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Novel Antibiotic Resistance Mechanisms in Clinical Bacteria: Implications for Treatment Strategies

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Annotation: The past few decades have seen the rapid emergence and spread of bacteria that are resistant to multiple antibiotics. This increased level of resistance has resulted in a number of challenges when it comes to treating infections in the clinic, and healthcare providers are finding themselves with limited options for treatment.

1. Introduction to Antibiotic Resistance

As such, infections caused by resistant bacteria have increased considerably, especially in patients with hospital-acquired infections. This trend towards multiple antibiotic resistance is clearly a cause of concern for public healthcare systems globally. Without cheap and effective antimicrobials, many aspects of modern medicine would be compromised. This includes such routine procedures as surgery, chemotherapy, transplantations, and the care of premature infants.

Resistance to antibiotics is as old as antibiotics themselves and has developed over time as a result of the continued use and misuse of antimicrobials. Nevertheless, antibiotic resistance has spread in

recent years, and in many cases, it is now a global problem. The molecular mechanisms of resistance have been revealed over the past three decades, and these studies have shown that bacteria possess a large and diverse range of ways to avoid the action of antimicrobial drugs. Many genes encoding the enzymes or proteins necessary for these processes have been cloned and sequenced, and, in some cases, the three-dimensional structure of the protein has been elucidated. These findings have given unique insights into the domains of enzymology and molecular genetics, furthering our understanding of the molecular machinery in living organisms. In addition, some researchers have turned this knowledge to good use and patented these proteins and enzymes for use in biotechnology and pharmaceuticals. How can we tackle antibiotics and antibiotic resistance?

1.1. Historical Context

Antibiotics, the treatment of choice for most bacterial diseases and infections, are the cornerstone of modern health care. In 1928, penicillin, the first antibiotic accessible to man, was discovered. The introduction of sulfa drugs in 1936 was an even greater potential triumph in clinical therapy. It was soon evident that sulfanilamide was remarkably successful in the management of streptococcal infections. In 1943, there were other even more potent sulfonamides such as sulfanilamide, aminosidin, and others that were effective in treatment.

1.1.1. Introduction Antibiotics are one of society's earliest success stories, which revolutionized drug treatment prior to extensive study by the end of the 19th century to determine the etiology of just about all communicable diseases. This was partially due to an ingenious, but simple, concept of obtaining selective toxicity toward bacteria by the use of materials produced by other organisms. In more than a hundred years, bacterial resistance to these drugs has begun to be established, causing the continual, increased, difficult, and untiring effort on the part of society to overcome resistance. A brief history of the key bacterial resistance advances and growth over the past 80 years is therefore an interesting example of improvement in antimicrobials vis-à-vis the continual pressure of resistance. With the exception of growth and variations, the area of antimicrobial research and discovery has undergone various rises and recessions. A more comprehensive scientific understanding and awareness of the historical developments in relation to antibiotic activities and evolution of resistance will be crucial for the framing of potential strategies. [1][2][3]

1.2. Current Global Situation

The rise of resistance in bacteria has a major impact on healthcare systems worldwide. Resistance to infections is now a major cause of concern. In the European Union, more than 400,000 infections caused by resistant bacteria and fungi have been recorded each year, causing 33,000 deaths. In the United States of America, more than 2.8 million infections caused by resistant bacteria are recorded each year, causing more than 35,000 deaths. In Japan, some 7,000 infections caused by resistant bacteria were recorded in 2019. In addition to the extra human burden resulting from resistance, there is also a significant economic impact. Breaks in the treatment of resistant infections lead to extended hospital admissions. Underfunded healthcare systems often allocate fewer resources and less effective drugs to fight resistant bacteria than the developed systems. In highly endemic settings, approximately 75% to 90% of the infected population carry at least one MDRO in developing countries. In non-public hospitals, fighting MDROs is usually not a priority because most people must pay for their treatment, and MDROs are considered a threat in public health hospitals. In public hospitals, MDROs account for 40% to 70% of all isolated packed material. International travel and trade speed up dissemination. As a result, resistance comes to the West from the developing world, and then it comes to those parts of the developing world. As a result of these resistant bacteria, the increased economic burden is up to 14 billion. Total resistance is estimated. In the USA, antibiotic resistance is estimated to kill more than 35,000 people every year due to excessive morbidity. It is estimated that an additional infections per year lead to additional hospital costs of US$ 76 million, losses resulting from lost working days of US$ 2.8 million, and a cumulative loss of quality of life of 300 QALYs. Just in India, an estimated 58,000 newborns died in 2012 from resistant sepsis.

Although the cost of antibiotic resistance is difficult to calculate, it was estimated that between 2002

and 2012, the required cost in the USA alone was US$ 1.67 to 10.1 billion. An additional 35,000 people die from antibiotic resistance in Europe. Age-related disabilities due to resistance are enormous. Antibiotic resistance has evolved as a major risk for humanity. Community policies are of great help in limiting the worldwide spread of resistance, as international trade and travel increase the scope of antimicrobial resistance at an unprecedented rate. National policies are not enough to control the outbreak. Global sharing of medicine-resistant pathogens is responsible for the spread of specific transmission dynamics. It is completely global because people who live in India may have resistance from the same strain when they travel to the USA through the UK. Resistance has been hypothesized and debated due to the dynamics of resistance patterns and the causative biological factors that are spreading. [4][5][6]

2. Mechanisms of Antibiotic Resistance

Bacteria evolve in a range of ways, and this adaptability allows them to develop strategies to evade the effects of antibiotics. The main mechanisms that are used for this purpose are well recognized and include alteration of drug targets to reduce the level of antibiotic binding and consequently limit drug effects, or enzymatic degradation of the antibiotics to minimize or abolish their activity. Other mechanisms include alteration of membrane permeability and active efflux of the antibiotics via transporters. Most of these mechanisms are encoded in the bacteria's genome and result in inherited resistance. This review aims to focus on more emerging resistance mechanisms that are less well studied, and the section below discusses a number of mechanisms that are known to be important for such conditions. Alteration of either the antibiotic target, typically in the bacterial cell, or the self-protection of the antibiotic-producing organism is where most work in the drug development area concentrates. Conventional mechanisms of resistance usually involve a reduced sensitivity to the drug that allows the resistant organisms to outcompete their susceptible counterparts either at the site of infection or during transit. Novel mechanisms include both inducible and spontaneous tolerance to high concentrations of the drug. The intrinsic levels of tolerance vary between strains of the same species and reflect complex changes in stress responses and the ability to establish a largely inactive, non-growing, dormant population embedded within a rapidly growing subpopulation. This minireview focuses on the mechanisms that bacteria use to resist the action of antibiotics aimed at killing them rather than stopping their growth. [7][8][9]

2.1. Conventional Mechanisms

Antibiotic resistance describes the ability of bacteria to survive in the presence of antibiotics or to continue growing once exposed to an antibiotic. Bacteria can fight antibiotics using many different mechanisms that have been well described. The traditional strategies include modification of a drug’s target, use of efflux pumps to remove the antibiotic from the cell, and inactivation of the antibiotic using enzymes. Examples of these mechanisms have been identified in a variety of bacterial species, including Gram positives such as methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus, and Gram negatives such as Pseudomonas aeruginosa and Salmonella.

Understanding the conventional mechanisms bacteria use to resist antibiotics is critical for microbiologists and clinicians, as it informs decisions regarding the most effective way to treat a bacterial infection. However, despite advances in our understanding of resistance mechanisms and how to counteract them, many of our oldest antibiotic classes are experiencing widespread bacterial resistance. Furthermore, few new classes of antibiotics have been discovered or come to market within the past 20 years, severely limiting our options to treat infections caused by multidrug-resistant bacteria. [10][11]

2.2. Novel Mechanisms

In an effort to identify novel mechanisms, researchers have implemented a series of enhanced strategies to provide further insight into the pool of novel strategies to emerge in the near future. Some of these have been achieved through research into the role of biofilms as a protective strategy

against conventional therapies and a source of novel resistance mechanisms. Gene-level investigations such as whole genome sequencing have helped to detect gene mutations that exist within certain bacterial species. More recent innovative strategies include research into the possibility that viruses encode resistance elements that could be horizontally transferred to clinical bacteria. Characterize the genetics, genomics, and molecular mechanisms of resistance and the impact this has on patient care. As a consequence of passing infection transmission barriers, these novel resistance mechanisms are of increasing relevance to public health. Determine their epidemiology and prevalence, possible associations with host and environment, and assess the risks for public and animal health. This should be followed by guidelines and recommendations for the best patient care and measures to be taken. While studying these novel mechanisms will undoubtedly offer valuable information, the further exploitation of treatment strategies needs to be approached with caution.

Mobile elements that move between plasmids and between every genome of the genera determined from the recent 21st century have made the genetic generation scenarios of bacteria more complex. A collaborative scientific approach is needed by the medical and microbiological communities to fully understand the barriers to the tolerance of bacteria that proliferate horizontally in a mammalian host to avoid such complex conditions. At least 57 different resistance gene cassettes have been revealed by decoding base pair-level reading of the gene cassettes. The new genes, which could of course encode new resistance mechanisms, are the ten gene cassettes whose protein products are not related to the gene products among them. A larger proportion of genes with multiple copies encoding resistance in different segments have also been recently discovered. One example, integron N-complete, contains 14 genes and 1,010 nucleotides within the 3′-CS of a 10-gene cassette array. With the exception of the five related aminoglycoside phosphotransferases, the 14 gene products are not related to each other or to any other protein, sequence, or structure documented. In addition, many of the simpler nucleotides encoding resistance have been entirely new. At least one of these plasmid- and transposon-encoded enzymes is not a serine enzyme but a very rare arginine-type β-lactamase. The majority of such plasmid converters prevent resistance from being engaged, although few are classic resistance genes. [12][13]

3. Detection and Surveillance of Antibiotic Resistance

Prompt detection and surveillance of antibiotic resistance are crucial in order to determine the best treatment strategies. The rising tide of clinically important antibiotic resistance mechanisms demands that we be ready for a rapid and vigilant response to new threats. Laboratory detection of resistant strains is an essential part of this task; the detection must be both accurate and as rapid as possible in order to guide targeted therapy. Commonly used laboratory tests report large numbers of isolates as susceptible on the basis of measurements of bacterial growth in media containing antibiotics. Tests have evolved from simple phenotypic assays based on the measurement of growth of bacteria on solid agar to complex molecular phenotypic assays that measure multiple bacterial traits in response to antibiotic exposure. Over the last 20 years, the use of molecular diagnostics based on direct detection of resistance genes in clinical specimens has become a standard part of the clinical laboratory armamentarium. To some extent, this has revolutionized the laboratory approach to bacterial infection. Surveillance for new mechanisms of resistance is an essential adjunct to the detection of resistance in complex indigenous or reference laboratories. This is particularly important in the detection of β-lactamase enzymes that can be difficult to detect or are often uncharacterized in their treatment profile. There are extensive national surveillance programs that have highlighted the increasing prevalence of multi-resistant Gram-negative bacteria and the clustering of certain resistance mechanisms in specific regions. These data have provided both longitudinal information for policy and service providers and the clinical niche for new antimicrobials. The next frontier of resistance surveillance will probably involve functional genomics and implementation of next-generation sequencing technology that allows sequencing of emerging resistance genes in clinically important outbreaks in real time in the clinical and public health sectors. Modeling and development of validated pipelines for data analysis and interpretation

are currently ongoing. Current caveats of the technology include high instrument and consumables costs, relatively high error rates of base calling, and limitations with upper and lower drug resistance gene detection thresholds. It is conceivable that functional genomics and nanopore-based sequencing will significantly accelerate timelines and decision-making in the clinical management of infections caused by multi-resistant bacteria. [14][15]

3.1. Laboratory Techniques

There are several techniques available in clinical laboratories to detect resistance. A summary of these methods and their limitations can be found in a table. Culture methods have been the traditional method used for the detection of resistance. Some examples of culture methods include disk diffusion, agar dilution, broth microdilution, E-test, and automated susceptibility testing. Culture methods detect resistance by isolating colonies that grow in the presence of antibiotics. Isolated colonies can then be further analyzed to determine the mechanism of resistance. Some of the mechanisms of resistance that can be identified include drug inactivation, altered target site, reduced drug concentration within the cell, increased efflux of drugs, and bypass mechanisms.

Molecular methods that can be used to detect resistance include polymerase chain reaction, next-generation sequencing, and proteomic methods. These methods can detect antibiotic resistance genes and chromosomal mutations in hours to days. Polymerase chain reaction is a prime example of a molecular method used to diagnose infectious diseases and has been very successful in doing so. There are several advantages and limitations associated with polymerase chain reaction and next-generation sequencing that have been well documented. One limitation is that these molecular methods cannot quantitate viable organisms. Several direct-from-blood polymerase chain reaction assays for the detection of microorganisms from a positive blood culture have become popular, being able to produce a result in a few hours. However, for many pathogens, including mycobacteria, they are expensive and cannot yet be applied to the direct detection of the pathogen in most types of patient specimens. Direct hybridization of RNA can also provide rapid results, but is generally applicable to a limited number of pathogens. Therefore, different techniques used in tandem can increase the likelihood of successful organism identification. A combination of techniques would also overcome the limitations that are specific to each of the assays.

Several methods to identify the mechanisms of drug resistance have been described recently. One approach involves phenotypic tests. Despite high levels of resistance, large populations of bacteria may be drug sensitive. This resistant subpopulation may be a marker for drug resistance. Single-cell analysis of a small population of resistant cells can provide insights into the mechanism of drug resistance. Results can be confirmed with known molecular tests, such as detection of a specific gene, or with a genetic knockout in combination with phenotypic analysis. Proteomics can be useful in analyzing mechanisms of drug action and resistance. Overexpression of efflux pumps or mutation of efflux pump-related genes can be assessed by a shift in protein intensity in time courses with drug treatment, for example. High-throughput next-generation studies have also been useful in looking into drug action and resistance. Some examples include knockouts or haploinsufficiency after drug exposure, epistasis studies of drug efficacy or mechanisms, or deep sequencing of bacterial populations treated with multiple antibiotics. [16][17]

3.2. Epidemiological Surveillance

Epidemiological surveillance is not only fundamental but also a global priority able to enforce AMR through resistance profiles, guidelines, and drug development for neglected pathogens both within and beyond national boundaries. Some worldwide professional and governmental networks ensure the monitoring and obtaining of quality data for early warning and tracking of the resistance profile of clinically relevant pathogens and zoonoses globally. Among these are various surveillance networks and organizations. Strategies and technical data sheets allowing countries to correctly play the role of reference in the international traffic of diagnosis and resistance monitoring can be found on relevant platforms. This is a peculiarity of a qualified prevention system able to counter antibiotic resistance exercised through control or an exceptional health declaration, stemming from a network

of international reference laboratories and national collaborating centers.

Moreover, in an international scenario, like the European one, collected data can be updated annually, daily, or weekly through shared communicative platforms with over 60 laboratories in 36 European countries, providing quality-controlled data from the network of national surveillance systems. The technological improvement makes possible a wide global surveillance system in real time, thanks not only to traditional repeated molecular typing but also through the definition of displacement in the same host. These tools combine with individual medical history and risk factors allowing the prevention of future resistance evolution through quick implementation of governmental policies or guidelines concerning risk society behaviors. On a daily basis, possible control procedures may need to be implemented at the farm level by veterinarians or in the community in humans with the inclusion of social care aspects. In conclusion, the development of antibiotic surveillance activities addresses different levels from the political, social, and environmental to the individual. In detail, in the clinical area, these data could enable the implementation of antimicrobial and diagnostic stewardship. The implementation of the strategies represents a good break in intrahospital outbreaks that impact the national and international epidemiological scenarios. [18][19]

4. Impact of Antibiotic Resistance on Clinical Practice

Antimicrobial resistance is increasingly affecting the clinical outcome of systemic infections. For an increasing number of infections, there are no therapeutic options left, and antibiotic options are limited in an alarming number of clinical syndromes. This infection is complicated in the clinic, posing significant challenges, especially at the pharmacovigilance and therapeutic levels. Many infected patients need long-term antibiotic therapy, involving complex multi-drug regimens. Effective oral options are available for only a few infections, particularly outside the approval of the antimicrobial indication, and are likely to be insufficient to treat critically infected patients from de-escalation or step-down therapy. Avoiding re-hospitalization or emergency department visits increases the morbidity of the affected patients and increases healthcare costs. Infections caused by multidrug-resistant bacteria are associated with increased lengths of hospital stay, hospital costs, and readmissions. In individuals, spontaneous bacterial infections can also lead to increased anxiety, increased psychological stress, and chronic diseases. The increasing number of bacteria resistant to antibiotics has had a major impact on the clinical outcome of many infections. There are several independent risk factors for a higher likelihood of healthcare issues. Antibiotic reputation may vary depending on the ambulance, hospital-based infections, and infection wards. When transcribed, some bacteria account for more than 10% of all infections. Some resistance mechanisms have been extensively evaluated in the hospital setting, and patients are considered to be at risk for these infections. It is possible to direct empirical antibiotic options for most patients, although local epidemiology should be continuously examined if a steady infection is suspected. In general, most often, most drugs target an escalating strategy to be severe and healthcare. [20][21]

4.1. Treatment Challenges

Though the emergence and spread of new antibiotic resistance mechanisms is a continuous and inevitable process, the current array of multidrug- and extensive drug-resistant Gram-negative pathogens poses treatment challenges that are unprecedented. These organisms are no longer sensitive to multiple drugs commonly used for empiric or active therapy, expanding the choice of treatment options to a limited number of agents which have, in turn, a high possibility of already being less efficacious because of the same resistance mechanisms. This requires that the choice of therapy be tailored to the infecting organism, ideally by at least two active drugs in combination in order to exploit their additive or synergistic effects or to limit the selection of further resistant strains. Infection due to a resistant pathogen is one of the drivers of inappropriate or prolonged therapy, both already associated with poor outcomes, and is independently related to a longer length of hospital stay. A healthcare cost analysis demonstrated that bloodstream infections were significantly associated with higher adjusted hospital costs compared to infections caused by non-

resistant strains and discussed an active empirical treatment duration with a de-escalation approach being possibly cost-effective. Those additional costs should also include the economic burden for society caused by the psychological stress of physicians and other healthcare professionals who experience a sense of powerlessness and discouragement from the increased difficulty or impossibility to cure. Illustrative of all these concepts is a case history of a female patient who was hospitalized with a first-time bloodstream infection. The patient sequentially received a total of three different treatment regimens, without a satisfactory clinical and microbiological response, developed an orbital abscess, and finally died. These complications can lead to the inability to provide adequately targeted therapy and may then require the involvement of other health professionals in an ad hoc multidisciplinary healthcare team, including but not limited to microbiology, infectious disease, clinical pharmacology or clinical pharmacy, internal medicine, infection control, and surgery. [22][23]

4.2. Economic Burden

It is estimated that antibiotic resistance adds approximately 50,000 days in hospital and yearly costs of £10,000 to antibiotic treatment for Gram-negative bloodstream infections in England. This is the cost of the hospital stay and does not include the cost of treatment with more complex interventions, sequelae, rehabilitation, or time away from work due to illness. When the economic burden of antibiotic resistance for the patient is investigated, it is estimated that in the United States the patient pays an extra $250 to $25,000 for the antibiotics and up to $16,000 in out-of-pocket expenses compared to those with susceptible bacteria. Furthermore, the cost of insurance increases for patient care with antibiotic-resistant bacteria, between $165 and $250 per year per person, due to the increased cost of care. It is also estimated that insurance costs in the United States will increase by 2030 to between $2.1 billion and $38.8 billion annually due to all antibiotic-resistant infections. The economic burden is further compounded by the downward financial implications on resources; an increase in the consumption of resources with £1.6 million in additional costs is estimated in England for the average hospital due to inpatient days and increasing twice patient needs for outpatient antimicrobial treatment. From a workforce perspective, productivity loss costs are also a result of antibiotic resistance. Physicians are faced with longer appointment times with patients and, worldwide, the cumulative productivity losses for the different scenarios range from $33.3 billion to $47.5 billion. It is estimated that in high-income countries, an additional spending of $52 billion to $127 billion is needed for global tuberculosis control by 2050 due to the increased incidence of drug resistance, leading to a cumulative GDP loss of up to $3.7 trillion. Given the abundance of costs at a societal level, calls are made for urgent economic evaluations to be conducted in order to inform more policy and funding decisions and reposition the resistance agenda as an economic resource tool in support of our public health value system. Economic evaluations differ from cost of illness studies in that they include both the value of health gains and the returns on the financial investments. Planetary economics investigates the long-term effects of antibiotic resistance to provide a basis for comparison of where the most financial resources should be spent in any field of policy, based on scientific evidence and its sustainability. In addressing the global coronavirus disease, the perils of having no economic evaluations in place at the onset of a public health issue became evident and how this paucity is related to a lack of adequate resource allocation by governments for pandemics. Therefore, for antibiotic resistance, looking only at individual costs, such as the cost of hospital stays or antibiotic resistance in only one bacterial species, is not addressing the level of evidence-based care needed to reduce the burgeoning antibiotic resistance epidemic. Economic evaluations, taking into account various scenarios related to any antibiotic-resistant bacteria, could assess where most capital should be allocated in what sector that we should and can afford as we are facing this imminent crisis. Without addressing economic concerns, we are excluding pillars of activity and could lose the game against antibiotic resistance. [24][25]

5. Innovative Treatment Strategies

Innovative Treatment Strategies. When traditional antibiotics no longer efficiently treat an infecting microorganism, it is paramount to consider alternative mechanisms of action, which ideally target

virulence factors while not interfering with the microbiome. Phage therapy is an alternative to traditional antibiotics that is currently being considered. Although it has been extensively used in the form of compassionate treatment, it has more recently developed into a better-defined and regulated therapeutic approach. A prime example herein is the use of the phage cocktail that proved effective against two critically ill patients with an extensively resistant infection. This custom-prepared phage cocktail contained nine different phages, each of which targeted a different virulent gene. Another individual patient, infected with a strain, was successfully treated with a phage-antibiotic combined therapy, in which the phage was administered at a low titer to avoid selecting for phage-resistant bacteria, after which he received ciprofloxacin.

There are also a few antibiotic adjuvants, which act by potentiating the activity of an antibiotic, which have recently been registered for clinical use. For instance, one adjuvant partially protects β-lactams from degradation by serine β-lactamase. Antibiotics become less prone to selection for resistance when degradation of the drug is prevented. Another example comprises inhibitors of β-lactamase: which, when respectively combined with certain antibiotics, lead to the recovery of the native, active antibiotic. Thus, the development of novel antibiotic adjuvants is important for the branching of new entry points into the existing pool of antibiotics. Repurposing of existing drugs in therapy is also of clinical relevance. When two established antibiotics are separately lowly effective, their combined therapy may prove to be beneficial; however, with the development of resistance, the effectiveness wanes. Another approach could encompass the repurposing of the non-antibiotic drug itself, which can also result in antagonistic effects, since the resistance is less, and the potency is often augmented by these new combinatorial therapies. For instance, the use of a calcium channel blocker, which inhibits efflux, alongside a certain antibiotic remains effective in treating infections, and thus, the bactericidal effect increases following the halt of efflux. Furthermore, another compound is also an efficient inhibitor of efflux and is used in hotspot countries against associated infections, reducing the use of another antibiotic; however, heart issues in some patients were noticed, and it was eventually replaced by non-toxic drug derivatives. These therapies hold potential for fighting against pathogen-derived resistance. Overall, the toxicity and effectiveness criteria of these drugs warrant additional extensive research in order to position this combinatorial therapy in the frontline battle against antibiotic resistance. Additionally, strict guidelines must be clearly delineated in order to ensure optimal efficacy and the reduction of potential toxicity risks through thorough clinical trials. Pharmaceutical research to foster creativity and innovation could unlock these therapeutic gems. [26][27]

5.1. Phage Therapy

Phage therapy has been suggested as a promising and complementary approach for the treatment of recalcitrant infectious diseases. Phages are viruses that infect only bacteria; they bind to the outer membrane of susceptible bacterial strains and multiply intracellularly, with the subsequent release from the cell when bacterial osmotic pressure is maximized, resulting in cell lysis. Given clinical interest, at least some phage therapy products have demonstrated efficacy in pilot human clinical trials. In a controlled study on the treatment of chronic rhinosinusitis, phage therapy was found to be as efficacious as standard-of-care treatment. Regulators worldwide also seem amenable to, or are exploring the acceptance of, phage therapy. The sole registered phage therapy clinical trial is currently recruiting patients with various infectious diseases with a phage therapy that will be adapted as per a patient's needs using phage sensitivity testing that can be done in vitro.

As of this writing, the greatest challenge to the uptake of phage therapy globally is the lack of regulatory clearance of phage therapy in various countries, particularly in the United States and Australia. In addition, uncertainty exists regarding the complete standardization and safety of proprietary phage therapy preparations. As bacteria can rapidly develop resistance to phages, they have a multitude of qualities that might indicate them as effective therapeutic options. Similarly, the fundamental aspects of phage therapy and the use of this mode of microbial killing mechanism are largely unexplored. In conclusion, one should consider developing phage therapy as an accurate, therapy-specific mechanism to deal effectively with bacterial recalcitrant and multidrug-resistant

infections. Long-term persistence of phage therapy in hospitals will hinge on, to a substantial degree, the production of clinical trial data that reliably indicate its safety and efficacy. More organized research is required on, and vital questions need to be addressed within the realms of, phage therapy regulatory oversight, including clinical administration, verifications of phage versus phage phylogenetic relationships, and trade-offs in phage therapy product administration. More attempts to develop such agents may prove helpful when added in conjunction with other antimicrobial strategies based on how uniform and functional they are. [28][29]

5.2. Antibiotic Adjuvants

Several adjuvants have been identified that can enhance antibiotic activity; however, the mechanism of action of inhibiting resistance mechanisms has only been demonstrated clearly for a handful. Thus far, inhibitors have been discovered for efflux pumps via efflux pump inhibitors, AmpC and extended-spectrum beta-lactamases with the use of beta-lactamase inhibitors, resistance by loss of porins by using outer membrane permeabilizers to allow antibiotic uptake, and cellular protection mechanisms such as quorum sensing or bacterial two-component systems. Trends can be observed in the effectiveness of combining adjuvants with antibiotics when combinations have been tested in vivo. Enhancing the activity of antibiotics using adjuvants is most effective when the inhibitor targets a resistance mechanism not yet present in the bacteria; for example, using efflux pump inhibitors in cells that do not express the offending efflux pump. This can delay the emergence of resistance to treatment in models and possibly in patients through the inhibition of de novo resistance mechanisms such as signal transduction pathways.

While many of the targets described have undergone significant assay evaluation, and a few even have human doses defined, there remain significant gaps in confirmation of the effect that these compounds may have in patients. An area that requires further study is dosing regimens that effectively achieve target site exposure. This is particularly important for adjuvant compounds targeted towards intracellular targets such as cell signaling. Scant information is available for the long-term treatment of chronic disease that has resulted in biofilm formation. Additionally, identifying the in vivo durability of inhibition of a target pathway is an important viability/feasibility parameter. As many advanced antimicrobial combination routes are at early stages of development, it remains to be seen how much success this group of compounds will achieve as adjuvants. However, the commercial attractiveness of further reducing the duration of therapy for antibiotics in infectious disease areas makes the development of adjuvant-inhibitor combinations an area of interest. A remaining issue is safety. Agents that target the activation or deactivation of intracellular effectors have potential off-target liabilities that need addressing upfront, partly from a safety viewpoint, but also to reduce the chance of lack of efficacy consequent to crosstalk in patients. A related practical problem relates to target validation. No good in vivo models for quorum sensing-based efficacy are currently available. So while demonstrating inhibition of quorum sensing-related phenotypes in vitro will be essential, this will in itself not guarantee in vivo efficacy. A potential for a pipeline validation issue also exists here, since it is unclear how quorum sensing antagonists function in combination with traditional antibiotics. Quorum sensing antagonists, as a rule, will probably only affect biofilm and may be developmentally advantageous only as a trapping strategy in a biofilm infection, for example. Thereby, the approach of targeting drug tolerance could only provide a benefit in the antibiotic combination context, facilitating better penetration and faster cell killing by the antibiotic, rather than being an adjuvant per se, although reflections on this issue are few. [30][31]

6. Future Directions in Antibiotic Development

6.1. Tailoring Treatment

Instead of the traditional one-size-fits-all approach, precision medicine is an emerging trend whereby treatments are personalized to match the requirements of individual patients. The development of targeted therapies for certain forms of cancer has demonstrated the potential of precision medicine in improving therapeutic outcomes. Advancements in genomic technologies

have revealed the diverse genetic backgrounds of pathogens, contributing to our understanding of the subtle genetic variations that may lead to significant differences in patient responses to different antimicrobials. Subsequently, next-generation sequencing technologies are now also being used as tools for the development of first- and second-line therapies that are tailored for individual patients. The very smallest of differences in genetic makeup can have significant impacts on treatment outcomes; thus, highly specific drugs may well become a powerful weapon in our anti-infective arsenal. These technologies primarily focus on targeting resistance mechanisms and are not yet used for the identification of novel antimicrobials, though they may help to identify novel candidates—targeting conserved essential cellular functions that are difficult to mutate using current methods.

6.2. New Tools for Discovery

Artificial intelligence is now being used in the discovery of small-molecule leads that may have antibiotic activities. Although candidate molecules are often expected to fail due to toxicity or develop resistance, AI can help with streamlining the candidate discovery pipeline, allowing predictions of antimicrobial resistance and toxicity to be tested, with an aim of developing efficacious drug candidates more rapidly. Improved techniques in single-cell biology will be an important tool for narrowing down the number of leads that need to be commercially synthesized, with the potential to make small-molecule screening and candidate discovery economical, thereby encouraging investment into this arena. High-throughput screening methods are accelerating the development of alternatives to classic small-molecule strategies, identifying antimicrobial peptides that can disarm and kill pathogens. Antimicrobial peptides are cationic, amphipathic peptides that make contact with the membranes of bacteria, lysing the cells; while these are still in early stage development for new drugs, they are being utilized for numerous applications in areas such as antimicrobial packaging and hospital equipment coatings.

6.1. Precision Medicine Approaches

Precision medicine represents a shift in the paradigm of multiple therapeutic strategies, providing tailor-made treatment for a single patient. This approach offers the opportunity to guide antibiotic therapy through a wide characterization of resistance, including essential genome-based insights, directly applicable in guiding the therapeutic strategy. Importantly, patient-derived diagnostic tools sophisticated for resistance determination are being developed and go far beyond sequencing bacterial genomes. Precision medicine is based on new diagnostic tools to reveal the potential of therapeutic targets and the sensitivity or resistance to different antibiotics in a given patient, representing the clinical application of medical genomics. The identification of an individual resistance pattern or target for specific patient infections represents a way to maximize therapeutic treatment by using the drugs that will target the causative agent, rather than therapy. Due to the poison-pill effect that antibiotics have on the human microbiome and the overall misguided attempts in antimicrobial therapy, some pioneering applications of precision medicine approaches are already being used to help treat infections. While increasing in volume and market penetration, new developments for the massive use of these technologies, including on a single-cell level, are still being developed. Notably, precision medicine marks a significant advance towards personalized therapy. From awareness surrounding biological heterogeneity, resulting in individual variation in disease susceptibility, disease progression, and quality and outcome of medications, a personalized approach offers new ways of assistance to each patient infected, pushing for personalized antibacterial therapy able to cure each patient effectively and without side effects. Although predictive diagnostics remain to be developed, a faster translation of available data is needed for a direct clinical approach. Although the development of new diagnostic methodologies in parallel with new therapeutic strategies is crucial, the effectiveness of ultimately also depends on government involvement in negotiating the cost-benefit of new antimicrobials for the public. In the absence of a modern managed care system, it will be challenging to rein in healthcare costs, regardless of technological advancement. [32][33]

6.2. Alternative Therapies

The stark rise in resistance to antibiotics has increased interest in the development of alternative treatments. Some have hypothesized that with the right adjuvant therapy, our susceptibility to illnesses may rise dramatically. Such therapies encompass a wide range of modalities including immunotherapy, antimicrobial peptides, greater use of vaccines, and many others to come. Diversifying the mechanisms of treatment in our collective toolbox could help reduce the spread of resistance by not over-relying on one type of antibiotic. The initial research for these therapies was against susceptible strains; however, recent research suggests that many of these therapies also show promise in combating antibiotic-resistant strains. In addition to reduced or unaffected efficacy against resistant strains, some of these therapies do harbor potential stumbling blocks, such as regulatory patient inclusion and ideal trial design. Alternative therapies are currently an underappreciated area with great potential, requiring great collaboration from many different fields to fully bring the war to MDR bacteria.

There is potential for revolutionizing the treatment of hard-to-treat and antibiotic-resistant strains to ensure proper progression and early implementation of such therapies. Currently, many novel study designs are underway under many of these umbrella headings, such as investigating attenuated bacteria to deliver cancer-specific antigens. A deeper understanding of the mechanisms of action and precise implementation of these therapies will be imperative. In addition, the potential for synergy with known or developing antibiotics will have the added effect of aiding novel drug development. However, considering the case fatality and high costs of MDR treatment and the superfluous risk of creating resistance, more refined approaches may be of use. Immunotherapies, probiotics, and bacteriophages could be activated for the elimination of resistant strains. Others could be explored for potential synergy and may help drive a new field of immune-stimulating therapies. [34][35]

7. Conclusion and Recommendations

Antibiotic resistance poses a severe threat to global public health; the situation is further exacerbated as pathogens increasingly deploy novel mechanisms to survive the action of clinically available antibiotics. The panel stresses the need for global action to be adopted by the R&D community, medical practitioners, and policymakers to confront and tackle the antibiotic resistance crisis. Increased collaborative ventures and transitions in policy are essential. National and global regulatory agencies should support and facilitate access to in vitro cell and systems-based tools that model human anatomy. Such models should be combined with genomic knowledge and be available across all clinical and research sectors, allowing the best utility to be made of any discovered candidate resistance mechanisms. Recommendations Improved Battery of Detection Methods. Develop new detection initiatives to improve the identification of antibiotic resistance. Next-generation culture-based and non-culture-based detection methods should be able to identify novel resistance mechanisms by reflecting the human situation more accurately. Evaluate the Feasibility of Mechanism-Based Antibiotics. Evaluate the feasibility and the added benefit of developing mechanism-based antibiotics for targets that are more conserved within a multidrug class, or investigate whether non-classical antibiotic approaches would be the best course of action. Revise Breakpoints. Evaluate strategies for the regulation of resistance against the current antibiotic classes, as these are needed for animal health as well as in humans. Educational Support, Surveillance, Correction of Policy, and Legislation coupled with Acceleration of Innovations. Research should also target the development and delivery of new antibiotics or alternative strategies for empirically difficult-to-treat patients. Antimicrobial stewardship programs should educate healthcare practices to adapt for differential first treatments based on a patient's risk profile and symptoms rather than the site of infection or a presumed resistance profile. Public health initiatives and policies should also be designed to reduce the indiscriminate systemic consumption of important antibiotics to ensure that final drugs are retained for future infectious disease outbreaks.

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