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Pharmacogenomic strategies against microbial resistance: from bright to bleak to innovative

The last decade saw an alarming increase in antibiotic resistance in infections, with more than 13 million deaths per year from infections. Counter strategies include hygiene, antibiotic restriction and new antibiotics such as quinupristin, linezolid, tigecycline, daptomycin and dalbavancin. Presently, pharmacogenomics with basic research is revealing new antimicrobial peptides and is applying old drugs in new ways to break resistance. New approaches with host-directed drug targeting emerge to circumvent resistance. A future systems perspective from large-scale molecular techniques and bioinformatic modeling allows pharmacogenomics to reveal new intervention angles. This includes the fight against resistance and its transmission, improved vaccines, disarmament of microbes and antibiotic options from novel molecular targets (lipids, RNA and carbohydrates). Such a system perspective is also essential for improved diagnostics and individualized medicine. However, an increase in public awareness and closer cooperation of industry and basic research are essential to turn research into powerful new drugs that will enable us to treat new arising infections in the future.

KEYWORDS: antibiotics • drug pipeline • genomics • infection • MRSA
• systems biology

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The great medical advance of antibiotics in the 1970s led to the hope that the book on infectious diseases would soon be closed [1]. However, Alexander Fleming's early warning of resistance development became true and antibiotic resistance is an escalating problem. This culminated in the 1990s, with the fear of an 'antibiotic armageddon' [2]. In 1998, 13 million people died of infectious diseases such as pneumonia (3.5 million), diarrheal disease (2.2 million) and TB (1.5 million), a quarter of all deaths worldwide, many occurring in developing countries [1]. Inappropriate prescription of antibiotics prompted resistance and increased infectious disease mortality also in developed countries. Aging populations, changes in behavior and a decline in the development of new antibiotics exacerbated a deteriorating situation.

Counter-strategies included educational and prior approval programs, computer-assisted decision-making and antibiotic cycling [2]. The Center for Disease Control (GA, USA) advocated four major pillars to combat antibacterial resistance: surveillance, prevention and control, research, and product development [1]. New research strategies considered bactericidal antisense agents [3] or inhibitors of essential enzymes, for example, enzymes against persistence in TB [4]. Direct antimicrobial screening programs started to shift towards rational

target-based strategies enabled by new genomic insights and technologies stimulated by the human genome project (e.g., expression profiling and proteomics) [5]. Since approximately 10 years ago there is a new hope that has been brought about by pharmacogenomics. In the broad sense used here it means the exploitation of genomic information for pharmacological purposes. This refers to both the genome of the host and the pathogen. Powerful post-genomic approaches exploit the genome information to measure, decipher and analyze the proteome, metabolome and transcriptome, including high-throughput techniques and different functional tests. Furthermore, this opens up the possibility to personalized medicine with the prediction of the individual outcome of specific therapies and a broad pharmacologic strategy considering both host and pathogen. Considering the present strategies against antibiotic resistance, how can this potential be better used in future?

Present strategies to fight resistance

In spite of numerous prevention efforts, multiresistance has increasingly spread from hospital to community-acquired diseases (e.g., methicillin-resistant *Staphylococcus aureus* [MRSA] and pneumococci) [6]. Other critical resistances have emerged in *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and glycopeptide-resistant enterococci [3]. The

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past decade delivered new antibiotics such as quinupristin/dalfopristin, linezolid, tigecycline, daptomycin and dalbavancin. The effectivity of β -lactams, streptogramins and quinolones with activity against MRSA, penicillin-resistant pneumococci and vancomycin resistant enterococci, is being investigated [6]. However, too few companies invest in new antibiotics owing to mergers and low profits from antibiotics [3].

Reviewing old drugs and strategies reveals new options, such as silver in dressings, nanogels and nanolotions to protect burns and wounds, this even protects against MRSA [7]. Furthermore, adjuvants such as β -lactamase inhibitors (e.g., clavulanic acid, tazobactam and sulbactam) restore susceptibility to conventional antibiotics [3]. Probiotic strategies out-compete pathogens by native and harmless bacteria with high potential as helpful protectants against dangerous infections. An ethical challenge is the fact that a suitable *Escherichia coli* Nissl strain might protect children in Africa from deadly diarrheas but would not be permitted in Western clinics owing to its similarity to pathogenic *E. coli*. Also bacteriophages have been applied to antibacterial treatment in different regions since approximately 100 years, but convincing safety and efficacy trials remain a concern. The administration of phage products, such as lysins, will be feasible in the near future [8].

Further research strategies re-examine different resistance mechanisms. Resistance by antibiotic uptake blocking or antibiotic secretion is countered by efflux pump inhibitors [3,9]. Other microbes escape antibiotic impact by turning into dormant states, which is attempted to be circumvented by growth elicitors [7].

Besides the modification of existing agents and the development of new synthetic compounds, current approaches focus on novel target and drug identification. In particular, genomic screening revealed new classes of drugs. Thus, antimicrobial peptides (AMPs) act in a wide variety of organisms as endogenous antibiotics. The majority of AMPs disrupt the integrity of microbial cells and stimulate nonspecific immune response, inflammation and bacterial clearance. Increased effectiveness and specificity of AMPs could be achieved by *in vitro* evolution and bioinformatics helped with computer simulations to find new candidates [10]. Problems in application are potential induction of cross-resistance to innate human peptides, toxicity and their instability to proteases. The risk of resistant 'super-bugs' against such peptides is low as they are physiological products acting in many ways.

Clinical studies involve MBI-226 (a synthetic cationic peptide) and the human histatin 5 derivative, P-113 [10]. Further AMPs have been proven to be effective in animal use. Acyldepsipeptides are one example of lipoproteins that do not inhibit bacteria but unleash their caseinolytic protease P. The resulting internal proteolysis kills Gram-positive bacteria [11]. Other proteins with antimicrobial effects have been identified in the past and are waiting for application: lysozymes (1922), histones (1942), calprotectin (2003), bactericidal/permeability-increasing protein from human neutrophils (2007), granulysin (2008), human lactoferrin (2008) and histidine-rich glycoprotein (2008) [10].

Previously, genomic screenings mainly targeted microbial virulence factors. To circumvent resistance, development targeting the host factors essential for pathogen entry, survival and replication has come to the forefront of research. Intracellular pathogens exploit various host factors critical for bacterial survival, which can be identified as a potential drug target by innovative strategies combining computational biology, genomics, transcriptomics and proteomics. Host signaling pathways are manipulated by intracellular pathogens for invasion. Pathway manipulation includes cytoskeleton components, clathrin- or receptor-mediated endocytosis to phagocytosis. Other pathogens such as plasmodium actively penetrate host cells, form an intracellular vacuole and shut off apoptosis. Different bacteria use virulence factors to subvert GTP-binding protein (G-protein) signaling, ubiquitination, and MAPK-signaling. Such host-directed drug-targeting studies have already provided a proof of concept in viral infections [9].

Future perspective

Regarding the immediate future, genomics and other 'omics techniques allow new results. Thus, rapid sequencing helps to show very similar MRSA strains in hospitals and to identify carriers of various multiresistant organisms all over the world [6]. However, apart from hygienic measures (e.g., patient isolation, clean hands and sufficient treatment personnel) antibiotics are important and antimicrobial usage in food, animals and general overuse promotes bacterial resistance in patients. Ranking should identify critical antibiotics and suggests use in only for humans, such as fluoroquinolones, macrolides, and third- and fourth-generation cephalosporins [12], as drug pipelines are expensive and easily dry out. Current Phase II/III trials against infections up until 2012 include only glycopeptides,

quinolones as well as improved trimethoprim treatment. Lipopeptides and oxazolidinones are arriving. On the market, antibiotics are cheap and only used for a short time per patient. There is no real incentive for company development, only Novartis (Surrey, UK) and Pfizer (NY, USA) remain committed, as well as several smaller companies. Noninnovative 'noninferiority drug trials' block the pipeline further. The search for new antibiotics must be intensified, including new antibiotic resources such as marine and soil genomes, innate immunity and antibiotic (artificial) peptides, lipids, carbohydrates and RNA (antisense RNAs against infections, ribozymes and aptamers [3]). Other approaches include the impairment of virulence factors, protection against toxins and vaccination [6].

Individualized antibiotic treatment has already been initiated by considering drug-metabolizing enzyme variants [13,14], but has to be extended by haplotyping, SNPs, gene copy number and the modeling of individual immune response. The analysis of mitochondria could even provide a personalized barcode for antibiotic therapy [15]. 'Pharmacometabolomic' approaches personalize drug treatment even further by combining predose metabolite profiling and chemometrics to model and predict the responses of the individual subject. A proof-of-principle was shown for paracetamol [16], but should be extended to antibiotics. Monte Carlo simulations could help to integrate data from pharmacokinetics, pharmacodynamics and pharmacogenomics to predict the optimal antibiotic doses needed to kill the particular pathogen with the least toxic effects [17].

A stronger systems biology perspective must now focus on the interaction between drug, host, infectious bacteria and environment to foster the long-term success of antibiotic strategies. These include drug combinations, dual drugs and multiple hit strategies by one drug on several pathways. Thus suppressive drug interactions can be analyzed and how they result in non-optimal microbial growth responses [18] with subsequent bacterial persistence. Recent work stopped bacterial conjugative DNA relaxase using biphosphonates, which are drugs clinically approved for bone diseases [19]. Variation in the microbial genome modulates disease occurrence and severity in the host [1], as is well known for strain variation in *S. aureus* [6] or *E. coli* [7]. However, this may now be turned into genomics based new drug design strategies, for example targeting AT-rich malaria parasites with AT-specific alkylating drugs [20]. Moreover, stopping

bacterial conjugation or targeting of specific nucleotide compositions in resistant strains will strongly reduce antibiotic resistance propagation. This is particularly important for mucoviscidosis and other diseases leading to long-term antibiotic treatment. New software tools such as STITCH2 [21] allow systematic searches to reveal new applications for old drugs used in combating infections. Thus, membrane modifiers, such as neuroleptics and anesthetics, lower resistance in severe infections such as sepsis [7].

Finally, as a systems perspective, host processes will be considered to ameliorate conditions in infectious disease and improve efficacy of treatment. This is well known for symptomatic therapy in sepsis but also includes prevention and blocking of rheumatic reactions and chronic inflammation [22]. This is even more important for eukaryotic parasites such as malaria and other apicomplexa. Thus, the damage to the brain in severe malaria is largely owing to immune response (and cytokine load) to the infection rather than infection *per se*. Moreover, for optimal therapy and to prevent resistance, the individual pharmacogenetics of drugs should be considered clearly more than been done previously, for instance regarding antimalarial drugs [23,24].

Conclusion

It is very clear that today an innovative systems perspective is apparent for pharmacogenomics treatment of infections. This new view will ultimately treat infections with unprecedented efficiency, personalized and long lasting, based on a fundamental understanding of all host–pathogen–drug interactions. However, the challenge is to translate these new insights into a concrete pipeline of sustainable drug development. The remedy will be a closer cooperation of industry and research and increased political and general awareness of the urgent necessity to search for new and better antibiotic drugs, taking also into account the improvement of old concepts and resources.

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