally present in the external environment due to the death and lysis of another bacterium.

Transduction occurs when bacteria-specific viruses (bacteriophages) transfer DNA between two closely related bacteria.

*rofessor Andersson continues: ‘This is just what we’ve been working on in our stud. We have developed a new method that permits identification of both the species and the resistance pattern of bacteria in urinary infections in less than four hours. By comparison, the resistance determination done at present takes one to two days.’*

*The method is based on highly sensitive, bacterium-specific measurement of bacterial growth in the absence and presence of various antibiotics. If the bacterium is resistant, it can multiply with antibiotic present; this is detected as a rise in the number of copies of a specific DNA sequence. If it is sensitive, on the other hand, no growth takes place. The researchers showed that the method could identify correctly both the bacteria and their resistance patterns in all the clinical samples analysed.*

*Anja Mezger, the principal author, says that the method is highly specific and sensitive, and can be automated for use in a clinical laboratory. What is more, it is entirely general in application and could, in principle, be used for all types of bacteria and antibiotics.*

*An instrument based on the method is currently being developed at Q-linea, a company in Uppsala of which Mats Nilsson was a co-founder. This instrument focuses on blood infections. Such infections are life-threatening and it is extremely important for effective treatment that the patient should start taking the correct antibiotic without delay. The company expects to launch a working instrument on the market in 2017.*

*‘We hope that the method can be used in the future at hospitals and health centres, so that the right treatment is given promptly, and also so that the use of antibiotics is reduced,’ says Dan Andersson.*

*The study was funded by the Swedish Governmental Agency for Innovation Systems (Vinnova) and the Swedish Research Council.*

***Result and observations***

*Though there is undoubtedly a significant human contribution to the evolution of bacterial resistance, there is also resistance that has occurred in nature absent human interference.*[*43*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b43-pmc-6-2014-025)*Resistances to first in class antibiotics such as penicillin G (****4****) and streptomycin (****24****), discovered during the golden age of antibiotics, were observed shortly after their initial isolation.*[*44*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b44-pmc-6-2014-025)*Though this is not always the case, this phenomenon is typical when examining the antibiotic arsenal as a whole.*[*42*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b42-pmc-6-2014-025)*With the advent of cloning and sequencing it was possible to trace β-lactamases to a large number of homologous, but distinct genes that were transferred vertically and horizontally throughout many microbial communities, directly between bacteria and indirectly mediated by the many bacteriophages that infect them.*[*45*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b45-pmc-6-2014-025)*Resistance genes can associate in clusters and be transferred together as well.*[*46*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b46-pmc-6-2014-025)*This kind of genetic diversity couldn’t have arisen in the time frame since penicillin’s discovery and indeed phylogenetic analysis suggested a more ancient root evolution of these enzymes.*[*47*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b47-pmc-6-2014-025)

*Resistance elements have even been found in bacterial DNA that was isolated for 30,000 years in permafrost.*[*48*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b48-pmc-6-2014-025)*Estimates based on the genetic divergence of antibiotic biosynthetic genes have suggested that some antibiotics could have evolved hundreds of millions of years ago.*[*49*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b49-pmc-6-2014-025)*Taken together this evidence suggests that bacteria have likely had a very long time to evolve resistance to many, if not all, natural product antibiotics, and therefore, resistance is highly likely to exist long before their discovery by man. Most soil bacteria exhibit some form of antibiotic resistance and many of them exhibit many resistance mechanisms even to antibiotics that they do not naturally produce.*[*50*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b50-pmc-6-2014-025)*It could be argued that these samples could be contaminated in a variety of ways including antibiotic runoff. However, this evidence is also supported by a number of studies that have found antibiotic resistant (in some cases highly resistant), commensal bacteria on both humans and animals from remote locales that have never been exposed to antibiotics through unnatural means.*[*51*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b51-pmc-6-2014-025)*Evolution of bacterial resistance to antibiotics is therefore a natural process and would exist even absent human mismanagement.*

*Human use (and misuse) of antibiotics has clearly put unnatural selective pressure on bacteria, which has accelerated their evolutionary process to the detriment of everyone. To address this problem, faster development of new antibiotics and more responsible use of currently antibiotics are clearly necessary* The use of antibiotics in animal feed stocks has also exacerbated the spread of resistance. Especially egregious is their use for non-curative reasons such as prophylaxis, metaphylaxis, and growth promotion which by one estimate accounted for 25–50% of all antibiotic consumption in the early 2000s.[25](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b25-pmc-6-2014-025) Other assessments within the US during the same time period estimated agricultural use to be much greater at 24.6 million pounds of antibiotics being given to animals for non-therapeutic purposes, 2 million pounds being used therapeutically on animals, and 3 million pounds being used in humans per year.[33](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b33-pmc-6-2014-025) Antibiotic use for growth promotion has been banned in the European Union (EU) since 2003[34](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b34-pmc-6-2014-025) and finally in 2012 the FDA banned the use of antibiotics in livestock without a veterinary prescription.[35](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b35-pmc-6-2014-025) There are still many countries where this practice remains unlegislated, however.

There is strong evidence that the use fluoroquinolones in food animals has led to the emergence of fluroquinolone resistant E. coli,[36](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b36-pmc-6-2014-025),[37](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b37-pmc-6-2014-025) Salmonella, and Campylobacter.[38](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b38-pmc-6-2014-025) The emergence of vancomycin resistant Enterococci (VRE) in Europe was tied to the use of the glycopeptide avoparcin in food animals.[39](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b39-pmc-6-2014-025)Avoparcin was banned in the EU in 1997, which resulted in a reduction in VRE there,[40](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b40-pmc-6-2014-025) but many members of critical antibiotic classes are still used for veterinary purposes. In a survey by the European Medicines Agency there was actually an increase in veterinary sales of fluoroquinolones and fourth generation cephalosporins from 2005 to 2009.[41](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b41-pmc-6-2014-025) The food industry’s use of antibiotics has not been strictly limited to livestock either. In the US, in 1996 for example, 300,000 pounds of the aminoglycoside streptomycin (**24**), and oxytetracyline were sprayed prophylactically on apples and pears.[42](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b42-pmc-6-2014-025) Waste runoff containing resistant bacteria or antibiotics from large corporate farms or agro-industrial plants is also a concern.[23](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b23-pmc-6-2014-025) This serves as a mobile means of exposure to antibiotics and the terrestrial locale provides an ideal environment for dissemination of resistance elements from pathogenic bacteria and potentially from soil bacteria as well.[23](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b23-pmc-6-2014-025)

Dangerous, antibiotic resistant bacteria have been observed with increasing frequency over the past several decades. In this review the factors that have been linked to this phenomenon are addressed. Profiles of bacterial species that are deemed to be particularly concerning at the present time are illustrated. Factors including economic impact, intrinsic and acquired drug resistance, morbidity and mortality rates, and means of infection are taken into account. Synchronously with the waxing of bacterial resistance there has been waning antibiotic development. The approaches that scientists are employing in the pursuit of new antibacterial agents are briefly described. The standings of established antibiotic classes as well as potentially emerging classes are assessed with an emphasis on molecules that have been clinically approved or are in advanced stages of development. Historical perspectives, mechanisms of action and resistance, spectrum of activity, and preeminent members of each class are discussed.

The number of new antibacterial agents has decreased steadily in the United States over the last several decades.[8](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b8-pmc-6-2014-025),[146](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b146-pmc-6-2014-025) Historically there has been a higher chance of success with the development of compounds that belong to already established antibiotic classes.[147](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b147-pmc-6-2014-025) Developmental risks are lower because of already proven microbiological assays to determine efficacy, known toxicological issues, and established regulatory routes for approval.[148](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b148-pmc-6-2014-025) Some scaffolds have been used particularly extensively. Between 1981 and 2005 cephalosporins, penicillins, quinolones, and macrolides accounted for 73% of all new antibiotics.[149](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b149-pmc-6-2014-025)

There is also a lack of diversity in the cellular target of all known antibiotics. Almost all clinically used antibiotics inhibit DNA, RNA, protein, or cell wall synthesis, and there are less than twenty-five molecular targets that account for their activity. Approximately half of all antibiotics target the cell wall.[150](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b150-pmc-6-2014-025) In some cases structurally distinct antibiotics, even from separate gene clusters, are known to bind the same sites.[151](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b151-pmc-6-2014-025),[152](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b152-pmc-6-2014-025) Comparative analysis of bacterial genomes has indicated that there are around 300 essential, highly conserved proteins that could potentially be new, broad spectrum drug targets.[153](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b153-pmc-6-2014-025)–[157](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b157-pmc-6-2014-025) Though studies have recently begun to identify many antibacterial agents with novel molecular targets, activity is insufficient for many of these to be developed without further modification.

The development of new antibiotics in existing classes is an absolutely essential exercise that has been encouraged even by the IDSA, a principal entity in the push for new scaffold development.[11](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b11-pmc-6-2014-025) However, new antibiotics that conform to established classes are often subject to at least some of the same resistances observed in previous members of the class. It is therefore also necessary to develop new antibiotic classes. There have only been six first in class antibiotics with totally novel scaffolds approved since the 1960s and all of these have been introduced in the past fifteen years, a thirty year innovation gap ([Fig. 2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/figure/f2-pmc-6-2014-025/)). It is worth noting that all of these were developed to combat gram-positive pathogens including M. tuberculosis and they all have very little or no activity against gram-negatives. The innovation gap remains for novel antibiotics with potent gram-negative activity.

Given the length of time bacteria as a whole have likely had to develop resistance to many natural product antibiotics coupled with the apparent ease with which many resistance genes are disseminated, developing totally synthetic antibiotics would appear to be an attractive strategy. To date, however, synthetic antibiotics are still extremely rare with the sulfa drugs, quinolones, oxazolidinones, and diarylquinolines being the only examples. They are outnumbered two to one by natural product antibiotics and their semi-synthetic derivatives,[42](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b42-pmc-6-2014-025),[160](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b160-pmc-6-2014-025) with development over the past few decades focused especially on the latter.[149](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b149-pmc-6-2014-025)Historically all synthetic classes, with the exception of diarylquinolines, were originally discovered outside of traditional antibiotic discovery programs. Sulfa drugs were originally developed as dyes, the first quinolone was an intermediate in the synthesis of chloroquine, a malaria drug, and oxazolidinones were originally developed to treat foliage diseases in plants.[62](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b62-pmc-6-2014-025)

Without a doubt one of the greatest challenges to finding new synthetic scaffolds is the issue of bacterial cell penetration. This is particularly true of gram-negatives, which are naturally resistant to many antibiotics because of outer membranes that keep many amphipathic drugs out as well as inner membranes and highly active efflux pumps that often recognize highly hydrophilic molecules.[161](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b161-pmc-6-2014-025),[162](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b162-pmc-6-2014-025) The difficulties of prokaryotic uptake often mean that antibiotics have to be administered at concentrations two to three orders of magnitude higher than therapeutics prescribed for most other diseases. This can impact the therapeutic window and lead to additional toxicity concerns.[163](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b163-pmc-6-2014-025)

The majority of antibiotics do not strictly adhere to the Lipinski rules, a series of soft rules governing the likelihood of a compound’s oral bioavailability and “drug like” character. In fact, several major antibiotic classes routinely break all of them. Notably, these rules were designed in the context of treating eukaryotic maladies. The establishment of a similar set of rules for antibacterials would greatly aid in antibiotics rational design and in the formation of compound libraries better suited for antibiotic screening purposes. There are no rules that have been routinely applied as of yet; however, some insights have started to be noted. Relatively hydrophilic compounds with masses below 600 Da tend to have good penetrance probably because of their ability to pass through outer membrane porins.[164](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b164-pmc-6-2014-025),[165](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b165-pmc-6-2014-025) MDR efflux pumps tend to recognize cations and hydrophobic compounds particularly well, whereas anions are generally not recognized.[166](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b166-pmc-6-2014-025)–[168](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b168-pmc-6-2014-025) The inclusion of atoms not usually found in nature like boron and fluorine have had successes, possibly again because of efflux pump evasion.[169](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b169-pmc-6-2014-025) Fluoroquinlones, at this point probably the most successful fully synthetic antibiotics, adhere to all of these observations.

Screening for synthetic leads has not conventionally been a successful method of discovery. Major high throughput synthetic screens and rational design campaigns of synthetic molecules have failed utterly in many cases to identify a single antibiotic.[170](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b170-pmc-6-2014-025) The wide spread failure of cell free target based screens in particular, which were an industry standard, has been implicated as one of the primary reasons for many major pharmaceutical companies movement away from antibiotics development.[170](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b170-pmc-6-2014-025)–[171](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b171-pmc-6-2014-025) Most scaffolds found at major pharmaceutical companies are optimized more for human eukaryotic targets and are not up to the disparate challenges associated with prokaryotic cellular uptake and evasion of rampant bacterial efflux mechanisms, particularly in gram-negative bacteria. Therefore target based screens of synthetic molecules will often lead to hits with high potency, but no real world utility.[43](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b43-pmc-6-2014-025),[62](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b62-pmc-6-2014-025) This is a drawback that rational design of synthetic molecules suffers from as well.[163](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b163-pmc-6-2014-025) Whole cell screens do not suffer from this disadvantage though.

Taking note of fact that all current synthetic antibiotics were originally discovered for other purposes, whole cell screens of libraries originally created by non-antibiotics programs have been done recently. These have been used to identify some promising new leads.[172](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b172-pmc-6-2014-025),[173](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b173-pmc-6-2014-025) Many hits on whole cell screens may exhibit narrow or even genus specific activity, as in the case of bedaquiline (**84**) though, which notably is the only synthetic, clinically approved antibiotic to our knowledge that was discovered by high throughput screens actually designed to identify antibiotics.[163](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b163-pmc-6-2014-025) For some particularly hard to treat pathogens this may be acceptable at this point though.

Taking whole cell screens a step further, in vivo screens have also gained some interest with the rationalization that metabolically activated prodrugs, like sulfonamides, may be overlooked in traditional in vitro screens. Using animal models would of course be prohibitive in any large screening process for obvious reasons. However, Caenorhabditis elegans, a nematode, can be infected with human pathogens to making it a passable model organism for in vivo screens. Screens using C. elegans have had hits, including some that have no in vitro activity.[174](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b174-pmc-6-2014-025),[175](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b175-pmc-6-2014-025)

Diversity oriented synthesis based approaches have been used to create promising totally synthetic molecules that more closely mimic microbial natural products.[176](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b176-pmc-6-2014-025) Combinatorial chemistry can be done to create libraries around known privileged scaffolds. Another approach is to do unbiased diversity oriented synthesis. This approach, coupled with subsequent SAR, has been used to find promising antibiotic candidates.[177](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b177-pmc-6-2014-025),[178](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b178-pmc-6-2014-025)

Natural products are a historically successful and still a very much viable option as new antibiotics. During the “golden age” of antibiotics many of the current antibiotic classes were discovered by systematic screening methods of Streptomyces introduced by Selman Waksman in the 1940s. There is reason to believe that many natural products are still as yet undiscovered. One recent estimate puts the number of discovered antibiotics as only 10% of the total from screened bacterial strains and only 1% from all microbes.[179](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b179-pmc-6-2014-025) Approximately two thirds of natural product antibiotics are isolated from terrestrial soil actinomycetes.[180](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b180-pmc-6-2014-025),[181](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b181-pmc-6-2014-025) Multiple classes of antibiotics are even known to be encountered within the same gene clusters.[182](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b182-pmc-6-2014-025) Finding a useable antibiotic in the milieu of compounds produced by these organisms is no easy feat though, especially given that the most commonly produced antibacterial molecules for these particular bacteria have all likely been already identified. It was recently estimated that with current technology 10[7](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b7-pmc-6-2014-025) actinomycete strains would have to be screened to discover the next novel antibiotics class.[49](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b49-pmc-6-2014-025) Given that a strain collection at a large pharmaceutical company may be around 50,000 isolates, this is no longer a feasible approach.[163](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b163-pmc-6-2014-025)

Exploration of bacteria from other ecological niches has recently yielded many promising new lead compounds, however. The producers of these include deep sea sediment actinomycetes,[183](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b183-pmc-6-2014-025) marine sponges and seaweeds (though these seem to actually be made by colonizing bacteria),[184](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b184-pmc-6-2014-025),[185](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b185-pmc-6-2014-025) bacterial symbionts of insects, ascidians, fungi,[186](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b186-pmc-6-2014-025)–[189](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b189-pmc-6-2014-025) and myxobacteria.[190](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b190-pmc-6-2014-025)

With the colossal advances in gene sequencing technology within last several decades, genomic prospecting has also begun. Genomic sequencing has in several cases identified silent operons that code for secondary metabolites within Streptomyces, some of which are not currently known to produce antimicrobial compounds.[191](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b191-pmc-6-2014-025),[192](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b192-pmc-6-2014-025) The proper conditions for realizing expression of these potential antibiotics in cultures can be difficult as antibiotic production may depend on a variety of factors including proper concentration of quorum sensing factors, which may be difficult to replicate.[193](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b193-pmc-6-2014-025)–[195](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b195-pmc-6-2014-025) Methods of manipulating these silent operons represent an active area of research. These approaches have thus far never realized more than the identification of several lead compounds per year though.[192](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b192-pmc-6-2014-025),[196](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b196-pmc-6-2014-025)–[199](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b199-pmc-6-2014-025)

Natural products screens have been touted over synthetic molecule screens both for the obviously superior number of compounds available and the fact that natural products have already been “prescreened” by evolution.[163](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b163-pmc-6-2014-025) It has traditionally been an often used approach, with whole cell empirical screening being the method of discovery of the majority of antibiotics used today.[171](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b171-pmc-6-2014-025) Whole cell screening does not aid in identification of mode of action, however, and this approach can be expensive. It is made even more so in the realm of natural products screening, particularly for antibiotics, as many antibiotic producing bacteria are difficult to culture (an estimated 99% of microbial species are uncultured).[200](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b200-pmc-6-2014-025),[201](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b201-pmc-6-2014-025)

Even more importantly, in current screens, many hits are actually previously discovered compounds. This is because of the pervasiveness of lateral transfer of antibiotic producing genes amongst terrestrial soil bacteria. One study estimated that 1 in 100 actinomycetes produce streptomycin (**24**), 1 in 250 tetracycline (**39**), 1 in 66,000 vancomycin (**52**), and 1 in 200,000 erythromycin (**31**).[49](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b49-pmc-6-2014-025) This phenomenon is not strictly limited to actinomycetes either.[202](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b202-pmc-6-2014-025)–[206](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b206-pmc-6-2014-025) Some members of the same antibiotic subclass can even be produced by extremely disparate organisms. Cephalosporins, for example, are produced by actinomycetes, proteobacteria, and fungi.[207](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b207-pmc-6-2014-025),[208](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b208-pmc-6-2014-025) Several methods have been developed to alleviate the problem of rediscovery. One strategy is to use strains resistant to commonly “rediscovered” antibiotics in the screening process. This has been done with wild type MRSA and with MDR E. faecium, which led to the discovery of many new promising compounds.[209](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b209-pmc-6-2014-025),[210](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b210-pmc-6-2014-025)

Target based natural products screens offer a useful counterpoint to whole cell screening. Target based screens do not suffer from all the drawbacks that these screens have when applied to synthetic molecules. Recently, several bioinformatics based, genome screening approaches have been used with some success.[211](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b211-pmc-6-2014-025)–[213](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b213-pmc-6-2014-025) It was previously mentioned that through genomic screening it has been estimated that there are hundreds of potential broad spectrum targets that no antibiotics have ever been developed for. Screens developed for these targets have the advantage that there are no false positives caused by already discovered antibiotics. Also, drugs developed for these targets may have less initial bacterial resistance than targets that already have selective pressure from many current antibiotics.

Hybrids of whole cell and cell free target based assays now exist as well. Whole cell reporter assays or whole cell target-based assays employ either cells or conditions that are engineered to report specific molecular events at sub-bactericidal concentrations, unlike traditional whole cell screens that simply look at cell death upon introduction of a compound or isolate. Antisense technology has also been used successfully to identify promising natural products. One particularly interesting discovery made using these techniques was platensimycin and other potent fatty acid biosynthesis inhibitors

Resistance usually occurs via hydrolysis of the β-lactam ring mediated by a wide range of β-lactamases. These enzymes have been divided into four classes by the Ambler classification system: class A (KPCs and most ESBLs), class B (MBLs), class C (AmpC β-lactamases), and class D (OXAs). Class A includes many enzymes that can hydrolyze penicillins and cephalosporins as well as some that can hydrolyze monobactams and KPCs that are capable of hydrolyzing carbapenems.[221](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b221-pmc-6-2014-025) The ESBLs from this class are plasmid mediated which has aided in their intra- and interspecies diffusion.[222](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b222-pmc-6-2014-025) MBLs use divalent cations such as zinc as cofactors. Many are encoded in class 1 integrons, typically on gene cassettes also coding for aminoglycoside modifying enzymes (AMEs), found on transposons facilitating their spread.[223](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b223-pmc-6-2014-025) MBLs inactivate many β-lactams including carbapenems and there are no currently improved inhibitors for them, but they have no activity against aztreonam (**18**), a monobactam.[224](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b224-pmc-6-2014-025) AmpC β-lactamases are typically chromosomally encoded. AmpC and other class C β-lactamases can inactivate many β-lactams including aztreonam (**18**) with preferential activity against cephalosporins, but they have no activity against carbapenems.[221](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b221-pmc-6-2014-025),[225](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b225-pmc-6-2014-025) Many OXAs are encoded on integrons.[226](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b226-pmc-6-2014-025)–[229](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b229-pmc-6-2014-025) Class D which is solely comprised of OXAs can hydrolyze cephalosporins and aztreonam (**18**) and some have carbapenemase activity as well.[221](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b221-pmc-6-2014-025),[230](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b230-pmc-6-2014-025) Though their activity isn’t as great as MBLs they are the most commonly found β-lactamase in Acinetobacter, which makes them particularly problematic.[222](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b222-pmc-6-2014-025)

Altered PBPs, especially in Streptococci, also occur.[231](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b231-pmc-6-2014-025) Methicillin (**5**) and other β-lactam resistances in MRSA are caused by production of low affinity PBP2a in greater than 90% of isolates.[232](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b232-pmc-6-2014-025) Likewise in S. pneumoniae resistance to β-lactams is commonly caused by expression of a variety of low affinity PBPs.[42](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b42-pmc-6-2014-025)Efflux by RND and ABC efflux pumps,[233](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b233-pmc-6-2014-025) and outer membrane impermeability[234](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/#b234-pmc-6-2014-025) can also cause resistance to β-lactams.

***Interpretations and conclusions***

*Several indicators have been used to measure antibiotic use at the group level; the question of which is best remains unresolved (*[*Table 1*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/table/T1/)*). The most commonly used metric is the defined daily dose (DDD), as proposed by the World Health Organization (WHO), generally expressed as DDDs per 100,000 population (for outpatient use) and DDDs per 1,000 patient-days (for inpatient use) (*[*7*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B7)*). This measure allows standardized comparisons between institutions or countries (or within an institution over time or between departments), and the data needed for calculation are easily available. However, there are several limitations to DDDs: this metric will over- or underestimate true antibiotic consumption if the administered daily dose differs significantly from the WHO-defined DDD, DDDs have not been determined for children or patients with renal failure, and the WHO occasionally updates DDDs, which complicates comparisons over time (*[*8*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B8)*–*[*10*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B10)*). Alternative measures for antibiotic consumption are days of therapy (DOT) for each antibiotic administered (e.g., 3 different antibiotics taken for 3 days each equals 9 DOTs) and length of therapy (LOT), also known as antimicrobial exposure time, which is the number of days in which a patient receives an antibiotic irrespective of the number of different drugs (e.g., 3 different antibiotics taken for 3 days each equals 3 LOTs) (*[*11*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B11)*). While LOT gives a more accurate estimation of the duration of therapy, neither LOT nor DOT reflects the dosage given, and both require individual-level data. As with DDD, DOT and LOT can be expressed as a density, i.e., DOT (or LOT) per 1,000 patient-days (*[*12*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B12)*,*[*13*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B13)*). A limitation of assessing antibiotic exposure is that all these measurements (DDD, DOT, and LOT) reflect the amount of drug prescribed; the amount taken by the patient may be less if compliance is imperfect, a problem that is relevant to studies conducted in outpatient settings.*

*Comparison of measures for reporting antibiotic use*a

*In addition to quantity, how other characteristics of antibiotic exposure are defined may influence results. First, prior antibiotic use can be described as a categorical variable (exposed or unexposed) or as a continuous variable (number of days of treatment). Hyle et al. evaluated risk factors for extended-spectrum-beta-lactamase (ESBL)-producing*Escherichia coli*and*Klebsiella*spp. by separately analyzing studies based on measurement of prior antibiotic use as a categorical versus a continuous variable (*[*14*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B14)*). They found that the use of third-generation cephalosporins was a risk factor for infection with ESBL-producing*E. coli*and*Klebsiella*spp. when antibiotic use was described as a continuous variable but not when antibiotic use was described as a categorical variable. Carmeli et al. examined antecedent treatments with different antibiotics as risk factors for vancomycin-resistant*Enterococcus*(VRE) infection (*[*15*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B15)*). They found some antibiotics to be risk factors when measured as a dichotomous variable and others to be risk factors when measured continuously by duration of therapy, which may suggest that different drug classes have different modes of selection for antibiotic resistance. Second, if antibiotic use is treated as a categorical variable, a minimum length of therapy should be defined. Hyle et al. (*[*16*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B16)*) studied risk factors for fluoroquinolone-resistant*Pseudomonas aeruginosa*. They constructed four models, each with a different definition of antibiotic exposure: (i) any use, (ii) >24 h of use, (iii) >48 h, and (iv) >72 h. In all models, prior fluoroquinolone use was an independent risk factor for fluoroquinolone-resistant*P. aeruginosa*; however, the strength of the association increased as the duration of use increased. Third, researchers must determine the time frame for exposure. Lipsitch reviewed studies of the association between antibiotic use and penicillin-resistant*Streptococcus pneumoniae*infection (*[*17*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B17)*). The period of exposure varied from use in the past 6 months (at most) to current use at the time of*S. pneumoniae*infection (at least). In general, associations were weaker in the studies that defined exposure with a wider time frame. Fourth, the exposure can be classified at the level of drug (e.g., ciprofloxacin), class (e.g., fluoroquinolones), or spectrum of activity (e.g., antipseudomonals). In two studies, different risk factors emerged as significant depending on whether antibiotic exposure was classified at the class level or at the spectrum level (*[*18*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B18)*,*[*19*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B19)*).*

*Studies may define their outcome as (i) the presence or absence of resistance to a given antibiotic, where the threshold for resistance can either include or exclude isolates with intermediate susceptibility to the chosen antibiotic (*[*20*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B20)*,*[*21*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B21)*); (ii) a change (e.g., 4-fold increase) in the MIC relative to the baseline MIC (*[*22*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B22)*); or (iii) the specific mechanism that confers resistance (*[*23*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B23)*,*[*24*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B24)*). Study findings regarding the association between antibiotic use and resistance may vary depending on the definition chosen. For example, Thiebaut et al. measured β-lactam antibiotic use and incidence of colonization with third-generation-cephalosporin-resistant*Enterobacteriaceae*(*[*25*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B25)*). When the outcome measure was cephalosporin resistance, no correlation was found; when the outcome was limited to ESBL-producing*Enterobacteriaceae*, a significant correlation was noticed.*

*A second consideration in defining the outcome is whether the organism is resistant to a single drug or to more than one drug (*[*26*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B26)*–*[*28*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B28)*). Bacteria may exhibit coresistance to different families of antibiotics, e.g., owing to the presence of multiple resistance genes on a single transferable genetic element (*[*29*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B29)*,*[*30*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B30)*). D'Agata et al. showed the differences in antibiotic exposure for subjects infected with*Pseudomonas aeruginosa*isolates resistant only to ciprofloxacin versus subjects infected with*P. aeruginosa*isolates resistant to ciprofloxacin and at least one other drug (*[*31*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B31)*). For the first group, prior fluoroquinolone use was the only significant antibiotic risk factor; the second group had significant exposure to carbapenems, cephalosporins, and gentamicin in addition to quinolones. Had those authors taken only ciprofloxacin resistance into consideration (without assessing resistance to other drugs), it would falsely appear that exposure to gentamicin, for example, is a risk factor for quinolone resistance.*

*A third consideration when measuring resistance is choosing which types of specimens to include. The four options are (i) surveillance cultures that detect colonization (usually performed for research or infection control purposes), (ii) any clinical cultures taken during routine care of the patient (which, if positive, do not necessarily indicate infection), (iii) microbiologically and clinically documented infections (i.e., a positive culture plus signs and symptoms of infection), or (iv) site-specific cultures (e.g., blood cultures) (*[*23*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B23)*,*[*32*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B32)*–*[*34*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B34)*). The last three options are more commonly available, but the risk factors identified by using these samples may in fact be risk factors for developing infections rather than risk factors for harboring resistant bacteria. Only the first option, surveillance cultures, will identify asymptomatic carriers. Using the other 3 options, asymptomatic carriers will be misclassified as “controls,” and the strength of the association between antibiotic use and resistance may be biased toward the null hypothesis. A key parameter is the prevalence of resistance. If resistance is rare, the probability of misclassification is low, and clinical samples may practically be used for studying antibiotic resistance. However, if resistance is common, it is preferable to use the outcome of colonization, which generally precedes infection and affects more patients (*[*35*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B35)*,*[*36*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B36)*).*

*Finally, antibiotic resistance can be measured in several ways (*[*37*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B37)*). The most common way is to measure the proportion of resistant isolates among all retrieved isolates. For example, a hospital's “antibiogram” may note that 20% of all enterococci detected in its laboratory are resistant to vancomycin. This approach is useful for the clinician who needs to prescribe empirical antibiotic therapy before the results of susceptibility testing are available. The problem with this method is that an increase in the proportion of organisms that are resistant may not necessarily reflect an increase in the absolute number (burden) of resistant organisms. An elegant demonstration of this discordance between metrics was presented by Burton et al. regarding methicillin-resistant*S. aureus*(MRSA) central line-associated bloodstream infections (BSIs) in U.S. intensive care units (ICUs) (*[*38*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B38)*). The overall proportion of*S. aureus*central line-associated BSIs due to MRSA increased by 25.8% between 1997 and 2007; however, in that same time period, the incidence of MRSA central line-associated BSIs declined by half (*[*Fig. 1*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/figure/F1/)*). The introduction of an antibiotic will decrease the number of susceptible organisms, so the proportion of resistant organisms will increase, even if there is no increase in the number of resistant isolates. For a public health professional who is interested in the consequences of antibiotic use, knowing the burden of resistance is most important. The best way to measure the burden of resistance is by using a rate*

*Although there is no doubt regarding the causal relationship between antibiotic use and resistance, defining and quantifying this for a given antibiotic and a given resistance are extremely difficult. In particular, it is difficult to control for all the confounding factors (known and unknown) that play a role in the development and spread of resistance. For example, different prognostic factors may influence the choice of a specific antibiotic and may also have an impact on the development of resistance (“confounding by indication”). Also, patient-to-patient transmission of resistant bacteria may be prevented by infection control measures and may be unrelated to the antibiotics prescribed. In addition, temporality may be uncertain: patients may be undiagnosed carriers of resistant bacteria before antibiotic exposure, or, on a population level, an increase in antibiotic use may be a response to an increase in antibiotic resistance rather than a trigger.*

*Nevertheless, research on antibiotic use and resistance supports a causal relationship by fulfilling the following criteria: (i) consistent association in different study populations, (ii) dose-effect relationships, (iii) concomitant variations (changes in antimicrobial use lead to parallel changes in the incidence of resistance), and (iv) biological plausibility based on experimental models (*[*39*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623381/#B39)*). In the microbiology laboratory, the identified infectious organisms are usually tested for their degree of resistance to various anti-infective substances in order to prevent the administration of ineffective treatments. The treating physician is usually informed of the test results with a report in which the activity of individual drugs against the isolated organism is categorized by one of the three terms "susceptible" (earlier term: "sensitive"), "intermediate," and "resistant." This information can be used to optimize treatment for the individual patient, while, in the aggregate, data of this type can be used to form a picture of the degree of resistance to each drug in the population at large. The latter is, in turn, an important criterion in the selection of antibiotics for the initial ("empirical") treatment of infectious diseases (*[*1*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2701059/#R1)*,*[*2*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2701059/#R2)*). As will be described in this article, the classification system for antibiotic effectiveness has recently been modified to enable the detection of more resistance mechanisms and to take better account of recent discoveries in the pharmacokinetics and pharmacodynamics of antimicrobial chemotherapeutic agents. The degree of certainty of the assessment has also been improved.*

*This review article will describe the basic elements in the determination of the drug resistance of infectious organisms and will point out the major changes that were recently introduced in an attempt to unify the criteria of assessment across Europe. It is based on a selective review of the relevant literature, and it reports on the results of the working sessions of three relevant committees: the CEN/ISO (Comité Européen de Normalisation/International Organization for Standardization) task force, the DIN subcommittee on chemotherapeutic testing methods, and the EUCAST (European Committee on Antimicrobial Susceptibility Testing).*

*An important task of medical microbiology is the phenotypic in vitro testing of antimicrobial substances for their effectiveness against infectious organisms. A variety of tests have been developed for this purpose. Thus, for example, the bactericidal activity of antibiotics can be described by the investigation of bactericidal kinetics or by the determination of the minimal bactericidal concentration of a particular antibiotic against a particular bacterial strain (*[*3*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2701059/#R3)*,*[*4*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2701059/#R4)*). Such tests generally provide a basic characterization of the interactions between the substance and the microorganism.*

*Data on the minimum inhibitory concentration (MIC) of various antibiotics used against the detected organism generally suffice for the assessment of therapeutic options. The MIC is defined as the minimum concentration of an antibiotic that is just barely able to prevent the further growth of the infectious organism in vitro. Testing techniques must be standardized to make the test results reproducible, because parameters such as the culture medium, microorganism inoculum size, and incubating temperature and duration can all influence the result (*[*5*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2701059/#R5)*). In Germany, the subcommittee on chemotherapeutic testing methods of the Medical Standards Committee (Normenausschuss Medizin, NAMed), a section of the German Institute for Standardization (Deutsches Institut für Normung, DIN), has devoted itself to this task for more than 40 years. Nonetheless, other standardized methods are used in Germany as well, namely those of the Clinical Laboratory Sciences Institute (CLSI) of the United States, formerly known as the National Committee on Clinical Laboratory Standards (NCCLS). In the last 3 years, and on the initiative of the DIN, a norm has been worked out and approved by the International Organization for Standardization (ISO 20776-1) (*[*6*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2701059/#R6)*) that is now considered valid all over the world. Microbouillon dilution has been chosen as the reference method for the determination of MIC (*[*table 1*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2701059/table/T1/)*). A further ISO norm has also been approved concerning quality criteria for derived testing procedures (ISO 20776-2) (*[*7*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2701059/#R7)*). This norm was necessary because other automatized and simplified tests are often performed instead of MIC determinations, particularly for reasons of cost. We show that most*Salmonella typhimurium*mutants resistant to streptomycin, rifampicin, and nalidixic acid are avirulent in mice. Of seven resistant mutants examined, six were avirulent and one was similar to the wild type in competition experiments in mice. The avirulent-resistant mutants rapidly accumulated various types of compensatory mutations that restored virulence without concomitant loss of resistance. Such second-site compensatory mutations were more common then reversion to the sensitive wild type. We infer from these results that a reduction in the use of antibiotics might not result in the disappearance of the resistant bacteria already present in human and environmental reservoirs. Thus, second-site compensatory mutations could increase the fitness of resistant bacteria and allow them to persist and compete successfully with sensitive strains even in an antibiotic-free environment.*

*During the last decade there has been an alarming increase in the appearance of antibiotic-resistant bacteria as a result of an increased use of antibiotics combined with the exceptional ability of bacteria to develop resistance. One strategy to reverse this development is to decrease the use of antibiotics to promote the disappearance of the resistant bacteria present in human and environmental reservoirs. Implicit in this reasoning is that resistance confers a cost on the bacteria, which results in a counter-selection against resistant strains in an antibiotic-free environment. An associated question and potential problem is whether the supposedly less fit, avirulent-resistant bacteria might accumulate compensatory mutations that restore fitness and virulence without loss of resistance, and thereby stabilize the resistant population.*

*In spite of the importance of these questions, there are few experiments that explicitly address them under the relevant conditions, i.e., in animal model systems using genetically defined bacterial strains (for a review, see ref.*[*1*](http://www.pnas.org/content/95/7/3949.full#ref-1)*). For example, it has been shown in*Escherichia coli*that carriage of resistance genes on a plasmid is associated with a decreased growth rate, and that these strains can accumulate chromosomal compensatory mutations that, by an unknown mechanism, compensate for the growth rate decrease (*[*2*](http://www.pnas.org/content/95/7/3949.full#ref-2)*,*[*3*](http://www.pnas.org/content/95/7/3949.full#ref-3)*). Likewise, it has been shown that slow-growing streptomycin-resistant mutants of*E. coli*can accumulate compensatory mutations that restore rapid growth under laboratory conditions without affecting the resistance (*[*4*](http://www.pnas.org/content/95/7/3949.full#ref-4)*). Interestingly, these compensatory mutations appear to create a genetic background in which the streptomycin-sensitive revertants have a strong selective disadvantage, implying that it would be difficult for an evolved resistant strain to become sensitive even in the absence of the antibiotic (*[*5*](http://www.pnas.org/content/95/7/3949.full#ref-5)*). There are also animal data which indicate that tetracycline-resistant*E. coli*persist in pigs long after the antibiotic has been removed, suggesting that the added burden of this particular resistance*in vivo*is in fact small (*[*6*](http://www.pnas.org/content/95/7/3949.full#ref-6)*). Finally, studies of human clinical isolates of*Mycobacterium tuberculosis*, which are isoniazid-resistant because of a defect in the catalase encoded by the*katG*gene, suggest that they accumulate mutations resulting in increased synthesis of an alkyl hydroxy peroxidase supposedly to compensate for the decrease in virulence caused by the loss of a functional catalase (*[*7*](http://www.pnas.org/content/95/7/3949.full#ref-7)*). However, this interpretation has been questioned recently (*[*8*](http://www.pnas.org/content/95/7/3949.full#ref-8)*).*

*In this study we examined the fitness of antibiotic-resistant*S. typhimurium*in mice. Our results indicate that most resistant mutants are less virulent than the wild type. However, the avirulent mutants rapidly accumulate various types of compensatory mutations that restore virulence to wild-type levels without loss of high-level resistance.*

*The virulence of the different resistant mutants and the sensitive wild-type bacteria was examined by competition experiments in BALB/c mice after intraperitoneal injection of the bacteria. Bacterial strains were constructed by transductional transfer of the resistance mutations into the*S. typhimurium*LT2 wild type to ensure isogenicity of the strains used for competitions. Bacteria were recovered from the spleens after various times, and as can be seen in Fig.*[*1*](http://www.pnas.org/content/95/7/3949.full#F1)*, the wild type outcompeted the restrictive SmR mutants as well as the RifR and NalR mutants in mice. Similar results were also seen when the bacteria were recovered from the livers (data not shown), indicating that these mutants are at a general disadvantage when grown in the reticuloendothelial system. In contrast, the nonrestrictive SmR mutant was similar compared with the wild type, and of the seven resistant mutants examined this was the only mutant that did not show a decreased growth rate in mice. In addition, as seen in Table*[*1*](http://www.pnas.org/content/95/7/3949.full#T1)*, most of the mutants had a slower growth rate than the wild type in LB medium.*

*Solutions to the problem and future plan*

*Use good hygiene! By washing your hands often and thoroughly with soap and water, you are helping to prevent disease - and therefore the need for antibiotics (see*[*Handwashing*](http://www.tufts.edu/med/apua/about_issue/handwashing.shtml)*). Additionally, cooking meat thoroughly and handling food hygienically will help to prevent food-borne illnesses. Also, you should take antibiotics only when necessary In institutions such as hospitals and nursing homes, these agents are useful and appropriate when used under strict guidelines for specific purposes. However, there is some concern that antibacterials could promote antibiotic resistance, (see*[*Antibacterial agents*](http://www.tufts.edu/med/apua/about_issue/agents.shtml)*for more information) and their usefulness by the general public is unproven. Some institutions, such as hospitals, have 'Antibiotic Policy' guidelines and antibiotic review committees, to ensure that antibiotic use in their institution is rational and does not compound the antibiotic resistance problem.

Governmental oversight of antibiotics varies widely from country to country. In some countries, antibiotics can be purchased 'over-the-counter,' that is, without a prescription from a doctor. Other countries require a doctor's prescription before a patient is allowed to purchase an antibiotic, although these laws are not always enforced. Antibiotics have also been sold over the Internet, a commerce mechanism with little governmental oversight that reaches across national borders.

Furthermore, food animals (animals raised for human consumption) are often given long-term, low-levels of antibiotics to promote growth. This antibiotic use represents a large fraction of the total antibiotic use in the industrialized world. A few governments restrict which antibiotics can be used for food animals, with the goal of preserving the most powerful antibiotics for treating human disease. The World Health Organization (WHO) has become quite concerned about the rising levels of resistant bacteria in all areas of the world. To provide some global coordination, WHO issued its*[*Global Strategy for Containment of Antimicrobial Resistance*](http://whqlibdoc.who.int/hq/2001/WHO_CDS_CSR_DRS_2001.2.pdf)*, a document aimed at policy-makers that urges governments to take action to help contain antibiotic resistance.

Developing nations need to focus on eliminating uncontrolled access to antibiotics and prevention measures such as improving sanitation, cleaning up water supplies and relieving overcrowding. These preventative measures, along with frequent hand washing, would ensure that people get sick less often, and would therefore pass on fewer resistant infections to others.

Industrialized countries need to focus on prevention measures such as frequent handwashing and limiting antibacterial use, developing vaccines that can protect certain vulnerable populations such as young children, controlling multi-resistant bacteria in hospitals and in the community, and reducing antibiotic use in animal farming and agriculture.

Experts agree that a global system for tracking antibiotic resistance is needed. It would serve as an indicator for recognizing "hot-spots" of resistance and measuring trends that can tell us if our educational programs or other solutions are having positive effects. To preserve the potency of existing antibiotics, overall antibiotic use must be decreased. Physicians, pharmacists, and the general public must avoid careless use of these valuable drugs. Antibiotics must be prescribed only for bacterial infections and in the proper dose for the correct amount of time.*[*Narrow spectrum*](http://www.tufts.edu/med/apua/about_issue/glossary.shtml#narr)*drugs should be chosen by doctors whenever possible to avoid destroying populations of beneficial bacteria along with the disease-causing bacteria. In addition, non-therapeutic uses of antibiotics in farm animals and agriculture should be eliminated. One approach taken by scientists to combat antibiotic resistance is to strengthen the action of existing antibiotics by modifying them so the bacterial enzymes that cause resistance cannot attack them. Alternately, "decoy" molecules can be used along with the antibiotic, so that the bacterium's resistance enzyme attacks the decoy molecule rather than the antibiotic. Decoy molecules such as clavulanic acid or sulbactam are already in use for blocking the beta-lactamase enzymes that destroy the penicillin family of drugs.

An alternative approach to the antibiotic resistance problem is to interfere with the mechanisms that promote resistance, rather than to attempt to kill the bacteria. For example, interfering with the duplication or movement of a bacterium's genetic material would eliminate the transfer of resistance genes between bacteria. National perceptions about antibiotics and resistance have undergone dramatic undulations over many decades, with attitudes shifting back and forth in rapid cycles, with each cycle sadly uninformed by the one before it. The initial availability of antibacterial therapy fundamentally transformed medicine from a diagnosis- and prognosis-focused field to an interventional field in which lives could be routinely saved. As early as 1948, the fate of infected patients had so dramatically improved that initial inklings of hubris arose regarding the potential defeat of bacterial infections [*[*1*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C1)*].*

*Shortly thereafter, penicillin resistance spread to become a substantial clinical problem, such that, by the 1950s, many of the gains of the prior decade were threatened [*[*2*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C2)*]. In response, investigators and industry discovered, developed, and deployed new antibiotic classes, restoring our overconfidence. By 1962, a Nobel laureate wrote, “One can think of the middle of the 20th century as the end of one of the most important social revolutions in history, the virtual elimination of the infectious diseases as a significant factor in social life” [*[*3*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C3)*].*

*Only 3 years later, the pendulum swung back. In 1965, a roundtable of some of the most prominent figures in the history of infectious diseases warned that antibiotic resistance was once again rising and that the pipeline of new antibiotics was waning and was probably insufficient to deal with the threat [*[*4*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C4)*]. Repeating the cycle, industry once again brought along multiple new antibiotics from the late 1960s through the early 1980s, such that the problem of infections was again believed solved. One of the giant figures of 20th century medicine wrote in 1978, “I cannot conceive of the need for … more Infectious Disease specialists … unless they spend their time culturing each other” [*[*5*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C5)*]. He repeated this sentiment at a keynote address at the annual meeting of the Infectious Diseases Society of America as late as 1985 [*[*6*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C6)*].*

*Thereafter, the antibacterial pipeline began to dry up. While resistance continued to spread, we no longer had new antimicrobial weapons to deal with the threat. As a result, in 2014, a full 82 years after the first patients were treated with a sulfa antibacterial agent [*[*7*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C7)*], we once again find ourselves at a crossroads in our struggle with microbes. It is time to break the cycle of denial and state the plain and simple reality: we will never win a war against microbes; there is no “endgame.”*

*The math is clear (Table*[*​(Table1).1*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/table/CIU392TB1/)*). Microbes will never stop adapting to whatever selective pressure we throw at them. As an example of the scope of the problem, recent national data from the United States indicate that among bacteria isolated in intensive care units, 10% of*Klebsiella*spp., 20% of*Pseudomonas aeruginosa,*and an astonishing 50% of*Acinetobacter baumannii*strains are resistant to carbapenems [*[*9*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C9)*]. Furthermore, a 2011 national survey of infectious diseases specialists conducted by the Emerging Infections Network found that more than 60% of specialists had seen a pan-resistant, untreatable bacterial infection within the prior year [*[*10*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C10)*]. In summary, we have learned that antibiotic resistance ranks up there with death and taxes as a third inevitable truth of life. The antibiotic resistance crisis is the predictable outcome of how we have developed and used antibiotics since their discovery. If we continue to develop, use, and protect antibiotics for the next 80 years in the same way we have in the past 80, the future of the resistance problem is easy to predict. Resistance will continue to emerge, our treatment options will continue to dwindle, and we will enter a postantibiotic era for an increasing number of infections. If we want to change the future state, and have long-term availability of effective antimicrobial therapy for infections, we need to think disruptively and challenge long-standing and sometimes cherished assumptions.*

*We have had the honor of working with Dr Bartlett as members of a team affectionately referred to as “Bartlett's Renegades” to describe specific changes that are necessary to combat antimicrobial resistance [*[*11*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C11)*,*[*12*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C12)*]. Our purpose here is not to restate those recommendations but rather to provide context for 7 specific tasks and to highlight 3 common themes that cut across all of these tasks.*

*The first common theme is that clinicians, academicians, and many public health officials have limited control over how medicine is practiced. Our influence is dependent on educating, building consensus, cajoling, and advocating. These necessary efforts will continue with respect to antibiotic resistance. However, we must have the courage to admit our limits. We believe, as Dr Bartlett has phrased it, that it is time to focus on “crossing the divide that separates us from those who own medicine—payors and regulators” (personal communication). We need to engage those who control medicine (and agriculture), help them understand the causes of and solutions to the antibiotic resistance crisis, and help them create interventions that will work long term.*

*The second theme is the need to move away from policies and procedures that rely exclusively on convincing persons to change their behavior. Physicians are generally well intentioned and seek to help, not harm, their patients. No one goes to work with an intention to misuse or abuse antibiotics. But we are also imperfect beings, subject to fear, confusion, pressure, and mistakes. We need technologies, automation, and economic incentives that will help hard-wire changes to the ways we develop, use, and protect antibiotics and help us overcome the mistakes individuals inevitably will otherwise continue to make.*

*The third theme is that the time for excuses has passed. New ideas that challenge the establishment have a tendency to generate an equal and opposite resistance that prevents their consideration or adoption. Excuses like, “it's too hard,” “that's not the way we do things,” or “it can't be done,” are easily conjured, but we must push through them. The alternative is to accept a future that is without effective antimicrobial therapy for an increasing number and diversity of infections. We must expand our thinking and aggressively explore new approaches. The second task is to reduce unnecessary agricultural antibiotic usage. A staggering 15 million kg (17 000 tons) of antibiotics are used in the United States every year, 80% in agriculture [*[*12*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C12)*]. This level of environmental contamination is simply unacceptable and will inevitably continue to drive resistance. We cannot continue to let industrial excuses prevent society from acting on the moral imperative to preserve antibiotic effectiveness.*

*Those in opposition try to confound the national dialogue by raising doubts as to whether the massive amounts of agricultural antibiotic use contributes to resistance or by suggesting that banning growth-promotional antibiotics in livestock will drive up the cost of meat production, but it has been well established that agricultural use of antibiotics contributes to resistance in human patients [*[*12*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C12)*]. Excuses about economic disaster or extensive harm to animals are belied by the experience in Europe. For example, Denmark banned growth-promotional antibiotics for livestock 15 years ago and not only experienced no disaster but had a nearly 15-fold increase in hog production after the ban [*[*13*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C13)*]. Excuses abound. Action is needed.*

### *Stop Abusing Antibiotics in Humans*

### *The third task is to modernize approaches to antimicrobial stewardship. The alarm that physicians and patients overuse antibiotics was first raised by Sir Alexander Fleming as early as 1945 [*[*14*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176319/#CIU392C14)*]. In the 69 years since, a variety of means of stewardship have been devised, but they primarily revolve around devising ways to change human behavior (eg, education, restriction). We need to continue such efforts. Ramp Up Infection Prevention*

*The fourth task is to modernize approaches to infection prevention. Efforts to improve hand washing rates are critical and must continue. We must also relieve the pressure on hand washing by employing automation and disinfection technology so that disaster does not ensue when individuals forget to wash their hands.*

 ***Conclusion***

*Microbes have been creating and defeating antibiotics for billions of years. Microorganisms are more tempered and judicious in their use of these molecules, perhaps explaining the long-term viability of antibiotics as effective growth inhibitors in nature. In contrast, in just 80 years of clinical use, humans have so abused antibiotics that we threaten their availability for future generations. Multiple generations of clinicians, scientists, and leaders have attempted to deal with complex forces that drive overuse and misuse of these drugs and the need to continue discovering new ones, but we have not yet achieved long-term solutions. It took billions of years for microbes to get it right. Perhaps 80 years on the scale of human societal evolution is simply not enough time for us to figure out how to optimally handle antibiotics.*

*Nearly 15 years ago, Nobel laureate Joshua Lederberg wrote, “The future of humanity and microbes will likely evolve as … episodes of our wits vs their genes” . With respect to our wits, despite past failings, there is reason for future optimism. The current high frequency of inappropriate antibiotic use could lessen dramatically over the coming decade thanks to major and rapidly evolving scientific advances among diagnostic and biomarker technology, and new policies and research reflecting a better understanding of the psychology driving inappropriate use. Future therapy could consist of some combination of specific antibody, organism-specific bacteriophage, small molecules (or antisense small interfering micro-RNAs) that inhibit specific virulence factors, and drugs that counter antibiotic resistance mechanisms (eg, new β-lactamase inhibitors, and blockade of efflux pumps).*

*In short, humans will have the tools to behave like bacteria that produce antibiotics. When threatened, the potential could exist for a short course, narrowly focused, customized, treatment package. Such an approach offers the promise of enhanced efficacy and reduced collateral damage in the form of drug-related adverse effects and resistance. Ultimately, long-term success may depend on a complete reconceptualization of our relationship with microbes, so that the eventual goal is to stop seeking their destruction and instead seek to achieve peaceful coexistence.*

*These principles are the result of innumerable hours of thought and dialogue, in large part driven by Dr Bartlett. No one in our field can claim to have done more to combat resistance, to educate policy makers and regulators, and to promote the principles herein laid out. We are indebted to him in ways that are difficult to describe and defy limit. We are card-carrying members of “Bartlett's Renegades,” and we call on others to join the movement to preserve and restore the life-saving public resource that is effective antibiotic therapy.*

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*(Our village LAHANDBUD JHARSUGUDA)*

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