ACTA MICROBIOLOGICA BULGARICA

**Review**

# Antibiotic Resistance - a World Challenge

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**Abstract**

Antibiotic resistance is a worldwide health problem that continues to increase. The European Union and World Health Organization declared the rapid development of antimicrobial resistance as one of the three greatest threats to human health. In this paper are presented current data from literature and data of our own, concerning the increasing antibiotic resistance in the world. There was registered a significant increase in the proportion of multiresistant bacteria to the main groups of antimicrobials used in the clinical practice - third-generation of cephalosporins, carbapenems, quinolones, and aminoglycosides. Among the so-called multidrug-resistant or pandrug-resistant bacteria are *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA, multi-drug resistant), extended-spectrum beta-lactamase (ESBL) producing *Klebsiella* species and *Escherichia coli*, and others. Indication for the significance of the problem in military settings is the determination of an increase in the number of reported multidrug-resistant *A. baumannii* in bloodstream and wound infections in US soldiers at military medical facilities in Iraq, Kuwait, and Afganistan. In this context, at the EU-US Summit in November 2009 were made decisions and developed strategies, which could be better addressed by cooperation between the United States and Europe for improving of the use of antibacterial drugs.

**Key words**: resistance, multidrug-resistant bacteria, ESBL-producing bacteria, MRSA, treatment

**Резюме**

Резистентността към антибиотци е световен здравен проблем. Специалистите от Европейския съюз (ЕС) и Световната здравна организация (СЗО) декларират, че бързото развитие на резистентност към антимикробни средства е една от трите най-големи заплахи за човешкото здраве. В настоящата работа представяме съвременни литературни и собствени данни за нарастващата резистентност на проблемни за болничната патология микроорганизми към антибиотици и химиотерапевтици. Регистрирани са значителен брой микроорганизми, резистентни към основни групи антимикробни средства, използувани понастоящем в клиничната практика като трета генерация цефалоспорини, карбапенеми, хинолонови производни и аминоглигозиди. Специално внимание заслужават т.н. множествено-резистентни бактерии, включващи *Acinetobacter baumannii*, метицилин-резистентни *Staphylococcus aureus* (MRSA), множествено-резистентни щамове *Klebsiella pneumoniae* и *Escherichia coli,* продуциращи широко-спектърни бета-лактамаци (ESBL) и карбапенемази. Индикация за значимостта на проблема за военната медицина, е и нарастването броя на съобщенията, свързани с изолирането на множествено-резистентни щамове *A. baumannii* като причинители на септични състояния и раневи инфекции в американски войниици от мисии в Ирак, Кувейт и Афганистан. В този смисъл, на среща на „върха" през 2009 г. между представители на Европейския съюз и САЩ, се взема решение за бъдещи стратегии, насочени към кооперирание усилията на специалистите за оптимизиране борбата с феномена антимикробна резистентност.

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The era of antibiotics is drawing to a close. In just a couple of generations, what once appeared to be miracle medicines, have been beaten into ineffectiveness by the bacteria, they were developed to knock out. Once, scientists hailed the end of infectious diseases. Now, the post-antibiotic apoca lypse is within sight. Sarah Boseley, The Guardian, UK. In 2009, the World Health Organization (WHO) declared that antibiotic resistance is one of the greatest threats to health on a global scale (Kaplan *et al.,* 2013).

**Introduction**

Antibiotic resistance is a worldwide public health problem that continues to grow. When penicillin became widely available during the World War II, it was a medical miracle, rapidly vanquishing the biggest wartime killer - infected wounds. But just four years after drug companies began mass-production of penicillin in 1943, microbes began to appear in a way that could resist it. The first microorganism to battle penicillin was *Staphylococcus aureus* (Lewis, 1995). Another type of penicillin resistant pneumonia, caused by *Streptococcus pneumoniae* surfaced in a remote village in Papua New Guinea in 1967. American military personnel in Southeast Asia were acquiring penicillin-resistant gonorrhea from prostitutes (Lewis, 1995) at about the same time. A hospital-acquired intestinal infection caused by the bacterium *Enterococcus faecium* joined the list of microorganisms that outwit penicillin in 1983. At present, resistance increased to a number of commonly used antibiotics. We have come to point certain infections (*Acinetobacter baumannii* infections) in the 1990s, that we do not have agents available for. According to the report in the New England Journal of Medicine from April 28, 1994, researchers have identified bacteria in patient samples that resist all currently available antibiotic drugs (Lewis, 1995). The data from the European Union shows that 25 000 deaths per year were attributed to infections caused by antibiotic-resistant bacteria, of which 66% were Gram (-) bacilli. The total number of additional hospital days required for treatment of resistant bacteria is 2.5 million days per year at a cost of 1.5 billion euros (www. medscape.com/ viewarticle/717606-3).

The aim of this study is to present current data about the resistance and multiresistance of bacteria, which are problematic for human health, to antimicrobials, their behavior in the infections and the strategies for improving of their treatment.

**Bacterial Resistance Weaponry**

The most serious, life-threatening infections are caused by a group of drug-resistant bacteria that the Infectious Diseases Society of America (IDSA) has labeled the „ESKAPE“ pathogens, because they effectively escape the effects of antibacterial drugs. Table 1. (http://www.medscape.com)

***Enterococcus sp.* infections**

Enterococci are intrinsically resistant to a broad range of antibiotics including cephalosporins, penicillins, sulfonamides, and low concentration of aminoglycosides. Based on our data (Fig.1), as a possibility for treatment of infections, caused by *E. faecalis*, continue to be vancomycin with 0.4% resistance for 2008, teicoplanin - 1.3%, linezolid - 2.4%, and a combination of ampicillin/ sulbactam - 0.22% (Savov *et al.,* 2010).

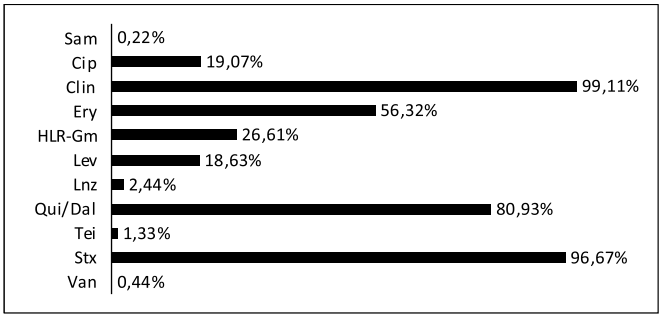


**Fig.1.** Resistance of *E. faecalis* to animicrobials (n-455) - 2008

Van - vancomycin, Stx - sulfam/trimet, Tei - teicoplanin, Qui/Dal - quinopristin/dalfopristin, Lnz - linezolid, Lev - levofloxacin, Ery - erytromycin, Clin - clindamycin,Cip - ciprofloxacin, Sam - ampicillin/sulbactam, HLR-Gm - high level resistance to gentamicin

Particularly virulent strains of *Enterococcus* that are resistant to vancomycin (vancomycin-resistant *Enterococcus* or VRE) have emerged in nosocomial infections of hospitalized patients especially in the US in the last two decades. (Fisher *et al*. 2009). Other developed countries such as the UK have been spared of this epidemic, and Singapore managed to halt an epidemic of VRE in 2005. VRE may be treated with quinupristin/dalfopristin (Synercid) with response rates of approximately 70% (Tunger *et al.*, 2004).

**Methicillin-Resistant *Staphylococcus aureus* (MRSA)**

Reports of methicillin-resistant *Staphylo-coccus aureus* (MRSA) - a potentially dangerous type of *Staphylococcus* bacteria that is resistant to certain antibiotics and may cause skin and other infections - in persons with no links to healthcare

**Table 1.** The most serious life-threatening infections

| | | |
|---|---|---|
| E | *Enterococcus faecium* | Third most common cause of healthcare-associated HCA BSI. Increasing resistance to vancomycin. |
| S | *Staphylococcus aureus (MRSA)* | Emerging resistance to current drugs and significant drug toxicities. Lack of oral agents for step-down therapy. |
| K | *Klebsiella*<br>*Escherichia coli*<br>*K. pneumoniae* | ESBL-producing organisms increasing in frequency and severity; associated with increasing mortality. *K. pneumoniae* carbapenemases causing severe infections in LTCF. Few active agents; nothing in development. |
| A | *Acinetobacter baumannii* | Increasing worldwide, recent surge reported in hospitals. Very high mortality. Carbapenem-resistant. |
| P | *Pseudomonas aeruginosa* | Increasing *P. aeruginosa* infections in the US and worldwide. Resistant to carbapenems, quinolones, aminoglycosides. |
| E | *Enterobacter* species | MDR HCA infections increasing; resistance via ESBLs, carbapenemases, and cephalosporinases. |

*HCA = healthcare associated; BSI = bloodstream infection; MRSA = methicillin resistant S aureus; ESBL = extended-spectrum beta-lactamase; LTCF = long-term care facility; MDR = multiple drug-resistant*

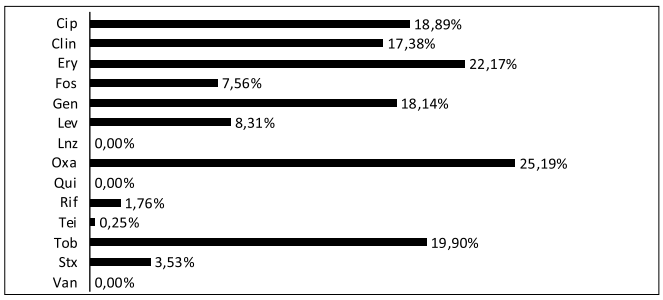


**Fig. 2.** Resistance of *S. aureus* to animicrobials (n-397) - 2008

Van - vancomycin, Stx - sulfam/trimet, Tob - tobramycin, Tei - teicoplanin, Rif - rifamycin, Qui/Dal - quinopristin/dalfopristin, Oxa - oxaccillin, Lnz - linezolid, Lev - levofloxacin,Gen - gentamicin, Fos - fosfomycin, Ery - erytromycin, Clin - clindamycin, Cip - ciprofloxacin

systems, have been observed with increasing frequency in the US and elsewhere around the globe.

The relative part of infections caused by MRSA varies in the different countries. According to Jewell, M. (Jewel, 1994), this type of infections is a big problem in Chicago hospitals, where the number of MRSA infections is about 60%. In Spain and in France this number is between 30-33%. (Herwaldt *et al*., 1995). The data for the Military Medical Academy in Sofia, Bulgaria showed that about 25% of the *S. aureus* infections were caused by MRSA (Fig.2) (Savov *et al.,* 2010). The options for treatment of this type of infections at the moment are: vancomycin, teicoplanin, linezolid, and tigecyclin.

**Multidrug-Resistant *E. coli* and *Klebsiella* sp**.

Multi-drug resistant, extended-spectrum beta-lactamases (ESBL) producing *Klebsiella* species and *Escherichia coli* have been isolated in hospitals throughout the world. ESBL positive strains are associated with increased mortality, because of the failure to treat infections, caused by ESBL positive organisms, due to the limited therapeutic choices (Kim *et al.,* 2002; Paterson *et al.,* 2005). Of all "EARSS-specific" pathogens, *E.coli* demonstrated the most worrying trends. *E. coli* isolates with multiple resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides were registered in Bulgaria in great proportions (EARSS 2002). The relative part of the *E. coli* strains, producing ESBL in the units of Military Medical Academy (MMA) in the last 3 years is about 20%. As compared to 2007, the relative part of *K. pneumoniae* strains, producing ESBL significantly increased from 53% in 2007 up to 67.8% in 2008 (Savov *et al.,* 2010). The results, presented in an extensive study performed in Bulgaria for a period of eight years (1996 - 2003), showed, that the most widespread enzymes found in *Enterobacteriaceae* belong to three groups - SHV-12, CTX-M-15, and CTX-M-3 as well (Markovska *et al.,* 2008). The possibility for treatment of such of infections stay only carbapenems and beta-lactams in combination with beta-lactamase inhibitors.

**Multidrug (Pandrug) Resistant *Acinetobacter baumannii* Infections**

*Acinetobacter baumannii* has emerged as one of the most troublesome pathogens for health care institutions on a global scale. Its clinical significance, especially over the last 15 years, has been propelled by its remarkable ability to acquire resistance determinants, making it one of the organisms threatening the current antibiotic era

(Davis *et al.,* 2005). The rapid global emergence of *A. baumannii* strains resistant to all β-lactams, including carbapenems, quinolones and other antimicrobials illustrates the potential of this organism to respond swiftly to changes in selective environmental pressure (Peleg *et al.,* 2008). After performing whole-genome sequencing of a clinical epidemic *A. baumannii* strain found in France (AYE), an 86-kb resistance island, one of the largest to be described thus far, was identified (AbaR1). Overall, 52 resistance genes were identified, and surprisingly, 45 (86.5%) were localized in the AbaR1 resistance island (Fournier *et al.,* 2006). The resistance to carbapenems at MMA in Sofia (85% to meropenem) (Fig. 3) is associated with the production of Oxa 23 and Oxa 58 carbapenemases, but not to metallo-beta-lactamases (Stoeva *et al.* 2009; Savov *et al.* 2010).

The resistance to quinolones was assessed at the DNA level for mutation detection in quinolone-resistance-determining regions (QRDRs) and the subsequent aminoacid substitution in the GyrA and/or the ParC enzymes. A strong correlation was found between quinolone resistance and mutations in gyrA codon 83 and/or in the parC gene (codons 80 or 84) (Deccache *et al.,* 2011).

Additionally, there is an indication of an increase in the number of reported *A. baumannii* bloodstream infections in soldiers at military medical facilities in Iraq, Kuwait, and Afganistan. Fifty-three percent of *A. baumannii* infections have been registered as a bloodstream infections at the Walter Reed Army Medical Center /WRAMC/, which is the major US site receiving casualties from the conflict in Iraq/Kuwait and in Afganistan (Hawley *et al.,* 2007; Hujer *et al.,* 2006).

The choice of antibacterials for treatment of such of infections is difficult. The most promising data with regard to *A. baumannii* are the benefits of a prolonged infusion of up to 3 hours and increasing of the dose of up to 2 g per every 8 hours of meropenem administration; the concentration, obtained by this way in serum of above 16 μg/ml for almost 60% of the time, supports the use of an extended meropenem infusion time for treating serious *A. baumannii* infections (Peleg *et al.,* 2008). The use of the polymyxins and tigecycline is very important for the treatment of serious infections with multidrug-resistant *A. baumannii,* however the Food and Drug Administration (FDA), the Clinical and Laboratory Standard Institute (CLSI), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have established no breakpoints for interpretation of antibiotic susceptibility testing of tigecycline against *A. baumannii*. These data suggest that caution should be taken in considering tigecycline treatment for *A. baumannii* infection in sites where drug levels may be suboptimal, such as the bloodstream (Peleg *et al.,* 2008). Unlike EUCAST and the British Society for Antimicrobial Chemotherapy (BSAC), the CLSI has established breakpoints for colistin and polymyxin B against *A. baumannii* (Peleg *et al.*, 2008). Clinical use of polymyxins against *A. baumannii* isolates proved to be extremely successful (Neonakis *et al.,* 2011). These antimicrobials have been tested extensively in combination with other agents against multiple drug-resistant *A. baumannii* - carbapenems, cefepime, amikacin, and others. Clinically, the combination of colistin with meropenem appears to be superior to the other agents (Neonakis *et al.,* 2011).

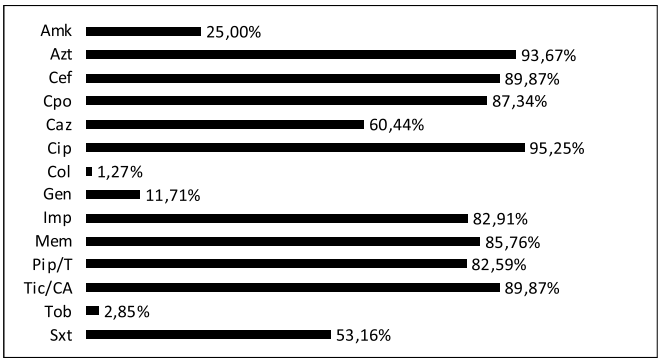


**Fig. 3.** Resistance of *A. baumannii* to animicrobials (n-530) - 2008

Sxt - sulfam/trimet, Tob - tobramycin, Pip/Taz - piperacilin/ tazobactam, Mem - meropenem, Imp - imipenem, Gen - gentamicin, Col - colistin, Cip - ciprofloxacin, Caz - ceftazidim, Cef - cefepim, Azt - aztreonam, Amk - amikacin, Cpo - cefpirom

## Multidrug-Resistant *Pseudomonas aeruginosa* Infections

The Gram (-) bacterium *Pseudomonas aeruginosa* is a significant opportunistic pathogen. Chronic infections due to this organism are prevalent in cystic fibrosis patients and are frequently recalcitrant to treatment. In addition to displaying high levels of intrinsic antibiotic resistance, *P. aeruginosa* frequently converts to a mucoid state resulting in a rapid adaptive resistance that accounts for the high failure rate of antibiotic therapy in eradicating these infections. *P. aeruginosa* is intrinsically resistant to the majority of antimicrobial compounds due to its selective ability to exclude various molecules from penetrating its outer membrane. It is a problem, because in many cases the *P. aeruginosa* strains isolated were multiresistant (with resistance to: piperacillin/tazobactam of 22.7%, cefepime - 51.5%, and ceftazidime -

46.9%). The level of the resistance to carbapenems is about 32.2% for imipenem and also 41.7% for meropenem (Fig. 4) (Savov *et al.,* 2010).

The spread of similar multiresistant strains is very important for big hospital complexes according also to Edalucci *et al.,* 2008. This multiresistance usually is connected with production of metallo-beta-lactamase (MBL) VIM-2 and also these widespread clones, responsible for human infections, belong to O11 and O12 serotypes (Edalucci *et al.,* 2008).

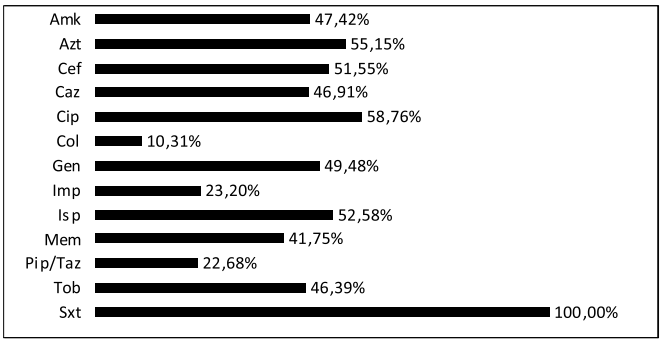


**Fig. 4.** Resistance of *P. aeruginosa* to animicrobials (n-277) - 2008

Sxt - sulfam/trimet, Tob - tobramycin, Pip/Taz - piperacilin/tazobactam, Mem - meropenem, Isp - isepamycin, Imp - imipenem, Gen - gentamicin, Col - colistin, Cip - ciprofloxacin, Caz - ceftazidim, Cef - cefepim, Azt - aztreonam, Amk - amikacin

Resistance to ciprofloxacin and aminoglycosides is also high - 58.8% and 47-49% respectively (Fig. 4). (Savov *et al.,* 2010).

## Multidrug-Resistant Bacteria Linked with New Delhi Metallo-Beta-Lactamase 1 (NDM-1)

A novel enzyme, described during 2009 - NDM-1 (New Delhi Metallo-Beta-Lactamase 1) (Yong *et al.,* 2009), which is encoded by blaNDM-1 gene, is increasingly dominant (Kumarasamy *et al*. 2010, www.phac-aspc.gc.ca)

The NDM-1 gene produces an enzyme which makes bacteria resistant to most antibiotics (fluoroquinolones, aminoglycosides and beta-lactams, including carbapenems (imipenem, meropenem, ertapenem, doripenem), except tigecycline and colistin. Carbapenems are powerful, broad-spectrum antibiotics, which are often considered to be the last line of defence against multi-resistant strains of bacteria, such as *E. coli* and *K. pneumoniae*. NDM-1 is strongly linked to India and Pakistan and many of the UK cases have recent medical exposure in the Indian-subcontinent (Kumararasamy *et al.,* 2010). All of the 21 UK producers comprise *K. pneumoniae* (14), *E. coli* (4), *Enterobacter spp.* (1), and *C. freundii* (2) from 18 patients and 16 hospitals scattered across England, and also one in Scotland. Forty strains with NDM-1 were isolated for 2009. In the US, three cases have been confirmed - in California, Illinois, and Massachusetts. Researchers believe all three patients picked up the resistant microorganism in hospitals in India (Kumararasamy *et al.,* 2010). The first positive for NDM-1 eleven local *E.coli* strains, proven by real time polymerase chain reaction (PCR) NDM-1 kit were isolated from clinical samples at the Military Medical Academy in Sofia, Bulgaria (Poirel *et al.,* 2014; Savov *et al.,* 2012).

## The Bacterial Challenge and Bad Practice

The discovery of antibiotics was a leap in modern medicine. However, the bacteria in particular have proven to be much more innovative and adaptive than scientists had imagined. Considering that the bacteria existed for a period of $3.8 \times 10^9$ years, while the antibiotics were used in the last 70 years, it is clear why there is no definitive success in bacterial infections' treatment (Hamilton-Miller, 1990). On the other hand, the bad practices and mismanagement have only exacerbated the situation. In 1998, in the US, it was estimated that there were 80 million prescriptions of antibiotics for human use - the equivalent of about 12 500 tons per one year (Yim, 2009). According to the American College of Physicians, 190 million doses of antibiotics are administered each day in the hospitals (www.acponline.org).

Among outpatients, more than 133 million courses of antibiotics are prescribed by doctors each year. It is estimated that 50 percent of the latter prescriptions are unnecessary since they are being prescribed for colds, coughs, and other viral infections. When animal and agricultural uses of antibiotics are added to human use, it is estimated that in the past 50 years, more than one million tons have been produced and disseminated (Yim, 2009). To combat the occurrence of resistant bacteria, pharmaceutical companies must constantly research, develop, and test new antimicrobials in order to maintain a pool of effective drugs on the market. Five years ago, there were approximately 150 antibiotics available to the public with new drugs appearing once every 8-10 years. This appears to be a substantial amount (Yim, 2009). However, these numbers are misleading because many of the targets of these drugs are similar. Since the drug development process is very expensive, pharmaceutical companies often concentrate on finding antimicrobials similar to the ones

already found, to reduce the risk of producing an unmarketable drug. This means that it is easy for a microorganism to develop resistance to a similar drug to which it already has resistance. Past and current strategies to combat resistance are not effective (Yim, 2009). In this sense, it can be concluded, that there is a crisis in the lack of new antibiotics to deal with the evolving and predictable problem of antibiotic resistance, and a critical need for agents active against multidrug-resistant Gram (-) bacilli (Top 10 Infectious disease publications in 2009) (http://www.medscape.com).

**Perspectives, Cooperation Against Resistant Bacteria**

The review of data from the European Union shows that 25 000 deaths per year were attributed to infections caused by antibiotic-resistant bacteria, of which 66% were Gram (-) bacilli. The total number of additional hospital days required for treatment of resistant bacteria is 2.5 million days per year at a cost of 1.5 billion euros (Top 10 Infectious disease publications in 2009) (http://www.medscape.com). Although this conclusion is not new, the document is extremely full of substantial data provided by the Antibiotic Availability Task Force from the Infectious Diseases Society of America (IDSA), which has catalogued much of these data over the past 5 years. The difference here is that the conclusion has particular meaning when it comes from the European equivalent of the CDC and the European equivalent of the US Food and Drug Administration (FDA) (Top 10 Infectious disease publications in 2009) (http://www.medscape.com). In this connection, at the EU-US Summit on November 3, 2009 in Washington, the president B. Obama, Jose Manuel Barroso, Fredric Reinfeldt, and Javier Solana were agreed to establish a transatlantic task force on urgent antimicrobial resistance issue (EU-US Summit agrees to form transatlantic task force on antimicrobial resistance.) (www.reactgroup.org).

The task force has to focus on appropriate therapeutic use of antimicrobial drugs in the medical and veterinary communities, prevention of both healthcare and community-associated drug resistant infections, and strategies for improving the pipeline of new antimicrobial drugs, which could be better addressed by intensified cooperation between the US and Europe. Following this, the IDSA Antibiotic Availability Task Force announced the necessity to achieve the development of ten new antibiotics within the next ten years (the 10 × ‘20 initiative), meaning that the aim is to develop 10 novel drugs for Gram (-) bacteria by the year 2020 (EU-US Summit agrees to form transatlantic task force on antimicrobial resistance), (Top 10 Infectious disease publications in 2009) (www.reactgroup.org, http://www.medscape.com). However, these numbers are misleading because many of these drug targets are similar.

Since the drug development process is very expensive, pharmaceutical companies often concentrate on finding antimicrobials similar to the ones already found to reduce the risk of producing an unmarketable drug. This means that it is easy for a microorganism to develop resistance to a similar drug to which it already has resistance (Yim, 2009). Past and current strategies to combat resistance are not effective.

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