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REVIEW ARTICLE

The Drug Resistance in Microorganism: A Review Report

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ABSTRACT

Multiple antibiotic resistance in bacteria was at first thought to be caused exclusively by the combination of several resistance genes, each coding for resistance to a single drug. More recently, it became clear that the activity of drug efflux pumps often achieves such phenotypes. Some of these efflux pumps exhibit an extremely wide specificity covering practically all antibiotics, chemotherapeutic agents, detergents, dyes, and other inhibitors, the exception perhaps being very hydrophilic compounds. Such efflux pumps work with exceptional efficiency in Gram-negative bacteria through their synergistic interaction with the outer membrane barrier. It is disturbing that the antibacterial agents of the most advanced type, which are unaffected by common resistance mechanisms, are the compounds whose use appears to select for MDR mutants that overproduce these efflux pumps of wide specificity.

Since the introduction of antibiotics, bacteria have not only evolved elegant resistance mechanisms to thwart their effect but have also evolved ways in which to disseminate themselves or their resistance genes to other susceptible bacteria. During the past few years, research has revealed not only how such resistance mechanisms have been able to evolve and to rapidly disseminate but also how bacteria have, in some cases, been able to adapt to this new burden of resistance with little or no cost to their fitness. Such adaptations make the control of these superbugs all the more difficult.

Keywords: Antibiotic Resistance, Drug Resistance, Multidrug-Resistant, Superbugs, Totally Drug-Resistant.

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INTRODUCTION

Antimicrobial resistance (AMR or AR) is the ability of a microbe to resist the effects of medication that once could successfully treat the microbe. The term antibiotic-resistance (AR or ABR) is a subset of AMR, as it applies only to bacteria becoming resistant to antibiotics. Resistant microbes are more difficult to treat, requiring alternative medications or higher doses of antimicrobials. These approaches may be more expensive, more toxic or both. Microbes resistant to multiple antimicrobials are called multidrug-resistant (MDR). Those considered extensively drug-resistant (XDR) or totally drug-resistant (TDR) are sometimes called "superbugs".

Resistance arises through one of three mechanisms: natural resistance in certain types of bacteria, genetic mutation, or by one species acquiring resistance from another. All classes of microbes can develop resistance. Preventive measures include only using antibiotics when needed, thereby stopping misuse of antibiotics or antimicrobials. Narrow-spectrum antibiotics are preferred over broad-spectrum antibiotics when possible, as effectively and accurately targeting specific organisms is less likely to cause resistance, as well as side effects. For people who take these medications at home, education about proper use is essential. Health care providers can minimize the spread of resistant infections through proper sanitation and hygiene, including handwashing and disinfecting between patients. They should encourage the same of the patient, visitors, and family members.[1]

Rising drug resistance is caused mainly by the use of antimicrobials in humans and other animals and spread of resistant strains between the two. Growing resistance has also been linked to the dumping of inadequately treated effluents from the pharmaceutical industry, especially in countries where bulk drugs are manufactured. Antibiotics increase selective pressure in bacterial populations, causing vulnerable bacteria to die; this increases the percentage of resistant bacteria which continue growing. Even at very low antibiotic levels, resistant bacteria can have a growth advantage and grow faster than vulnerable bacteria. With resistance to antibiotics becoming more common, there is greater need for alternative treatments. Calls for new antibiotic therapies have been issued, but new drug development is becoming rarer.

Antimicrobial resistance is increasing globally because of greater access to antibiotic drugs in developing countries. Estimates are that 700,000 to several million deaths result per year. Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics, and at least 23,000 people die as a result. There are public calls for global collective action to address the threat that includes

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proposals for international treaties on antimicrobial resistance. Worldwide antibiotic resistance is not completely identified, but poorer countries with weaker healthcare systems are more affected.[2]

Brief History of Antibiotics Development

Antibiotics are natural or synthetic chemicals that can affect the survival of microorganisms by inhibiting their growth or killing them. Many molds and mold-like bacteria have rather remarkable bacterial-inhibiting abilities. The modern history of antibiotics devolved as follows: In recorded history and probably before: Fermenting yeasts and fungi that comprise the sediment in all grain-synthesized alcohol products were the source of many medicinal effects.

1. In the 1870s: John Burdon Sanderson, Joseph Lister, William Roberts, and John Tyndall had each described the antagonistic activity of a certain mold to bacterial growth. The mold was of the same genus as Penicillium.
2. In 1896: Ernest Duchesne demonstrated a similar bacteriostatic (that is, controlling or limiting the growth of bacteria) effect from Penicillium molds.
3. In 1899: Rudolph Emmerich and Otto Low used the residues left after cultivating the bacterium Pseudomonas pyocyanin to kill other bacteria (an example of "antibiosis").
4. By the end of the 19th century: The idea of isolating a chemical that would directly impede bacterial growth was widespread among medical researchers across Europe (Lord Joseph Lister, Paul Ehrlich and others).
5. In 1908: The German I. G. Fabre Industries prepared whole batteries of synthetic chemicals, truly antibacterial drugs in the sulfa family.
6. In the early 1920s: Andre Grate and Sara Dath discovered that some Penicillium molds had direct bacteria-killing properties.
7. In 1928: After nearly half a century of research and investigation by others, Sir Arthur Fleming made his momentous and serendipitous discovery of penicillin – a natural product of fermentation (a mold) representing the most potent and nontoxic chemical agent for controlling bacterial infections. Penicillin remains a laboratory discovery unused in humans for the next 1213 years.
8. In 1932: Gerhard Domagk synthesized Prontosil (2.4-diaminoazobenzene-4'-sulfonamide) to treat streptococcal infections in mice and humans.
9. In 1933: First reports on sulfanilamide.
10. In 1933-1936: J. Trefouel, F. Nitri and D. Bovet demonstrated that para-aminobenzenesulfonamid (or "sulfa" for short), a derivative of Prontosil, was more effective and less toxic than its parent form.
11. In 1937: Sulfas were first used in the USA to treat urinary tract infections and in Japan for treating dysentery. An early derivative of the sulfa drugs, Gentrisin (3.4-dimethyl-5- sulfanilamidoisoxazole) is till used to treat bladder infections.
12. In 1938: First reports on penicillin.
13. In 1939: Rene Dubos discovered Gramicidin, a potent but highly toxic antibiotic with dramatic effects against gram-positive bacteria.
14. In the early 1940s: Selman Waksman found or named over twenty potential antibiotics (other than penicillin) and later identified 200 of them, including streptomycin, chloramphenicol, tetracyclines, and erythromycin.
15. In 1941: Sir Howard W. Florey and Ernst B. Chain produced penicillin in clinically useful quantities and were the first to use it on humans.
16. In 1940: Chain and Abraham suspecting the limitations of penicillin reported on the existence of a bacterial enzyme (penicillinase) with the ability to inactivate penicillin. This is one of the earliest indications of antibiotic-resistant bacteria.
17. In 1942: Sir Alexander Fleming, the discoverer of penicillin, warned the medical profession about the appearance of antibiotic resistance among the staphylococci.
18. In or about 1943: Discovery of an enzyme in bacteria that could break off the molecular core of penicillin, the beta-lactam ring.
19. In 1944: Several researchers (Howard W. Florey, Ernst H. Chain, H. W. Florey) decried the misuse of antibiotics.
20. In 1945: Giuseppe Brotzu isolated a fungus in the genus Cephalosporium that produced a novel chemical with antibiotic properties ("cephalosporin"), which resisted attacks by bacterial enzymes (called beta-lactamases) that were fast undermining the effectiveness of penicillin.
21. In 1947: A Chloromycetes organism was identified as the source of chloramphenicol, one of the most potent antibiotics known.
22. 1947-1948: The antibiotic Cephalosporin is invented.
23. In 1952: Tsutomu Watanabe isolated an organism that was simultaneously resistant to several different antibiotics.
24. In the mid-1950s: Thomas H. Jukes and E. L. Robert Stokstad co-discovered the antibiotic stimulant effect in animal feed. (However, Dr. Maxwell Finland and his co-workers warned against the possible effects on human health.)
25. In 1959: K. Ochai and co-workers reported that the genes for antibiotic resistance could be passed intact from Schigella to Escherichia coli (the major bacterium of the human intestinal tract).

26. In 1964-1966: it was discovered in England that in an epidemic of Salmonella diarrhea in both calves and people, an appreciable number of human Salmonella strains (22%) showed the same pattern of antibiotic resistance found in the calves' Salmonella.
27. In 1975-1976: The antibiotic Carbapenem is invented.
28. In 1977: MM Mc Connell found identical patterns of antibiotic resistance by the organism Salmonella. Wien in different countries in North Africa, Europe and Asia.
29. In 1990: The antibiotic Fluoroquinolones is invented.
30. Since 1990: No new antibiotic was invented.[3]

Antibiotic Mechanisms of Action and Mechanisms of Resistance

Antimicrobial agents are substances that are capable of killing or inhibiting the growth of microorganisms. The term "antimicrobial" includes antibiotics as well as other chemicals and compounds that may be used to kill microorganisms. The term "antibiotic" refers to substances that are naturally produced by certain fungi and bacteria, as well as similar substances that are created synthetically.[4]

There are several different mechanisms through which antibiotics inhibit or kill microorganisms. Because bacteria become resistant to antibiotics by disrupting or rendering these mechanisms ineffective, it is important to understand the function of the different antibiotic. It is also important to consider that while a good antibiotic should destroy the microorganism it targets, it would ideally not be harmful to human cells. One of the most common mechanisms of action is of targeting the cell wall, which is present in bacteria (or prokaryotic cells) but absent in humans (or eukaryotic cells). In bacteria classified as gram-positive, the cell wall is composed of a layer of peptidoglycan and an internal cell membrane. Gram negative bacteria also have an internal membrane and a cell wall made of peptidoglycan, but they possess an additional external cell membrane as well. This external cell membrane can sometimes help to shield gram-negative bacteria from antibiotics that can disrupt the peptidoglycan that composes the cell wall.[5]

Peptidoglycan is a compound that is not present in human cells, so it is ideal for antibiotics. The beta-lactam antibiotics function by disrupting how the peptidoglycan molecules link together to form the cell wall. Penicillin's and cephalosporins do this by binding to enzymes that assemble the precursor components of the peptidoglycan molecule. Other antibiotics, such as cabapenems, prevent peptidoglycan molecules from linking together to form the cell wall. Vancomycin functions by binding to a precursor molecule to peptidoglycan, which disrupts the microorganism's ability to create new peptidoglycan molecules. Due to vancomycin's large size, it cannot cross through the porins of the outer cell membrane of gram-negative bacteria, so it is only used to treat infections caused by gram positive organisms.[6]

Disrupting the formation or linking of the peptidoglycan molecules disrupts the structuralintegrity of the cell, an action that can lead to a collapsing or bursting of the cell wall, resulting in the death of the microorganism. For this reason, beta-lactams are classified as bactericidal drugs.[7]

Like beta-lactams, polymyxins also disrupt the cell's structural integrity and are bactericidal, but polymyxins function by targeting the outer cell membrane of gram-negative bacteria. A compound called lipopolysaccharide is a structural component of the outer cell membrane. This compound is negatively charged. It is normally stabilized by calcium and magnesium, which possess a positive charge. Polymyxins have a positive charge that is stronger than the charge of calcium or magnesium, so this drug can bind to the lipopolysaccharide with a stronger affinity than calcium and magnesium. When the drug binds, it removes the calcium and magnesium, causing a disturbance in the cell membrane. Cell death occurs due to the loss of integrity of the outer cell membrane.[8]

In order to create proteins or cellular products that the cell requires to survive, all cells must first copy their DNA into an RNA molecule that can be translated into a protein. Many antibiotics function by disrupting the process of DNA replication. Initially, a DNA strand is unwound by an enzyme called helicase. Topoisomerase II (DNA gyrase) helps to stabilize the DNA molecule and relieve the strain from being unwound by helicase. A class of antibiotics called the Quinolones target the complex of DNA and topoisomerase II. The drug binds to this complex and prevents further DNA replication from occurring. Normally, after a DNA strand is unwound, an RNA copy is made of the nucleus' DNA. This process is facilitated by RNA polymerase. While both human cells and bacterial cells utilize RNA polymerase, the bacterial RNA polymerase differs slightly from that found in eukaryotic cells. The drug Rifampin works by binding the RNA polymerase molecule, thus preventing the RNA chain of nucleotide bases from elongating. Rifampin is the only drug currently available that is able to bind RNA polymerase, and stop the transcription of RNA. Rifampin can be bactericidal or bacteriostatic depending on the concentration of the drug that is administered.[9]

After the DNA is copied into a complementary RNA strand, the RNA exits the nucleus and complexes with ribosomes, which are capable of converting the RNA code of nucleotide bases to a functional protein molecule. Bacterial cells contain ribosomes composed of the 30S and the 50S ribosomal subunit, while eukaryotic cells

contain ribosomes made from a 40S and a 60S ribosomal subunit. Antibiotics can target the 30S or the 50S ribosomal subunit. Aminoglycosides and Tetracyclines target the 30S subunit. The binding of an aminoglycoside to the 30S subunit prevents the docking of transfer RNA, which leads to incomplete or incorrect proteins. Tetracyclines block how the RNA molecule rotates into the ribosome, which causes the RNA molecule to be released prematurely, leaving an incomplete peptide. It is important to note that these effects are reversible, and if the drug concentration diminishes in the patient, then the microorganisms will be able to function as normal. Thus, these drugs are bacteriostatic.[10]

Sulfonamides also function by disrupting the production of DNA and RNA, but in a different way. Folic acid is a compound that is used in the synthesis of nucleotides. Many bacteria convert para-aminobenzoic acid to folic acid. Sulfonamides are very similar in structure to para-aminobenzoic acid; therefore, they can bind to an enzyme involved in the conversion of para-aminobenzoic acid to folic acid. This slows the cell's production of folic acid, thus producing DNA and RNA, an action that can restrict the growth of the microorganism. Trimethoprim functions very similarly to sulfonamides, but it binds to a different enzyme involved in folic acid production. Both of these antibiotics disrupt the production of a metabolic product the microorganism needs and they are both bacteriostatic.

While there are many different ways in which antibiotics can kill or inhibit microorganisms, there are also many mechanisms of resistance that microorganisms sinnately possess or have developed over time. It is possible that through one mechanism, an organism can become resistant to many different classes of antibiotics, especially if the antibiotics function in a similar way. Sometimes resistance can be shared between individual bacteria through the production of "resistance plasmids," which are pieces of DNA capable of being transferred from one cell to another (Clewell, 2014). Understanding these mechanisms of drug resistance is essential to understanding why drug resistance is a growing problem. One way a cell may gain resistance to an antibiotic is by making an enzyme that renders the drug inactive or decreases the functionality of the antibiotic. An example of this is beta-lactamases, which are capable of breaking the beta-lactam rings of penicillin and other beta-lactam antibiotics. Breaking the beta-lactam ring stops the antibiotic from being able to attach to the peptidoglycan precursors. It will be less likely that penicillin or other similar drugs will be able to disrupt the integrity of the cell wall, as long as the organism produces beta-lactamases. This method of resistance can be transferred from one bacterium to another through the production of R-plasmids, and is common in strains of methicillin-resistant Staphylococcus aureus.[11]

Another common way of interfering with antibiotics is by preventing the entry of the drug into the cell. Gram-negative bacteria have an external cell membrane, and drugs must pass through the cell porins, which are channels that span the outer membrane and allow the entry and exit of materials into or out of the cell. To enter the cell or interact with the cell wall, the drugs must be able to pass through the porins. A gene mutation can result in altered porins, usually by changing the electrical charge or the physical structure. These changes can make it more difficult for an antibiotic to enter the cell. The antibiotic is still functionally active, but it is now unable to reach its target site. A microorganism can develop resistance to multiple drug classes at once in this manner. Gram negative bacteria are innately resistant to large drugs like vancomycin, which is too large to pass through the porin even before a mutation occurs.[12]

Many antibiotics act by binding to a target molecule that is a component of the microorganism. A microorganism can decrease the effectiveness of a drug if the target molecule changes slightly in its structure. If the structure of the target changes, then the antibiotic may no longer be able to bind to the target molecule. For example, tetracyclines block the transfer RNA access site by binding to it. Slight changes in the access site may result in microbial resistance to tetracyclines. Another mechanism through which microorganisms can become resistant to tetracyclines is by utilizing an efflux pump. An efflux pump is a biological pump that can force the antibiotic out of the cell, so that it cannot reach or stay in contact with its target. This method of antimicrobial resistance may often create resistance to more than one class of antibiotics.[13]

MECHANISMS AND ORGANISMS

Bacteria

The four main mechanisms by which bacteria exhibit resistance to antibiotics are:

1. Drug inactivation or modification: for example, enzymatic deactivation of penicillin G in some penicillin-resistant bacteria through the production of β-lactamases. Most usually, the protective enzymes produced by the bacterial cell will add an acetyl or phosphate group to a specific site on the antibiotic, which will reduce its ability to bind to the bacterial ribosomes and disrupt protein synthesis.[14]
2. Alteration of target- or binding site: for example, alteration of PBP—the binding target site of penicillin's—in MRSA and other penicillin-resistant bacteria. Another protective mechanism found

among bacterial species is ribosomal protection proteins. These proteins protect the bacterial cell from antibiotics that target the cell's ribosomes to hinder protein synthesis. The mechanism involves binding the ribosomal protection proteins to the ribosomes of the bacterial cell, which in turn variations its conformational shape. This allows the ribosomes to continue synthesizing proteins essential to the cell while preventing antibiotics from binding to the ribosome to inhibit protein synthesis.[15]

3. Alteration of metabolic pathway: for example, some sulfonamide-resistant bacteria do not necessitate para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides, instead, like mammalian cells, they turn to use preformed folic acid.[16]
4. Reduced drug accumulation: by decreasing drug permeability or increasing active efflux (pumping out) of the drugs across the cell surface.[17] These pumps within the cellular membrane of convinced bacterial species are used to pump antibiotics out of the cell before they are able to do any damage. They are often activated by a specific substrate associated with an antibiotic[18] as in fluoroquinolone resistance.[19]

In gram-negative bacteria, plasmid-mediated resistance genes produce proteins that can bind to DNA gyrase, carrying it from the action of quinolones. Lastly, mutations at key sites in DNA gyrase or topoisomerase IV can decrease their binding affinity to quinolones, decreasing the drug's effectiveness.[20]

Some bacteria are naturally resistant to convinced antibiotics; for example, gram-negative bacteria are resistant to most β-lactam antibiotics due to the presence of β-lactamase. Antibiotic fight can also be acquired as a result of either genetic mutation or horizontal gene transfer.[21] Although mutations are rare, with spontaneous mutations in the pathogen genome happening at a rate of about 1 in 10^5 to 1 in 10^8 per chromosomal replication,[22] the fact that bacteria reproduce at a high rate allows for the effect to be significant. Given that lifespans and production of new cohorts can be on a timescale of mere hours, a new (de novo) change in a parent cell can quickly become an inherited mutation of widespread prevalence, resulting in the microevolution of a fully resistant colony. However, chromosomal mutations also confer a cost of fitness. For example, a ribosomal mutation may keep a bacterial cell by changing the antibiotic's binding site nonetheless will also slow protein synthesis.[23] manifesting, in slower growth rate.[24] Moreover, some adaptive mutations can propagate not only through inheritance but also through horizontal gene transfer. The most common mechanism of horizontal gene assignment is the transferring of plasmids resonant antibiotic resistance genes among bacteria of the same or different species via conjugation. However, bacteria can also acquire resistance through transformation, as in *Streptococcus pneumoniae* up taking of naked fragments of extracellular DNA that contain antibiotic resistance genes to streptomycin,[25] through transduction, as in the bacteriophage-mediated transfer of tetracycline resistance genes among strains of *S. pyogenes*,[26] or through gene transfer agents, which are particles produced by the host cell that resemble bacteriophage structures and are capable of transferring DNA.[27]

Antibiotic resistance can be introduced affectedly into a microorganism through laboratory protocols, sometimes used as a selectable marker to scrutinize gene transfer mechanisms or to identify individuals that absorbed a piece of DNA that included the resistance gene and another gene of interest.[28]

Recent findings show no necessity of large populaces of bacteria for the appearance of antibiotic resistance. Small populations of *E. coli* in an antibiotic gradient can become resistant. Any heterogeneous environment with respect to nutrient and antibiotic gradients may facilitate antibiotic resistance in small bacterial populations. Researchers hypothesize that the mechanism of resistance advance is based on four SNP mutations in the genome of *E. coli* produced by the gradient of antibiotics.[29]

In recent years, the emergence and spread of β-lactamases called carbapenemases has become a major health crisis.[30] One such carbapenem's is New Delhi Metallo beta-lactamase 1 (NDM-1),[31] an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics. The most common bacteria that make this enzyme are gram-negative such as *Escherichia coli* and *Klebsiella pneumoniae*, but the gene for NDM-1 can spread from one strain of microbes to another by horizontal gene transfer.[32]

Viruses

Specific antiviral drugs are used to treat some viral infections. These drugs prevent viruses from reproducing by preventing essential stages of the virus's replication cycle in diseased cells. Antivirals are used to treat HIV, hepatitis B, hepatitis C, influenza, herpes viruses, including varicella-zoster virus, cytomegalovirus, and Epstein-Barr virus. With each virus, around strains have become resistant to the administered drugs.

Antiviral drugs typically target key components of viral reproduction; for example, oseltamivir targets influenza neuraminidase, while guanosine analogs inhibit viral DNA polymerase. Resistance to antivirals is thus acquired through mutations in the genes that encode the drugs' protein bulls. Resistance to HIV antivirals is

problematic, and even multi-drug resistant strains have evolved. One source of resistance is that many current HIV drugs, with NRTIs and NNRTIs, bull reverse transcriptase; however, HIV-1 reverse transcriptase is highly error-prone and thus mutations conferring resistance arise rapidly. Resistant strains of the HIV virus emerge rapidly if only one antiviral drug is used. Using three or more drugs together, termed combination therapy, has helped to control this unruly, but new drugs are needed because of the continuing emergence of drug-resistant HIV strains.[33]

Fungi

Infections by fungi are a cause of in height morbidity and mortality in immunocompromised persons, such as those with HIV/AIDS, tuberculosis or receiving chemotherapy. The fungi candida, *Cryptococcus neoformans* , and *Aspergillus fumigatus* cause most of these infections and antifungal resistance occurs in all of them. Multi-drug resistance in fungi is increasing because of the widespread use of antifungal drugs to treat infections in immunocompromised individuals. Of particular note, Fluconazole-resistant Candida species have been dyed as a growing problem by the CDC. More than 20 species of Candida can cause Candidiasis infection, the most common of which is *Candida albicans*. Candida yeasts normally inhabit the skin and mucous membranes without causing infection. However, overgrowth of Candida can lead to Candidiasis. Some Candida strains are becoming resistant to first-line and second-line antifungal managers such as azoles and echinocandins.[34]

Parasites

The protozoan parasites that cause the diseases malaria, trypanosomiasis, toxoplasmosis, cryptosporidiosis, and leishmaniasis are important hominid pathogens.Malarial parasites that are resistant to the drugs currently available to infections are common, which has led to increased efforts to progress new drugs.Resistance to recently developed drugs such as artemisinin has also been reported. The problem of drug resistance in malaria has ambitious efforts to develop vaccines.[35]

Trypanosomes are parasitic protozoa that cause African trypanosomiasis and Chagas disease (American trypanosomiasis). There are no vaccines to avert these infections so drugs such as pentamidine and suramin, benznidazole and nifurtimox are used to treat infections. These drugs are effective but infections caused by resistant parasites have been reported. Leishmaniasis is instigated by protozoa and is important public health tricky worldwide, especially in subtropical and tropical countries. Drug resistance has "become a major concern".[36]

Resistant Escherichia coli

E. coli are gram-negative, facultative anaerobes that are most commonly commensal but can also be pathogenic. Pathogenic draining can produce theoretically deadly toxins including enterohemorrhagic-verodoxin (Shiga-like toxin), which causes hemolytic-uremic syndrome and renal failure.[40] This toxin was originally gained from a prophage.[37]

Traditionally *E. Coli* has stood one of the most widely antibiotic susceptibles of the *Enterobacteriaceae* family. Recently, though, horizontal gene transmission has allowed for the rise of highly resistant strains. *E. coli* resistance is worrying because they are the most common gram-negative bacterial infections in humans. The occurrence of strains with extended-spectrum β-lactamases (ESBLs) talking resistance to third-generation cephalosporins has been gradually rising in Europe.[42] ESBL positive strains in bacteremia have also shown high cross-resistance to fluoroquinolones (>80%) and gentamicin (25) (>40%).[43]Although still fairly uncommon, *E. coli* on multiple landforms have also acquired the New Delhi Metallo-β-lactamase-1 (NDM-1) enzyme from *K. pneumoniae*, which confers broad resistance to all β-lactams including carbapenems with the exception of the monobactam, aztreonam (**18**). Fluoroquinolone resistance is also common among *E. coli*. Bacteria overexpressing *FomA* and *FomB* plasmidic genes are capable of inactivating fosfomycin through phosphorylation. *E. coli* are also the most frequently zoonotic pathogens discussed herein. *E. coli* O157:H7, an enterohemmorhagic strain, has been associated with many zoonotic outbreaks and incidences of food borne illness, including a 1999 outbreak in the US that infected at least 127 people.Another enterohemmorhagic strain, *E. coli* O104:H4, infected over 3,800 people in Germany in 2011 causing 54 fatalities.[38]

Diminished Pharmaceutical Investment

A flagging interest in antibiotics by the pharmacological industry is one factor that has contributed to an increased occurrence of hard-to-treat bacterial infections. In 2004 for example, only 1.6% of drugs in clinical development by the world's 15 largest drug companies were antibiotics. This reduced output of antibiotics has several causes. Antibiotics schedules are typically administered for very limited durations making them far less profitable than drugs used to treat chronic diseases. Further, newly approved drugs for most other ailments are closely prescribed, whereas new antibiotics are typically held in fallback and only prescribed for infections that more established antibiotics can't treat. This policy helps delay the emergence of resistant strains, but it also limits initial speculation return. A market

saturated with generic participants and the inevitable growth of bacterial resistance exacerbates this profit discrepancy as compared to other drugs in the long term.[39]

Regulatory hurdles have also hushed the interest of major pharmaceutical companies. The tolerance of adverse side effects has recently been decreased for numerous drug classes, including antibiotics. Sanction requirements during clinical trials have escalated in most cases from a demonstration of noninferiority to superiority, and at times a lack of clear trial guidelines for antibiotics, in particular, have stifled growth. Pharmaceutical companies are presented with a paradox wherein federal agencies issue calls for antibiotic development while concomitantly other centralized agencies enact policies limiting the appeal of that very development.[40]

These factors have made investment in antibiotics expansion too high risk, and with the cost at an estimated $1.7 billion per drug, with too little potential reward for many large pharmaceutical companies. A metric called net present value (NPV) has been developed for pharmaceutical companies to determine the best avenues of investment at a given time. NPV is a risk-adjusted measure of a drug's projected future revenues ignoring initial development investment and other projected future expenses. A characteristic NPV for an injectable antibiotic may be around 100, which is somewhat unattractive compared to a typical cancer drug, around 300, or a neuroscience drug around 720.[41]

Since 1998 AstraZeneca, GlaxoSmithKline, Merck, Johnson and Johnson, and Pfizer/Wyeth have been the only major pharmacological companies to develop a past antibiotic phase I clinical trials. Sanofi Aventis, Eli Lilly, Bristol-Myers Squibb, GlaxoSmithKline, Proctor and Gamble, Roche, and Wyeth have all greatly curtailed, rejected, or spun off their antibiotic Research and Development divisions. In fact, as of 2013, there are only four transnational pharmaceutical companies with antibiotics divisions left.[42]

No government agency has ever successfully discovered and developed an antibiotic, and there have been no indications that any will contribute the resources necessary for such an endeavor anytime in the near future. As a consequence, much of what is currently being done in antibiotic development in the western world is done in small pharmaceutical companies, biotech entities, and academic institutions. A number of large pharmaceutical companies still play a central role in antibiotic development in Japan, however.[43]

Policies have recently been enacted and incentives are offered in a determination to reverse this exodus from antibiotic Research and Development. Agencies including the World Health Organization (WHO), the European Center for Disease Prevention and Control (ECDC), the Infectious Diseases Society of America (IDSA), and even the US Congress have gotten involved.

In the 111th congress the Generating Antibiotic Incentives Now (GAIN) Act and the Strategies to Address Antimicrobial Resistance (STAAR) Act were introduced. In 2011 the US government gave $94 million in government funding for the development of the structurally novel antibiotic candidate, Anacor's GSK-052 (though its experimental trials were subsequently halted in 2012) and also $67 million for Teatraphase's TP-434 (eravacycline 44), a next-generation fluorocyclineat present in phase III trials. Even the FDA has recently publically acknowledged that there is an antibiotic crisis.[44]

Chronic Clinical Over-prescription and Public Misconceptions

The other factor fueling antibiotic resistance is the evolution and dissemination of resistance factors within bacterial populations. There is a plethora of means by which humans have inadvertently accelerated the evolution of bacterial resistance. The over-prescription of antibiotics by surgeons for symptoms that in many cases may not be caused by bacteria has historically been one such problematic policy. In recent years steps have been taken to limit antibiotic over-prescription, however. In surveys of doctor's visits in 1995 compared to 2005, the percentage that resulted in antibiotic prescriptions cut universally for symptoms including ear infections, colds, bronchitis, sore throats, and sinusitis.[45]

Despite these positive leanings, the Center for Disease Control and Prevention (CDC) recently estimated that approximately 50% of antibiotics are still prescribed unnecessarily in the US at a yearly cost of $1.1 billion. Antibiotic stewardship programs are becoming more commonplace in hospital settings and have been correlated in many belongings to significant reductions in some strains of resistant bacteria.[46]

Despite these successes, only 48% of US infirmaries have adopted stewardship policies to date and numbers are unquestionably even lower in the majority of developing countries. Varied methodologies in measuring antibiotic consumption in US hospitals have been an undermining factor even where stewardship policies are enacted.[60] Along with overall reductions to antibiotic usage, cycling usage between antibiotic classes, using combination therapies, and avoiding use of comprehensive spectrum and last resort antibiotics whenever possible, have also been implemented as strategies to avoid the evolutionary pressure that accelerates resistance.[47]

Overly long or improper behavior regimens may also, in some cases exert unnecessary evolutionary pressure on bacteria.This can lead to acquired drug resistance in which a minority resistant bacterial phenotype can find themselves in a less competitive, and therefore more advantageous environment as a phenotypically sensitive majority is slaughtered off.Outpatient antibiotic use has been directly tied to macrolide resistance in *Streptococcus pyogens* and penicillin resistance in *Streptoccous pneumoniae*.

More restrictive policies regarding casualty regimes have resulted in the decline of certain resistant isolates in both Finland and France.[48] A lack of public knowledge about antibiotics has also led to their overuse. In a 2009 European survey, of those who had taken antibiotics within the last year, 20% claimed to have taken them for a virus, a viral malady. Only 36% of those surveyed answered correctly, that antibiotics do not kill viruses. This particular variability of misuse is especially problematic in countries where antibiotics can be obtained without prescriptions. Europe has instituted an Antibiotics Awareness Day annually on 18 November in an effort to raise public knowledge.

Misuse by the Food Industry

The use of antibiotics in animal feedstocks has also exacerbated the spread of resistance. Especially egregious is their use for non-curative reasons such as prophylaxis, metaphylaxis, and growth preferment, which by one estimate accounted for 25–50% of all antibiotic consumption in the early 2000s.

Other assessments within the US during the same time period estimated agricultural use to be much greater at 24.6 mountain 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. Antibiotic use for growth promotion has been banned in the European Union (EU) since 2003, and finally, in 2012, the FDA banned the use of antibiotics in livestock without a veterinary prescription.[49]

There are still many nation-states where this practice remains unlegislated, however. There is strong evidence that the use of fluoroquinolones in food animals has led to the emergence of fluoroquinolone resistant *E. coli*, *Salmonella*, and *Campylobacter*. The emergence of vancomycin impervious *Enterococci* (VRE) in Europe was tied to the use of the glycopeptide avoparcin in nourishment animals. Avoparcin was banned in the EU in 1997, which resulted in a reduction in VRE there,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 rummage sale of fluoroquinolones and fourth-generation cephalosporins from 2005 to 2009.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 oxytetracycline were sprayed prophylactically on apples and pears. Waste runoff containing resistant bacteria or antibiotics from large corporate farms or agro-industrial plants is also a concern.This serves as a mobile means of exposure to antibiotics. The terrestrial locale provides an ideal environment for disseminating resistance elements from pathogenic bacteria and potentially from soil bacteria.[50]

Human Independent Resistance

Though there is undoubtedly a significant human contribution to bacterial resistance evolution, there is also resistance that has occurred in nature absent human interference. Resistances to first in session antibiotics such as penicillin G (4) and streptomycin (24), discovered during the golden age of antibiotics, were observed shortly after their initial isolation.Though this is not always the case, this phenomenon is typical when examining the antibiotic battery as a whole.

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. Resistance genes can associate in clusters and be transferred together as well. This kind of genetic diversity couldn't have arisen in the time frame since penicillin's discovery and undeniably, the phylogenetic analysis suggested a more ancient root evolution of these enzymes.[51]

Resistance elements have even been found in bacterial DNA that was isolated for 30,000 years in permafrost. Estimates based on the genetic divergence of antibiotic biosynthetic RNA components have suggested that some antibiotics could have evolved hundreds of millions of years ago.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 countless resistance mechanisms even to antibiotics that they do not naturally produce. 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 places that have never been exposed to antibiotics through unnatural means.[52] Therefore, the evolution of bacterial resistance to antibiotics is 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-fangled antibiotics and more responsible custom of current antibiotics are clearly necessary.

Emergent Bacterial Threats[53]

Bacterial threats fast facts

- **20%** – Proportion of people that are persistent carriers of *S. aureus*
- **$3 billion** – Annual healthcare costs associated with MRSA in US
- **19,000** – Deaths per year caused by MRSA in the US
- **61%** – Vancomycin (52) resistance rate of *E. faecium* in the US
- **40%** – *S. pneumoniae* strains resistant to penicillin.
- **50%** – Chance of contracting C. difficile with >4 week hospital stays
- **1.3 million** – Worldwide deaths caused by TB per year
- **$483,000** – Average cost of XDR-TB treatment
- **30%** – Increase in carbapenem-resistant A. baumannii strains from 1995–2004
- **30%** – Quinolone resistance rate for *Enterobacter*
- **700,000** – *N. gonnorhoeae* infections in the US per year
- **15.5%** – HAI incidence rate in developing countries.

CONCLUSION

Rapidly emerging resistant bacteria threaten the extraordinary health benefits that have been achieved with antibiotics. This crisis is global, reflecting the worldwide overuse of these drugs and the lack of development of new antibiotic agents by pharmaceutical companies to address the challenge. Antibiotic-resistant infections place substantial health and economic burden on the US health care system and population. Coordinated efforts to implement new policies, renew research efforts, and pursue steps to manage the crisis are greatly needed.

Many years of stagnant development and the alarming rise of bacterial resistance fueled by irresponsible policies and practices has created an undeniably dangerous quandary for the field of antibiotics research. Recent efforts by diverse groups, including scientists, medical doctors, and even in some cases politicians, have shed light on this predicament, however. The approval of five new classes of antibiotics since the turn of the century to combat the emergent resistant gram-positive pathogens of the 1990s was a step in the right direction. Advances in scientific technology have provided the tools necessary for the discovery of new antibiotic classes and the improvement of already established ones to combat the largely unchecked rise of resistant gram-negative pathogens. It remains to be seen whether these encouraging developments will flower with increases in funding and the backing of major pharmaceutical companies into an antibiotic renaissance or if they will wilt, paving the way for a dreaded "post-antibiotic" era.

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