Developmental Approaches to Antimicrobial Agents for the Battle Against Pathogens

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The battle between humanity and pathogenic microorganisms has continued since the beginning of the history of mankind on the earth. Many times this perilous relationship has unbalanced in favour of microbial pathogens resulting in outbreaks and holocausts, or benefit of humankind resulting in control of the pathogens and recovery of their hazards.

In this regard, finding various antimicrobial agents for specific targets has always been the most promising strategy for combating pathogens and a lot of antimicrobial natural and synthetic compounds such as antibiotics, bacteriocins, antibacterials, antifungals, antivirals and antiprotozoals have been discovered for decades.

Recently increasing resistance rates to antimicrobial agents in pathogenic microorganisms, mainly characterized by infections with high mortality levels both in hospitals and the community, has drawn attention to the developmental approaches and research studies on antimicrobial agents. Thus, the development of new molecules and new formulations against resistant pathogenic microorganisms is considered to be of great importance to human health.

For this aim, the present study was conducted to review brief history of antimicrobial-pathogen interactions, sources of antimicrobials, related international databases, developmental approaches to antimicrobial agents and current trends in the research studies.

Keywords: Antimicrobial Agents; Pathogenic Microorganisms; R&D

1. Antimicrobials at a glance

The word antimicrobial was originated from the Greek words anti (against), mikros (little) and bios (life) and covers the all kind of natural, semisynthetic or synthetic agents that kill microorganisms (microbiocidal agents) or inhibit their growth (microbiostatic agents). The targets of antimicrobials are fundamentally grouped into bacteria, fungi, protozoa and viruses. According to their acting capabilities against these targets, antimicrobial agents can be also further subdivided into antibacterials, antifungals, antiprotozoals and antivirals [1–5].

Antiseptics and disinfectants, which are commonly used for a broad range of topical and hard-surface applications, constitute an important group of antimicrobials and an essential part of infection control practices. As a definition, antiseptic is a general term describing chemical agents that inactivate microorganisms in or on living tissues. Similarly disinfectant is a general term describing similar chemicals inactivating microorganisms, but generally is used on inanimate objects or surfaces. Both of these antimicrobial agents have been extensively used in clinics, hospitals and other health care settings for centuries and effectively provided antisepsis, disinfection, prevention, and even cleaning and sterilization in some cases [6–7]. Table 1 shows several active antimicrobial chemicals commonly used in antiseptic and/or disinfectant formulations, and their intended clinical uses.

In chemotherapy, antimicrobials are especially defined as all kind of natural, semisynthetic or synthetic agents that inactivate microorganisms but cause little or no damage to the host. This definition distinctly distinguishes chemotherapeutic antimicrobials from the others generally used in antiseptics and disinfectants. These types of antimicrobials with their unique medication potential are commonly considered as the most essential ones for the battle against pathogenic microorganisms and their harmful effects on humankind. Thus, the vast majority of antimicrobial researches have been focused on chemotherapeutic antimicrobials for many years [1, 3–5, 8–11].

Although all remarkable achievements for antimicrobial chemotherapy were done in the last century, history of the first practices as old as the long-established history of human diseases. In these initial treatment practices, people of ancient Serbia, China, Egypt and Greece unconsciously used old moldy bread to heal wounds and protect from infections more than 2000 years ago. At approximately 1550 BC, Egyptians used a mixture, which includes honey, lard and lint, for similar purposes. In these times, there was no any information about presence of microorganisms responsible for illness and people believed that these treatments persuade the spirits or the gods to heal suffering in the patients. Many similar trends had gone on in a similar way until the latter half of 19th century when microorganisms were found to be key factors responsible for many of infectious illnesses [5].

The enlightenment began with the discovery of microorganisms in 1674 by Antonie van Leeuwenhoek and continued with an improving knowledge on several microorganisms cause a variety of infectious diseases. These revelations drastically changed the research route to the antimicrobials. As a result of partly intensive efforts and partly fortuity, E. von Freudenreich discovered antimicrobial activity of the blue pigments from *Pseudomonas aeruginosa* in 1888. Rudolf Emmerich and Oscar Löw investigated this phenomenon further and named the active substance as “pyocyanase”

originated from *Bacillus pyocyaneus*, the earlier name of *Pseudomonas aeruginosa* and commonly used that time. Pyocyanase was the first antimicrobial agent to be performed clinical trials in hospitals in 1889. Paul Ehrlich synthesized Salvarsan, another initial antimicrobial agent and a remedy for syphilis, in 1910. Ten years later, in 1920, Alexander Fleming reported an antimicrobial substance, initially described from chicken egg by Laschtschenko in 1909, in human tears and nasal mucus. He later named this antimicrobial compound as lysozyme in 1922 due to its lytic activity on bacterial cells. In 1935, Gerhard Domagk discovered and developed the first sulfonamides, synthetic antimicrobial compounds with limitations in terms of safety and efficacy. Sulfonamidochrysoidine (KI-730), a synthetic red dye, was the first commercially available antibacterial and marketed under the brand name Prontosil. Although there were important use limitations, discovery of sulfonamides triggered off the golden age of antimicrobials and pave the way for discovery of antibiotics. Therefore, the period till Domagk has been acknowledged as the pre-antibiotic era until today [1, 3–5, 8–11]. Figure 1 shows an outlook for the timeline of antimicrobials before the pre-antibiotic era and the golden age of antimicrobials.

Table 1 Several antimicrobial agents commonly used in antiseptics and disinfectants, and their intended clinical uses.

Main Group	Examples	Intended Use
Alcohols	Ethanol Isopropanol	Antisepsis Disinfection Preservation
Aldehydes	Glutaraldehyde Formaldehyde	Disinfection Sterilization Preservation
Anilides	Triclocarban	Antisepsis
Biguanides	Chlorhexidine Alexidine	Antisepsis Antiplaque agents Disinfection Preservation
Bisphenols	Triclosan Hexachlorophene	Antisepsis Antiplaque agents Preservation
Diamidines	Propamidine Dibromopropamidine	Antisepsis Preservation
Halogen-releasing agents	Chlorine compounds Iodine compounds	Antisepsis Disinfection Cleaning
Halophenols	Chloroxylenol (PCMX)	Antisepsis Preservation
Heavy metal derivatives	Silver compounds Mercury compounds	Antisepsis Disinfection Preservation
Peroxygens	Hydrogen peroxide Ozone Peracetic acid	Disinfection Sterilization
Phenols and cresols	Phenol Cresol	Disinfection Preservation
Quaternary ammonium compounds	Cetrimide Benzalkonium chloride	Antisepsis Cleaning Disinfection Preservation
Vapor-Phase sterilants	Ethylene oxide Formaldehyde Hydrogen peroxide	Disinfection Sterilization

*Table 1 adapted from McDonnell & Russell 1999 with some minor modifications [6].

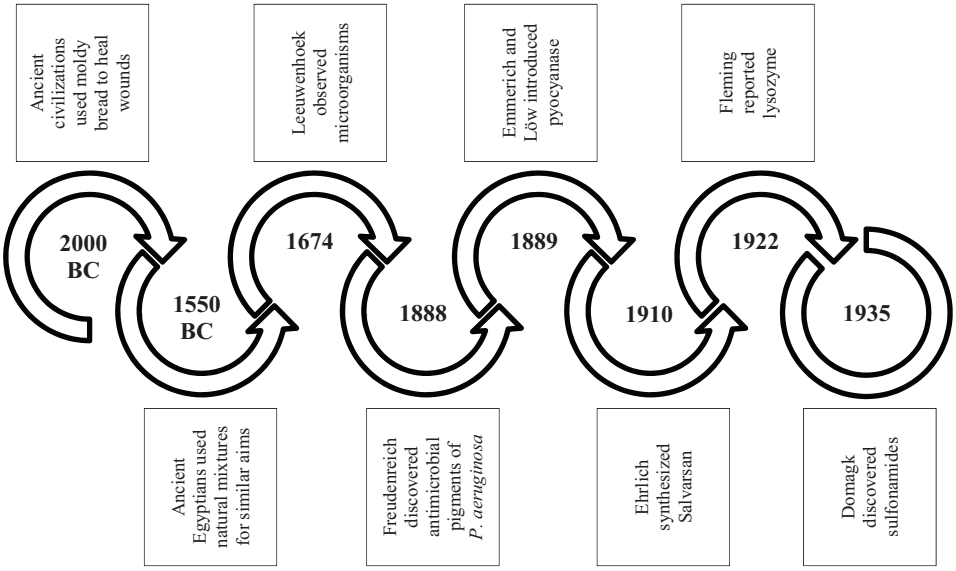

Fig. 1 An outlook for the pre-antibiotic era of antimicrobials.

The antibiotic era initially began with the discovery of penicillin by Alexander Fleming in 1928 and entirely revealed with the introduction of penicillin as a therapeutic agent against microbial infections by Howard Florey and Ernst Boris Chain in 1942. In that time, these advancements saved millions of lives around the world, penicillin was called as the miracle drug of the 20th century and Fleming, Florey and Chain shared the Nobel Prize for Physiology and Medicine in 1945. Since that time, antibiotics have been the most effective antimicrobials in chemotherapy and many types of antibiotics have been discovered and introduced for clinical applications during the past years [1, 3–5, 8–11].

In the present day, novel natural, semi-synthetic or synthetic antibiotic derivatives continue to be discovered and introduced, but this process has been slowing down day by day.

2. Antimicrobial resistance

2.1 Emergence of antimicrobial resistance

As mentioned before, a number of new antimicrobial agents were discovered and introduced for clinical use in the second half of the 20th century. Although these drugs saved millions of lives during the past years, the emergence of the resistance to these antimicrobial agents has always been an important concern since the beginning of the antimicrobial era [3–23].

As an example for the initial emergence of antimicrobial resistance, *Staphylococcus aureus* was one of the firstly realized resistant strains. Sensitive *S. aureus* strains rapidly acquired resistance to sulfonamides after these drugs were used. Then, penicillin was effectively used for the battle against this bacterium, but resistant strains with penicillinase producing capability emerged in the 1950s. In the following years, penicillinase-stable methicillin came into clinical use in 1960 for solving this resistance problem. However, this attempt failed by reason of isolation of methicillin-resistant *S. aureus* (MRSA) in 1961. Since that time, MRSA-associated nosocomial infections, also known as hospital-acquired infection (HAI), have become a social problem. Besides MRSA strains, vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) have been other threats for the public health care. Until today, many attempts and efforts have been done to defeat the resistant *S. aureus* strains and many groups of new antimicrobials discovered for this aim. Although there are several effective antimicrobial agents against resistant *S. aureus* infections, the concerns have taken on more importance recently. Similarly, *Streptococcus pneumoniae*, originally susceptible to penicillin, acquired resistance to antimicrobials and penicillin-intermediate *S. pneumoniae* (PISP) was detected in the latter half of 1960s. Ten years later, in the latter half of 1970s, penicillin- resistant *S. pneumoniae* (PRSP) strains were found. In the 1980s, ß-lactamase-producing strains of *Haemophilus influenzae*, resistant to ampicillin, were found. After this, ß-lactamase-negative ampicillin-resistant strains of *H. influenzae* (BLNAR), emerges a worse scenario, were detected in 1990s. Another important resistant bacterium was *Pseudomonas aeruginosa*, whose several strains resistant to anyone of many antimicrobial agents in the beginning. But, new *P. aeruginosa* strains resistant to all of three classes of antimicrobials including carbapenems, quinolones, and aminoglycosides have been determined recently [3–23].

As seen looking back on the history of antimicrobials, there is a strict connection between development of new antimicrobial agents and emergence of drug-resistant microorganisms [3–23]. In the frame of this connection, Figure 2 summarizes developmental trends of antimicrobial agents and emergence of resistant bacteria in a historical perspective.

Table 2 Discovery and introduction history of chemotherapeutic antimicrobials.

Period	Discovery/Introduction	Year
Before 1930	Penicillin discovered	1928
1930s	Sulfanomides discovered	1935
	Gramicidin discovered	1939
1940s	Penicillin introduced	1942
	Streptomycin discovered	1943
	Bacitracin discovered	1943
	Cephalosporins discovered	1945
	Chloramphenicol discovered	1947
	Chlorotetracycline discovered	1947
	Neomycin discovered	1949
1950s	Oxytetracycline discovered	1950
	Erythromycin discovered	1952
	Vancomycin discovered	1956
	Kanamycin discovered	1957
1960s	Methicillin introduced	1960
	Ampicillin introduced	1961
	Spectinomycin reported	1961
	Gentmicin discovered	1963
	Cephalosporins introduced	1964
	Vancomycin introduced	1964
	Doxycycline introduced	1966
	Clindamycin reported	1967
1970s	Rifampicin introduced	1971
	Tobramycin discovered	1971
	Cephamycins discovered	1972
	Minocycline introduced	1972
	Cotrimoxazole introduced	1974
	Amikacin introduced	1976
1980s	Amoxicillin-clavulanate introduced	1984
	Imipinem/cilastin introduced	1987
	Ciprofloxacin introduced	1987
1990s	Azithromycin introduced	1993
	Quinupristin/dalfopristin introduced	1999
2000 onwards	Linezolid introduced	2000
	Cefditoren introduced	2002
	Daptomycin introduced	2003
	Telithromycin introduced	2004
	Tigecycline introduced	2005

2.2 Mechanisms of antimicrobial activity and resistance

A well understanding on mechanisms of antimicrobial resistance can be achieved only via knowing modes of antimicrobial action. Basically an antimicrobial agent may interfere with cell wall synthesis, interfere with the cytoplasmic membrane, inhibit protein synthesis, interfere with nucleic acid synthesis or inhibit a metabolic pathway in the target cell and eventually cause microbiocidal or microbiostatic effects [24].

In the interference with cell wall synthesis, antimicrobial agent blocks peptidoglycan synthesis and cause a bactericidal effect. For example, both in Gram-positive and Gram-negative bacteria, ß-lactams bind to penicillin binding proteins (PBSs), key factors for cell wall synthesis, inhibit their functions and cause cell lysis on account of defective cell walls [24].

In the interference with the cytoplasmic membrane, an antimicrobial agent such as polymyxin binds to the cytoplasmic membrane and leads to disruption and destabilization, and eventually cell death [24].

Many of antimicrobials interfere with protein synthesis. For example, tetracyclines (e.g. tetracycline, minocycline, doxycycline) bind to the 30S subunit of the ribosome, block the attachment of tRNA and lead a bacteriostatic action.

Aminoglycosides also bind to the 30S subunit of the ribosome and cause bactericidal effects. Macrolides and chloramphenicol bind to the 50S ribosomal subunit and lead bacteriostatic effects. Differently, linezolid binds to a site on the 23S rRNA and inhibits formation of 70S initiation complex [24].

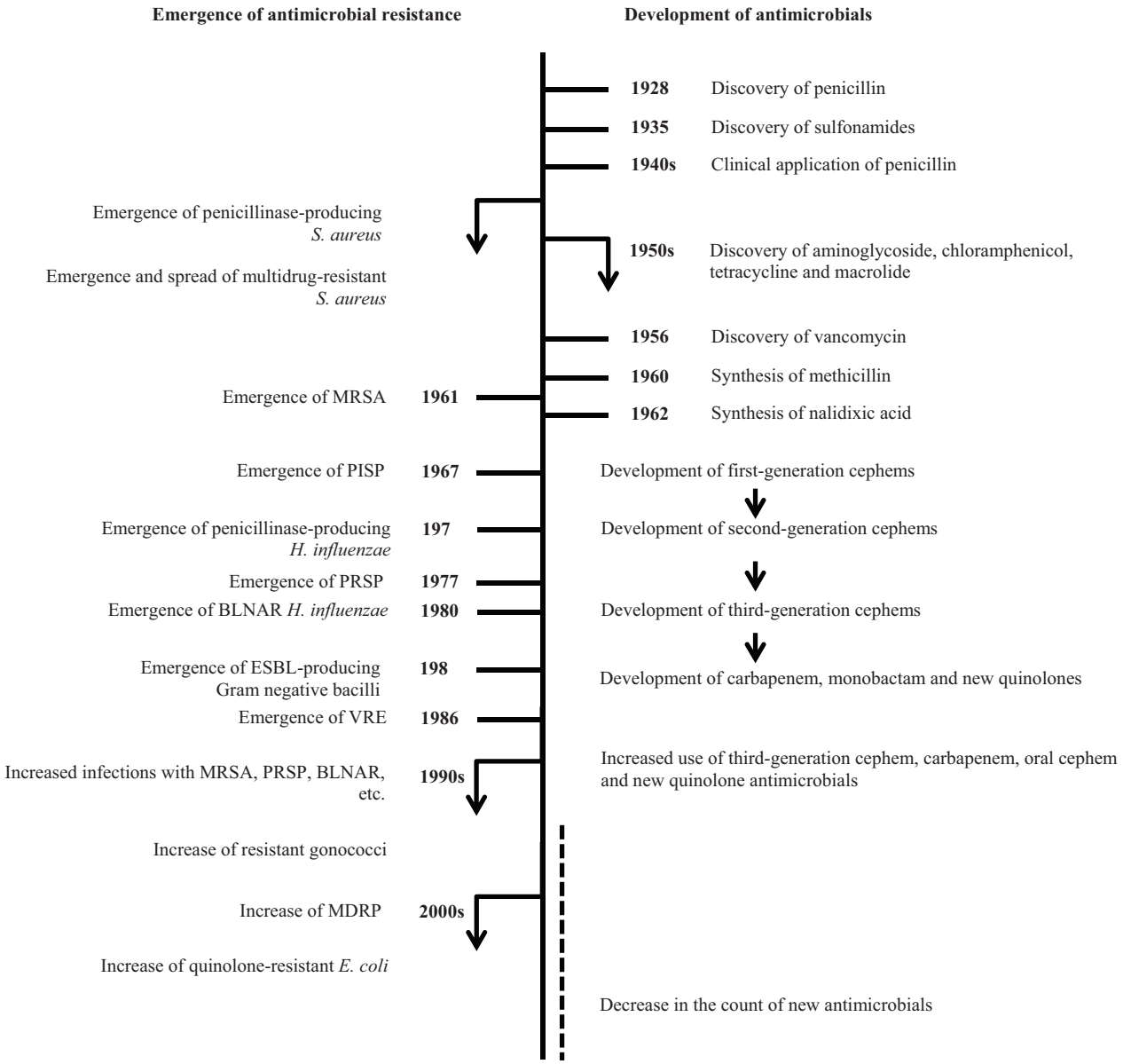

Fig. 2 Developmental trends of antimicrobial agents and emergence of resistant bacteria (adapted from Saga & Yamaguchi) [10].

In the interference with nucleic acid synthesis, antimicrobial agents such as fluoroquinolones or rifampin interfere with several enzymes related to nucleic acid synthesis such as DNA gyrase or DNA-dependent RNA polymerase, inhibit nucleic acid synthesis and cause cell death [24].

In the inhibition of a metabolic pathway, antimicrobial agent may act as a structural analog of a key metabolite in a critical pathway or as an inhibitor for a critical enzyme. For example, sulfonamides act as structural analogs of para-aminobenzoic acid (PABA), but trimethoprim inhibits dihyrofolate reductase. Both sulfonamides and trimethoprim block the folic acid synthesis pathway and cause bacteriostatic effect [24].

Depending on these modes of actions, mechanisms of antimicrobial resistance include: a) production of enzymes such as ß-lactamases and aminoglycoside-modifying enzymes that destroy or modify the antimicrobial agent; b) alteration of bacterial outer membrane permeability that inhibit the entrance of antimicrobial agents into the target cell; c) alteration of targets such as PBPs, ribosomes or enzymes; d) activity of efflux pumps; and e) alteration of specific pathways. Some species have these mechanisms intrinsically; some can also acquire resistance by genetic events such as mutation, conjugation, transformation, transduction and transposition. By any means whatsoever, antimicrobial

resistance has been one of the most important issues in the antimicrobial history and it has driven antimicrobial drug development studies [24]. Table 3 summarizes some of antimicrobial resistance reports and their mechanisms.

Table 3 Some examples for the mechanisms of antimicrobial resistance.

Antimicrobial agent	Resistance reported	Mechanism of resistance
Penicillin G	1940s	Penicillinase production
Streptomycin	1947	Mutation in ribosomal protein
Tetracycline	1952	Efflux
Penicillin and tetracycline in *N. gonnorhae* and enterobacteriaceae	1976-1980	Plasmid-encoded broad spectrum ß-lactamases and tetracycline efflux pump
Mehicillin and all ß-lactams in *S. aureus*	1961	MecA (penicillin-binding protein 2a)
Nalidixic acid	1966	Topoisomerase mutations
Gentamicin	1969	Aminoglycoside modifying enzyme
Cefotaxime	1981 1983	AmpC ß-lactamase Extended spectrum ß-lactamases (ESBLs)
Linezolid	1983	23S RNA mutation

*Table 3 adapted from Bush 2004 with some minor modifications [9].

3. Recent trends in antimicrobial agent research

3.1 Principal antibacterial drug discovery approaches

Looking back on the history of antimicrobial agent researches, three important antibacterial drug discovery strategies, whole-cell (non-target-based) antibacterial screening, *in vitro* high-throughput screening (HTS), and structure-based drug discovery (SBDD), have reached to day since the 1940s (Figs. 3–4). The whole-cell (non-target-based) antibacterial screening, known as also empirical whole cell screening, is the primal approach in the antimicrobial drug discovery strategies. From the 1940s, many of the known antibacterial agents were originally discovered as a result of empirical whole cell screening of natural products or synthetic chemical libraries. In more recent programs of this approach, research efforts have especially focused on the synthetic compound libraries. HTS is a target-based approach and closely related with genomics. Hundreds of highly conserved broad-spectrum targets with little mammalian homology have been determined since the sequencing of the whole genome of *Haemophilus influenzae* in 1995. SBDD is a new *in silico* approach to antimicrobial drug discovery and includes the component technologies of virtual high-throughput screening (VHTS) and fragment-based drug discovery (FBDD) [11, 25–31].

Although there are severe differences among these approaches, all of them aims discovery of chemically novel leads and they fall back on supporting technologies such as classical microbiological methods; bacterial physiology, biochemistry and genetics to identify new drug targets; genomics to identify further novel targets; and structural elucidation of targets [11].

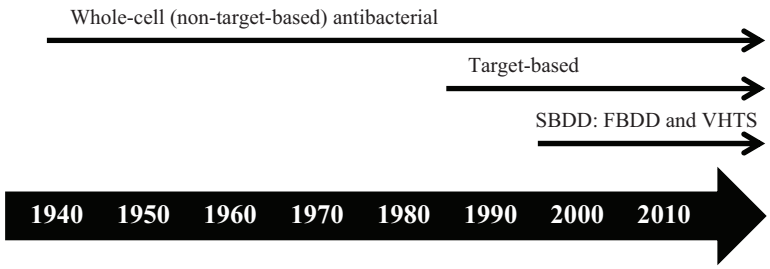

Fig. 3 Principal research and development strategies from 1940 to the present day (adapted from Chopra 2012 with some minor modifications) [11].

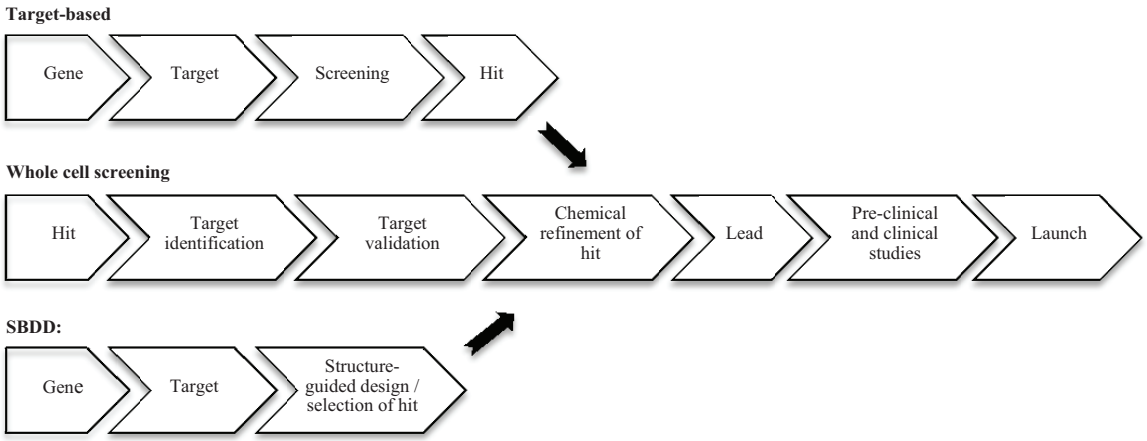

Fig. 4 The three principal research and development strategies currently utilized for the generation of antimicrobial lead structures (adapted from Chopra 2012) [11].

3.2 Databases and bioinformatics resources of antimicrobial researches

During the past years various databases and bioinformatics resources were developed and introduced to researchers around the world. In the development process of new antimicrobial agents, databases and bioinformatics tools containing genomic, proteomic and functional information have been essential and helpful since the beginning of the 2000s. Today, there are many of these effective tools that generally serve specific demands in antimicrobial researches [32]. Some of them and their general properties were summarized in Table 4.

Table 4 Some examples for Databases and bioinformatics resources of antimicrobial researches.

Database	Summary
UniProt	Resource for protein sequence and annotation data
The Protein Data Bank	A worldwide repository for the processing and distribution of 3D biological macromolecular structure data
AMSDb	Antimicrobial sequences database
ANTIMIC	Database of Antimicrobial Peptides
APD2	The Antimicrobial Peptide Database
CAMP	Collection of Antimicrobial Peptides
APPDb	Antimicrobial Peptide and Protein Database
AMPer	AmpC ß-lactamase Extended spectrum ß-lactamases (ESBLs)
AMPer	A database and discovery tool for antimicrobial peptides, based on hidden Markov models and the SwissProt databank
Peptaibol	Peptaibol Database
SAPD	Synthetic Antibiotic Peptides Database
Defensins	Defensins Knowledgebase
CyBase	A database of cyclic protein sequence and structure
PenBase	The Shrimp Penaeidin Database
TB Database	Database for tuberculosis research
BACTIBASE	A data repository of bacteriocin natural antimicrobial peptides

*Table 4 adapted from Hammami and Fliss 2010 with some minor modifications [32].

Table 4 Some examples for Databases and bioinformatics resources of antimicrobial researches (continued).

Database	Summary
PhytAMP	A database dedicated to plant antimicrobial peptides
RAPD	A database of recombinantly-produced antimicrobial peptides
BAGEL	A genome mining tool for putative bacteriocin gene clusters detection
AMICBASE	Contains information on the antimicrobial and toxicological properties of natural compounds produced by microorganisms and higher plants
Novel Antibiotics DataBase	Contains substances reported first in the Journal of Antibiotics
A/OL	Antimicrobial compounds, applications in the food field

*Table 4 adapted from Hammami and Fliss 2010 with some minor modifications [32].

3.3 Natural products as the future scaffolds for new antimicrobials

Increasing emergence of resistant microorganisms to antimicrobials accompanied by fails on development of new effective chemical drugs has revived scientific interests in natural products for over last two decades. Only seven new chemical entities have been approved for treatment of bacterial infections in the past decade. In this regard, the role of natural products has gained more attention in the modern drug discovery studies and a lot of various natural antimicrobial agents such as bacteriocins, antimicrobial peptides, and arylomycins have been discovered as a result of dense research efforts [2, 33-35].

4. Final Remarks and conclusions

This recent analysis clearly shows that there is an urgent need for new antimicrobials for the battle against pathogens. In this phenomenon, especially emergence of resistant microorganisms has been driving antimicrobial drug development researches and related trends since the beginnings of 1940s. To achieve resistance challenges in the near future, a well-understanding on the modes of antimicrobial action, the mechanisms of resistance, the principal antimicrobial drug discovery trends and related supporting tools is indispensible.

On the other hand, today, it would better be remembered that the inappropriate use of antimicrobial agents cause the selection of resistant pathogens and only the proper use of currently available drugs may help to minimize the spread of resistance.

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