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MINIREVIEW

The Decline of Antibiotic Era – New Approaches for Antibacterial Drug Discovery

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Abstract

Infectious diseases still remain the main cause of human premature deaths; especially in developing countries. The emergence and spread of pathogenic bacteria resistant to many antibiotics (multidrug-resistant strains) have created the need for the development of novel therapeutic agents. Only two new classes of antibiotics of novel mechanisms of action (linezolid and daptomycin) have been introduced into the market during the last three decades. The recent progress in molecular biology and bacterial genome analysis has had an enormous impact on antibacterial drug research. This review presents new achievements in searching a new bacterial essential genes, a potential targets for antibacterial drugs. Application of metagenomics strategy is also shown. Some recent technologies aimed at development of anti-pathogenic drugs such as inhibitors of quorum sensing process or histidine kinases are also discussed. Extensive research efforts have provided many details concerning structure of bacterial proteins playing an important role in pathogenesis such as adherence proteins or toxins, what allowed searching for antitoxin drugs or drugs interfering with bacterial adhesion. As an example, the review focuses on anthrax therapies under development. Additionally, the article presents the progress in phage therapy; using bacteriophages or their products such as lysins in antibacterial therapy.

Key words: antibiotics, bacteriophages, infection diseases, genomics, horizontal gene transfer, protein structure

Introduction

The World Human Organization reports yearly death of around 15 million people caused by infectious diseases. It is estimated that in one hour 1500 people die due diseases developed from microbial infections, over half of them are children under 5 years of age. Additionally, many illnesses regarded as non-related to microbial infections, mainly cancer, were found to be a consequence of chronic infections.

Based on the review of literature performed in the year 2000, 1415 species of human pathogens (including 538 bacteria and rickettsia, 217 viruses and prions, 66 protozoa, 307 fungi and 287 intestinal worms) were identified (Taylor *et al.*, 2001). Over 90% of death cases were caused by illnesses, which in the era of antibiotics and vaccines, and in the face of the enormous scientific progress should be, at least to some degree, under our control. Among them are illnesses of the respiratory system, tuberculosis, diarrhea, malaria, measles and most recently – AIDS. The listed diseases are responsible for over half of the cases of premature death, mainly among children and adults under 40 years of age.

Apart from "old" pathogens, against which we still are unable to fight effectively, a new group of the so-called emerging pathogens (including such bacterial species as *Legionella pneumophila*, *Campylobacter jejuni*, *Bartonella henselae*, *Helicobacter pylori*, *Borrelia burgdorferi*) has been distinguished. A large threat to humans is posed by yet another group, termed re-emerging pathogens, which are well-known microorganisms that by horizontal gene transfer have acquired new features increasing, in a significant way, their virulence. Among human pathogens, 175 species (12% of all so far identified) were classified to both groups. The majority of them (75%) are zoonotic pathogens (Woolhouse, 2002).

Antibiotics resistance

Without doubt antibiotics were the most effective form of antibacterial therapy introduced into medical practice in the XX century. The "golden era" of

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antibiotics falls into the years 1940–1960, when most of the presently applied therapeutics was introduced into medical clinics. During the last thirty years, only two new classes of antibiotics have been introduced onto the market – daptomycin, a cyclic lipopeptide antibiotic obtained from *Streptomyces roseosporus*, and linezolid, a synthetic antibiotic belonging to oxazolidinones. While the first antibiotic affects the cell wall, the second one inhibits the process of translational initiation. Despite the fact that both drugs were described in the 80s of the XX century, their administration was allowed only in the years 2003 and 2000, respectively. Both drugs were found effective in therapy of infections caused by methicillin- and/or vancomycin-resistant strains of Gram-positive bacteria, *e.g.* MRSA or VRE (Pucci, 2006; Wright, 2007).

The increasing antibiotic-resistance of many bacterial strains is a serious medical and economical problem. This phenomenon is due to improper antibiotic administration in treatment of human infections as well as preventional application of the same antibiotic drugs to evade epidemics in livestocks. Since 1989, epidemics of typhoid fever caused by *Salmonella* strains resistant to many antibiotics were observed in 11 countries. Another noted epidemic is dysentery, which is frequently caused by *Shigella* strains that are resistant to two most commonly used antibiotics in therapy. Furthermore, around 80% of gonorrhea cases were connected with infections by *Neisseria gonorrhoea* strains resistant to penicillin, which was, until recently, the most frequently applied antibiotic in treatment of this disease. Currently, treatment of many bacterial infectious diseases demands application of costly drugs of the new generation, which surely are not in the range of developing countries that exhibit a low *per capita* income and a minimal part of their budget spent on healthcare. Effective therapy of one patient infected with a multidrug-resistant *M. tuberculosis* costs $1500 – $4000 and the average cost of therapy of one child with respiratory infection has increased from $5 to $40, due to the necessity of using new generation antibiotics. Additional problems are posed by bacterial strains, which are involved in hospital infections and exhibit resistance to many antibiotics. These strains are a direct cause of thousands of deaths by attacking sick individuals, often recuperating from serious operations, who are especially prone to bacterial infections. In the USA alone yearly around 14000 people die from infections caused by antibiotic-resistant hospital strains.

MDR (multi-drug resistant) bacterial strains, termed also "superbugs", are divided into two groups – "re-emerging" pathogens, which are well-known pathogens that have acquired genes conditioning resistance to antibiotic drugs, and opportunistic pathogens that exist in the natural environments and cause serious disease symptoms in humans with reduced immune resistance. The first group comprises, among others, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRA). Representatives of the second group are, *e.g. Pseudomonas aeruginosa* and *Acetinobacter baumannii.* Medicine is helpless in case of certain infections. Bacterial strains resistant towards all known antibiotics have already been isolated, *e.g.* strains of *Klebsiella pneumoniae* or *Acinetobacter baumannii* (Wright, 2007). *A. baumannii* is a Gram-negative bacterium commonly found in soil or water, which until the late 70s of the XX century did not constitute any danger to humans. Currently, around 2–10% of hospital infections in Europe caused by Gram-negative bacteria are invoked by this bacterium. *A. baumannii* strains are characterized by the ability to acquire antibiotic resistance *via* horizontal gene transfer (HGT; by plasmid, transposon or integron acquisition). Multi-drug resistant strains were isolated from wounded American soldiers during the Vietnam War, in Kuwait or from the army personnel stationing in Iraq and Afghanistan. Fournier *et al.* (2006) has conducted comparitional analysis of genomes of two *A. baumannii* strains: AYE (genome size 3.9 Mb) resistant to numerous antibiotics, isolated during an epidemic in France, and an antibiotic-sensitive SDF strain (genome size 3.2 Mb). The genome of the AYE strain carries 52 genes, which potentially encode proteins implicated in antibacterial drug resistance. Yet, only seven genes of this type were found in the SDF genome. Moreover, 86% of "resistance" genes of the AYE strain are clustered on the 86 kb-resistance island. Detailed bioinformatics analysis showed that genes localized on the "resistance" island were acquired relatively not long ago from such bacterial species as *Salmonella*, *Pseudomonas* or *Escherichia.* However, the same genomic region of the SDF strain carries a 20 kb-genomic island, which does not contain genes conferring resistance to antibacterial drugs (Fournier *et al.*, 2006; Roberts, 2006).

Another example of the role of HGT in dissemination of antibiotic-resistance genes was presented in the work of Welch *et al.* (2007) concerning MDR of a *Yersinia pestis* strain, etiological agent of plaque. *Y. pestis,* which has cause death of around 200 million people during several pandemics, is currently regarded as a re-emerging pathogen. Still, occasionally local plaque epidemics burst out in different parts of the world. The majority of *Y. pestis* strains are sensitive to antibacterial drugs. Yet, in 1995 in Madagascar, a *Y. pestis* strain was isolated, termed later MDR IP275, and found to carry a plasmid – pIP 1202 (182 913 bp), that conferred a MDR phenotype. The nucleotide sequence of the plasmid was compared with nucleotide sequences of two other plasmids, also responsible for

the MDR phenotype – the pSN254 plasmid of *Salmonella enterica* sv. Newport SL254 and the pYR71 plasmid isolated from the cells of a fish pathogen, *Yersinia ruckeri* YR71. *In silico* analysis showed that all three plasmids contain a "similar" DNA region (113320 bp in size, 135 synthetic genes with a similar codon usage and 90–100% of nucleotide sequence identity). This DNA fragment constitutes the core of genetic information of all three plasmids and carries genes responsible for the replication process, plasmid maintenance as well as conjugal transfer. Moreover, the region contains four sites into which DNA fragments carrying genes conferring resistance to antibacterial drugs were integrated. Additionally, plasmids with DNA regions of similar nucleotide sequences and structure were isolated from numerous enterobacterial cells obtained from meat samples from several US states (Welch *et al.*, 2007).

Human campylobacteriosis constitute a serious public health concern. Recently *Campylobacter* bacteraemia has a high mortality rate mainly due to increase resistance among *Campylobacter* isolates to two antibiotics commonly used in therapy (fluoroquinolones and macrolides). The contaminated poultry meat is the main source of human's infection at least in developed countries. The most reasonable and effective way to restrict the amount of human's infection seems to be the decrease the level of chicken's intestinal tract colonization by *Campylobacter.* A large numbers of possible intervention at farm level and during processing have been recommended. Their effectiveness and efficiency was evaluated by CARMA (*Campylobacter* Risk Management and Assessment) project (Havelaar *et al.*, 2007). Vaccination of chicken will be probably the most effective way to prevent human campylobacteriosis. Although several attempts have been recently undertaken to construct effective chicken vaccine no such vaccine is commercially available (Sizemore *et al.*, 2006; Wyszynska *et al.*, 2004; for review see de Zoete *et al.*, 2007).

In the last years, the term "antibiotic resistome" was introduced. It characterizes a set of microbial genes conferring resistance to antibiotics present in the pan-microbial genome (set of all bacterial genes). The resistome is constituted not only by "resistance" genes present in the genomes of pathogenic and non-pathogenic strains, but also by genes circulating in the bacterial population, which can potentially evolve and generate products conferring antibiotics resistance. *In silico* analyses as well as structural studies of proteins supply examples of an increasing number of "resistance" genes (Wright, 2007).

The few examples presented above forcibly document that bacterial infectious diseases still pose a serious challenge, not only for medical services. Strategies limiting bacterial infections demand the development of new prophylactic approaches as well as diagnostic and therapeutic methods. Moreover, sick and death rates can sometimes be reduced without high financial efforts, such as using insecticide-soaked mosquito nets to prevent malaria. Yet, often such measures turn out to be impossible to implement in a global scale.

"Essential genes" – use in therapy

The genomic era began in the year 1995 when the first, complete bacterial genomic sequence of *Haemophilus influenzae* was published. Since that moment, a distinct change in the quality of microbial genetic studies can be observed. In effect, analyses of single genes leads presently to global analyses of microbial cells while analyses of full genetic sequences, whole transcriptomes as well as total protein content or networks of protein-protein interactions is directed to the genome, transcriptome, proteome and interactome, respectively. It is believed that soon most probably complete genetic sequences of over 1000 microbial strains will be discovered (data from year 2008). Results obtained from these analyses (*in silico* methods, microarray studies) supply data helpful in searching for new targets of antibacterial drug activity.

Classical antibiotics are characterized as compounds, which influence microbial life processes, without harming the host cells. Their main mechanism of action is based on blocking the cell wall synthesis as well as replication or translation inhibition. Metagenomic techniques based on direct cloning of DNA present in natural environments allowed identifying several new antibiotics (Handelsman, 2004). Unraveling the genome nucleotide sequences of many pathogenic bacteria allowed searching for new potential genes/protein targets of novel drugs. Microbial genomes were screened for the presence of genes essential for cell survival. Yet, it should be mentioned that not every gene or its product that is essential for survival of the pathogenic microorganism will be effective as target for therapeutic action. The methodology of this type of studies comprises various mutagenization strategies (transpositional mutagenesis, plasmid-integration mutagenesis, allele exchange, STM mutagenesis or IVET strategies). The number of genes potentially essential for cell survival is usually significantly higher in pathogenic than in non-pathogenic bacterial genomes, *e.g.* in *Bacillus subtilis* 271 among 4101 genes are essential, in *Escherichia coli* 620 out of 4279, in *Mycoplasma genitalium* 256–350 out of 484 and in *Helicobacter pylori* 344 out of 1552. Nonetheless, interpretation of experimental data should be done with great care since often the inability to obtain a mutant in a certain gene is not due to the lethality of the process but to technical difficulties.

Only construction of conditional mutants or applying antisense RNA strategies supplies reliable results. It should be also taken into consideration that genes potentially useful in therapy should be expressed *in vivo*, when the pathogen enters the host organism. Development of global analyses of mRNA (transcriptome analysis using microarrays) and bacterial cell proteins (two-directional electrophoresis linked with mass spectrometry) allows analyzing microbial transcriptomes and proteomes in different environmental conditions. Additional study strategies, such as IVET techniques (*in vivo expression technology*), allow to identify microbial genes active only when the pathogen is in the host organism. In the last years, many scientific papers have been published which characterized genes essential for survival of various species of pathogenic microorganisms (exemplary review papers are Freiberg and Brotz-Oesterhelt, 2005 and Pucci, 2006).

One of the novel drugs that most probably will be available on the market soon is the peptide deformylase (PDF) inhibitor. Peptide deformylase – an iron-dependent metalloenzyme, is essential for survival of prokaryotic cells and participates in the posttranslational processing of proteins, specifically in removing the formyl group from the N-terminal methionine of the protein chain (Yuan *et al.*, 2001). In most genomes, genes encoding PDF and another enzyme – FMT, which is a formyltransferase that adds the formyl group to the methionine of the N-terminal part of the chain, are co-transcribed. PDF was described in 1968 by Adams (1968); yet, only development of genetic engineering and methodologies able to overcome the instability of the enzyme allowed overproducing the protein in *E. coli* cells and resolving its structure (in 1997) (Chan *et al.*, 1997). All gathered experimental data led to the synthesis of several PDF inhibitors, proven later to be active both *in vitro* and *in vivo* (Wang *et al.*, 2006). Two drugs – BB-83698 Oscient Pharmaceutical and LMB415 Novatris Pharmaceutical, which currently undergo clinical trials, were found to exhibit activity against many Gram-positive bacterial species as well as against several Gram-negative microorganisms. Bacteria sensitive to the PDF inhibitor include many pathogens of the respiratory system. Advances in genomic methods allowed detailed characterization of PDF-encoding genes from different microorganisms. The number of paralogous genes in different genomes as well as the organization of PDF-encoding regions was also determined. In several bacterial genomes, *e.g.* in *S. pneumoniae* or *B. subtilis*, two paralogous genes were identified; yet, they were not always active. *In silico* modeling and crystallographic analyses allowed establishing the structure of many PDFs. Despite significant differences in amino acid sequences, all examined enzymes contained three, relatively closely localized and conserved motifs (signature sequences), which were determined to form structures responsible for binding metal ions. Analyses of eukaryotic genomes did not confirm the earlier assumptions that PDF is unique for prokaryotic organisms. Although the enzymatic activity of PDF is not observed in the cytosol of eukaryotic cells, the gene was detected, *e.g.* in the human genome as well as in plants and in several parasites. The human PDF contains a signaling sequence directing the protein to the mitochondria. However, the C-terminus of many mitochondrial proteins contains a formylated methionine (resulting from FMT activity), indicating low PDF activity in this cellular compartment. Elucidation of the mechanisms of action, phylogenesis and physiological role of human PDF is a prerequisite for determining the safety of drug administration (Yuan and White, 2006).

Antipathogenic drugs based on protein structure analysis

Not all novel drugs exhibit bactericidal or bacteriostatic activity blocking the basic life functions of bacterial cells. Increasing attention is devoted to analyses, which aim at constructing drugs that inhibit the mechanisms of microbial pathogenesis. This class of drugs includes adhesion inhibitors, compounds influencing the "quorum-sensing" process or modulating pathogen biofilm formation as well as inhibitors of two-component systems mediating the process of signal transduction in response to environmental changes.

Adhesion is one of the first stages of pathogenesis characteristic for numerous pathogenic microorganisms, especially those that pass through the host mucosal surface. The adhesion process is based on interaction between the bacterial adhesin and specific receptors on the eukaryotic cell surface. Bacterial adhesins are proteins which are either connected with the cell envelope or are components of structures present on the cell surface (*e.g.* adhesion fimbrie). Resolution of the structure of many bacterial adhesins as well as identification of respective host receptors permitted structural studies and construction of inhibitors blocking adhesin-host receptor interactions (Cazzola *et al.*, 2003; Niemann *et al.*, 2004). Among bacterial virulence factors are proteins from the LPXTG protein family, which constitute a numerous group of virulent factors in Gram-positive bacteria. Proteins of this type were identified in the genomes of *Listeria monocytogenes* (41 proteins) and *Staphylococcus aureus* (10 proteins). Their characteristic feature is that they are processed by a mechanism, which, in its final stage, anchors them to the cellular envelope though covalent bonds. LPXTG proteins contain a sorting signal which is localized in their C-terminal part

and consists of the LPXTG motif, preceded by an approximately 20 AA, hydrophobic region, and a long domain containing positively charged amino acids. The sorting signal retains the protein within the cell envelope, which is followed by cleavage of the peptide bond between threonine and glycine within the LPXTG motif and subsequent amide bond formation between the free threonine carboxyl group and peptide precursor of the cell wall. The process of microbial cell wall-anchoring of LPXTG proteins is catalyzed by sortases – enzymes with transpeptidase activity. Hence, sortase inhibitors could be effective in therapy of diseases caused by Gram-positive antibiotic-resistant microorganisms (Marraffini *et al.*, 2006).

Resolution of the three dimensional structure of both structural and accessory proteins that participate in assembly of adhesion organelles (*e.g.* Pap-type pili of uropathogenic *E. coli*) will allow developing their specific inhibitors. Periplasmic chaperone proteins or certain outer-membrane proteins, like the PapC protein in the case of Pap-type pili – a decisive factor of the assembly of individual compounds of the adhesion structure, are just examples of potential targets of inhibitor activity (Piatek *et al.*, 2005; Sauer *et al.*, 2004). Pathogenic bacteria produce many adhesins, which recognize various receptors; hence, drugs that do not kill the microorganism but inhibit adhesion, crucial stage of pathogenesis, could assist in other types of therapy.

Another group of proteins produced by pathogenic bacteria, which could be a potential target for new drugs, are autotransporters, classified as proteins of the type V transport system. So far, their presence was determined only in proteomes of pathogenic microorganisms. Autotransporters are multifunctional proteins localized on the cell surface, which participate at various stages of pathogenesis (adhesion, interaction with ECM components, inhibition of complement components). Moreover, structure of many autotransporters was recently resolved facilitating the search for their inhibitors (Girard and Mourez, 2006).

Advanced studies on the mechanism of action and the structure of the anthrax toxin led in the last years to proposing many therapeutic methods. Humans can be diagnosed with three types of anthrax: cutaneous, inhalation (termed also the wool-sorters' disease) and, the least frequently occurring – gastrointestinal type. Skin anthrax develops when anthrax spores enter an abrasion in the skin. It is characterized by relatively mild symptoms and, when diagnosed promptly, is curable upon antibiotic treatment. Inhalation anthrax proceeds in a different manner – disease symptoms develop very rapidly (one day after infection) and are highly fatal (over 90% of mortality). Anthrax spores, after reaching the lungs are internalized by lung macrophages, are transformed into the vegetative form, producing the threatening toxin. Macrophages serve as vehicles which at the same time spread and release into the blood stream a large number of the pathogenic cells as well as the toxin, itself. Two main *B. anthracis* virulence factors are encoded by genes localized on two plasmids – pXO1 and pXO2. pXO2 carries genes responsible for the synthesis of a polypeptide envelope, which protects bacteria from phagocytosis and the activity of the complement. The pXO1 plasmid contains genes responsible for synthesis of the anthrax toxin. The toxin belongs to the AB toxin group and is made up of three subunits – the edema factor (EF), the protective antigen factor (PA) and the lethal factor (LF). The three proteins are transported independently through microbial cell wall and assembled on the surface of targeted cells into a mature toxin. The PA antigen, after binding to a specific receptor (transmembrane proteins ANTXR1/ANTXR2 – anthrax toxin receptor), undergoes proteolytic processing by a specific protease. The N-terminal fragment (PA_{20}) is cleaved off, while the PA_{63} fragment remains attached to the receptor and undergoes subsequent oligomerization. Only in this form PA binds the LF and EF lethal factors. Successively, the formed complex is internalized by endocytosis. Due to the low endosomal pH, the PA fragment dissociates and penetrates the membrane. In result, a pore is formed through which LF and EF lethal subunits are translocated to the cytosol of mammalian cells. There, both factors reveal their enzymatic activities. The EF factor, being an adenylate cyclase dependent on the level of calcium ions and calmodulin concentration, increases the concentration of c-AMP, while the LE factor (zinc-dependent protease) inhibits several signal-transducing pathways by proteolytic cleavage of MAPKK (mitogen-activated protein kinase kinase). The common activity of both factors leads to cell death (Moayeri and Leppla, 2004; Mourez *et al.*, 2002). Hence, the anthrax toxin and its receptors are the main target of anti-anthrax drugs activity. Currently, after resolving the structure of all proteins constituting the mature toxin and its receptors, development of new drugs preventing high mortality is possible. Therapeutic strategies, presently in the phase of *in vitro* studies or tests on animal models, include polyclonal anti-PA antibodies, a synthetic peptide that competes with LE for the heptamer-binding site, mutated PA subunit that binds, but does not translocate LE, soluble receptor (sATR), a synthetic peptide that binds domain I of both receptors, and low-molecular compounds (β cyclodextrin), inhibiting pore-formation by the PA subunit (Basha *et al.*, 2006; Karginov *et al.*, 2005; Karginov *et al.*, 2006; Mourez *et al.*, 2002; Scobie *et al.*, 2007). Similarly, many new antitoxin drugs are currently tested for the ability to inhibit at various stages the activity of such dangerous toxins as the botuline toxin or ricin (Rainey and Young, 2004).

Antipathogenic drugs – influence the processes of signal transduction

Bacterial cells must constantly react to environmental changes. The most common mechanism of information transduction is the two-component system, which senses environmental signals, translates them and, in effect, modulates transcription of specific genes. In its simplest form the two-component system consists of two proteins – the sensor, which receives the signal and the regulator, which binds with DNA and acts as a transcriptional activator or repressor, depending on the type of promoter sequence. Signal transmission is driven by translocation of the phosphate group of the donor ATP from the histidine of the sensor protein exhibiting kinase activity, to the aspartic acid of the regulator protein, which causes its conformational change and binding to specific nucleotide sequences in promoter regions. Dephosphorylation causes the system to return to its initial state. The reaction speed of the system as well as the type of regulated process is highly diverse. Microbial two-component systems employ many proteins, which additionally can communicate between each other providing a great precision of the response (Beier and Gross, 2006). Expression of genes encoding virulence factors as well as genes providing antibiotic resistance of certain microbial pathogenic species, *e.g.* to vancomycin and polymyxin, is regulated by two-component systems. Development of structural genomics as well as resolution of three dimensional structures of proteins from two-component systems, especially of histidine kinases, initiated studies aimed at developing molecules blocking their activity (Matsushita and Janda, 2002).

Quorum sensing (QS) is the ability to sense cell density of bacterial populations and in turn coordinate gene expression. Many processes, including virulence, conjugation and natural transformation, biofilm formation, and production of secondary metabolites, are regulated by the "quorum-sensing" phenomena. Recently conducted analyses have shown that biofilm formation plays a key role in development of symptoms of around 80% infectious diseases (Davies, 2003; Parsek and Singh, 2003). Usually, the role of signaling molecules in Gram-negative bacteria is performed by N-acyl homoserine lactones (AHL), termed type I autoinductors (AI-1), low-molecular compounds able to cross the cell wall. The system is regulated by two proteins – LuxI (AI-1 synthase) and the LuxR regulator protein. When signaling molecules exceed the threshold concentration level, they bind to LuxR, which leads to its oligomerization and, in consequence, activates expression of genes that are the target of the QS system (Whitehead *et al.*, 2001). Potentially all three elements of the system – elements of the AHL synthesis pathway, signaling molecules and regulator proteins, can serve as drug targets. The QS system was most extensive examined using various inhibitors and different study models (mouse model, *C. elegans* model) in respect to *Pseudomonas aeruginosa.* Infections with this opportunistic pathogen are the main death cause of patients diagnosed with mucoviscidosis. The ability of the pathogen to form biofilm in a significant way reduces the effectiveness of the human defense systems and antibiotic therapy (Lyczak *et al.*, 2000; Venturi, 2006). Obtained experimental data encourage further studies. Yet, so far, none of the QS system inhibitors shown efficient on animal models comply with the safety demands posed for human therapeutics (Rasmussen and Givskov, 2006). Nonetheless, application of metagenomic strategies allows seeking new signal molecules of the QS system (Handelsman, 2004).

Application of bacteriophages and their products in therapy of infectious diseases

The idea of using bacteriophages as therapeutic antibacterial factors was considered ever since their discovery. Due to incomplete understanding of bacteriophage biology, ambiguous experimental results on animals and lack of documentation proving its safety, this type of therapy was abandoned for the benefit of antibiotic therapy. Presently, sequencing of phage genomes allows avoiding potential complications of bacteriophage therapy, mainly by detecting the presence of genes encoding toxins or virulence factors. Identification of phage receptors, which mutation can lead to the development of a phage-resistant bacterial population, has also significant importance. Several bacteriophage preparations are already applied in protection of plant- and animal-derived food products (Listex^{TM} P100; AgriPhage, LMP102) (Liu *et al.*, 2004; Petty *et al.*, 2007; Skurnik and Strauch, 2006).

High hopes are also connected with application of bacteriophage-encoded proteins, mainly lysins – bacterial cell-wall degrading enzymes which activity leads to the release of phage progeny, as therapeutics. Most lysins possess two domains – the N-terminal fragment carrying enzymatic activity, and the C-terminal fragment responsible for recognition of specific receptors. Lysins, in contrast to antibiotics, exhibit high specificity and are not active on physiological flora. Several lysins, cloned and overproduced in *E. coli* cells, were found efficient against such pathogens as *B. anthracis, Enterococcus* sp. or group B streptococci (Cheng *et al.*, 2005; Yoong *et al.*, 2004; Yoong *et al.*, 2006). It is believed that also other phage-encoded proteins, *e.g.* exopolisaccharide-degrading enzymes or components degrading bacterial

membranes, will also find application in antibacterial therapy. The sequencing of 26 *Staphylococcus aureus* phage genomes resulted in identification of many proteins able to inhibit the growth of this pathogen (Liu *et al.*, 2004).

Note: The references cited are representative and are not intended to be comprehensive.

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