Curr Treat Options Infect Dis (2019) 11:42–57
DOI 10.1007/s40506-019-0181-4

Neglected Tropical Diseases (A Sanchez, Section Editor)

Bacterial Antibiotic Resistance: on the Cusp of a Post-antibiotic World

Kameron Sprigg, B.Sc.
Carolynn E. Pietrangeli, Ph.D.[*]

Address
[*]Department of Health Sciences, Brock University, 1812 Sir Isaac Brock Way, St.
Catharines, ON, L2S 3A1, Canada
Email: cpietrangeli@brocku.ca

This article is part of the Topical Collection on *Neglected Tropical Diseases*

The online version of this article (https://doi.org/10.1007/s40506-019-0181-4) contains supplementary material, which is available to authorized users.

Keywords Antibiotic resistance · Antibiotic stewardship · Multi-drug-resistance · Extreme drug resistance · *Klebsiella pneumoniae* carbapenemase · Carbapenem-resistant Enterobacteriaceae

Abstract

Purpose of review The specific aim of this article is to provide evidence that antibiotic resistance (AR) has become a human disease unto itself and to describe the current means of preventing, treating, and reversing AR in individuals and in affected populations.

Recent findings An ever-increasing number of infections are being classified as multidrug resistant (MDR). Low and middle-income countries are most likely to increase the spread of AR due to limited healthcare infrastructure coupled with policies that promote unregulated access to antibiotics. The genetic basis for AR has become more thoroughly understood as efforts move toward a global big data approach to surveille and implement effective public health measures.

Summary Antibiotic Stewardship (AS) programs are critical to prevent the spread of AR. As resistant pathogens reside in patients for many years after they are initially integrated into the microbiome, the potential for future infection increases substantially. This property contributes to the conclusion that AR is in and of itself a human disease that must be treated appropriately. While all bacteria are at risk to become AR, *Staphylococcus*, *Klebsiella*, *Mycobacterium*, *Acinetobacter*, and *Pseudomonas* represent the greatest challenges in infectious disease treatment as rapid AR acquisition leads to MDR infections.

Introduction

By 1943, penicillin, the first antibiotic developed for mass distribution, was being widely used for combat-associated infections and for the treatment of sexually-transmitted infections among wartime troops. At that time, Dr. Alexander Fleming, who, in 1928, originally isolated *Penicillin rubens* from a mold-contaminated petri dishes plated with bacteria, was already warning of impending antibiotic resistance. By 1960, methicillin-resistant *Staphylococcus aureus* (MRSA) had emerged as a major threat to effective hospital use of antibiotics, opening a gateway to multiple drug resistance [1].

Today, the contribution of antibiotic misuse as well as their widespread use in livestock to the development of antibiotic resistance (AR) among human populations is fully recognized. There is also heightened awareness of the trend toward infection of healthy members of the population with highly virulent multi-drug-resistant strains, and the dangerous threat of transmission of pandrug-resistant (PDR) *Mycobacterium tuberculosis*. The success against antibiotic resistance depends upon the concerted, cooperative effort of basic microbiologists, epidemiologists, and educators of health care providers and the public.

The World Health Organization (WHO) has acknowledged the seriousness of AR and has developed a series of standard recommendations for halting the spread of AR at a global level. The Pan-Canadian Public Health Network defines *Antibiotic Stewardship* as the planning and management of resources to limit the development of AR [2]. The Canadian stewardship program is based on the development of core competencies in leadership, intervention, monitoring, and research, encompassing both human and food animal practices. A parallel program is sponsored in the USA by the Centers for Disease Control and Prevention (CDC) [3].

The present review describes the basic mechanisms of bacterial AR, delineates the most important pathogens in which AR is a serious threat, discusses strategies to reduce AR, presents current developments in terms of diagnostics and treatment approaches, and concludes with a summary of the research needed to address AR.

Genetic transfer of antibiotic resistance among bacteria

Excluding intrinsic resistance, acquisition of AR occurs through genetic transfer mechanisms and natural adaptation under selective pressure from antibiotics. Any resistance that is not intrinsic to the species may be easily transferred between bacteria through horizontal mechanisms.

Vertical gene transfer is responsible for AR occurring within a single bacterial species. Horizontal mechanisms including conjugation, transformation, and transduction are responsible for inter-species and inter-genera resistance (Table 1). The larger the microbial burden, and the more consistent and concentrated the antibiotic usage is, the faster AR develops [4].

Vertical gene transfer

The only endogenous method for acquiring AR in bacteria is through random mutations in the bacterium's genome that provide a survival advantage over its colony members. This bacterium then passes on its resistance to offspring, creating bacterial progeny that survive under the selective pressure of antibiotics [5]. Individual level mutations can then be applied to other species in the same environment through horizontal mechanisms.

Table 1. Genetic transfer of antibiotic resistance (AR).

Vertical gene transfer	Random mutation in the bacterial genome	Persistence of resistance genes occurs under antibiotic pressure [4, 5]
Horizontal gene transfer		
Conjugation -Plasmid donation -Transposable elements -Integrons	Direct donation of AR genes between bacteria -Plasmid donation is most prevalent	All bacterial species are capable of conjugation
Transformation	Random transfer of resistance genes from damaged bacteria to competent bacteria in the population	*Streptococcus*, *Acinetobacter*, *Pseudomonas* species are known to acquire resistance through this mechanism
Transduction	-Indirect transfer of AR genes within a bacterial colony, mediated by bacteriophage	-Dominant mode of AR development among bacteria found in wastewater treatment plants and in agricultural areas [6]

Conjugation

Plasmids are a well-known mechanism of genetic exchange among bacteria. These strands of DNA often contain antibiotic resistance genes and are easily passed from F+ bacteria to F– bacteria [7]. Conjugative plasmids include the genetic code required to achieve cell-cell transfer of the plasmid. Mobilizable plasmids are smaller and are transferred in the presence of a conjugative plasmid. In both cases, the pilus is formed as part of the plasmid's replication process [7].

Integrons are found in both plasmids and within the bacterial chromosome. These are regions of a gene that promote insertion of single genes, known as gene cassettes. Addition of multiple gene cassettes together can add significant AR properties to the bacteria. Gene cassettes lack promoter regions; therefore, they are activated only when they have been inserted into the integron [8]. Under constant selective pressure, these integrons and gene cassettes become more stable, leading to long-term integration of antibiotic resistance genes. Integrons also operate in cases of other temporary environmental functions [8].

Transformation

Transformation is a random process in which the contents of dead bacterial cells are released to the immediate environment. Competent bacteria including *Streptococcus*, *Acinetobacter* and *Pseudomonas* take up free DNA and incorporate it into their own genome [7].

Transduction

This process is a bacteriophage-mediated mechanism of transmitting genetic information between bacteria. The phage life cycle can occur either through the lytic pathway or the lysogenic pathway. In either case, the phage both incorporates its own DNA into the bacterium and may accept new DNA from the host. The subsequent infection of new bacteria then leads to a cyclic process wherein AR genes are transferred throughout a bacterial colony or nearby species [6].

Drug-resistant pathogens in the modern health care system

Acinetobacter baumannii

Acinetobacter baumannii is an opportunistic pathogen that causes pneumonia, meningitis, urinary tract infections, bacteremia, post-operative infections, and septicemia [9, 10; Table 2]. It is most commonly acquired nosocomially, and is transmitted via aerosol and through contact with infected persons [9, 10]. While relatively non-virulent, *Acinetobacter baumannii* has become relevant in the modern healthcare system due to its exceptional ability to develop resistance to antibiotic treatment throughout the course of infection [11–13]. At least 13 novel genes have been identified within the last 3 years in the genus, which are responsible for resistance to antibiotics across all antibiotic classifications [14, 15]. This demonstrates the wide capacity for *Acinetobacter baumannii* AR potential. The genus is capable of producing biofilms [16]. There have been reports of

Table 2. Examples of notable pathogenic antibiotic resistance (AR) trends

Pathogen	Virulence	Antibiotic resistance	Individual concerns
Acinetobacter baumannii	Opportunistic pathogen	-Widespread resistance to multiple classes of antibiotic resistance. -Identified as critical priority by WHO	Rapid acquisition of AR genes
Klebsiella pneumoniae	Opportunistic pathogen	-Extensive resistance to beta-lactams including last resort therapies	-Generation of novel AR genes and transmission of these to other genera
		-Identified as critical priority by WHO with other Enterobacteriaceae	-MDR resistant with potential to develop PDR
Pseudomonas aeruginosa	Opportunistic pathogen	-High intrinsic resistance to antibiotics due to extensive efflux pump gene expression	-Acquiring resistance within the treatment period
		-Identified as critical priority by WHO	-Increased toxicity associated with AR status
Staphylococcus aureus	Sometimes opportunistic pathogen	-MRSA and VRSA strains are becoming more common with increased resistance to other antibiotics	-Deadly infection, particularly in immunocompromised patients and in toxic shock syndrome events
	Often a primary pathogen	-Identified as high priority by WHO	-Rapid increase in AR throughout the *Staphylococcus* population
Mycobacterium tuberculosis	Primary pathogen	-XDR-TB and PDR-TB strains have already been isolated	-Deadly infection with widespread AR
	Often an opportunist (e.g., with HIV/AIDS)	-Previously identified as a critical priority by WHO	-Ease of transmission and recurrent infections

WHO World Health Organization, *MDR* multidrug resistant, *MRSA* methicillin-resistant *Staphylococcus aureus*, *VRSA* vancomycin-resistant *Staphylococcus aureus*, *XDR-TB* extensively drug-resistant tuberculosis, *PDR-TB* pandrug-resistant tuberculosis

Acinetobacter baumannii developing AR within the span of a single infection and its treatment [17].

Klebsiella pneumoniae

Klebsiella pneumoniae is an opportunistic infection resulting in diarrhea, post-operative infections, pneumonia, bacteremia, septicemia, and meningitis. It is an endogenous bacterium that can become pathogenic when the host is immunocompromised and is commonly acquired through hospital interactions [18, 19]. MGR-B is a recently identified gene in *Klebsiella* genera that increases resistance to colistin, while simultaneously increasing the pathogen's virulence by modifying the lipid bilayer [20]. In 50% of XDR *Pseudomonas* cases, all lines of treatment have failed including last resort treatments such as colistin [21]. This serves as an example of a further danger associated with antibiotic resistance—some genes act to increase both virulence and resistance to treatment.

Klebsiella was the first bacteria isolated with *Klebsiella*-producing carbapenemase (KPC)—a carbapenem resistance gene that has since spread to other bacteria, beginning the threat of carbapenem-resistant enterobacteriaceae [19]. KPC is highly prevalent in India and Southeast Asia, using these regions as hotspots for dissemination locally and internationally. Further, 93% of species produce at least one extended-spectrum beta-lactamase (ESBL) [21], granting resistance to penicillins, carbapenems, and cephalosporins. Finally, *Klebsiella* has been shown in multiple institutions across Europe to recently express resistance as high as 80% against nearly all classes of antibiotics [22], compared with rates as low as only 15% in 2010. In Europe, there is a higher concentration of antibiotic-resistant *Klebsiella* in Southern countries including Turkey, Italy, and Greece, with as much as 50-fold greater rate of AR compared with northern countries such as the United Kingdom, Greece, and Sweden [23].

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic pathogen that causes pneumonia, septicemia, urinary tract infections, gastrointestinal infections, and skin infections. It is acquired through soil, contaminated water, and contact with infected animals and nosocomially. It has intrinsic resistance to many different antibiotics due to its extensive system of efflux pumps [24]. *P. aeruginosa* has been associated with increased virulence when antibiotic resistance is present. ExoS and ToxA are two toxins which are secreted in greater quantity when ESBLs are also expressed, which cause excess inflammation by inducing T cells [25]. Multiple studies have indicated that like *A. baumannii*, *Pseudomonas* is capable of activating or acquiring AR within a week [26, 27] of selective pressure.

Staphylococcus aureus

Staphylococcus aureus is a high priority pathogen in the WHO's latest antibiotic resistance global assessment [28]. Some strains exist commensally in the human microbiome and are opportunistic pathogens. Others are primary pathogens. *Staphylococcus* causes pneumonia, food poisoning, meningitis, endocarditis, toxic shock syndrome, and wound infections. There are two AR considerations in the genus, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA).

Ninety-nine per cent (99%) of MRSA isolates are positive for at least two toxins and 100% express at least one form of adhesion protein, both of which are responsible for the genus' virulence [29]. Toxic shock syndrome is caused by one of these toxins, which overstimulates the immune response. In 2005, MRSA with resistance to vancomycin (VRSA strains) or carbapenem resistance was consistently reported as less than 30% of MDR *Staphylococcus* strains. In 2016, this was reported to be as high as 60%, with concomitant increases in KPC and other ESBLs [30]. In Ontario, Canada, the number of MRSA infections increased from 9000 to 26,500 cases, a 2.94-fold jump between 2000 and 2015 [31]. Over this time, the increase in resistant infections was extended to cephalosporins, fluoroquinolones, and tetracyclines [31].

Mycobacterium tuberculosis

Mycobacterium tuberculosis, the etiologic agent of tuberculosis (TB), is the world's deadliest bacteria infecting 10.4 million people in 2016, killing 1.7 million people [28]. TB is transmitted via respiratory droplets expelled by infected persons. The infection is chronic and often relapses in patients who have been treated "successfully" [32]. The pathogen's reproductive cycle is very slow, which contributes to the exceptional ability of *M. tuberculosis* to develop and maintain AR even in active infection of a single patient [33].

Among the possible AR mechanisms, *M. tuberculosis* most often employs efflux pumps to remove antibiotics from the cell. At least 32 isolated gene islands encode various types of pumps, which may account for the highly variable resistance profile of *M. tuberculosis* [33]. Typically, these efflux pumps are one of the MFS, ABC, RND, or SMR families [34].

Different strains of *M. tuberculosis* occupying different geographic areas contribute to the bacterial resistance profile in varying ways. In Beijing, there is a higher mutation rate compared with the average across the rest of the globe, potentially allowing for more rapid development of AR [35]. There has been an increasing rate of extensively drug-resistant tuberculosis (XDR-TB) across the globe. This was first seen in South Africa in conjunction with a high population of HIV+ patients [36, 37] but has since expanded to include a pandrug-resistant (PDR) strain in Laos [38].

Transmission of AR within patients

AR can be viewed from two perspectives. The first interprets AR as a property of bacteria that complicates treatment. The second is the description of AR as a disease in and of itself. According to the latter, AR is then a disease that can be transmitted between patients and is associated with *its own clinical outcomes* [39, 40], which can vary drastically from their non-AR counterparts, despite stemming from the same bacterial species [41].

The microbiome provides a repository of commensal bacteria that act as a harbor for antibiotic resistance genes. When patients are treated with antibiotics, the microbiome is subject to the same pressure. This causes surviving commensal bacteria to become antibiotic resistant. Upon proliferation, AR is spread within the host's microbiome [42]. These resistance genes can then be horizontally transmitted to new infections later in life. Likewise, AR infections can transmit these resistance genes to the rest of the microbiome [43]. Based on the rate of genetic mutation in bacteria, it can take more than 10 years for resistance to be lost, even in the absence of continued selective pressure [44]. In this sense, AR is a chronic disease with the potential to worsen the outcome of future infections. Future prospective cohort studies on this topic would expand the understanding of the epidemiological features of AR itself.

Geography and antibiotic resistance hot spots

Geographic location, the local economy and population dynamics (globalization), and infrastructure exert major impact on the development and spread of AR. Of these, a region's infrastructure and economy have the strongest influence.

Presently, highly developed countries have sufficient infrastructure to contain the spread and mitigate the effects of AR. Much of western Europe, North America, and Japan are examples of regions with sufficient health infrastructure to avoid uncontrollable development of AR. These regions use antibiotics with some restrictions and are able to clear most infections (even MDR cases), due to the abundance of second, third, and last line of antibiotic treatments. Despite their advancement, these regions can harbor isolated pockets that become hotspots. For example, in Canada, there are hot spots of MRSA found in indigenous communities, as well as endemic KPC enterobacteriaceae in the Brampton region in Ontario.

Middle-income countries, such as Turkey, South Africa, Brazil, China, and India, pose a greater threat to the development of AR. South Africa, for example, has the highest prevalence of XDR-TB [36]. China, particularly southern China, is prone to repeated MRSA outbreaks [28]. These countries have the resources to produce and acquire antibiotics, but the infrastructure is lacking in proper implementation, regulation, or public health measures. Lack of these capabilities leads to increased use of antibiotics and to the development of AR against first-line drugs. The primary use of second-line antibiotics and drugs of last resort increases the risk of MDR when these treatments are not completely effective [45].

In general, low to middle-income countries, including many in Africa, do not have the health infrastructure to administer antibiotics on a wide scale and do not contribute significantly to the development of AR. However, countries that are close to hot spot nations acquire AR through migration. These strains are then easily transmitted through these nations' poor healthcare infrastructure [46]. Globalization is responsible for the spread of AR between countries. Airplane ventilators spread bacteria among passengers, who then have the potential to carry pathogens to their communities [47].

Limiting the spread of antibiotic resistance

The World Health Organization has acknowledged the seriousness of AR by developing six steps to contain its spread [48]. Implementation of these six steps is the standard that nations and companies must strive for to make progress in halting the spread of AR.

National plan

The first of the six steps is to recruit governments to facilitate the implementation of the following five steps. Even if the development of novel antibiotics were a current priority for pharmaceutical manufacturers, resistance would be sure to develop. One potentially more effective approach may be to establish alliances between governments and academic institutions to develop new rationally designed antibiotics. Finally, it is important to recognize that any newly developed antibiotics will be used as second, third, or last lines of treatment [46].

Since this recommendation was made by the WHO, some governments have begun leading the battle against AR. For example, in 2016, then US President Obama signed an order allocating US $160 million to the research and development of new tools to combat AR, including the promotion of antibiotic stewardship [49].

Surveillance and laboratory capacity

Tracking AR is complicated by the diversity of its origin and the environments in which it develops, as well as the breadth of bacterial species involved. This diversity underscores the need for widespread, accurate testing and diagnostic procedures [50, 51].

Surveillance of AR is directly linked to the operational capacity of laboratories. Three techniques are used to diagnose an AR bacterial strain: bacterial genome sequencing, minimal inhibitory concentration (MIC) of the drug, and clinical observation of failed antibiotic treatment. Genome sequencing is both the most rapid and most costly method.

Access to effective treatment

Access to effective antibiotic treatment may be limited by two broad practices. In some countries, including India, substandard antibiotics are sold without prescription, promoting their misuse and the generation of resistant bacterial strains. In other areas, including some African and Middle Eastern countries, fraudulent antibiotics containing little or no active ingredient are sold, leaving patients untreated (or minimally treated) and susceptible to the development of AR [45].

Another factor prevalent in LMICs is the widespread use of second- and third-line antibiotics, such as carbapenems. The use of carbapenems in beta-lactam-resistant infections has led to the development of AR to carbapenems via the common mechanism of KPC. This practice of using second-line treatments as first-line defense against bacterial infection is used in neonates and in vulnerable populations in hospital intensive care units. [45].

Antibiotic stewardship

Antibiotic stewardship (AS) describes a variety of programs aimed at reducing the spread of AR through discerning treatment regimens. While AS applies in all settings, healthcare and agriculture are the first targets for this practice because they are the principal contributors to AR.

While AS programs have been implemented as research studies across the globe, their widespread adoption has yet to take place. Even in the face of mixed results of AS programs based on the region and intervention being implemented, in all cases, these programs have been shown to economically benefit the hospitals in which they exist [52–55]. For example, one 4-year study in South Africa found that although there was a large upfront cost to reducing antibiotic usage (in the form of training hospital staff and making laboratory upgrades), each year there was a decrease in antibiotic usage of 18% and a decrease in costs of treatment by at least 40% [56]. For this reason, hospitals should consider implementing these programs on a large scale, so that funds can be redirected toward other AR prevention practices or healthcare directives.

Knowledge dissemination is key to reduce AR levels and to promote stewardship. Extensive research has revealed that knowledge regarding antibiotic resistance is suboptimal and sometimes erroneous, even among trained pharmacists and nurses [57, 58]. One example of this being 75% of fourth year pharmacy students in a UK pharmacy program believing antibiotic resistance cannot develop if antibiotics are administered properly [57]. Given the lack of general knowledge among professionals in the field, it is not surprising that up to 70% of the general public does not perceive antibiotic resistance to be a problem [58].

One basic stratagem in stewardship is the use of narrow-spectrum vs. broad-spectrum antibiotics. This has been shown to prevent the spread of AR by limiting the number of species pressured to adapt to survive [59].

AS programs either reduce overall antibiotic use by alternating treatment between patients, or by limiting the use of one specific class of antibiotic. Results of separate systematic reviews that investigated use reduction of all hospital-prescribed quinolones revealed that the prevalence of quinolone-MDR infections of *Clostridium difficile*, MRSA, and ESBL gram-positive species declined by 75%, 24%, and 17% respectively [60]. In some cases, there was no observed effect of quinolone reduction on the prevalence of resistance among some *Klebsiella* or *Escherichia* infections [61•].

Enhance infection prevention and control

The simplest approach currently being employed to prevent infection is hand washing. Hand washing is an intervention that can be adopted to some degree in nearly every region and has been shown to significantly decrease the spread of healthcare-associated infections by up to 50% [62]. Preventing infection on a large scale in the community is subject to compliance and policy implementation. Some other interventions that can be applied include vaccination, education, smoking cessation, and self-management of healthy-living.

Research and development

The final step in preventing further spread and managing AR is to invest further in research and development of interventions, treatments, diagnostics, and public health policy. Interventions including AS have been subject to research for over a decade but have yet to be implemented on a large scale. This step is meant to promote global adoption of AS strategies and the development of new antibiotics for use.

Areas of urgent research include evaluation of public health policies aiming to curb the spread of AR. Similarly, implementation research looking into hand hygiene and other effective interventions is seriously needed [62].

Diagnostics as a treatment against antibiotic resistance

If infections are treated immediately with narrow-spectrum antibiotics and if patient outcomes improve, the duration of hospital stays may be reduced and the spread of AR would be contained [63–65]. If this is not the case, the patient's life may be at risk and clinical decisions are of monumental importance.

Genomic analysis is the way to move forward in diagnosis of AR infections. It is faster and more precise than clinical observation [66]. There are several key improvements necessary to develop effective genomic diagnostic techniques, including development of a bioinformatic databases capable of predicting clinical results based on genomic information, as well as further reduction in the size, cost, complexity, and time required for genomic sequencing [67, 68].

For the successful development of new and effective diagnostics based on genetic material, a predictive database able to determine the specifics of resistance from amino acid sequences is necessary. At present, there have been at least three attempts at this [69–71]. However, all of these require manual entry of data and are imprecisely linked to the literature. Further, the literature is relatively sparse on data regarding the genetic markers for AR in bacteria.

The best attempt at establishing a central database of bacterial AR is the Comprehensive Antibiotic Resistance Database (CARD; [72••]. Under this framework, there would be a central, global repository of information that can be accessed by clinicians and researchers across the globe. These groups would upload data they collect using free software [72••]. These data can then be compared with what is phenotypically and clinically observed on a global basis. Due to bacterial genetic mutations, any predictive model must be able to accurately account for variability. A probabilistic graphic model (PGM) is one way to do this. A PGM has already been used successfully in genetics research in various chronic diseases [73]. PGMs have been shown to account for messy or incomplete datasets and still predict phenotypes with high accuracy. Sensitivity and specificity are key considerations in implementing a PGM, as homology between AR proteins and non-AR proteins is sometimes significant [74].

Equally important is to develop a tool capable of performing accurate, rapid, cost-effective genomic analysis at the point of care (PoC). Currently, the most advanced technology appropriate for use in bacteria is the size of a small microwave oven, costs about US $250,000, and requires up to a week to process a bacterial genome [75]. This is not feasible for widespread distribution in any country. Promising advancements have been made in basic research using a pH detection system specific for particular AR genes at only 40 °C [76–79].

When antibiotic treatment fails

When antibiotics fail, the next steps are to attempt treatment using other antibiotics. However, this process is counterproductive, as it only exacerbates the spread of AR, which will emerge in future infections. This is readily apparent when considering the emergence of KPC in India. For two reasons, alternatives to antibiotics such as phage therapy [80] and use of bacterial predators [81] are being investigated. Firstly, to limit the spread of AR, and secondly, to provide a treatment option for the nearly 700,000 PDR cases that occur each year [82].

Resource competition

This approach seeks to treat AR itself, instead of the infections that contain AR. As previously discussed, there is a decay rate of resistance genes within bacteria. Research has shown that there are conditions which select for non-resistant strains of bacteria. The investigators allowed easy access to AR genes through plasmids that came at a high energy cost to the bacteria. The approach focuses on the loss of the plasmid—and resistance—when selective pressure is not present [83]. A great advantage of this technique is 15 times faster than the standard rate of resistance decay in a bacterial population [84]. In its current form, this treatment may be best applied in cases of chronic infections, such as MDR or XDR TB, which can persist for multiple years [85].

Conclusions

Antibiotics have shifted the practice of medicine and have redefined global human health. Both the chemistry of antibiotics and the inherent adaptability of bacteria have made the emergence of antibiotic resistance an inevitability. Indeed, following his discovery, Alexander Fleming predicted the occurrence of antibiotic resistance. The careless and prolific use of antibiotics has since brought us to the cusp of a potentially dangerous post-antibiotic world.

Antibiotic resistance follows the paradigm in health that those least prepared and able to deal with crisis are those most deeply affected. However, AR extends beyond borders, affecting the UK, Canada, and the USA as well as India, South Africa, China, and the countries of the developing world. Antibiotic resistance is itself a feedback cycle where treatment against infections is necessary, and yet that treatment causes its own ineffectiveness. As this pandemic spreads and becomes more potent, health systems across the globe will become strained trying to cope with the increasing morbidity and mortality of once easily treated infections.

A fundamental shift in perception of AR needs to take place. The perspective taken by the clinical and scientific community is of AR as a condition of bacteria. What is easily overlooked is the behavior of AR as its own human disease, with its own patient outcomes, costs, and distribution independent of any single bacterial infection. Indeed, AR exists in human populations through the microbiome, whether that be commensal or pathogenic and as demonstrated by *Staphylococcus aureus* and *Klebsiella* *pneumoniae*, AR genes are associated with higher virulence. Therefore, healthcare systems must move toward AR reduction strategies, in addition to AR management as seen in AS programs.

Compliance with ethical standards

Conflict of interest
Kameron Sprigg declares that there are no competing interests.
Carolynn E. Pietrangeli declares that there are no competing interests.

Human and animal rights and informed consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Bushake L, A Brief History of antibiotic resistance [Internet]. Medical Daily. 2018 [cited 2 June 2018]. Available from: https://www.medicaldaily.com/antibiotic-resistance-history-373773. Accessed 2 Jun 2018.
2. Khan F, Arthur J, Maidment L, Blue D. Advancing antimicrobial stewardship: summary of the 2015 CIDSC report. Can Commun Dis Rep. 2016;42(11):238–41. https://doi.org/10.14745/ccdr.v42i11a04.
3. Antibiotic Stewardship | Antibiotic/antimicrobial resistance | CDC [Internet]. Cdc.gov. 2018 [cited 15 June 2018]. Available from: https://www.cdc.gov/drugresistance/solutions-initiative/antibiotic-stewardship.html. Accessed 15 Jun 2018.
4. Hughes D, Andersson D. Selection of resistance at lethal and non-lethal antibiotic concentrations. Curr Opin Microbiol. 2012;15(5):555–60. https://doi.org/10.1016/j.mib.2012.07.005.
5. Goossens H. Antibiotic consumption and link to resistance. Clin Microbiol Infect. 2009;15:12–5. https://doi.org/10.1111/j.1469-0691.2009.02725.x.
6. Balcazar J. Bacteriophages as vehicles for antibiotic resistance genes in the environment. PLoS Pathog. 2014;10(7):e1004219. https://doi.org/10.1371/journal.ppat.1004219.
7. Karp G, Iwasa J, Marshall W. Cell and Molecular Biology. Hoboken, NJ: John Wiley & Sons, Inc.; 2015.
8. Toleman M, Bennett P, Walsh T. ISCR elements: novel gene-capturing systems of the 21st century? Microbiol Mol Biol Rev. 2006;70(2):296–316. https://doi.org/10.1128/MMBR.00048-05.
9. Das I, Lambert P, Hill D, Noy M, Bion J, Elliott T. Carbapenem-resistant *Acinetobacter* and role of curtains in an outbreak in intensive care units. J Hosp Infec. 2002;50(2):110–4. https://doi.org/10.1053/jhin.2001.1127.
10. Kumar A, Randhawa V, Nirupam N, Rai Y, Saili A. Risk factors for carbapenem-resistant *Acinetobacter baumanii* blood stream infections in a neonatal intensive care unit, Delhi, India. J Infec in Dev Ctries. 2014;8(08):1049–54. https://doi.org/10.3855/jidc.4248.
11. Gozutok F, Mutlu Sariguzel F, Celik I, Berk E, Aydin B, Guzel D. Investigation of antimicrobial resistance rates of *Acinetobacter baumannii* strains from nosocomial infections. Ankem Derg. 2013;27(1):7–12. https://doi.org/10.5222/ankem.2013.007.
12. Kireççí E, Kireççí M, Aksu M. Investigation of the antibiotic susceptibility of *Acinetobacter baumannii* strains

isolated from clinical samples. Türk Mikrobiol Cemiy Derg. 2015;13(4):266–70.

13. Khajuria A. Molecular characterization of carbapenem resistant isolates of *Acinetobacter baumannii* in an intensive care unit of a tertiary care centre at Central India. J Clin Diagn Res. 2014;8(5):DC38–40. https://doi.org/10.7860/JCDR/2014/7749.4398.
14. Wang H, Wang J, Yu P, Ge P, Jiang Y, Xu R, et al. Identification of antibiotic resistance genes in the multidrug-resistant *Acinetobacter baumannii* strain, MDR-SHH02, using whole-genome sequencing. Int J Mol Med. 2016;39(2):364–72.
15. Buyuk A, Yilmaz F, Gul Yurtsever S, Hosgor Limoncu M. Antibiotic resistance profiles and genotypes of *Acinetobacter baumannii* isolates and in vitro interactions of various antibiotics in combination with tigecycline and colistin. Turk J Pharma Sci. 2017;14(1):13–8. https://doi.org/10.4274/tjps.44127.
16. Khyriem A, Lyngdoh W, Banik A, Choudhury B, Bhattacharyya P, Gurung J. Association of biofilm production with multidrug resistance among clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from intensive care unit. Indian J of Crit Care Med. 2013;17(4):214–8. https://doi.org/10.4103/0972-5229.118416.
17. Nunez M, Simarro E, Martinez-Campos L, Ruiz J, Gomez J, Perez J. Evolution of resistance among clinical isolates of *Acinetobacter* over a 6-year period. Eur J Clin Microbiol Infect Dis. 1999;18(4):292–5. https://doi.org/10.1007/s100960050280.
18. Luo Y, Wang Y, Ye L, Yang J. Molecular epidemiology and virulence factors of pyogenic liver abscess causing *Klebsiella pneumoniae* in China. Clin Microbiol Infect. 2014;20(11):O818–24. https://doi.org/10.1111/1469-0691.12664.
19. Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Revs. 2017;41(3):252–75.
20. Kidd T, Mills G, Sá-Pessoa J, Dumigan A, Frank C, Insua J, et al. A Klebsiella pneumoniae antibiotic resistance mechanism that subdues host defences and promotes virulence. EMBO Molecular Medicine. 2017;9:430–47. https://doi.org/10.15252/emmm.201607336.
21. Padmini N, Ajilda A, Sivakumar N, Selvakumar G. Extended spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: critical tools for antibiotic resistance pattern. J Basic Microbiol. 2017;57(6):460–70. https://doi.org/10.1002/jobm.201700008.
22. Sahu M, Siddharth B, Choudhury A, Vishnubhatla S, Singh S, Menon R, et al. Incidence, microbiological profile of nosocomial infections, and their antibiotic resistance patterns in a high volume cardiac surgical intensive care unit. Ann Card Anaesth. 2016;19(2):281–7. https://doi.org/10.4103/0971-9784.179625.
23. Summary of the latest data on antibiotic resistance in the European Union [Internet]. European Union; 2018 p. 1–8. Available from: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Documents/antibiotic-resistance-in-EU-summary.pdf. Accessed 30 Jun 2018.
24. Ruh E, Gazi U, Güvenir M, Süer K, Çakır N. Antibiotic resistance rates of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated from a university-affiliated hospital in North Cyprus. Turk Bull Hyg Exper Biol. 2016;73(4):333–44. https://doi.org/10.5505/TurkHijyen.2016.82653.
25. Ullah W, Qasim M, Rahman H, Jie Y, Muhammad N. Beta-lactamase-producing *Pseudomonas aeruginosa:* phenotypic characteristics and molecular identification of virulence genes. J Chin Med Assoc. 2017;80(3):173–7. https://doi.org/10.1016/j.jcma.2016.08.011.
26. Feng Y, Hodiamont C, van Hest R, Brul S, Schultsz C, ter Kuile B. Development of antibiotic resistance during simulated treatment of *Pseudomonas aeruginosa* in chemostats. PLoS One. 2016;11(2):e0149310. https://doi.org/10.1371/journal.pone.0149310.
27. Swetha C, Babu A, Rao K, Bharathy S, Supriya R, Rao T. A study on the antimicrobial resistant patterns of *Pseudomonas Aeruginosa* isolated from raw milk samples in and around Tirupati, Andhra Pradesh. Asian J Dairy and Food Res. 2017;36(02):100–5. https://doi.org/10.18805/ajdfr.v36i02.7951.
28. Antimicrobial resistance: global report on surveillance [internet]. WHO; 2014. Available from: http://apps.who.int/iris/bitstream/handle/10665/112642/9789241564748_eng.pdf;jsessionid=674CF15427C38E6822B8A0BAF121ED71?sequence=1. Accessed 30 Jun 2018.
29. Cheraghi S, Pourgholi L, Shafaati M, Fesharaki S, Jalali A, Nosrati R, et al. Analysis of virulence genes and accessory gene regulator (agr) types among methicillin-resistant *Staphylococcus aureus* strains in Iran. J of Glob Antimicrob Resist. 2017;10:315–20. https://doi.org/10.1016/j.jgar.2017.06.009.
30. Gostev V, Kruglov A, Kalinogorskaya O, Dmitrenko O, Khokhlova O, Yamamoto T, et al. Molecular epidemiology and antibiotic resistance of methicillin-resistant *Staphylococcus aureus* circulating in the Russian Federation. Infect Genet Evol. 2017;53:189–94. https://doi.org/10.1016/j.meegid.2017.06.006.
31. Ontario Agency for Health Protection and Promotion (Public Health Ontario), Provincial Infectious Diseases Advisory Committee. Infection prevention and control for clinical office practice. 1st revision ed. Toronto, ON: Queen's Printer for Ontario; 2015.
32. Hawn T, Shah J, Kalman D. New tricks for old dogs: countering antibiotic resistance in tuberculosis with host-directed therapeutics. Immuno Revs. 2015;264(1):344–62.
33. Fonseca J, Knight G, McHugh T. The complex evolution of antibiotic resistance in *Mycobacterium tuberculosis*. Intl J of Infec Dis. 2015;32:94–100.
34. Liu F, Hu Y, Wang Q, Li H, Gao G, Liu C, et al. Comparative genomic analysis of *Mycobacterium tuberculosis*

clinical isolates. BMC Genomics. 2014;15(1):469. https://doi.org/10.1186/1471-2164-15-469.

35. Nguyen L. Antibiotic resistance mechanisms in *M. tuberculosis*: an update. Arch Toxicol. 2016;90(7):1585–604. https://doi.org/10.1007/s00204-016-1727-6.
36. Gandhi N, Moll A, Sturm A, Pawinski R, Govender T, Lalloo U, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet. 2006;368(9547):1575–80. https://doi.org/10.1016/S0140-6736(06)69573-1.
37. O'Donnell M, Padayatchi N, Kvasnovsky C, Werner L, Master I, Horsburgh C. Treatment outcomes for extensively drug-resistant tuberculosis and HIV co-infection. Emerg Infect Dis. 2013;19(3):416–24. https://doi.org/10.3201/eid1903.120998.
38. Iem V, Somphavong S, Buisson Y, Steenkeste N, Breysse F, Chomarat M, et al. Resistance of *Mycobacterium tuberculosis* to antibiotics in Lao PDR: first multicentric study conducted in 3 hospitals. BMC Infect Dis. 2013;13(1):275. https://doi.org/10.1186/1471-2334-13-275.
39. Shoji H, Maeda M, Shirakura T, Takuma T, Ugajin K, Fukuchi K, et al. More accurate measurement of vancomycin minimum inhibitory concentration indicates poor outcomes in meticillin-resistant *Staphylococcus aureus* bacteraemia. Int J Antimicrob Agents. 2015;46(5):532–7.
40. Matar M, Moghnieh R, Alothman A, Althaqafi A, Alenazi T, Farahat F, et al. Treatment patterns, resource utilization, and outcomes among hospitalized patients with methicillin-resistant *Staphylococcus aureus* complicated skin and soft tissue infections in Lebanon and Saudi Arabia. Infec and Drug Resist. 2017;10:43–8. https://doi.org/10.2147/IDR.S97415.
41. Menzin J, Marton J, Meyers J, Carson R, Rothermel C, Friedman M. Inpatient treatment patterns, outcomes, and costs of skin and skin structure infections because of *Staphylococcus aureus*. Am J Infec Cntl. 2010;38:44–9.
42. Blake D, Hillman K, Fenlon D, Low J. Transfer of antibiotic resistance between commensal and pathogenic members of the *Enterobacteriaceae* under ileal conditions. J Appl Microbiol. 2003;95:428–36. https://doi.org/10.1046/j.1365-2672.2003.01988.x.
43. Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. PNAC. 1999;96:1152–6.
44. Martinez JL, Baquero F. Mutation frequencies and antibiotic resistance. Antimicrob Agents Chemother. 2000;44:1771–7. https://doi.org/10.1128/AAC.44.7.1771-1777.2000.
45. Bebell LM, Muiru AN. Antibiotic use and emerging resistance. Glob Heart. 2014;9:347–58. https://doi.org/10.1016/j.gheart.2014.08.009.
46. Theuretzbacher U. Global antibacterial resistance: the never-ending story. J of Glob Antimicrob Resist. 2013;1:63–9. https://doi.org/10.1016/j.jgar.2013.03.010.
47. Pugliese G, Favero MS. Airplane cabin air recirculation and risk of upper respiratory tract infection. Infect Control Hosp Epidemiol. 2002;23:554. https://doi.org/10.1017/S0195941700081297.
48. Leung E, Weil DE, Raviglione M, Nakatani H. The WHO policy package to combat antimicrobial resistance. Bull World Health Organ. 2011;89:390–2. https://doi.org/10.2471/BLT.11.088435.
49. FACT SHEET. Obama administration releases National Action Plan to combat antibiotic- resistant bacteria. Washington D.C.: the white house; 2017.
50. Schürch AC, Schaik WV. Challenges and opportunities for whole-genome sequencing-based surveillance of antibiotic resistance. Ann N Y Acad Sci. 2017;1388:108–20. https://doi.org/10.1111/nyas.13310.
51. Le T-T, Nadimpalli M, Wu J, Heaney CD, Stewart JR. Challenges in estimating characteristics of *Staphylococcus aureus* nasal carriage among humans enrolled in surveillance studies. Front Public Health. 2018;6:163.
52. Kimura T, Uda A, Sakaue T, Yamashita K, Nishioka T, Nishimura S, et al. Long-term efficacy of comprehensive multidisciplinary antibiotic stewardship programs centered on weekly prospective audit and feedback. Infection. 2017;46:215–24.
53. Liu L, Liu B, Li Y, Zhang W. Successful control of resistance in *Pseudomonas aeruginosa* using antibiotic stewardship and infection control programs at a Chinese university hospital: a 6-year prospective study. Infec & Drug Resist. 2018;Volume 11:637–46. https://doi.org/10.2147/IDR.S163853.
54. Murni IK, Duke T, Kinney S, Daley AJ, Soenarto Y. Reducing hospital-acquired infections and improving the rational use of antibiotics in a developing country: an effectiveness study. Arch Dis Child. 2014;100:454–9.
55. Lawes T, Lopez-Lozano J-M, Nebot CA, Macartney G, Subbarao-Sharma R, Dare CR, et al. Effects of national antibiotic stewardship and infection control strategies on hospital-associated and community-associated meticillin-resistant *Staphylococcus aureus* infections across a region of Scotland: a non-linear time-series study. Lancet Infect Dis. 2015;15:1438–49. https://doi.org/10.1016/S1473-3099(15)00315-1.
56. Boyles TH, Naicker V, Rawoot N, Raubenheimer PJ, Eick B, Mendelson M. Sustained reduction in antibiotic consumption in a South African public sector hospital; four year outcomes from the Groote Schuur Hospital antibiotic stewardship program. South African Med J. 2017;107:115–8. https://doi.org/10.7196/SAMJ.2017.v107i2.12067.
57. Inácio J, Barnes L-M, Jeffs S, Castanheira P, Wiseman M, Inácio S, et al. Master of pharmacy students' knowledge and awareness of antibiotic use, resistance and stewardship. Curr in Pharm Teach & Learn. 2017;9:551–9. https://doi.org/10.1016/j.cptl.2017.03.021.

58. Minen MT, Duquaine D, Marx MA, Weiss D. A survey of knowledge, attitudes, and beliefs of medical students concerning antimicrobial use and resistance. Microb Drug Resist. 2010;16:285–9. https://doi.org/10.1089/mdr.2010.0009.
59. Garau J, Nicolau DP, Wullt B, Bassetti M. Antibiotic stewardship challenges in the management of community-acquired infections for prevention of escalating antibiotic resistance. J of Glob Antimicrob Resist. 2014;2:245–53. https://doi.org/10.1016/j.jgar.2014.08.002.
60. Butt AA, Navasero CS, Thomas B, Marri SA, Katheeri HA, Thani AA, et al. Antibiotic prescription patterns for upper respiratory tract infections in the outpatient Qatari population in the private sector. Intl J Infec Dis. 2017;55:20–3.
61.• Pitiriga V, Vrioni G, Saroglou G, Tsakris A. The impact of antibiotic stewardship programs in combating quinolone resistance: a systematic review and recommendations for more efficient interventions. Adv Ther. 2017;34:854–65. https://doi.org/10.1007/s12325-017-0514-y

This study demonstrates the health and economic benefits seen by hospitals implementing antibiotic stewardship programs in a thorough systematic review, as well as offering ideas to improve on techniques being used today.

62. Tartari E, Pires D, Pittet D. Clean your hands 5th May 2017: fight antibiotic resistance - its in your hands. Antimicrob Resist Infec Cntrl. 2017;45(4):342.
63. Lushniak BD. Antibiotic resistance: a public health crisis. Public Health Rep. 2014;129:314–6. https://doi.org/10.1177/003335491412900402.
64. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Peterson LE, et al. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia. J of Infec. 2014;69:216–25. https://doi.org/10.1016/j.jinf.2014.05.005.
65. Okeke IN, Peeling RW, Goossens H, Auckenthaler R, Olmsted SS, de Lavison JF, et al. Diagnostics as essential tools for containing antibacterial resistance. Drug Resist Updat. 2011;14:95–106. https://doi.org/10.1016/j.drup.2011.02.002.
66. Niwa T, Watanabe T, Suzuki K, Hayashi H, Ohta H, Nakayama A, et al. Early optimization of antimicrobial therapy improves clinical outcomes of patients administered agents targeting methicillin-resistant *Staphylococcus aureus*. J Clin Pharm Ther. 2015;41:19–25.
67. Köser CU, Ellington MJ, Peacock SJ. Whole-genome sequencing to control antimicrobial resistance. Trends Genet. 2014;30:401–7. https://doi.org/10.1016/j.tig.2014.07.003.
68. Reddy PN, Srirama K, Dirisala VR. An update on clinical burden, diagnostic tools, and therapeutic options of *Staphylococcus aureus*. Infec Dis: Res and Treat. 2017;10:117991611770399.
69. Peeling RW, Boeras DI, Nkengasong J. Re-imagining the future of diagnosis of neglected tropical diseases. Compu and Struct Biotech J. 2017;15:271–4. https://doi.org/10.1016/j.csbj.2017.02.003.
70. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother. 2010;54:969–76. https://doi.org/10.1128/AAC.01009-09.
71. Liu B, Pop M. ARDB—antibiotic resistance genes database. Nucleic Acids Res. 2009;37:D443–7. https://doi.org/10.1093/nar/gkn656.
72.•• Mcarthur AG, Wright GD. Bioinformatics of antimicrobial resistance in the age of molecular epidemiology. Curr Op Microbiol. 2015;27:45–50.

This study outlines a feasible framework for implementing global data collection as a tool for surveillance and diagnostic capacity of antibiotic resistance in new infections. https://doi.org/10.1016/j.mib.2015.07.004.

73. Mourad R, Sinoquet C, Leray P. Probabilistic graphical models for genetic association studies. Briefings in Bioinf. 2011;13:20–33.
74. Morar M, Wright GD. The genomic enzymology of antibiotic resistance. Annu Rev Genet. 2010;44:25–51. https://doi.org/10.1146/annurev-genet-102209-163517.
75. Illumina. Personal correspondence - MiniSeq sequencer small affordable benchtop sequencer. 2017. https://www.illumina.com/systems/sequencing-platforms/miniseq.html. Accessed 16 Jan 2019.
76. Farrar JS, Wittwer CT. Extreme PCR: efficient and specific DNA amplification in 15-60 seconds. Clin Chem. 2014;61:145–53.
77. Tanner NA, Zhang Y, Evans TC. Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes. BioTechniques. 2015;58:59–68. https://doi.org/10.2144/000114253.
78. Hu C, Kalsi S, Zeimpekis I, Sun K, Ashburn P, Turner C, et al. Ultra-fast electronic detection of antimicrobial resistance genes using isothermal amplification and thin film transistor sensors. Biosens Bioelectron. 2017;96:281–7. https://doi.org/10.1016/j.bios.2017.05.016.
79. Credo GM, Su X, Wu K, Elibol OH, Liu DJ, Reddy B, et al. Label-free electrical detection of pyrophosphate generated from DNA polymerase reactions on field-effect devices. Analyst. 2012;137:1351–62. https://doi.org/10.1039/c2an15930a.
80. Górski A, Międzybrodzki R, Weber-Dąbrowska B, Fortuna W, Letkiewicz S, Rogóż P, et al. Phage therapy: combating infections with potential for evolving from merely a treatment for complications to targeting diseases. Front Microbiol. 2016;7:1515. https://doi.org/10.3389/fmicb.2016.01515.
81. Atterbury RJ, Hobley L, Till R, Lambert C, Capeness MJ, Lerner TR, et al. Effects of orally administered *Bdellovibrio bacteriovorus* on the well-being and *Salmonella* colonization of young chicks. Appl Environ Microbiol. 2011;77:5794–803. https://doi.org/10.1128/AEM.00426-11.

82. O'Neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Antimicrob Resist. 2014;1:1–14.
83. Gomes ALC, Galagan JE, Segrè D. Resource competition may lead to effective treatment of antibiotic resistant infections. PLoS One. 2013;8:e80775. https://doi.org/10.1371/journal.pone.0080775.
84. Palmer AC, Angelino E, Kishony R. Erratum: corrigendum: chemical decay of an antibiotic inverts selection for resistance. Nat Chem Biol. 2010;6:244–9. https://doi.org/10.1038/nchembio0310-244a.
85. Ahuja SD, Ashkin D, Avendano M, Banerjee R, Bauer M, et al. Multidrug resistant pulmonary tuberculosis treatment regimens and patient outcomes: An Individual patient data meta-analysis of 9,153 patients. PLoS Med. 2012;9(8):e10001300. https://doi.org/10.1371/lornal.pmed.10001300.