Review Article
Volume 8:6, 2022

Journal of Pharmacognosy & Natural Products

ISSN: 2472-0992 Open Access

Trends in the Design and Discovery of Antibiotics using Natural Products: Bioresources and Capacity Building in Africa

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Abstract

The problem of increase in antibiotic resistance is often linked with the slow pace at which new antibiotic compounds are discovered and developed. Among several other factors, the problem has been exacerbated by most pharmaceutical companies losing interest in new drug discovery and development process owing to insurmountable cost barriers of the drug research and development for novel antibiotic drugs. Most large pharmaceutical companies have abandoned natural product screening for drugs in favour of high throughput screening of chemical libraries consisting of laboratory parallel and massively synthesized small molecules, made by combinatorial chemistry approach, which have often culminated in failure. Recent advances in analytical chemistry, chemical synthetic methods, computational chemistry, computational biophysics, computational biology, genomics, proteomics, bioinformatics and microbiology, are making screening of natural products once more amenable to drug discovery. This review focuses on the advances and technologies currently available for discovery, design and development of novel antibiotic drugs for clinical practices, and the current status, progress and capacity building for exploration of the rich biodiversity in Africa. In addition, strategies involving public-private partnerships including sharing and pooling together of resources-biological, technical and financial, for screening of natural products, as well as technology transfer and expertise sharing across Africa are discussed.

Keywords: Antibiotic resistance • Drug discovery • Natural product • Plant antibiotics • Microbial • Resistance-modifying agents • Virtual screening • Metagenomics • Antibiotics • Combinatorial chemistry • Diversity-oriented synthesis • Computational biology • Computational chemistry • Computational biophysics

Introduction

Antibiotics are substances that either kill or inhibit microbial growth. The problem of increase in antibiotic resistance is often linked with the slow pace at which new antibiotic compounds are discovered, developed and approved for treatment and accelerated by inappropriate use of antibiotics. This problem has been exacerbated by most pharmaceutical companies losing interest in new drug discovery owing to insurmountable cost barriers of drug discovery research and development for novel antibiotic drugs. Currently, the cost for developing new drugs to delivering the drug to the market is estimated at USD 2.6 billion, according to the tufts study [1].

The pace of discovery and development of new antibiotics has not been commensurate with the increasing antibiotic resistance. Over the last 40 years, only two new antibiotic classes have been approved, that is daptomycin, a lipopeptide isolated from *Streptomyces roseosporus* and linezolid, a synthetic oxazolidone. This slow pace in discovery of novel antibiotic compounds and eventually new drugs worsen the problem of antibiotic resistance because resistance genes are positively selected against even the most recently approved antibiotics, in microbial populations thus the wide-spread nature of the problem. The shortage in novel antibiotic classes developed is the biggest stumbling block in efforts to combating the increasing antibiotic resistance. This calls for focused efforts on the search for new antibiotics.

One of the main contributors to the lag in novel antibiotic development is rediscovery of the same classes of antibiotic compounds, time and again. These represent the low hanging fruits, and thus frequently encountered in screening efforts. In order to

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Received: 23-Sep-2021, Manuscript No. jpnp-21-001-PreQc-22; **Editor assigned:** 27-Sep-2021, PreQC No. jpnp-21-001-PreQc-22 (PQ); **Reviewed:** 11-Oct-2021, QC No. jpnp-21-001-PreQc-22; **Revised**: 04-Jul-2022, Manuscript No. jpnp-21-001-PreQc-22 (R); **Published**: 02-Sep-2022, DOI: 10.37421/2472-0992.2022.8.190.

to be able to find novel antibiotic compounds, a deeper screening is required. This requires new tools and approaches to bioprospecting, than those already being used. Advances in genomics, proteomics and transcriptomics are opening new horizons and promise [2]. There are by far vast potentials in more than 99% of the microbial world awaiting bioprospecting. Thus, we have only scratched the surface. The basic challenge has often been the difficulty of growing in lab cultures most of the "uncultivable" microbes. However, with novel tools and techniques to grow microbes, scientists are now gaining access to more distinct microbes and their unique secondary metabolites with unprecedented potential for development into novel clinically important antibiotics. Plants present another resource for novel antibiotics, though success of discovering antibiotics of clinical importance from plants have been low.

Africa is rich in bioresources that can be explored for novel antibiotics. In this review we discuss the trends in drug discovery with the particular emphasis on the relevance of screening natural products and natural products derivatives from microbes and plants, for novel classes of antibiotics as the solution to the increasing problem of antibiotic resistance. We also discuss recent advances that are relevant for natural product research. In addition, this review focuses on the exploration of African biodiversity, especially the unexploited microbes and plants for new antibiotic agents and clinical antibiotics. The strategies such as sharing and pooling together of resources and expertise across Africa for bioprospecting are discussed.

Natural products are secondary metabolites. Secondary metabolites are organic compounds synthesized by living organisms that are not required for growth, reproduction and development, but rather produced as a response to the abiotic environmental stimuli, thus enabling the organism to survive harsh conditions such as humidity and temperature extremes, herbivory, drought, salinity, radiation and wounding. It is important to mention here that secondary metabolites are not essential for survival; however they confer an evolutionary advantage to the organism producing them.

Generally speaking, natural products have been optimized through evolution as biologically relevant compounds and serve as excellent sources of compounds of medicinal value. In addition natural products act as scaffolds for designing new bioactive compounds for drug discovery. Molecular scaffold is that most basic part or substructure of the molecule, that determines its shape, influences global molecular properties and determine the activity of the parent molecule. Natural products inspire drug discovery by providing scaffolds for pharmacologically relevant molecules.

Screening of natural products has contributed by far most of the medicines in use today. Over one-thirds of all new molecular entities approved by the US Food and Drug Authority (FDA) are natural products or NP-derived compounds. With most NP being derived from mammals, then microbes and plants in that order. Antibiotic substances from natural products account for more than one half of the antibiotics currently registered by the FDA. Natural products continue to be the source of antibiotic compounds discovery. All major antibiotics, with the exception of quinolones and sulfonamides (both synthetic) and DNA gyrase inhibitors, are all derived from natural products. It is hoped that most lead structures will continue to be derived from natural products.

In its infancy, drug discovery involved mainly crude chemical and physical methods for isolation, separation and purification of the secondary metabolites, followed by biological assays to investigate bioactivity of the natural products. Currently drug discovery is a multi-faceted discipline drawing insights and expertise from botanical, molecular, phytochemical and biological techniques. Other disciplines that find application in drug design and discovery include bioinformatics, genomics, proteomics and computational chemistry.

The golden era of antibiotics discovery marked the introduction of most important antibiotics in use today. Most of these have been discovered by screening natural products. Antibiotics discovered by screening natural products were polymyxin, tetracyclines, chloramphenicol, lincomycin, erythromycin, streptomycin, ristocetin, sulfazecin (monobactam), thienamycin, penicillin G, penicillin V, cephalosporin C, clavulanic acid, vancomycin, cephamycin, SQ 26, 180 (monobactum) and oleandomycin. Most of these were isolated from microbes. However, with plants, no novel antibiotic of clinical significance has been discovered.

In folklore medicine plants were mostly used as a source of medicines. The use of plant natural products for therapeutical purposes for as long as human has existed provide clues that these products might contain pharmacologically active principles. Indeed, most drugs in use today have been isolated from natural products for which ethnomedicinal uses were well elaborated.

Most pharmaceutical companies abandoned natural product screening and turned to other platforms for drug discovery; and instead opted High Throughput Screening (HTS) of small synthetic molecules produced by combinatorial chemistry. These HTS screening methods were used in combination with other approaches, such as the application of bioinformatics in target identification and validation; and more generally for *in silico* drug discovery. However, because of the failure of HTS and the realization that these small molecules lacked the complexity of drugs derived from natural products, the libraries would later be expanded to include Natural Products (NP), their derivatives, or synthetic analogues (mimics). Even so, HTS was consistently met with only meager success. Screening natural products is now left mostly to academic and research institutions.

Antibiotics are isolated from nature, or chemically synthesized, or made through semi-synthesis. The processes from isolation or synthesis of compounds to testing and identifying those with promising bioactivity, to drugs is called drug discovery [3]. Drug discovery proceeds from lead discovery, through lead validation, to lead optimization. A number of approaches are currently being used for lead discovery, These includes screening of a collection of libraries of natural compounds, screening of large corporate small molecules libraries synthesized by

combinatorial chemistry; rational drug design and *in silico* screening.

This paradigm shift has been the case because of the inherent difficulties in preparing natural product libraries, when compared with the screening libraries chiefly composed of small synthetic organic molecules made form combinatorial chemistry syntheses. Also the high rate of re-discovery of the same bioactive compounds from natural products sources has discouraged the efforts to interrogate the natural product resources for novel lead compounds. It is astonishing however that these libraries are increasingly being designed to mimic natural products, though natural products themselves are generally overlooked. Another approach, the *in silico* drug discovery, did not expedite the drug discovery process and cut down the cost, as anticipated. This was thought possible through by-passing the expensive laboratory and field work for target discovery, validation and drug development, the approach has not been accompanied by the successes forecasted so far, as evident in the outcomes that only a handful of drugs have been discovered by these methods [4]. This new platform resulted into only a handful of new drug compounds being discovered despite immense screening campaigns. For this reason screening of natural products for medicinal compounds declined.

It is important to point out that the main reasons rational drug design and HTS did not turn very promising outputs despite the massive investments were that the small molecules used in screening had to obey the Lipinski rule of five on drug-like properties (pharmacokinetics) so that the molecules that pass the criteria would be screened against purified targets. Although a significant number of these small molecules would pass through these screens, most of them would not be developed into drugs because they could not penetrate bacterial cell membranes. As you can see, this contrasts with the success stories of *in vivo* screening.

It is encouraging however, to learn that technological advances in next generation sequencing, medicinal chemistry; combinatorial chemistry and bioinformatics are making it possible to overcome barriers to natural-product-based drug discovery. In addition, massive strides in the progress of biomedical technologies have provided the solution to the problems of structure elucidation and that of rediscovery of known secondary metabolites through dereplication, which can be excluded right at the stages of screening of crude extract, thus avoiding unnecessary cost used to rediscover known pharmacologically active natural products. For instance, the presence of novel tools such as NMR is making easier structural elucidation and help uncovering new structures of novel bioactive compounds. Also, new developments in chemical biology have revolutionalized target elucidation. Computational tools are already pushing frontiers in natural product research, making screening of natural products once more feasible than it was in the past and may provide more target hits and lead compounds than previous approaches were able to provide.

As far as resources for bioprospecting are concerned, only a very small fraction of nature has been screened, not to mention that most of the aquatic organisms, plants and animals have not been screened for antibiotic compounds; plus 99% of microbes not yet explored because they cannot be cultured. This represents the unexploited reservoir of immense chemical diversity of biologically relevant molecules that are stocked in nature waiting to be explored. This represents an area of immense potential in the discovery of new leads compounds.

Literature Review

The increasing concerns of the rise in antibiotic drug resistance

Antibiotic resistance is a natural phenomenon. However, overuse and misuse of antibiotics, poor infection control, inadequate sanitary conditions and inappropriate food handling accelerate the increase in antibiotic resistance. For instance, the widespread use and misuse of antibiotics for instance massive uses of antibiotics in agriculture and haphazard disposal of antibiotics to the environment have been shown to cause the increase in prevalence of resistant strains.

The concern with antibiotic resistance stems from its rapid emergence followed through by its global spread at a rapid rate, resulting in failures of current antibiotics to treat effectively common hospital and community acquired infections. This means more deaths as a result. Death rate for people having infections that are drug-resistant is double the number of people infected with susceptible strains [5]. Also, costs required to treat diseases caused by drug-resistant strains are considerably higher than treating normal cases.

The widespread increase of antibacterial resistance is epitomized by the 'ESKAPE' organisms which include *Enterococcus* sp., *Staphylococcus aureus*, *Klebsiella* spp, *Acinetobacterbaumannii*, *Pseudomonas aureginosa* and *Enterobacter* sp. All of which are resistant to antibiotics and cause difficult-to-treat nosocomial infections. The problem is particularly worse with some gram negative bacteria such as *A. bamaunnii* which are resistant to virtually all antibiotics. Multidrug-resistant tuberculosis is also increasing fast with 480,000 new cases reported in 2014 by WHO surveillance.

The rise in antibiotic resistance paints a gloomy picture for modern medicine. Without effective anti-infective treatment, many standard medical treatments will fail. This problem is likely to culminate into the post-antibiotic era if deliberate efforts involving all the stakeholders are not taken. In this respect, antibiotic stewardship which means best antibiotic practices is very important to slowing down the spread of antibiotic resistance. Other equally important measures to combating antibacterial resistance include the development of new antibiotics, effective resistance surveillance tools and diagnostics tests. New drugs are needed to keep up with resistant bacteria.

The lag in antibiotic discovery and development

The period between 1950s and 1970s is known as the golden era of antibiotic discovery because this period saw the introduction of most novel classes of antibiotics into clinical practice. Years after the antibiotics golden era have been marked by a hiatus in antibiotic discovery and development. The number of new chemical entities approvals has gone down significantly. Research and development of new antibiotic drugs have been faced with higher attrition and failure rates for investigational compounds, and the cost of launching new drug has skyrocketed as a result. The cost of launching the new drug to the market has doubled in the past decade to about US$ 2.6 billion.

The plausible reasons for this downward trend for antibiotic discovery and development are the changes in the regulatory environment in drug development, the increased drug safety restrictions, the prohibitively high cost of new drug discovery research and development measured in terms of market prospects of the new drug developed; and the failure of modern drug discovery approaches and technologies to expedite drug discovery process as previously anticipated.

For the past five decades, the need for new antibiotics has largely been met by semi-synthesis of new antibiotics tailored after the natural product scaffolds of antibiotic compounds discovered in the middle of the 20th century. Following 40 years without a new antibiotic class there followed approvals of new classes of antibiotics between 2000s and the early 2010s. For instance, in 2003 a new antibiotic, daptomycin, discovered from the soil bacteria, *Streptomyces roseosporus* was approved. Later in 2013, a new polyketide, anthracimycin was discovered from the marine *Streptomyces* bacteria. Later, fidaxomycin isolated from the actinomycete *Dactylosporangium aurantiacum*; active against *Clostridium difficile* was discovered. However, the problem is that the discovery of new antibiotics has not been able to keep pace with the development of resistance, plus resistance is mounting even against recently introduced drugs such as linezolid. In another case, resistance against daptomycin is shown to be mounting in glycopeptide-resistant enterococci. This represents a general scenario where microbes are adapting to the selective pressure those antibiotics pose on them [6].

Antibiotics in the pipeline

The antibiotic pipeline is drying up. In contrast, the demand for novel antibiotics is increasing fast in the light of ever rising concern of antibiotic resistance. Even so, only a few antibiotics are in the pipeline, most of these undergoing innovations of incremental nature, that is, modification and optimization of the currently used antibiotics resulting into the same classes of antibiotics with minor improvements. The demand for novel antibiotic classes is based on the premise that pathogens will not be able to develop resistance against them as fast as they can against antibiotic classes already prescribed in clinical practices.

As of September 2015, approximately 39 new antibiotics were in clinical development. However, the attrition rate is high such that only 20% of candidates that enter phase-I clinical trials will be approved for clinical practices. As of present greater promise lies with NCE and NME in clinical development, that are effective against antibiotic resistant gram negative bacteria, especially those of the ESKAPE group because they have evolved several resistance mechanisms leaving only fewer treatment options.

The report presented by the Pew Charitable Trust on December 17, 2015 revealed drugs in clinical phase-I; which included a monosulfactam BAL30072. This drug had activity against resistant gram-negative ESKAPE bacteria and urgent CDC threat pathogens. Another compound with promising activity in phase I was a combination Aztreonam/Avibactam7 (ATM-AVI)-a combination of monobactam and a novel β-lactamase inhibitor; while ceftaroline/avibactama, cephalosporin/novel β-lactamase inhibitor combination, now in phase-II, has a potential indication for bacterial infections. Drugs in phase-III with potential indication against gram negative ESKAPE pathogens and urgent CDC threat pathogens included plazomicin; a combination imipenem/cilastatin plus relebactam (MK-7655) (which is a carbapenem plus a novel β-lactamase inhibitor); eravacycline (tetracycline) and carbavance (RPX7009 plus meropenem)-a combination of meropenem and a novel boronic β-lactamase inhibitor. Last year (on 25th Feburary) saw the approval avycaz, (ceftazidime+avibactam)-a combination of a cephalosporin and a novel β-lactamase inhibitor with expected activity against ESKAPE bacteria and urgent CDC threat pathogens. Avycaz was approved for complicated UTIs, pneumonia, bacteremia among others. Most antibiotics in clinical trials are mostly modifications of the existing antibiotics or synergetic combinations with other antibiotics or β-lactamase inhibitors. For example, avycaz is a combination of an antibiotic cephalosporin with a novel β-lactamase inhibitor (Figure 1).

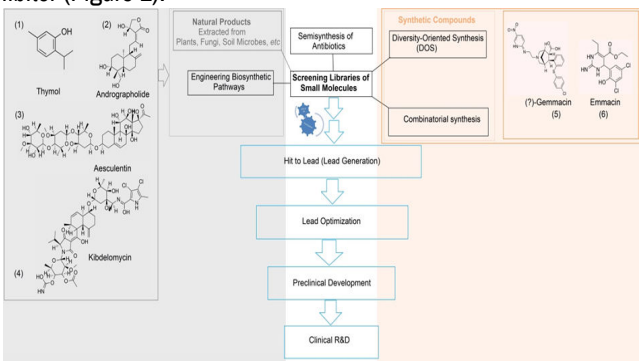

Figure 1: The conceptual diagram summarizes the different approaches that have been used for the antibiotic drug design and discovery.

Innovation in antibiotics as a strategy to overcome antibiotic resistance

A number of strategies have been put in place to overcome antibiotic resistance. They involve as many different approaches as their mechanisms and targets. Some of the strategies discussed (Figure 1) focus on improvements of innovative

or incremental nature on drugs that are currently being prescribed in clinical practice, to make them more effective.

Drug modifications and antibiotic improvement: Since discovery of new antibiotics and its subsequent development and approval is a daunting task, modifications of the current drugs to make New Molecular Entities (NME) seems a logical approach to prolong the medical 'life' of the current antibiotics. NMEs are drugs containing active moieties that have been approved by the FDA. Innovation of the existing antibiotics to improve pharmacokinetic and pharmacodynamic properties of the drug, such as potency, bioavailability and reduce or eliminate side effects to make NMEs is a good approach to overcome antibacterial resistance.

Take penicillins, for instance. Penicillins enjoyed a lot of successes as an effective antibiotic. However, emerging resistance to penicillins would have turned them obsolete if it were not for the semisynthetic penicillins. Semisynthetic penicillins have broadened the spectrum of activity against bacteria, have improved features that make them suitable for use as either parenteral or oral drugs; and are cheaper than most other antibiotics. In another instance, ampicillin-a semisynthetic derivative of penicillin, exhibits greater bioactivity than either benzyl penicillin or phenoxymethyl penicillin. Though not resistant to the MRSA that emerged later, semisynthetic derivatives of penicillin such as flucoxacillin, methicillin and dicloxacillin were effective treatment against the β-lactamase resistant bacteria than were their predecessors.

In addition, synthetic biology, medicinal chemical modifications of scaffolds of natural products and *de novo* synthesis of new natural-product inspired scaffolds can lead into the generation of screening libraries to use as resources for HTS. Chemo-biosynthesis makes possible chemical and biological syntheses approaches for structural modification of antibiotics, for instance by introducing altered building blocks to mutant microorganisms to enable mutasynthesis.

The processes above are applicable in a range of diverse antibiotics produced by different bacteria. Most common approach used to generate semisynthetic antibiotics is to introduce analogues of precursors in the fermentation cultures of microbes producing antibiotics. In a Mutational Biosynthesis (MBS) study, emblematic of the general processes used to generate structurally diverse analogues of antibiotics, either streptamine or epistreptamine used as synthetic analogues of precursors, were introduced to a culture of mutant strain of *Streptomyces fradiae* blocked for the synthesis deoxystreptamine [7]. The products of the fermentation process were the aminoglycoside antibiotic analogues of neomycin, hybrimycins. Analogous approaches have been applied on macrolide antibiotics to synthesize analogues. A macrolide, flurithromycin, an analogue of erythromycin A has been produced by introducing a semi-synthetic precursor 8-fluoroerythronolide B introduced into culture of *Saccharospolyspora erythrea* idiothroph blocked in the synthesis of aglycone.

Another approach, termed precursor-directed biosynthesis supplements the natural product chemical diversity. Precursor-directed biosynthesis involves the introduction of the synthetic structural scaffold moieties analogous to the natural core structures into the fermentation culture of wild type strain, which utilizes biosynthetic pathways to produce novel analogues. Several examples of synthetic oligoketides introduced into mutant strains of genetically engineered *E.coli* or *S. coelicolor* to produce novel and unnatural polyketides leading to novel derivatives of erythromycin A. Precursor-directed biosynthesis holds vast potential as a means to produce molecular analogues of antibiotics with added pool of structurally diverse molecules to screen for antibiotic activity or modify the activity of the known antibiotics.

Engineering biosynthetic pathways to produce new antibiotics: One of the problems facing natural product drug discovery is the issues of access and supply of the biological resources. It is time costly and a cumbersome task to isolate natural products from biological resources. Some important secondary metabolites are produced in small quantities. Also, synthesizing highly complex natural products through total chemical synthesis is quite challenging and a formidable task. However combinatorial biosynthesis offers the means to extend the diversity of the complex natural products by synthesizing analogues of the natural products with good bioactivity or less side effects. Engineering the metabolic pathways, such as β-lactam production to produce larger titers of the antibiotic of interest presents the opportunity to solve the problem of supply and access, but also produce cheaper drugs (Figure 1).

Understanding the biosynthetic pathways provide clues to increasing the antibiotic titre. A classical example of this is the use of a *Penicillium chrysogenum* BW 1890 strain with a high copy number of penicillin biosynthetic genes causing overexpression and subsequently high quantities of penicillin. Also, the use of a strong promoter with penicillin biosynthetic genes resulted in a rise in transcriptional levels and increased penicillin biosynthesis.

Recombinant DNA techniques have been used to clone genes or gene clusters encoding metabolic pathways synthesizing antibiotics, into heterologous hosts amenable to genetic modifications, such as *Streptomyces* sp. known for its genetic alterability. Genes encoding secondary metabolites reside in chromosomes and are termed structural genes. One classical example is the production of hybrid antibiotics by combining genes encoding Polyketide Synthases (PKS). Polyketides are synthesized by decarboxylative condensation of acyl thioesters, especially acetyl CoA, malonyl CoA, methylmalonyl-CoA and ethyl malonyl CoA. Enzymes that catalyze the process are the polyketide synthases.

In one study, genes and gene clusters encoding erythromycin A biosynthetic machinery of *Saccharopolyspora erythraea* was genetically engineered in a vector, *E.coli* vector to produce erythromycin A analogues. This was done because *S. erythraea* was not amenable to genetic manipulations. However, using *E.coli* as a heterologous host it was possible to express an insert of altered biosynthetic gene cluster from *S. erythreae*

in *E.coli*. This study demonstrated the substrate flexibility and promiscuity of enzymes in catalyzing reactions using analogues of precursors in various metabolic pathways. This proof of principle drives combinatorial biosynthesis.

The first hybrid antibiotic was made this way. In the process, a recombinant strain of *Streptomyces* sp., expressing gene clusters was transferred from strains encoding actinorhodin, granamycin and medermycin biosynthesis, culminating in the production of two hybrid antibiotics-mederrhodin A and B, analogs of medermycin, with mederrhodin A (6-hydroxymederrhodin) having the same bioactivity as medermycin and mederrhodin B showing no any bioactivity.

The approaches are therefore integrative, incorporating inputs from synthetic biology which engineers the genome of the host to optimize features required for the target biosynthetic pathways, synthetic chemistry that introduces synthetic analogue precursors or synthetic scaffolds and integrated-omics to produce diverse and novel derivatives of secondary metabolites for screening.

A combination of β-lactamase inhibitors (BLI) with β-lactam antibiotics: Resistance to most antibiotics is mounting, partly due to production by some pathogenic bacteria of rare or previously unknown *β*-lactamases-enzymes that break down β-lactam antibiotics such as penicillins, cephalosporins, monobactams and carbapenems. Therefore one strategy that offers ways to overcome resistance would be to use a combination of a β-lactam antibiotic with a BLI in order to extend its spectrum of antibacterial activity. Like the antibiotics, the BLI have faced slow development over years. BLI that are currently in use are clavulanic acid; that is commonly used in combination with amoxicillin; sulbactam with ampicillin; and avibactam-an approved BLI used in combination with ceftazidine. Investment in BLI promises to revive and extend the life of β-lactam antibiotics that are currently becoming more and more ineffective against resistant bacterial strains producing β-lactamase. The potential of BLI to alleviate the problem of resistance by β-lactamase and extended spectrum β-lactamase producing bacteria is manifested in the increased investment in research and development of β-lactam inhibitors. A number of pharmaceutical companies are developing their BLIs. At the time of writing this review, Merck Company was recruiting for clinical trials phase-III for its BLI, relebactam. BLI show a potential to restrict resistance development in other pathogenic bacteria such as *Clostridium difficile* and vancomycin resistant enterococci. However, it is important to mention that BLIs are more effective against gram negative bacteria because their resistance mechanisms involve production of β-lactamases, whereas resistance mechanisms in most gram positive bacteria is based on them producing penicillin binding proteins with variations on their structures reducing binding affinity to penicillins [8].

Although these improvements in antibiotics have produced effective antibiotics, sooner or later antibiotic resistance develops against these drugs. The sustainable solution would therefore be to explore for novel classes of antibiotics with new mechanisms of action.

Combination therapies: The synergistic effect of a combination of antibiotics has shown promising *in vitro* results for antibiotic activity against resistant strains. For instance, the combination of an aminoglycoside (or a fluoroquinolone) and a β-lactam antibiotics is effective against gram negative bacteria *in vitro*. This approach offers possibilities for new ways to combat antibacterial drug resistance, and is recommended for treating sepsis and septic shock caused by severe infection with multi drug resistant gram negative bacteria. Although a last resort for treating resistant strains, collistin is a component of most effective combinations.

Screening natural products for novel antibiotic compounds

One of the reasons that the discovery of novel drugs from nature has been slow is the problem of rediscovering the same compounds from nature. However, despite the rich biodiversity accessible for screening in Africa, there are many bioprospecting studies still being done without dereplication, thus leading to futile investment of time and resources to rediscover known compounds. Therefore, in order to improve the efficiency in natural product bioprospecting dereplication of natural products that have been isolated previously is necessary to avoid rediscovering the same compounds. Dereplication is the rapid identification of known compounds in a crude mixture of a sample possessing bioactivity prior to isolation, with the purpose of avoiding the isolation of compounds already known (Figure 2).

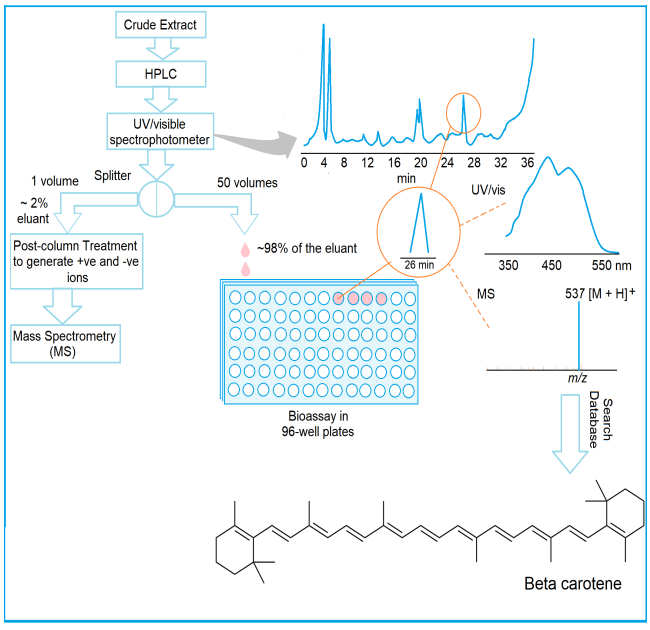

Figure 2: The conceptual depiction of the dereplication process used in natural product drug discovery to avoid rediscovering already known compounds.

Screening plants for antibiotic compounds: Plants have unparalleled high chemical diversity. This high chemical diversity compensates for the presence of an array of pharmacologically inactive secondary metabolites present in plants. This chemical diversity, shaped through eons of evolution has horned plant chemical defenses. The chemical diversity in plants makes them a good screening resource against

against a number of targets, to generate hits and eventually active lead structures for drug discovery. This is attested to by the fact that 25%-50% of pharmaceutical drugs are plant-derived.

Although random selection and screening of plant resources has produced some important clinical drugs, traditionally, ethnomedical records have been the basis of selection for plant materials used for screening in drug discovery. Taking as an instance the screening campaigns by The National Cancer Institute (NCI) of a staggering > 120000 plants for novel anticancer agents, the massive screening efforts faced diminished returns, leading to a handful clinical drugs Taxol® and Camptothecin. On the other hand ethnomedical reports have been really helpful in narrowing down the scope in a number of plants to be screened to find active leads. Given the high chemical diversity of plants, coupled to the fact that only a few of the secondary metabolites are active, success rates are smaller when plants are randomly selected and screened without prior knowledge of the pharmacological activity, that hint on the possible presence of medicinal compounds of interest.

Higher plants consist of approximately 250000 species, but only 5%-15% of these have been screened for medicinal secondary metabolites leaving out vast resources yet to be exploited for potential lead candidates for drug discovery. Plant antibiotic compounds are distributed across different classes of secondary metabolites. These are flavonoids, such as the isoflavonoids, tannins, quinones, phenols, terpenes, flavonoids or alkaloids. Potent pharmacological activity of some alkaloids has led to their use as drugs. Alkaloids are amines derived from amino acids; and contain nitrogen atom in a heterocyclic ring. Alkaloids consist of compounds that possess antibiotic activity. These are such as sanguinarine and berberine. Alkaloids have been shown to exhibit effective bioactivity against *S. aureus*, *S. mutans*, *Microsporum gypseum*, *M. canis* and *Trichophyton rubrum*, with MIC ranging from 16µg ml^{-1} to 400µg ml^{-1} in one study. Examples of alkaloids include berberine from *Berberis vulgaris*, benzyl benzoate (used as a scabicide) from many plants, andrographolide from *Andrographis paniculata*, emetin (an amoebicide) from *Cephaolisipocacuanha*, Hemsleyadin (bacillary dysentery) from *Hemsleya amabilis*, thymol (a topical antifungal) from *Thymus vulgaris*. Another important alkaloid showing antibacterial activity is a diterpene ferruginol, isolated from the redwood *Sequoia sempervirens*. Ferruginol has excellent antibiotic activity against *B. brevis* and a good bioactivity against other gram negative and gram positive bacteria.

Andrographolide is a diterpene lactone that is isolated from the leaves and stems of *Andrographis paniculata*. It is used to treat bacillary dysentery. Berberine is a quaternary ammonium alkaloid salt isolated from a number of species, such as *Berberis vulgaris*, *Berberis aristata*, *Berberis aquifolium*, *Hydratis canadensis*, *Xanthoriza simpliciana*, *Phellodendrona murense*, *Coptischinensis*, *Tinospora cordifolia*, *Argemone Mexicana* and *Eschscholzia californi*a. Berberine is used to treat bacillary dysentery.

Tannins are a class of secondary metabolites with diverse structures; some of which possess antibiotic activity. Examples of tannins discussed here are epicatechin, epigallocatechin gallate, epicatechin gallate and ellagitannin. Epigallocatechin gallate has shown a significant antibiotic activity against a number of bacteria such as *Stenotrophomas maltophila*, a pathogen causing nosocomial infection, has potential in treatment of MRSA and methicillin-sensitive *S. aureus*. Epicatechin gallate has attracted much attention in research due to its ability to reverse methicillin resistance in pathogenic bacteria such as *S. aureus*, and thus presents prospects for combating antibiotic resistance in MRSA [9].

Coumarins are important plant antibiotics. Coumarins exhibit broad-spectrum antibiotic activities against bacteria. In one study synthetic and naturally-occurring coumarins were tested against clinical isolates of MRSA either alone or in combination with clinical antibiotics. Results revealed that coumarins are resistance modifying agents, yielding antibiotic activity against MRSA even matching that of vancomycin when coumarins are combined with oxacillin 8-iodo-5,7-dihydroxycoumar. One important coumarin with significant antibacterial activity is Asphodelin A, an arylcoumarin isolated from *Asphodelus microcarpus*.

Isocytisoside and eucalyptin are flavonoids with excellent antibacterial activity. Isocytisoside isolated from *Aquilegia vulgaris* exhibit antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Eucalyptin, isolated from Eucalyptus sp., exhibits potent antibiotic activities too. Flavonoids are built from flavones. Some flavones such as Luteolin and hydroxybiflavonol show potent antibiotic activity. Luteolin is isolated from a number of different plant species such as *Chresta scapigera*, *Achillea tenuifolia* and *Soymida febrifuga*. Luteolin and luteolin derivatives demonstrate excellent *in vitro* activity against gram positive bacteria, such as *Bacillus subtilis*, *Staphylococcus* sp. and *Salmonella typhimurium*.

Terpenes with antibiotic activity include episiferol and 1-oxoferruginol. 1-oxoferruginol has been isolated from the roots of *Salvia* sp., and exhibited bioactivity against *B. subtilis*, *P. aureginosa*, *P. mirabilis* and *S. epidermidis*. Hemsleyadin is tetracyclotriterpenoid characterized by a bitter taste. Hemsleyadin is used to treat bacillary dysentery. Wang, et al. [10], showed the bioactivity of Hemsleyadin against *Shigella flexineri* to be excellent. In addition Hemsleyadin exhibited higher bacteriostatic activity than chloramphenicol, against *Salmonella typhi*, *Shigella dysenteriae* and *Staphylococcus aureus* than chloramphenicol.

Thymol a monoterpene phenol, extracted from *Thymus vulgaris* possesses antibiotic activity, for instance against *Pseudomonas aureginosa*. Thymol shows synergetic effect when used in combination with penicillins, thereby reducing antibiotic resistance to penicillins. Another interesting bioactivity of thymol is its antifungal activity against fluconazole-resistant fungus such as *Candida* sp.

Although the mechanism of plant antibiotics is not yet fully understood, studies suggest they have effect of increasing the

permeability of efflux pumps. Despite the wide spectrum of bioactivities and the diversity of antibiotic agents synthesized by plants, no single antibiotic compound of plant origin has been developed for clinical uses, one reason being that the minimum inhibitory concentrations for most plant antibiotic compounds exceeds 1000 µg/ml and are therefore of no clinical relevance. This is partly because plants, unlike fungi or microbes do not possess specialized chemical defense against microbes and as a result resort to the use of toxic metabolites or synergistic combination of a plethora of secondary metabolite for defenses. However, experimental evidence has proved the utility of plant-derived antibiotics as Resistance Modifying Agents (RMA) when used in combinations with other antibiotics to kill or inhibit microbial growth [11].

However, plant natural products could find their way into clinical settings by developing more potent derivatives or synthetic analogues of known secondary metabolites with promising bioactivities. A number of efforts are being done to realize this. For instance, the development of luteolin derivatives has yielded derivatives displaying a significant *in vitro* activity against some selected bacteria. In addition, plant antibiotic substances can be used as resistance-modifying agents in synergetic combinations with clinical antibiotics to make bacteria resistant to these drugs susceptible. Generally, increasing the diversity of natural-product like compounds through combinatorial chemistry and diversity-oriented synthesis is currently considered a promising approach to generating candidate molecules for screening.

Genomics Approaches to Antibiotic Drug Discovery from Microbes: Discovery of therapeutic target has been a hindrance for drug discovery process. Genome sequencing provides information about genes and proteins that are disease modifying and therefore potential targets for lead discovery [12]. Indeed the time for lead discovery and validation has been considerably decreased with the increase on genomic information on possible drug targets. Genomics and proteomics provide the key to decode and understand the various metabolic processes and biosynthetic networks in cells, coordinated by a network of signaling pathways. Processes that are involved in disease mechanisms are particularly a key to identifying novel targets to screen for lead discovery.

The advances in genomics and bioinformatics has revolutionalized drug discovery in a number of ways. The completion of the sequencing of the first bacterial genome sequence *Haemophilus influenza*, in 1995 highlighted the potentials of using structure-based approach to drug discovery through the identification of the possible proteins and gene targets for drugs that is the "druggable" genome. In addition, genomics could be integrated in the drug discovery process to understand aspects of safety assessment. The subsequent years marked several completed genomic sequences of pathogenic bacteria. This information is crucial in identifying genes encoding virulence factors since these factors are the possible targets for drugs. The genomic sequences of most pathogenic bacteria revealed highly conserved motifs that could be exploited as targets for broad-spectrum antibiotics [13]. One example of such highly conserved protein motif that has a potential for broad-spectrum antibiotic and anti-parasitic drug target is Peptide Deformylase (PDF). Post-genomic era has resulted in the increase of the number of therapeutical targets. As far as structure-based drug design is concerned, the more the number of pathogens sequenced, the greater the number of archived drug targets against which *in silico* screening of compounds, synthetic, semi-synthetic or natural, can be done. This is anticipated to increases chances of finding target hits and subsequently lead drug candidates. In addition, genomics and proteomics has increased our understanding of disease mechanisms and revealing more targets that were previously unknown [14].

The genome of a cell is relatively static when compared with its proteome. The dynamic nature of proteome is such that time and place in which proteins are expressed, the level of expression and post-translational modification of proteins, correspond with changes in cellular environments. Indeed, the post translational modification of proteins has been shown to be a molecular marker in some diseases, thus intensifying the interest on proteomics in pharmaceutical industries and in medicine [15].

Genome mining holds an immense potential to revolutionize drug discovery. Genome mining is searching the genome for DNA sequences coding for enzymes that catalyze the biosynthesis of a particular product. Analysis of genomes of several microbes has revealed the presence of cryptic gene clusters, which encode unknown and novel secondary metabolites. These represent a potential to unveil novel classes of compounds.

Efforts to exploit the potential of secondary metabolites encoded by cryptic gene clusters, for novel drug prospecting is exemplified in the work conducted of Challis, et al. [16], their work comprised the identification of the cryptic gene clusters in *Streptomyces coelicolor* M145 that encoded proteins involved in biosynthesis of complex natural products that are unknown. The research focused on predicting the structure of the natural product that would be produced by a gene cluster, based on substrate recognition by the NRPS adenylation domain. This work would serve as the basis for identification, subsequent isolation and purification and chemical characterization of a novel compound coelichelin.

Furthermore, combinatorial biosynthesis, which focuses on genes involved in metabolic pathways for the biosynthesis of antibiotics has opened new possibilities for redesigning antibiotic structures by altering enzymes that are involved in the biosynthesis of antibiotics thus promising to produce antibiotics that will overcome resistance to existing antibiotics. This represents another approach in antibiotic drug discovery. Given that most antibiotics have been isolated from microbes, especially actinomycetes, unveiling "hidden" secondary metabolites from microbial genome mining holds promises for increased chemical diversity in the biologically relevant chemical space, and a potential for new drug lead for novel antibiotics [17].

Natural products are still promising in term of resources with the potential to meet the challenge of increasing antibiotic resistance. The search for better antibiotics to address the challenge still goes on. Indeed 34% of drugs approved by the US FDA between 1981 and 2010 were natural products or direct derivatives of natural products [18]. However, when it comes to prospecting microbial biodiversity, the challenge encountered is that an estimated 85%-99% of bacteria and archaea are unculturable. The unculturable bacteria, also called the microbial dark matter, are very little explored, and yet offer unlimited potential as a source of antibiotics. The large proportion of archaea and bacteria that researchers have not been able to culture hinders efforts and progress to prospect for novel secondary metabolites might be useful in lead discovery.

The term "unculturable" is not a precise one, because as conditions required for the growth of these microbes become known, it becomes possible to grown them in the laboratory. Several studies represent efforts to culture microbes that once have been termed "unculturable". However, despite these relentless efforts, there is still a huge number of "unculturable" microbes. This inadequacy of present culture techniques to culture most microorganisms requires the new approaches in growing microbes, such as culture-dependent techniques and new tools for growing microbes in their natural environment. Microbes have been grown in their environments and divide to form colonies. In one study, a 96-chamber multichannel device, the iChip was used to culture bacterium *Eleftheria terrae*, previously considered unculturable. This bacterium was shown to produce an antibiotic teixobactin that can kill Methicillin-Resistant *Staphylococcus aureus* (MRSA) in mice; and *Clostridium dificile colitis*, the most common gastrointestinal tract infection. This ability to grow bacteria in their natural environments (using fluidics system, for instance) has opened new doors of opportunity to explore and screen the untapped natural products reservoir of unculturable bacteria. Given the large proportions of unculturable bacteria the approach could provide unlimited chemical diversity for novel antibiotic compounds. In addition, because metagenomic approaches enable the isolation and characterization of microbial communities using environmental DNA, isolation of the DNA can be used to study what microbes are in the environment and what they are doing thus identifying the key processes and metabolites from the microbial communities [19].

In Africa, despite the rich biodiversity, the lack of expertise in drug discovery and development, the lack of funding; and absence of high quality laboratories may be a hindrance to realizing novel antibiotics from this region of the world. However, pooling resources in this case and getting involved in initiatives for intensive bioprospecting may bring out desirable results in terms of novel antibiotics that make their way to clinics. Furthermore, the use of computational approaches may be very instrumental in the pre-clinical stages of discovery and design and development of lead compounds.

Diverse screening libraries: Combinatorial synthesis and diversity-oriented synthesis

Combinatorial chemistry is a massive and parallel synthesis of small molecules to make screening libraries. To overcome the problem of a small number of natural product screening samples, combinatorial synthesis was developed. The use of combinatorial chemistry to generate small molecules has been vastly employed since the 1990's in drug discovery process. Because of the prospects and the potential combinatorial chemistry seemed to present for drug discovery, the interest on natural product screening decreased [20]. Later, larger pharmaceutical companies left NP drug discovery to academia and research facilities. Unfortunately, these small organic synthetic molecules, designed to obey the Lipinski rule of five for drug-likeness have not been able to get through to clinical stages. So far, a handful drugs such as sorafenib (first oral multikinase inhibitor) derived from combinatorial chemistry leads have been approved.

One reason for the failure is that HTS approach to screen the small molecules mostly used purified targets, *in vitro*, not taking into considerations other important properties such as the permeability of the compounds through cell membranes. In most cases a drug will have to cross cell membranes before reaching its target(s). This is one reason why most small molecules generated by combinatorial chemistry failed after they seemed promising following high throughput screens. When compared with approved drugs, synthetic molecules produced by combinatorial chemistry lack drug-like properties. Despite an infinite number of compounds synthesized, these small compounds occupy a limited chemical space lacking structural rigidity and chirality characterizing approved drugs. The focus is therefore now shifting to diversity-oriented syntheses, to extending the chemical space of compounds produced instead of the sheer immensity of compound numbers [21]. One important case study is the development of the antibiotic linezolid, an oxazolidone that function as a protein synthesis inhibitor. On the other hand natural products are more "drug-like", with more chiral centers, being sterically more complex, possessing more rings and bridgehead atoms; having higher molecular weight and incorporating more oxygen and less nitrogen and halogens or sulphur in their structures. Although combinatorial chemistry has not been successful in drug discovery, it has been effective in development and optimization of natural product pharmacophores and is therefore an important strategy to incorporate in natural product drug development.

Combinatorial chemistry was adopted for lead discovery mainly due to the inherent hurdles in preparing a natural product-based library enough to keep the pace of vastly increased screening capacity of the HTS. Another problem that arose was that molecules produced lacked drug-like properties, were mostly flat and achiral resulting into failure on screens. Since the main problem with combinatorial chemistry small synthetic molecules was that they were not complex enough to make successful hits, incorporating diversity oriented synthesis provided more diverse NP-like molecules for biological screens. One solution

to this problem would be to base combinatorial chemistry syntheses approaches on natural product inspired scaffolds or produce synthetic highly diverse, structurally complex molecules with drug-like molecules [22].

Diversity Oriented Synthesis (DOS) is a diversity driven approach to synthesize simultaneously and efficiently more than one compound in order to solve a complex problem. DOS focuses on skeletal diversity for compound library. In order to increase the chemical diversity lacking in combinatorial chemistry, DOS combines not only the variations of the building blocks, but also varied scaffolds, varied, stereochemistry, functional groups and the molecular framework to produce the highest possible structural diversity that generates drug-like molecules to screen for novel drug leads. More importantly, DOS utilizes biologically relevant privileged structures from natural products because these are biologically more relevant, to generate skeletal diversity using natural scaffolds. DOS has resulted into highly diverse and structurally more complex molecules with sp3-hybridized carbons and more stereogenic centers yielding drug-like molecules that give rise to bioactive leads. The structural diversity is important because compounds with the same structure present a similar biological profile in terms of activity. Increasing the structural diversity of compound collections through DOS increases their relevance for lead discovery. It is possible to use natural products as starting points for lead discovery in natural-product-based Diversity Oriented Synthesis (DOS).

The application of DOS in the discovery of antibacterial agents is showing some promise, as exemplified in the discovery of gemmacin and emmacin. Emmacin, a dihydrofolate reductase inhibitor, was developed by diversity oriented synthesis of more than 200 structurally diverse compounds; later screened against methicillin-resistant strain of *Staphylococcus aureus* called EMRSA-16. Emmacin exhibited an excellent bioactivity against MRSA. Gemmacin showed strong antibacterial activity against MRSA strains, equal to that of oxacillin and erythromycin. Further synthesis of a focused library around the *cis*-fused bicyclic amine core scaffold of gemmacin produced gemmacin B with even greater activity against EMRSA [23].

Looking at the trends in the discovery of antibiotics, natural products still remain as relevant as it has been in the past, because compared to other approaches; NPs stand out as the best source of the biologically relevant chemical diversity in the chemical space, useful for the search of novel drugs. Even approaches such as combinatorial chemistry initially designed to produce diverse small molecules for high throughput screening could not generate enough molecules with biological relevance for screening. Although DOS was later developed as a modification of the random combinatorial chemistry approach to use scaffolds from natural products for generating the diversity, still the approach did not meet its expectation in yielding a good number of novel drugs. Therefore, again we turn back to natural products as the source of novel compounds, others with a probable novel mechanism of action, for screening against targets for microbes in order to overcome the problem of antibiotic resistance. We also look at the role Africa can play in the process of design and discovery of novel antibiotics, using the rich Africa's biodiversity.

Capacity building for bioprospecting in africa and antibiotics discovery

The problem of antibiotic resistance is global. Africa and most other developing countries on the other hand are more affected by infectious diseases than the rest of the world. This being the problem in Africa as it is for the rest of the world, it is important that we in Africa take a responsibility to contribute towards solving the problem. Although most of the large pharmaceutical companies are located in the resource-rich developed countries, antibiotics are very much required in developing countries where infectious diseases are a more serious problem. Even more challenging is the fact that most pharmaceutical companies are not very much interested in the search of novel more potent antibiotics by the screening of natural products because of the problem of rediscovering the same compounds from screening libraries of natural products, and also partly because of the perceived cost-benefits analysis, that the amount of resources invested in the discovery, design and development of antibiotics would not recuperate the costs incurred, and therefore does not provide the financial motive to drive the process. However, through dereplication process can be used to remove the compounds and the natural product drug discovery and development can once more be feasible.

To compound the challenges, most research in developing countries including most African countries has been donor-funded, who in turn define the scope and direction of the process of research and development. We propose a more sustainable approach for the Africa in the discovery of novel antibiotics [24].

Firstly, we discuss whether or not there is a clear vision, motivation, policies, expertise and other resources, such as equipped laboratories and sufficient funding for research and development. Are there initiatives for antibiotics discovery in Africa? Is funding adequate? In the next section we discuss the prospects for antibiotic discovery in Africa, the vision, funding and other resources available for antibiotic drug discovery; and how they can be put into feasible use for the best results (Figure 3).

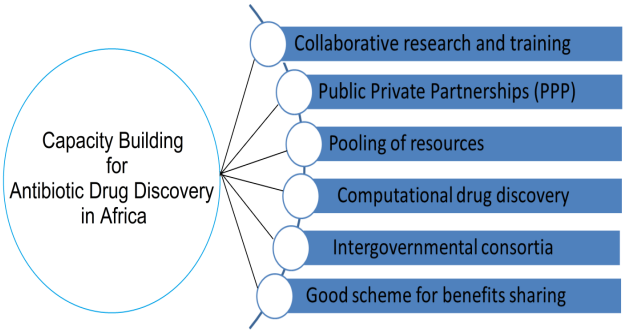

Figure 3: Summary of the approaches used in capacity building for drug discovery in Africa.

Bioprospecting unique biodiversity: Africa has a rich biodiversity. Bioprospecting rarely explored environments is especially

more likely to result in isolation of novel compounds and avoid redundancy in classes of compounds isolated. Therefore, screening unique biodiversity resources has a higher probability of yielding novel classes of compounds. The African unique biodiversity extending from plants such as *Terminalia bentzoe*, endemic in Mauritius, to the bacterium actinomycetes isolated from underexploited disserts in Egypt there is an abundance of bioresources with a potential for applications to treat antibiotic resistant infections. Other excellent examples include the isolation of a novel bacterial gyrase inhibitor, kibdelomycin from soil bacterium *Kibdelosporangium banguiesis* in the Central African Republic and the discovery of an antibiotic platensimycin isolated from *Streptomyces platensis* from soils of the Eastern Cape, South Africa. The identification of unique biological resources has been an integral part in bioprospecting of unique Africa biodiversity. This is because the screening of unique and unexplored biodiversity resources is more likely to yield novel compounds to screen for bioactivity. For instance, extreme environments of hot springs have not been fully exploited for natural products, but soil samples from these places have been shown to be a potential source of secondary metabolites that could give important antimicrobial lead compounds. Hot springs are found in many countries in Africa, such as Uganda, Zambia, Tanzania, South Africa, Egypt, Algeria, Rwanda and Nigeria. These extreme environments habour unique microbes that produce unique compound classes.

Research collaborations and training for capacity building: Evidently African countries are rich in bioresources for screening. There are also several research units in Africa involved in natural product isolation, purification and structure elucidation for natural products. Despite this knowledge no single drug has been developed in Africa, from its unique biodiversity due to lack of expertise in modern drug discovery techniques such as expertise in computational tools, medicinal chemistry, drug metabolism, and the ADME/Tox pharmacokinetic studies. This requires initiatives on the part of the continent to have more scientists trained in the outlined disciplines to increase its human resource capital important for the work of drug design, discovery and development. One example is the case of the University of Queensland's Community for Open Antibiotic Drug Discovery (CO-ADD). CO-ADD invests in collaborations within and outside Africa, thus benefiting from expertise from across borders, that is, within and outside Africa. This also allows sharing of other resources such as biological samples and sophisticated and expensive laboratory equipment that might be rare in Africa. Another such drug discovery and development centre is H3D, hosted by The University of Cape town from since 2011. H3D focuses on modern drug discovery and focuses in the areas of medicinal chemistry, pharmacokinetics and pharmacogenetics.

Pooling resources for bioprospecting: Furthermore, pooling resources between governments and international pharmaceutical companies and academia in a pubic private partnership can help in resource mobilization to expedite natural product drug discovery programmes. Due to the global nature of problem of antibiotic resistance, the idea of having intergovernmental consortia to oversee funding of innovative research and development, control, regulation and distribution of novel antibiotics is a good idea. This drive to pool resources is exemplified in Sanofi and Fraunhoufer institute of molecular biology, merging efforts through the establishment of natural product Excellency centres to search for novel anti-infectives. Also collaborations to share technology and expertise have been seen as a way to expedite drug discovery. This is exemplified in Sanofi and warp drive bio. Africa and other developing countries could follow this excellent example, where the best strategy would be to refrain from considering things only in monetary terms, for instance, receiving money in return for the bioresources exchanged, but rather to allow the collaborations which will lead to capacity building of the institutions and personnel in these developing countries. The collaborations are likely to be more fruitful if they allow training of the scientists in the developing partners, to get a critical mass of skilled scientists; and possibly the acquisition of equipment for collaborative research, with the focus to eventually develop sustainability and self-sufficiency in bioprospecting projects leading to novel drugs.

Despite screening efforts, bioprospecting for novel antibiotic compounds from plants has not yielded a single clinical drug in Africa, probably because most methods used for screening for activity used have not been reproducible, such as the agar diffusion methods. In addition most researchers have been trying to isolate pure compounds whereas plant metabolites work better as synergetic combinations. Studies for antibiotic activities in Africa have mostly focused on bioactivity, but most have not proceeded to cytotoxicity, acute toxicity and pharmacokinetic studies to realize the potential of crude extracts or purified compounds from natural products.

Since most bioprospecting studies have focused on evidence from ethnomedicine practices, plants have mainly been the object of bioprospecting. However, comparatively microbes offer far greater advantages than plants for antibiotic drugs prospecting. In addition to microbes being amenable to genetic manipulation to allow expression of biosynthetic gene clusters their massive production of secondary metabolites through fermentation insures a steady supply of the required material. On the contrary, issues of supply of plant materials, high cost of semi-synthesis using, and the insurmountable cost barriers for total synthesis have plants and most other biological resources unreliably scarce for screening and drug discovery programmes. The current developments in biotechnology, genomics, medicinal and synthetic chemistry make it easier, cheaper and quicker to preferably search novel lead compounds from microbial sources. However, the question of what source is suitable for novel medicines will not be settled by cost considerations alone, but rather by the realizing the solutions to baffling problem of antibiotic resistance through any conceivable means.

Most bioprospecting is currently performed on a small scale by numerous academic groups throughout the world. Very few large pharmaceutical companies have maintained a strong presence in natural product-based drug discovery. One reason for the decline could be the appetite for bioprospecting by pharmaceutical

development companies has diminished since the Rio Earth Summit in 1992, partly because of the complexities relating to access and benefit-sharing, often in the absence of adequate national regulatory clarity and institutional capacity. Various technical problems undoubtedly exist with the screening and isolation of natural products, but the rewards for overcoming them would seem to justify the effort required. Technical procedures such as purification and identification of natural products are believed to be difficult and slow: High throughput separation methods coupled with sensitive analytical techniques can resolve this.

Discussion

Future perspectives

As computers become more powerful, computational methods will be increasingly used for drug design, discovery and development. Computational approaches are more cost-effective for preclinical drug design, discovery and development because they allow *in silico* generation of hit and lead compounds for testing, lead optimization and the *in silico* prediction of ADME/Tox properties of leads. Force fields have been refined to include polarizability, such as the Druid and the AMOEBA force fields. Virtual screening, an *in silico* equivalent of high throughput screening of compound libraries, makes it possible to reduce the number of compounds to test in a wet lab from thousands and millions to a few hundreds or tens. Computational approaches in drug design and discovery include tools for protein modeling, virtual screening, molecular dynamics and. Furthermore, Machine Learning approaches are already currently employed in antibiotic drug discovery. A good example is a promising lead compound halicin identified by machine learning approach by the Stokes, et al. [25], and ready for clinical phases. Furthermore, as computers become more faster and algorithms more accurate and refined, the application of computational drug design and discovery is becoming more feasible. Computers are also becoming more affordable for institutions even in developing countries. Approaches such as virtual screening can be used to screen a number of natural products from databases and narrow down to a few compounds to screen in wet lab. Furthermore, the results can be refined through other approaches such as the application of molecular dynamics simulations and trajectory analysis in the accurate determination of binding energy and other variables and parameters.

The process of natural product drug discovery can be improved in resource-poor countries. The best strategy is to use the available resources feasibly, which would include collaborations with the global scientific community and the sharing of resources such as screening libraries, scientific equipment, expertise, computational resources and funding. Furthermore, the framework for the Intellectual Property Rights (IPR) and indigeneous knowledge should be such as to allow the setting of an environment that allows a more agreeable benefit sharing scheme between the indigeneous people and pharmaceutical companies.

Conclusion

Antibiotic resistance is a naturally occurring phenomenon. The problem has however been exacerbated by poor practices in antibiotic stewardship, particularly excessive and wide spread use of particular antibiotics. There are a number of ways to combat the problem of antibiotic resistance. However, the role of drug discovery in combating antibiotic resistance is key to obtaining a more logical solution towards solving the problem. A number of approaches to antibiotic discovery have been discussed in this review. It is important to mention here that most antibiotics were discovered serendipitously from natural products; and despite advances in drug design approaches, most of them have not yielded clinically important antibiotics. It might be that these modern drug discovery approaches are still at infant stage and still learning from failures and perfecting the art. Only time will attest to this truth. Most clinically important classes of antibiotics have been isolated from microbes, making microorganisms the most important source of antibiotics in use today. Most researchers have advocated on the need to find more screening resources among living organisms citing the fact that only a fraction of living organisms has been screened for drugs, let alone antibiotics. In the case of microbes for instance, only 1% are culturable making those that have been screened for antibiotics probably < 1%. This is just the top of an iceberg, as some put it, representing vast reservoir of microbes for which there are probably novel classes of compounds with novel antibiotic mechanisms. Other sources of materials for screening are the fungi, plants and animals. In the case of plants, no single antibiotic substance discovered from plants has been developed into clinically useful antibiotics. However, several studies show that plants have the potential to be used as resistance modifying agents. In addition, although plants have not been very promising as a source of clinically useful antibiotics, only the fraction of plants has already been screened, leaving extremely large number of plants unexplored for the presence of novel compounds with excellent antibiotic activity. If this potential from the screening of living organisms alone is harvested it might make it possible for the problem of antibiotic resistance to be completely eliminated. If anything, the lesson learnt in the course of antibiotic drug discovery is the utility of integrated approaches, borrowing expertise from several fields has in antibiotic drug discovery.

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How to cite this article: Mteremkoa, Denis, Ernest Mbega, Jaffu Chilongola and Musa Chach "Trends in the Design and Discovery of Antibiotics using Natural Products: Bioresources and Capacity Building in Africa." *J Pharmacogn Nat Prod* 8 (2022): 190.