

Antimicrobial Adjuvants Drug Discovery, the Challenge of Avoid the Resistance and Recover the Susceptibility of Multidrug-Resistant Strains

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Abstract

Currently, the emergence of multidrug resistant microorganisms as *Acinetobacter baumannii* and *Mycobacterium tuberculosis* as well as new drug resistant forms of *Neisseria gonorrhoeae* in conjunction with the evolving of resistance genes as the KPC (class A *Klebsiella pneumoniae*) and NDM-1 (New Delhi metallo- β -lactamase 1) carbapenemases are a public health threat that requires great efforts in prevention, treatment and diagnosis. For that reason, the InterAcademy Panel and InterAcademy Medical Panel statement (IAP-IAMP) included in their Global recommendations call for action to tackle antimicrobial resistance under the following item "Encourage industry innovation and public-private collaborative research and development programs for therapeutics, diagnostics, and vaccines". In this way, the development of novel antimicrobial drugs is not only necessary, is a priority the search and development of new pharmacological strategies for prevent antimicrobial resistance emergence, these strategies should have the ability of improve the current anti-infective therapy in combination, and recuperate the susceptibility of multidrug-resistant strains to the antibiotics. Thereupon, the introduction of screening platforms in a rational *in vitro* antimicrobial adjuvants drug discovery program is an important approach that will allow the detection of new chemical entities that can inhibit resistant mechanisms specifically and without side effects, looking for increase the microbicidal effect and prevent the development of subsequent mutations to anti-infective therapy. The aim of this review is to explore antibiotic adjuvants as therapeutic plan of action that can be developed for the discovery of new compounds that make bacteria more susceptible to anti-infectives by improving their efficacy.

Keywords: Antimicrobial resistance; Adjuvants; Drug discovery; Screening platforms

Introduction

Antibiotic-resistant strains are increasingly prevalent in hospitals and the community. Between antibiotic-resistant agents the major threats are constituted by methicillin-resistant *Staphylococcus aureus* (MRSA) that cause ~19,000 deaths by year in the U.S with a health care cost of \$3-4 billion. Equally, multidrug-resistant (MDR) and pan-drug-resistant (PDR) gram-negative bacteria as *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, MDR and extensively-drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (MDR-TB and XDR-TB) have increased cases in recent years persistently [1]. Therefore, the development of new antimicrobial agents is required to fight against these pathogens, but unfortunately the outcome in this regard has been slow, which suggests that strategies to discover new drugs should be reanalyzed [2]. Also, an important concern for to be considered is that acquired resistance is an adaptive response to the drug, because the indiscriminate use of antibiotics can cause selective pressure that induce resistance mechanism activation in microbes, which continue for many generations [3-5]. In this order of ideas antibiotics cause adaptive responses in microorganisms known as SOS repair system which not only acting on DNA also on the cell wall [4,6].

The most known mechanisms of resistance in microbes are increase of drug efflux pumps, enzymatic inactivation, target site modification, and biofilm formation [7,8], in response to environmental aggressions as well as anti-infective therapies [9,10]. For to decrease the emergence and transmission of antibiotic resistance, it is imperative to develop new anti-infective drugs with the ability of to inhibit drug efflux pumps as well as quorum sensing process and biofilm, also know more about biological mechanisms of induced resistance [11,12]. Equally, in the surveillance and prevention of the appearance of non-heritable resistance, is important to deep in the ways of antibiotic

tolerance [13-15]. In the same way, employ *in vitro* pharmacokinetic/ pharmacodynamic models to improve and control dosing regimens may decrease the emergence of resistance during anti-infective therapy [16-18]. Reduce the consequences of antibiotic resistance requires a multidisciplinary view in a translational medicine program, that includes surveillance of rational use of antimicrobial agents and control of the dissemination of resistant microorganisms between hospitals and the community, likewise develop strategies that involving approaches from the pharmacokinetics, pharmacodynamics and pharmacogenomics [19,20]. For that reason an interesting approach would be discover new chemical entities that inhibits antimicrobial resistance mechanisms, consigned in a rational program of antimicrobial adjuvants drug discovery with the aim of develop new drug combinations that permits new antibiotic strategies in clinical practice [21,22].

Antibiotic adjuvants

Among the therapeutic strategies that can be developed, antibiotic adjuvants, considered as compounds that make bacteria more susceptible to anti-infective by improving their efficacy is an interesting approach [23]. Adjuvants can be antibiotics, non-antibiotics, and molecules that can inhibit resistance genes and adaptive responses in microbial cells [24]. This synergism with antimicrobial activity can happen in several

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ways such as: inhibition of antibiotic resistance elements involved in SOS response (DNA repair, mutagenesis) [25], increase in income of the antibiotic into the cell through the membrane, efflux pumps inhibition and altering the biological adaptive capacity of resistant cells [26]. For to do this, antibiotics can be combined with other molecules that are not, but having the ability to inhibit these resistance mechanisms as are: β -lactamases, cell wall thickening, biofilm formation, and efflux protein activation (Figure 1) [20]. Therefore, the design and development of antibiotic adjuvants can increase the microbicidal effect and prevent the development of subsequent mutations to anti-infective therapy [26]. This combination strategy have been demonstrated as in the case of trimethoprim and sulfa drugs [27], and introduction of β -lactamase inhibitors (clavulanate, sulbactam and tazobactam) [28]; more recently a mixture of cefuroxime and ticlopidine with synergistic activity against MRSA have been demonstrated, increasing the antimicrobial activity of cephalosporins [29].

Antimicrobial synergism

In this order of ideas for fight against infections caused by MDR microorganisms is necessary develop new therapies that consider the combination of various medications during the treatment time, taking into account the potential drug interactions during the drug development process. This strategy has been used successfully in anticancer treatment, HIV infections, complicated cases of malaria and the treatment of *Mycobacterium tuberculosis* infections [20], in clinical medicine this approach is routinely employed in order to obtain clinical improvement in difficult to cure infections. Nevertheless despite this, side effects are present, for that reason the combination between antibacterials and nontoxic antimicrobial adjuvants can be an interesting choice in search of a new type of synergism combination therapy whose main objective is to provide anti-infective medical care without causing resistance. The principal targets of these adjuvants for develop synergistic interactions would be microbial signaling, microbial

effector molecules, virulence factors, resistance gene expression and horizontal transfer [30].

Antimicrobial targets for antimicrobial adjuvants

Using current methodologies of molecular biology has been possible to identify 265-350 anti-bacterial targets. Of which 60% are present in every pathogenic bacteria, but no more than 20 can be inhibited by the drugs available on the market [31]. For that reason, antimicrobial drug screening have been focused in antimicrobial target discovery, leaving aside whole cell assays that can identify molecules that can cross cellular barriers and inhibit biological processes [32]. In consequence, *in vitro* cell free screening was not successful [31]. So it is now recommended, combine antimicrobial targets approach with whole cell assays, with the end of increase the detection of compounds able to inhibit microbial cultures with a mechanism of action determined [33]. In this way, in the following excerpts the intention is to describe what would be the appropriate targets to develop a platform of this type, together with the corresponding phenotype cell assay.

Antibiotics combination FIC index

For determinate the susceptibility of bacteria and fungi to new antimicrobial products is necessary apply a uniform terminology based in the standards set forth in European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Clinical and Laboratory Standard Institute (CLSI) from USA. Using these standards is possible determinate four antimicrobial activities of the products in combination: indifference, additive, synergism and antagonism. The observed increase in activity always has to be confirmed using time-kill curve methods for to analyze the synergism and their bactericidal activity. Finally, the fractional inhibitory concentration (FIC) index is an important predictor to begin the evaluation of promising antimicrobial adjuvants in combination [34].

Cell Wall and Membrane

Enhancing the permeability of the cell wall

The initial bacterial defense mechanism is the control of membrane permeability with the end of decrease the intracellular concentration of antibiotics and biocides [35]. Equally, in gram-negative bacteria has been demonstrated that the outer membrane permeation is an initial step in the bactericidal activity of antimicrobial compounds [36]. Constituting the membrane disruption as a key target in the development of new drugs with potent bactericidal without emergence of resistance [37]. The membrane disruption can be obtained using essential oils, detergents, surfactants, polymyxins, daptomycin and antimicrobial peptides [38,39]. This target can be promising for the development of new antiseptics and disinfectant agents for eradicate MDR microorganisms from hospital environment [40].

Efflux pump inhibitors

One of the biological systems that control the intracellular concentration of anti-infective drugs are efflux pumps, which makes the search for molecules with efflux pump inhibition (EPIs) activity an important strategy to restore antimicrobial activity of existing antibiotics. The families of efflux transporters are composed by MFS (major facilitator superfamily), MATE (multi-drug and toxic compound extrusion), RND (resistance nodulation cell division) superfamily, SMR (small multi-drug-resistance), and ABC (ATP-binding cassette) transporters [41]. EPIs can be used inhibiting several of these efflux transporters families as adjuvants in antibiotic treatments affecting

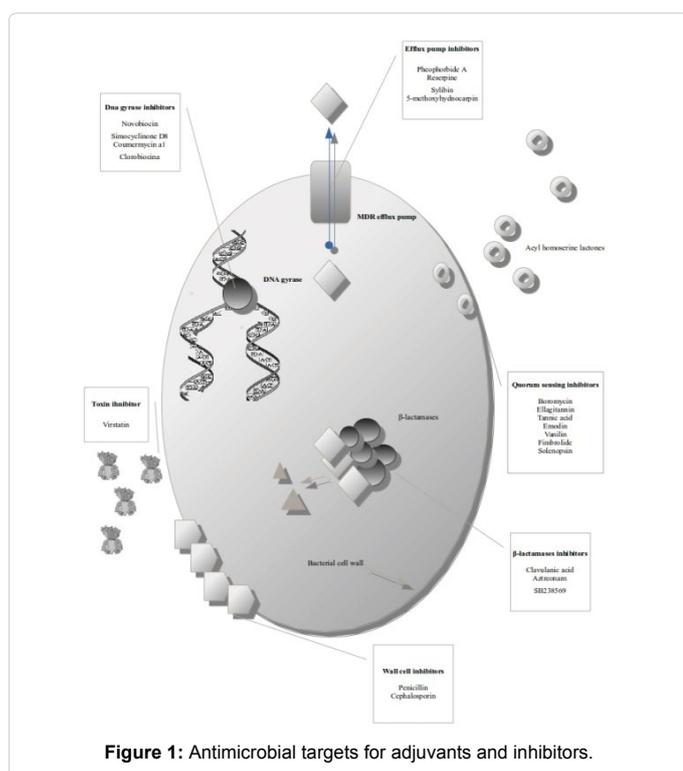


Figure 1: Antimicrobial targets for adjuvants and inhibitors.

bacterial survival, virulence and pathogenicity [42]. In this way, the efflux pump inhibitor verapamil has been evaluated using in vivo models as an adjuvant showing activity in antimicrobial treatment and increasing bactericidal activity [43,44].

ROS production induction

It has been observed in several studies that bactericidal antibiotics cause cell destruction by production of reactive oxygen species (ROS) [45]. ROS can damage DNA, RNA, proteins and lipids. So it has been proposed as strategy to increase antibiotic efficacy induce ROS production in microbial cells as well as inhibit detoxification and repair systems [23,46]. Likewise, is an important approach take advantage of the oxidative stress response for improving anti-infective therapies, giving way to the development of a new class of molecules [47]. Interestingly, there is connection between oxidative stress response and anti-infective drug action regulated by SoxRS and give antimicrobial resistance, which explain why antioxidants, such as vitamin C and glutathione increase antimicrobial activity of quinolones and aminoglycosides and suppress mutagenesis that produce quinolone resistance [48,49].

Virulence Factors Inhibition

Toxin inhibition

The production of toxins is an important virulence factor in bacteria and fungi. That target host cells by binding to specific glycolipids in the cell membrane. The inhibition of this interaction can prevent the potential tissue damage [50]. The most common microbial toxins described are: aflatoxin B1, fumonisins, and ochratoxin A produced by fungi; cholera toxin produced by *Vibrio cholerae* bacteria; Shiga toxins produced by *E. coli* bacteria; staphylococcal enterotoxins produced by *Staphylococcus aureus* bacteria and listeriolysin O (LLO) produced by *Listeria monocytogenes* [51]. This anti-virulence strategy looking for protects hosts cells against the adverse effects of toxins is an innovative approach for the control of infection and decrease the development of resistance [52]. The disadvantage of use of toxin inhibitors can be the toxin over-expression, increasing the virulence [53]. In this way, the most promising compound detected has been virstatin that inhibits ToxT dimerization of cholera toxin in *Vibrio cholerae* [54].

Anti-beta-lactamase

β -lactamases are enzymes that catalyze the hydrolysis of β -lactam ring of the antibiotics inactivating. Up to date, more than 700 of these enzymes have been detected and are classified according to the type of target antibiotic in four groups, A-D (class A penicillins, class C cephalosporins, class D oxacillin and class B for have two zinc ions at its catalytic site are called metallo- β -lactamases) [55]. Thanks to the success obtained with clavulanic acid, it is now a well-established principle that the combination of a β -lactamase inhibitor (as an adjuvant) with a β -lactam antibiotic enhances the efficacy. For that reason, there is an interesting research field in the development and search of novel β -lactamase inhibitors for co-administration with β -lactams [56].

Anti-quorum sensing

Bacterial communication is developed across production and detection of signaling molecules which belongs to quorum sensing (QS) process that regulates the formation of virulence factors, antibiotic production and biofilm formation [57]. A compound with anti-QS activity should be able of inhibit signal biosynthesis, block activity and decrease production of acyl homoserine lactones (AHL), induce enzymatic signal degradation and inhibit the reception of signal molecules [58], as well as mimic AHL for induce altered response [59].

Therefore, QS is a fascinating target for the development of novel anti-infective medications in combination with antibiotics [57].

Replication and Resistance Gene Transfer

DNA synthesis

DNA gyrase and topoisomerase IV are crucial in DNA synthesis, constituting in important therapeutic targets. Because, ATP-dependent supercoiling of DNA is catalyzed by DNA gyrase being essential in DNA replication, transcription, and recombination and in the regulation of chromosome supercoiling [60]. In this way, fluoroquinolones have the ability of inhibit bacterial DNA gyrase and topoisomerase IV binding to the enzyme-DNA complex in most bacterial species causing cell death by the accumulation of double-stranded DNA fragments. Currently the challenge is developing and discovers new chemical entities better than fluoroquinolones, but do not develop resistance [61].

Gene transfer inhibition

Horizontal gene transfer is defined as transportation of DNA fragments from one organism to another and subsequent incorporation, causing changes in their genetic structure. This DNA transfer is considered a critical mechanism whereby antibiotic resistance and virulence factors are acquired in bacterial communities. The pharmacological inhibition of transfer can be a crucial approach in the blocking of antimicrobial resistance spread, in this order of ideas antibiotics, polycyclic chemicals, and crude extracts have shown the ability of inhibit the transportation of the DNA fragments with resistance mechanisms in bacteria, preventing propagation genetic and making them more susceptible to the action of antibiotics [62].

Anti-plasmid

The eradication of plasmids that transport antibiotic resistant genes can turn again microbial populations in susceptible to antimicrobials and recovering the spectrum of activity [63]. Drugs such as promethazine and peppermint oil and its main constituent, menthol have eliminated plasmids that carry resistance genes [63,64], which makes compounds with antiplasmid activity as interesting adjuvant antimicrobial agents in the fight against drug resistance.

Antimicrobial Adjuvants Drug Screening Program

Is necessary the search and development of new pharmacological strategies for prevent antimicrobial resistance emergence, these strategies should have the ability of improve the current anti-infective therapy in combination, and recuperate the susceptibility of multidrug-resistant strains to the antibiotics. In this way, the introduction of screening platforms looking for a rational *in vitro* antimicrobial adjuvants drug discovery is an important approach that will allow the detection of new chemical entities that can inhibit resistant mechanisms specifically and without side effects [65]. In total should be implemented a mode of action (MOA) screening platform in combination with whole cell assays for natural and chemical library evaluation (Figure 2) [66].

Antimicrobial screening platforms to detect drugs that target microbial cell wall or membrane

The fast development of resistance makes many therapeutic targets lose their clinical efficacy. The envelope of Gram-negative species and mycobacteria presents a barrier for penetration, making it difficult to discover broad spectrum compounds. Increasing penetration of a large set of compounds and ranking them will enable the deduction of rules of penetration [67]. Thus, are necessary implement new agents that increase the permeability of the bacterial cell membrane,

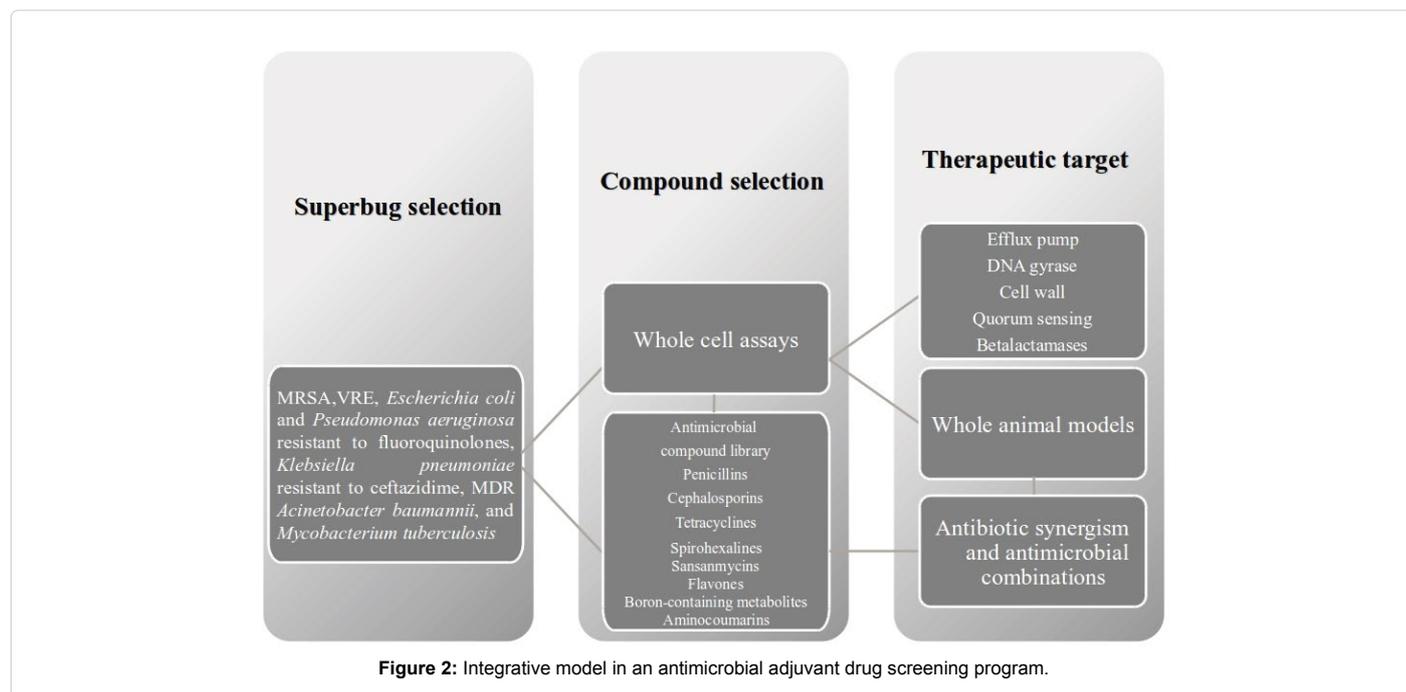


Figure 2: Integrative model in an antimicrobial adjuvant drug screening program.

which will make the majority of targets available to drug effects [37,67]. This strategy should be focused in three screening platforms looking for cell permeabilizers and efflux pump inhibitors and ROS production inducers, with the end of identify molecules that increase the penetration of antibiotics and keep the intracellular concentrations, decreasing antibiotic resistance emergence [68].

Antimicrobial screening platforms to detect drugs that target microbial virulence factors

Have been demonstrated that targeting key virulence factors can improve the healing in infectious models in vivo. The combination of an anti-virulence agent with a conventional antibiotic to achieve synergism is necessary for avoid the resistance selection [53]. Anti-virulence drugs are an important research field that can impact in drug development enhancing the anti-infective drug efficacy. These anti-virulence drugs decrease the pathogenicity of the etiological agent weakening the selection for resistance induced by antibiotics [69]. This strategy should be focused in three screening platforms looking for toxin inhibitors, biofilm and quorum sensing inhibitors and anti-betalactamase compounds, with the end of identify new molecules with anti-virulence activity [70].

Antimicrobial screening platforms to detect drugs that inhibit DNA replication and gene transfer

Microorganisms use mutation and horizontal gene transfer for diversify its genome. Antibiotic treatment stimulates mutagenesis in microbial populations; these mutations confer antibiotic resistance and can be disseminated to other individuals. For that reason is important identify new chemical entities that block the selection of the resistant mutants and further growth [71]. The development, acquisition and spread of antibiotic resistance in clinical environments have high impact in the clinical outcome to antimicrobial therapy around the world. The resistance acquired genetically by horizontal gene transfer or gene mutation is manifested phenotypically by target modification and over-expression of efflux pumps, blocking the transfer of genetic

information is possible to reuse antibiotic resistance that have become obsolete [72]. This strategy should be focused in antiplasmid screening platforms and DNA gyrase inhibition looking for obtains non-antibiotic adjuvants that become susceptible the MDR microorganisms to low concentrations of antibiotics. Equally, mutant prevention concentration (MPC) testing that represents the minimal dose that prevents the emergence of resistant mutants and mutant selection window of the compounds in combination should be performed [73].

Antimicrobial screening platforms to detect drugs that inhibit bacterial dormancy and persistence

Microbial persisters are tolerant to several bactericidal antibiotics; this phenomenon is named multidrug tolerance (MDT). These persisters not killed by antibiotics could revert to replicating forms (reverters), producing recurrent infections, for that reason is necessary new antimicrobial drugs with bactericidal activity against persisters cells [74]. Persisters are bacterial cells that are multidrug tolerant as a consequence of a transient switch [75]. Persister cells are responsible for the intractability of many bacterial infections to antibiotic treatment, which often result in recurrent courses of antibiotics [76]. Also, dormancy in biofilms and persister cells presence are the cause of the reduced susceptibility of biofilms to antibiotics [77]. This strategy should be focused in develop type I and type II persister cell assays of exponential- and stationary-phase cultures, for detection of anti-persisters chemical entities [78]. Equally, must be implemented an *in vitro* model of mycobacterial dormancy with colorimetric methods for determinate anti-dormancy and anti-tubercular activity [79].

In vitro whole animal infection model using *C. elegans* antimicrobial screening platforms to evaluate antimicrobial activity and determine toxicity

Drug discovery is a challenging research field. Research with the use of *Caenorhabditis elegans* has demonstrated great results and holds a promising future. The nematode is an excellent tool for the researcher and provides many valuable experimental elements. The

many advantages of their use, including easy handling, the option for high throughput assays and the possibility of study of the interactions between the host and the pathogen have made the *C. elegans* a promising tool for preclinical drug discovery [80]. The nematode *C. elegans* is a whole animal model system for identifying small molecules with in vivo anti-infective properties. *C. elegans* can be infected with a broad range of human pathogens (*Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Candida albicans*) [81,82]. Because of their small size, *C. elegans* nematodes can be placed in 96- or 384-well plates for high throughput experiments. This model offers distinct advantages for antimicrobial discovery as it provides the possibility to identify compounds with no antimicrobial activity *in vitro*, but which may target functions only important for in vivo survival or virulence [83].

Evaluate antimicrobial activity against MDR microorganisms using the screening platforms implemented of non-antibiotics compounds, essential oils as well as their formulations and derivate in combination with existing antibiotics

Non-antibiotics and essential oils have demonstrated to enhance the activity of antimicrobial drugs [83,84], also, phenothiazines and essential oils possess plasmid inhibition activities which can stop the transmission of resistance to other populations [64,85], examining the response of MDR microorganisms against these combinations is possible induce that we can be in the presence of a therapeutic alternative for treatment of these diseases which are destroying thousands of lives per year throughout the world. In previous reports it has been suggested that phenothiazines can be used as antimicrobial adjuvants. Between them chlorpromazine and amitriptyline have reduced or reversed resistance of clinical isolates to penicillin, tobramycin and cefuroxim [86]. Also, promazine, promethazine, prochlorperazine, methdilazine, fluphenazine, trimiprazine, trifluoperazine, triflupromazine, thioridazine and flupenthixol have shown antimicrobial activity against a broad spectrum of microorganisms [85,87]. Equally there are reports about the activity of essential oils as antibiotic resistance-modifying [88]. Essential oils from *Pelargonium graveolens*, *Lantana camara*, *Hyptis martiusii* and *Croton zehntneri* have shown enhance the antimicrobial activity against resistant microorganisms in combination with other molecules as aminoglycosides (amikacin, kanamycin and gentamicin) so they have potential to be used as antimicrobial adjuvants [89,90]. This strategy should be focused in the evaluation of promising antimicrobial adjuvants in combination looking for clinical application, for this aim will be used the previously implemented screening platforms and will employ non-antibiotics drugs, essential oils both new formulations and their derivatives.

The Importance of Natural Products as Source of New Compounds Chemically Diverse in Antimicrobial Adjuvants Discovery

So et al. [91] described the bottlenecks in R&D pipeline for novel antibacterial drugs and categorized in the following items:

Lead identification

Currently is considered that high throughput screening approach for novel antimicrobial drug candidates has had very little performance in comparison to other public health problems. Because, the current whole cell drug screens have been unsuccessful [82].

Medicinal chemistry

Since the proposal of rational anti-infective chemotherapy through the search for selective inhibitors of microbial enzymes, the process of

description and development in to clone genes and manipulate bacterial strains have achieved to enhance whole-cell phenotype screens for inhibitors of specific targets, and become antimicrobial drug discovery toward screening target-directed, seeking to increase the druggability [92]. But, slowing the process of transforming these leads into drugs that can enter in controlled clinical trials.

Crossing the valley of death

In translational research this term is used for describe the chasm caused by the absence of communication between clinical and basic scientists that prevents those discoveries of biomedical researchers reach to the patients [93]. In this valley the successful depends of control of risks: Scientific, Intellectual Property, Market, and Regulatory [94]. In antimicrobial drug discovery the success during lead optimization depends largely in the medicinal chemistry approach developed, and this influences the costs [93,94].

Regulatory approval

Types of clinical trials as well as enrolling adequate numbers of patients should be identified for to control costs and guidelines of regulatory agencies should be discussed [95].

Reimbursement

Reimbursement is a controversial aspect of antibiotic development especially for the duration of treatment (10-14 days), for that reason is very important look for a balance between research, regulation and economics [14,91].

Natural products for antibacterial drug screening has had a significant decrease in the obtainment of new leads, since the advent of high throughput screening methods, and the increase in its replacement of screening of chemical libraries from combinatorial chemistry [96]. Limiting antibacterial discovery to screening of this material, although the chemical diversity of natural products is greater [97-99]. Where soil has been the most explored environment with the highest number of compounds isolated in the search for natural products with antimicrobial activity [100]. Within this diversity the largest producers of antimicrobials are streptomycetes, bacillus, and myxococcus species [98]. Microorganisms have been the largest natural source of drugs used at present, in where 45% are produced by actinomycetes, 38% by fungi and 17% by unicellular bacteria [101,102]. Berdy [103] has described that the currently decrease in the research in isolation of new microbial metabolites could be due to the mix of the following factors:

- Human factor (disparagement of the microbes by the use of chemical libraries).
- Scientific mistake (due to constraints of high throughput screening methods and the absence of chemical diversity of the compounds from combinatorial synthesis).
- Costs and regulations of an antimicrobial drug discovery program [103].

To solve the weaknesses of search of bioactive microbial metabolite and optimize the rate of the discoveries is important to develop the following:

Seek new promising sources of natural products (e.g. Endophytes, essential oils) [104,105].

Develop new screening methods more selective and effective, with the ability of predict clinical response of the selected compounds [106].

Implement and use other models for study pathogenesis and therapeutics of infectious diseases [107].

Equally, is necessary to develop new drugs that acting not necessarily to eliminate the infectious agent in the individual patient, also for to prevent antibiotic resistance in specific environments weakening the evolutionary possibilities of the biological elements involved as it is chemical communication, which makes different microorganisms deliver a coordinated response to environmental stimuli [108-110].

Conclusion

Antimicrobial drug resistance is one of the greatest threats to the health of people, and antibiotic discovery is a growing need. In this way is necessary design strategies in screening for the detection and development of anti-infective, but these new approaches require combination of target-based activity with whole cell inhibition, with the end of obtain antimicrobial drugs with novel mechanisms of action. Antibiotics adjuvants that use the mechanisms involved in antibiotic resistance or virulence is a promising approach for new “magic bullets” and prevents the emergence of resistance. Also, this strategy is complementary to the pursuit of synergistic combinations. Currently, the principal limitation of studies in drug is the selection of bacterial strains and determinate the induction of mutation developed by drugs. In this research field the design of the antimicrobial screening strategy is focused in the detection of drugs that target microbial cell wall or membrane, microbial virulence factors, DNA replication and gene transfer, *C. elegans* infection model and bacterial dormancy and persistence, this in combination of clinical microbiology assays of antimicrobial susceptibility testing, checkerboard analyses, and mutant prevention concentration (MPC) for obtain a mutant selection window of the compounds in combination against MDR pathogens. Because the urgent need of new antibiotics require projects focused in application and development of new products that have shown activity as antimicrobial adjuvants with promising application in prevention of hospital-acquired infections. The challenges to be applied in this research will provide new therapeutic alternatives in the control and treatment in infectious diseases caused by MDR microorganisms. The concept of antibiotic adjuvants is an important strategy that look for recover the lost susceptibility of the etiological agents to the current anti-bacterial therapy, in this order of ideas the obsolete compounds can be used again in new formulations and combinations, because is necessary to remember that resistance is not an isolated phenomenon, it is a public health problem in all its aspects has the same causes that this exploration seeks to overcome into new products.

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