

## Antibiotic resistance of bacterial pathogens: the magnitude of the problem from two perspectives - Romanian and worldwide

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### Abstract

Despite the medical concerns arising from the emergence of antibiotic resistance, a regress in developing new antibiotic drugs has been registered in the past decades. Therefore, for the next years, the main objective is to slow down the development and spread of antibiotic resistance. At the same time new and alternative strategies are needed to identify novel classes of antibiotics.

In this perspective, the present review aims to discuss the magnitude of the emergence of bacterial resistance to approved antibiotics. The first section briefly describes the main mechanisms of resistance – an important issue to be considered for the discovery and design of new effective antibiotics. The next sections deal with the statistical information on antibiotic resistance. International surveillance studies on resistance and antibiotic consumption showed significant variation related to geographic location, type of population and community level care, sampling and analyses procedures. Despite these influences there is a worrying worldwide evolution regarding the development of resistant and multiresistant isolates of pathogens. Strategies to reduce antibiotic resistance should be adapted to each country, region and hospital database. We conclude by considering other issues, such as supervision, infection control policies and educational programs based on enhanced hygiene and decrease of the misuse and abuse of antibiotics, as equally important for the management of the resistance phenomenon.

**Keywords:** antibiotic resistance, mechanism of resistance, antimicrobial agents, drug discovery

### Introduction

Resistance of pathogenic organisms to approved antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases. The increased use/misuse of antibiotics in human medicine, veterinary and agriculture is mainly contributing to the phenomenon. There is an alarming increase of antibiotic resistance of bacteria that cause either community infections or hospital - acquired infections. Of particular interest are the multidrug resistant pathogens, e.g. *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant MRSA, penicillin-resistant *Streptococcus pneumoniae* (PRSP), vancomycin-resistant *Enterococcus* (VRE), and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* (ALEKSHUN & al. [1]). Resistance to methycillin and vancomycin is most commonly developed in nosocomial infections and non-hospital units. Penicillin-resistant *Streptococcus pneumoniae* is frequently detected in pediatric units. Hospital infections with methycillin-resistant *Staphylococcus aureus* occur most frequently in patients with invasive medical handling or immune-

suppressed patients with prolonged treatment in health care centres or dialysis (GOULET & al. [2]). Community infections are more virulent and spread faster, causing more severe health problems (AUPERT & al. [3], HAUTE AUTORITE DE SANTÉ 2008 [4]).

As the success to develop new classes of antibiotics with novel mode of action has been compromised in the last decades, pharmaceutical companies abandoned or drastically decreased investments in the antibiotic research (FOX [5]). It seems that partnerships of big pharmaceutical companies with biotechnology organizations might be a way to move forward in this field (BARRETT [6]). In the absence of major new classes of antibiotics, other strategies have to be considered in order to minimize the development of resistance, such as the restrictive and educational antibiotic stewardship programmes (GOULD [7]).

The present review describes the main genetic and molecular mechanisms of bacterial resistance to antibiotics and focuses on two perspectives of facing antibiotic resistance, based on international and national surveillance studies, providing statistical information on this phenomenon.

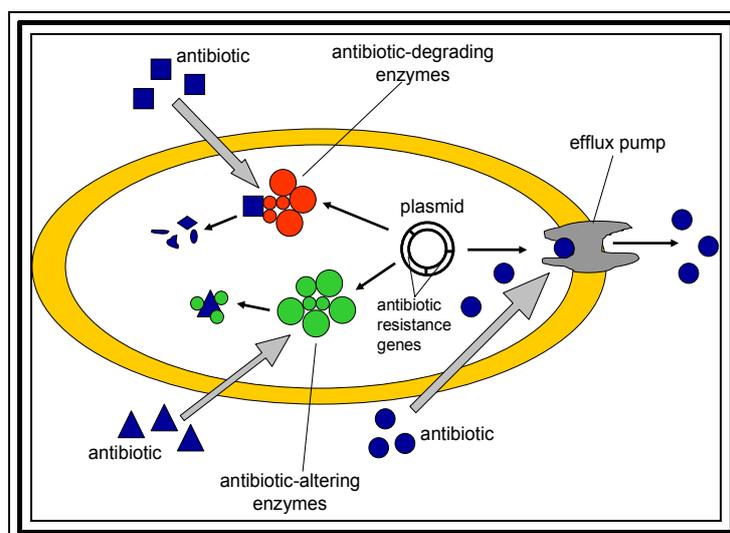
### Mechanism of antibiotic resistance

The present section of the review will give a brief background on the mechanisms by which bacteria acquire resistance to antimicrobial drugs.

Mechanism of resistance may arise in bacteria over months or years (DAVIES [8]). As bacteria have developed resistance to every class of antibacterial drugs, the main objective is to slow down the development and spread of antibiotic resistance.

The literature describes several genetic and molecular mechanisms of bacterial resistance to antibiotics as following (see Figure 1):

1. Modification of the bio-molecular target for antimicrobials either by a spontaneous mutation of the gene encoding the target or by substitution of the target function by an exogenous gene (ALEKSHUN & al. [1])
2. Reduction of the intracellular antibiotic concentration in bacteria, by its efflux outside from the cell through bacterial trans-membrane efflux pumps (efflux pumps that recognizes specific antimicrobials and multidrug efflux pumps) (ALEKSHUN & al. [1], LEVY [9])
3. Enzymatic inactivation of antibiotic, *e.g.*  $\beta$ -lactamases which hydrolyze  $\beta$ -lactams (ALEKSHUN & al. [1], BARBER [10], JACOBY & al. [11])



**Figure 1.** Main mechanisms of bacterial resistance to antimicrobial drugs (adapted from *Todar 2009*) (TODAR [12]).

A single strain of bacteria can select multiple and additional kinds of resistance mechanisms. This selection led to multidrug resistance – a worldwide problem which compromises treatment of infections.

Bacterial multidrug resistance is acquired through chromosomal mutations or exchange of extra chromosomal elements from other bacteria in the environment. Bacteria can acquire foreign genetic material by different processes, *e.g.* transformation (incorporation of free DNA segments into their chromosome), transduction (via bacteriophages) or conjugation (via plasmids and conjugative transposons) (ALEKSHUN & al. [1]).

Efflux pumps that expel antibiotics outside from the cell were first described for tetracyclines (MCMURRY & al. [13]). Regarding the bacterial efflux pumps based on proteins, they belong to the following proteins families: superfamily ABC (ATP binding cassette), families SMR (small multidrug resistance), MF (major facilitator), MATE (multi antimicrobial extrusion) and RND (resistance nodulation division) (KAATZ [14]). These efflux pumps differ by size, number of trans-membrane segments and mode of action. Most efflux pumps use the proton motive force as main energy source, while some particular pumps are based on ATP hydrolysis. Little is known about the structure of the pumps, but progress has been made using X-ray analysis (MURAKAMI & al. [15]). Research studies of the efflux pumps that recognize specific antibiotics, such as tetracyclines and macrolides, proposed a novel approach by developing an antibiotic compound that is not substrate of these specific pumps but has antibacterial activity against resistant bacterial strains (MRSA, VRE, PRSP). These studies led to a new antibiotic with expanded broad-spectrum activity, tigecycline, recently approved by the United States Food and Drug Administration (FDA) for clinical trials (PROJAN [16], JONES & al. [17]). Other studies on multidrug efflux pumps demonstrated the involvement of monomeric proteins with 12  $\alpha$ -helices capable to penetrate the membranes, resulting in the efflux of antibiotics either by ATP hydrolysis or by proton motive force (NIKAIDO [18], LOMOVSKAYA & al. [19]). The efflux pumps that expel multiple types of antibiotics mainly contribute to the multidrug resistance in bacteria.

The enzymatic inactivation of antibiotics represents another mechanism of antibiotic resistance. Enzymes that modify antibacterial drugs are divided into two general classes: (1) antibiotic-degrading enzymes, such as  $\beta$ -lactamase; (2) antibiotic-altering enzymes that perform chemical transformations of antibiotics by acetylation, phosphorylation, adenylation, glycosylation, hydroxylation, such as macrolide- and aminoglycoside-modifying enzymes, or flavin-dependent mono-oxygenase modifying tetracycline. There are few clinically used antibiotics acting as inhibitors of class A  $\beta$ -lactamases, such as clavulanic acid used in combination with amoxicillin or ticarcillin, and sulfone inhibitors (tazobactam and sulbactam) (SINGH [20]). In the past years, an increasing number of clinical bacterial isolates that contain genes of class C  $\beta$ -lactamases has been registered (WINOKUR & al. [21]), for which new inhibitors with improved spectrum are needed (MILLER & al. [22], KUMAR & al. [23]). A less number of clinical bacterial isolates produces metallo  $\beta$ -lactamases capable of the hydrolysis of all  $\beta$ -lactams. Research programs targeting zinc  $\beta$ -lactamase provided a new inhibitor, thiomandelic acid that can be used efficiently in combination with carbapenem – the known antibiotic resistant to other classes of  $\beta$ -lactamases (MOLLARD & al. [24]).

Bacteria can acquire resistance by different of the above mentioned mechanisms. Understanding the possible mechanisms of resistance can lead to a rational design of new antimicrobial drugs urgently needed to treat infectious diseases. Not at least, issues such as permeabilization of cytoplasmic and outer bacterial membranes have to be considered for the antimicrobial efficacy (WALSH [25]).

### Data on antimicrobial resistance of pathogens

There is a common opinion among researchers that an increased consumption of antibiotics mainly contributes to the rapid spread of bacterial resistance. Use of antimicrobials determines a selection pressure on pathogens, some of them being killed, others becoming adapted (antibiotic resistance).

Attempts to limit this problem which generates a great impact on antibiotic efficacy over time should be based on a more rational use of antibiotics, in particular broad-spectrum antibiotics, through education programs, guidelines and protocols, and on the implementation of infection control policies.

There are considerable differences between geographical regions regarding issues such as antibiotic consumption and bacterial resistance to antibiotics.

This section of the review will outline the existing surveillance data on antibiotic resistance and usage and the importance of continuing a genuine and integrated surveillance.

#### *Antibiotic resistance: International data and usage*

Understanding the international patterns of antibiotic resistance is an important key for the control of the spread of resistance. We are far from establishing efficient resistance correlations among countries as each region practises different sampling strategies, laboratory processing, diverse standards for defining a strain as resistant. However, there is a high geographical variability of bacterial resistance to specific antibiotics, as shown in Table 1.

**Table 1.** Regional variability in antimicrobial resistance rates of common pathogens (REINERT & al. [26], FERECHE & al. [27], ELSEVIERS & al. [28], BEAN & al. [29], VLIEGHE & al. [30]).

Antimicrobial agent	Resistance rates as reported in different studies (%)		
	Europe	U.S.A.	others
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	>30	>30	>30 (Australia, Asia Pacific)
Vancomycin-resistant <i>Enterococcus</i> (VRE)	<2	>10	<2 (Latin America, Asia Pacific)
Penicillin-resistant <i>Streptococcus pneumoniae</i> (PRP)	<12	14 18 to vancomycin 25 to penicillin 18 to erythromycin	>12 (Asia Pacific) <12 (Latin America) 13.7 (Central Africa)
multidrug-resistant <i>Pseudomonas aeruginosa</i> (MDR)	5	26 to ciprofloxacin/ofloxacin 30 to Levofloxacin 9 to ceftazidime	8 (Latin America) >18 (China)
Ampicillin-resistant <i>Haemophilus influenzae</i>	14-19	26-33	16 (Asia, Latin America)
Ampicillin-resistant <i>Escherichia coli</i>	40-60 55 (London) 10 resistance to fluoroquinolone	4 to quinolone 1 to 3 <sup>rd</sup> -gen. cephalosporines	>80 (China) 77.1 (Central Africa)
<i>Helicobacter pylori</i>	3-15 to macrolides 10-50 to metronidasol		
<i>Mycobacterium tuberculosis</i>	MDR strains: 5-14, in particular in Eastern Europe		5-14 (Russia, Iran, China) MDR 19 (Moldova) MDR 17 (India)
<i>Klebsiella pneumoniae</i>	UK: 14 resistance to 3 <sup>rd</sup> -gen. cephalosporines	4 resistance to 3 <sup>rd</sup> -gen. cephalosporines	42.7 to Ampicillin/Amoxicillin (Central Africa) 80.3 to Trimethoprim/Sulfamethoxazole (Central Africa) 3.3 to 3 <sup>rd</sup> -gen. cephalosporines (Central Africa)

The present data on the resistance of the most common pathogens to specific antibiotics illustrate that medicine is facing great problems of antibiotic therapy.

The first international longitudinal multi-learning research study regarding the national, regional and global susceptibilities to antibiotics – the “Alexander Project” (1998-2000) – investigated the susceptibility of most common respiratory pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* to different antibiotics (FELMINGHAM & al. [31]). The published results showed a high proportion of penicillin-resistant pneumococci in France (57.9%) and Spain (40.7%) compared to Germany, Czech Republic and Slovakia (5.9%-6.2%). Data were in accordance with the results of other European studies, e.g. the International Surveillance Study 1998-1999. Similarly, there is a high pneumococcal resistance to macrolides in France, Italy and Spain.

Through another large project – the European Antimicrobial Resistance Surveillance System (EARSS) – data on the antibiotic susceptibilities of *S. pneumoniae*, *Staphylococcus aureus*, *E. coli* and *Enterococcus faecalis* were collected from European countries since 1999 (EARSS Annual Report 2004 [32]). Surveillance data from 2004 to 2006 showed a low pneumococcal susceptibility to penicillin and erythromycin, particularly in Southern Europe. In 2007, EARSS published data on the resistance and co-resistance of clinical isolates of *Klebsiella pneumoniae*. These data showed a more common development of co-resistance of *Klebsiella pneumoniae* to three antibiotic classes than to only one or two classes (EARSS Annual Report 2007 [33]).

In U.S. most of the available resistance data come from two types of projects: (1) the National Nosocomial Infections Surveillance System (NNIS) for hospital-acquired infections; and (2) the Active Bacterial Core Surveillance (ABC) for community infections. From all the surveillance programs from 1999 to 2002 emerged an increased resistance prevalence to the following strains: MRSA (36%), VRE (10%), PRP (17), *Enterobacter* spp. resistant to the third generation of cephalosporins (19%), *Shigella* spp. resistant to trimethoprim/sulfamethoxazole (53%) (NNIS Report 2001, ABC Report 2001 [34]). By comparing these results with data coming from surveillance programs in another region like China (1999-2001), there are significant differences. In China, antibiotic resistance is higher for the most common investigated bacteria, reaching 73% resistance of *Streptococcus pneumoniae* to erythromycin, while resistance to VRE is not encountered (ZHANG & al. [35]). This is probably due to the influence of the volume of antibiotic usage.

There are several European projects that aimed to collect data on the consumption of antibiotics. The European Study on Antibiotic Consumption (ESAC) collected data from 1997 to 2002 from 24 European countries. The study showed a substantial increase in the consumption of fluoroquinolones and amoxicillin-clavulanic acid, and a decrease in the use of narrow-spectrum antibiotics, e.g. penicillin, erythromycin, quinolones and sulfonamides. In Northern Europe there was a small variation related to seasons and a lower consumption of antibiotics compared to Southern Europe. The ESAC data from 2004-2005 on resistance of *S. pneumoniae* strains collected in winter from patients with community-acquired infections from 15 European countries correlated the results of the level of resistance to the volume of antibiotic usage. For all countries, a significant correlation between the total consumption of antibiotics and the developed resistance of pathogens to those drugs was found. The strongest correlation was established between the consumption of  $\beta$ -lactams and pneumococcal resistance to these antibiotics (RIEDEL & al. [36]).

Several data reported that people of Southern European countries consume on average up to three times more antibiotics than populations in Northern Europe (SONG [37]).

Despite limitations to studies on antimicrobial resistance in different regions there is to be noted a worrying evolution worldwide of resistant and multi-resistant isolates of

pathogens. As globalization increases the crossing of the borders, a critical question of transferring antibiotic resistance from one nation to another, arises. Therefore, an efficient integration of international response is needed to control infections (FIDLER [38]).

***Antibiotic resistance: National data and usage***

Romania is an ESAC member and also a participant in the EARSS – a surveillance and information system which provides comparable and validated data on the prevalence and spread of major invasive bacteria and antimicrobial resistance in Europe. Data from national laboratories have been reported to EARSS since 2002, and the results have shown a wide increase of antibiotic resistance of certain microbial strains following the trends from 2002 to 2007 (EARSS Annual Report 2007 [33]). Considering the 2007 EARSS Report as the most complete database in the field, we have summarized relevant statistics for Romania by comparison with other EU countries (see Table 2).

**Table 2.** Rate of resistance of several bacterial strains to approved antibiotics, as reported by Romania to EARSS in 2007 ([33]), compared to data reported by other EU countries.

Clinical isolates	Class of antibiotics	Rate of resistance (%)	
		Romania	Other EU countries
<i>Streptococcus pneumoniae</i>	penicillin	25-50	Northern countries: 1-5
	erythromycin	10-25	5-10
<i>Staphylococcus aureus</i>	oxacillin/methicillin	25-50	Northern countries: 1-5
<i>Escherichia coli</i>	3 <sup>rd</sup> gen. cephalosporins	25-50	Northern countries, France: 1-5
	fluoroquinolones	25-50 (similar to Spain)	Northern countries, France: 10-25 Turkey > 50
	aminoglycosides	25-50 (similar to Turkey)	Central EU: 5-10
<i>Klebsiella pneumoniae</i>	3 <sup>rd</sup> gen. cephalosporins	>50 (similar to Bulgaria, Greece)	Northern countries: 1-5
	fluoroquinolones	10-25 (similar to France, Spain, Turkey, UK)	Northern countries: 1-10
	aminoglycosides	>50 (similar to Bulgaria, Greece)	Northern countries: 1-5
	carbapenems	< 1	most EU countries: < 1

As noticed, Northern countries reported significant lower antibiotic resistance, while high prevalence of resistant strains (*K. pneumoniae*) to 3<sup>rd</sup> generation cephalosporins, fluoroquinolones and aminoglycosides became evident in Central and South-Eastern Europe.

National research studies in the field established antibiotic susceptibilities for bacteria involved in most commonly infectious diseases, such as skin, urinary and enteric infections with high prevalence in hospitals and ambulatories. Therefore, we will refer here to some representative results from the following regional studies: Sibiu, Iasi, Timisoara, Brasov.

The regional study from Sibiu district investigated the antibiotics susceptibility of 345 *Salmonella* strains isolated from patients hospitalized with enteric infections, during 2004-2008 (HILMA & al. [39]). The isolated strains belong 42% to the B serological group and 58% to the D one. The results on *Salmonella* strains resistant to the investigated antibiotics presented in Table 3, showed significant increase of resistance to amoxicillin, tetracycline and nalidixic acid.

**Table 3.** Resistance of *Salmonella* strains to antibiotics (HILMA & al. [39]).

Antibiotic	Resistance during 2004-2008 (%)				
	2004	2005	2006	2007	2008
Amoxicillin	5.5	7.2	20.8	20.4	21.1
Augmentin	3.7	5.6	14.8	15.7	15.4
Cefuroxim	1.4	3.8	7.6	10.5	0
Gentamicin	0	0	0	0	0
Tetracycline	16.5	23.8	25	30	31.2
Colistin	7.4	14.2	4.2	6.5	4.2
Chloramphenicol	11.9	20.8	10.8	9.5	3.2
Nalidixic Acid	3.8	5.85	20.8	31.3	55.8
Ciprofloxacin	2.4	3.2	1.2	1.8	0
Cotrimoxazol	11.1	14.3	8.4	8.4	9.8
Furazolidon	4.7	8.4	6.5	4.4	1.4

Another regional study (Iasi district) investigated the resistance of coagulase-negative staphylococci (CoNS) with clinical significance, isolated in the Iasi Infectious Diseases Hospital (DORNEANU & al. [40]). The most frequently encountered CoNS species were *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. Bacterial resistance to oxacillin was high, as 55% for *S. epidermidis* and 76.9% for *S. haemolyticus* respectively, representing a serious problem in the management of CoNS infections. Methicillin-resistant CoNS were also resistant to fluoroquinolones (60%), gentamycin (60%), erythromycin (63.3%) and tetracycline (60%). The most important alternative for the therapy of infections produced by methicillin-resistant CoNS strains remains the use of vancomycin and the FDA approved combination of antibiotics quinupristin-dalfopristin.

The regional studies from Timisoara district refer to the multidrug resistant bacteria involved in the skin and urinary community-acquired infections. *Staphylococcus aureus* responsible for skin and soft-tissue infections belongs to MRSA strains resistant to oxacillin (33.33%), tetracycline (52.17%), and erythromycin (29.17%), which are non- $\beta$ -lactam-antibiotics (NEGRU & al. [41]). Another study reported by the same authors found a significant resistance of *Enterobacter* spp. (*K. pneumoniae*, *E. coli*, *Serratia*, *Proteus*) isolated from urine, to fluoroquinolones (NEGRU & al. [42]).

Other evaluation studies of the dynamics of antibiotic resistance of *Enterobacter* spp. isolated in Brasov Hospital, showed a high increase of resistance to ampicillin (85%), and ampicillin-clavulanic acid (74.5%) (IDOMIR & al. [43]).

Referring to the antibiotic consumption in Romania, the report of the national producer (*SC Antibiotice SA, Iasi*) showed an increasing trend in antimicrobial drugs sales during 2005-2009 [44]. The most representative quantitative increase in drug sales for the first semester of 2009 compared to the similar period of 2008 was registered for the following antimicrobials (commercial name): CEFORT® (50%), CEFTAMIL® (30%), AMOXIPLUS® (100%), AMIPLUS® (30%), CIPROQUIN® (21%) and EFICEF® (27%) [44]. This report showed that the company sales are comparable to those of the national pharmaceutical market.

According to ESAC, countries belonging to South-Eastern Europe have the highest antibiotic consumption compared to the much lower consumption in Northern Europe and the Russian Federation (ESAC [45]).

## The discovery of new antimicrobial drugs to address the problem of antibiotic resistance

The antimicrobial research is facing two major medical concerns: (1) increase of multiple drug resistant bacteria; (2) pharmaceutical company abandonments of the research for identification of new classes of antibiotics.

From the gold period of the discovery of natural antibiotics, antimicrobial research focused on analogues of the existing and proven classes of drugs in order to improve their spectrum of activity against bacterial pathogens and/or to increase their antibacterial activity (SENTHILKUMAR & al. [46], MASSARI & al. [47], EKAMBARAM & al. [48], KAGLIWAL & al. [49]). The route of analogues continues to be a successful one for antibacterial drugs discovery, as shown by marketed antibiotic derivatives of tetracyclines, macrolides and ketolides. Unfortunately, in the last decades only two new classes of antibiotics entered the marketplace, oxazolidinones (BARBACHYN & al. [50]) and cyclic lipopeptides (KERN & al. [51]). Consequently, new approaches are needed for accelerating the antibiotics discovery, either by target-based approach focusing several targets in parallel/targets encoded by multiple genes, or new strategies. There are several promising alternative routes to be considered, *e.g.* targeting non-multiplying bacteria, non-cultivable bacteria, or bacteriophages (COATES & al. [52]).

It is known that research for discovery of new bioactive molecules involves high costs, risks, side effects and long preclinical and clinical trials. The drug candidate selected from the complex drug discovery process will enter the development phases, which are time-consuming and expensive. Clinical trials of antimicrobial compounds are different from clinical evaluation of other drugs, and more complex because of the interactions of drug candidate, patient and pathogenic organism that have to be evaluated.

Table 4 presents several potential antibiotic drugs in development either by large pharmaceutical companies or by small biotech companies (BARRETT [6], ABBANAT & al. [53], TANASEANU & al. [54], MURRAY & al. [55], MURALIDHARAN & al. [56]).

**Table 4.** List of potential antibacterial agents in different phases of clinical trials [6], [53-56].

Name of the antibiotic drug	Class of antibiotics	Name of the Company
<i>Antibacterials developed by pharma companies</i>		
Garenoxacin	Quinolone	Schering-Plough/Toyoma
CS-023	Carbapenem	Sankyo/Roche
Tigecycline	Tetracycline; Glycylcycline	Wyeth
<i>Antibacterials developed by biotech companies</i>		
Iclaprim	Diaminopyridine	Arpida/Roche
Faropenem	Carbapenem	Replidyne/Suntory
PPI-0903M and TAK-599	Cephalosporin	Takeda/Peninsula
MBI 594AN	Indolicidin peptide	Migenix
Ramoplanin	Glycolipo-depsipeptide	Oscient/Vicuron
Oritavancin	Glycopeptide	InterMune/Lilly
MC-207110	Peptide	Essential Therapeutics/Daiichi
Dalbavancin	Glycopeptide	Vicuron/Aventis
TD-6424	Glycopeptide	Theravance

## Conclusions

As bacteria have developed different strategies to inhibit the effects of antibiotics, the identification of the resistance mechanism may help in the discovery and design of new antimicrobial agents.

European studies on antibiotic resistance and consumption show significant variation between different countries, most of them related to the difference between drug consumption and hospital infection control. Resistance to antibiotics varies widely depending on geographic location, type of population and community level care or testing procedures. Romanian data on antimicrobial resistance show high levels, above 25%, and a constantly increasing trend in the last years, that is quite common in Southern and Eastern Europe, in particular for *Streptococcus pneumoniae* strains non-susceptible to penicillin. The dynamic EARSS picture of this issue demonstrates differences between European countries, meaning particular strategies in limiting antibiotic consumption. An increasing problem all over Europe and U.S. is still MRSA strains. Combined resistance is also a frequent occurrence and seems to become the dominant threat. As carbapenems seem to be still effective against *Klebsiella pneumoniae* in most countries, a responsible prescribing of it in hospitals and in ambulatory care should be introduced.

Studies on antibiotic resistance and usage are important for establishing the mechanism of microbial resistance to antibiotics, the reference points for the current medical practice and the possibility to develop medium and long term strategies in order to slow down the emergence of resistance and to optimize the treatment of bacterial infections. Supervision is essential for the management of antimicrobial resistance. Monitoring the magnitude and trend of this phenomenon offers useful data for developing intervention measures and assessment of their impact.

In the absence of efficient new classes of antibiotics, other issues such as educational programs based on enhanced hygiene and decrease of the misuse and abuse of antibiotics, must be reconsidered.

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