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The global need for effective antibiotics—Moving towards concerted action

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ABSTRACT

Antibiotic resistance has emerged as one of the greatest global health challenges to be addressed in the 21st Century. The risk of widespread antibiotic resistance threatens to mitigate the positive changes made in modernizing healthcare systems; therefore, fresh approaches are essential, as well as new and effective antibacterial drugs. In a globalized world, a spectrum of different interventions and health technologies must be employed to contain antibiotic resistance. Finding ways of accelerating the development of new drugs and diagnostic tools is one strategy, as is better surveillance of antibiotic resistance and ways of improving use of existing antibiotics. Moreover, a framework to regulate use is called for to avoid that potential new antibiotics are squandered. Finally, the ongoing pandemic spread of resistant bacteria illustrates that the problem can only be addressed through international cooperation and thus that any new strategy to manage antibiotic resistance must take into consideration issues of global access and affordability.

Antibiotics are among the most important medical discoveries and their introduction represents a remarkable success story. However, the extensive use and misuse of antibiotics have resulted in selection and worldwide spread of antibiotic resistant bacteria and we now face an immediate risk of entering a post-antibiotic era where our medical advances are lost. Within just a few years, we may very well be faced with unimaginable setbacks, medically, socially, and economically, unless we react now. Antibiotics are indispensable in virtually all modern medicine; for example major surgery, organ transplantation, treatment of preterm babies and cancer chemotherapy would not be possible without effective treatment and prevention of bacterial infections.

The emergence of antibiotic resistance is further complicated by the fact that bacteria and their resistance genes are travelling faster and further. Resistant and multiresistant bacteria pose a risk to people everywhere. A study from Tanzania showed a 43.5% mortality from bloodstream infections caused by Gram-negative bacteria and antibiotic resistance was a predictor of fatal outcome (Blomberg et al., 2007). Antibiotic resistance is not only costly in terms of human suffering but also in monetary terms. Presently, at least 25,000 patients in Europe die per year because their bacterial infections are not treatable with available antibiotics at the estimated cost of more than 1.5 billion EUR annually (ECDC/EMEA, 2009). The overall positive trend of economic development in low and middle-income countries also brings about increased availability and demand of antibiotics, which exacerbate the already excessive consumption around the world. Simultaneously, with the increasing level of resistance to first line drugs, antibiotic resistance leads to the need for more costly second and third line drugs which often are unaffordable to many in low-income countries. In the dual problem of access and excess, the challenges lie in reducing irrational use and improving access without ruining the antibiotic effectiveness – a global public good.

Reducing the spread of bacterial infections, using existing antibiotics correctly and developing new antibiotics are literally a matter of life and death, and should be regarded as a collective responsibility. As stated by Elinor Ostrom, 2009 Nobel Laureate in Economic Sciences and Tercentenary Linnaeus Honorary Doctor of Uppsala University, Sweden, "the issue is comparable to that of climate change in the sense that both phenomena involve non-renewable global resources, both are caused by human activity and are intrinsically linked to our behavior. The problem can only be addressed through international cooperation". Another similarity with the issue of climate change is that if we fail to turn the tide, all countries will be affected, but the poorest countries will suffer the earliest and the most. On the other hand, when it comes to global community response the difference could not be greater. While climate change is at the very top of political agendas throughout the world, antibiotic resistance has been conspicuously absent. The ongoing pandemic spread of resistant bacteria illustrates that the problem can only be addressed through international cooperation.

To promote international collaboration, several resolutions concerning antibiotic resistance have been adopted by the World Health Assembly (WHA). In 2000 the WHO presented a global strat-

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egy for the containment of antimicrobial resistance, calling for a multidisciplinary and coordinated approach. However, sufficient financial and human resources to implement the strategy were never provided. In 2005, the WHO member states requested the director general to strengthen the leadership role of the WHO in containing antimicrobial resistance and to provide technical support. Moreover, the same resolution urges the member states to ensure the development of a coherent, comprehensive and integrated national approach. Still to this date, little has been done to implement the global strategy. The links between well-meant strategies at the global level and the uptake by national governments and agencies are unfortunately very weak. Stronger leadership and coordination by the WHO is urgently needed, coupled with the building of strong public awareness in order to translate recommendations into action.

A wide range of measures is needed to ensure that currently available antibiotics remain effective as long as possible. This can be achieved primarily by means of greater awareness among the public, health care professionals and the food- and agriculture sector regarding the importance of rational use of these medicines as well as ways to prevent infections and spread of antibiotic resistant bacteria. However, in parallel with these measures we urgently need to address the serious lack of new antibiotics in the drug pipeline (Freire-Moran et al., 2011) as well as investigate novel drugs as alternatives to traditional antibiotics (Fernebro, 2011).

In order to kick-start the discussions for how to incentivize the research and development of new antibiotics, Sweden initiated an expert conference during its Presidency of the European Union in 2009. The results of conference entitled "Innovative Incentives for Effective Antibacterials" led to a set of conclusions by the European Health Minister which included a call to the EU Commission to develop an EU Action Plan on Antibiotic Resistance. This plan will include among a number of other important issues, concrete proposals concerning incentives to develop new effective antibiotics. The plan is to be presented in November 2011. Moreover, during the Swedish EU Presidency, a transatlantic taskforce (EU and US) on antimicrobial resistance (TATFAR) was established which also will address the need to reinvigorate the research and development pipeline for novel antibiotics.

To keep the momentum of these discussions and developments and to further deepen the dialogue on the need for new antibiotics, ReAct—Action on Antibiotic Resistance (www.reactgroup.org) arranged a global conference in Uppsala, Sweden in September 2010 on "The global need for effective antibiotics—Moving towards concerted action". The conference gathered 200 participants from around the world, representing 45 countries and many leading stakeholders—civil society, academia, pharmaceutical industry, governments, and supranational organizations. Among many important contributions at the conference the European Federation of Pharmaceutical Industries and Associations (EFPIA) gave a clear signal that return of investment on research and development of new antibiotics will have to be delinked from market sales in order to boost necessary innovation while yet limiting the use of antibiotics. This will require a new business model where private and public sectors cooperate (So et al., 2011). Moreover, there is a clear understanding and commitment from EFPIA to make any future new antibiotics globally accessible and affordable (Bergström, 2011).

While developing a new model for the development of novel classes of antibiotics, several other tracks need to be explored in parallel as we must ensure that potential new antibiotics are not squandered in the future. It is also necessary to make better use of the antibiotics that are available today by looking more closely at dosage, treatment duration and drug combinations (Mouton et al., 2011). In addition, diagnostic tests are under-used as tools for resistance containment and need to be developed to provide rapid and reliable results (Okeke et al., 2011). Lastly, all these measures require improved global surveillance of resistance to generate the data on which to base priorities and ensure a needs-driven research and development process (Grundmann et al., 2011).

A fundamentally changed view of antibiotics is urgently needed. Antibiotics must be viewed as a global public good. ReAct strongly believes that for current and future generations to have access to effective prevention and treatment of bacterial infections as part of their right to health, all of us need to act now. The window of opportunity is rapidly closing. Managing the resistance problem requires political action and awareness of decision makers to promote research and implementation of global strategies for action.

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The global need for effective antibiotics—A summary of plenary presentations☆

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ABSTRACT

To highlight the global need for effective antibiotics and explore possible concerted actions for change, cross-cutting plenary sessions served to frame the program of the conference. These sessions contained presentations on the present state of antibacterial resistance and the availability, the use and misuse of antibiotics. A number of possible actions were discussed, such as rational use of and access to antibiotics from various perspectives. The roles of vaccines and diagnostics were touched upon and followed by in depth discussions on supply-side bottlenecks with their scientific, regulatory and financial challenges. The value chain for research and development (R&D) of antibiotics has to be reengineered if we are to realize the development of much needed new antibiotics. This challenge will require a multitude of actions, some of which are related to changing the financial realities of antibiotics and interventions by global and regional institutions.

1. Background

1.1. Setting the scene: the global picture of antibiotic resistance

The antibiotic era in clinical medicine was launched more than 70 years ago with the introduction of sulfonamides. However, the major breakthrough was the mass production of penicillin during the Second World War. Professor Otto Cars, chairman of Action on Antibiotic Resistance (ReAct), Sweden, underlined that the stunning success of penicillin which meant a drastic increase in survival from bacterial infections, clearly changed the world (Fletcher, 1984). However, there were already early warning signs of what was to come. As early as 1942, René Dubos predicted that bacterial resistance should be expected. "Rather than counter bacterial resistance with even more potent weapons", he argued that we should, "seek instead more peaceful coexistence with pathogens" (Moberg, 1996). When Alexander Fleming (Fleming, 1945) received the Nobel Prize in 1945, he noted, "it is not difficult to make microbes resistant to penicillin".

Resistance develops by spontaneous mutations or through horizontal transfer of resistance genes. In large bacterial populations (e.g., the gut flora) small subpopulations of resistant bacteria will be selected and amplified by antibiotic treatment that kills susceptible bacteria. The selection process has been ongoing since the beginning of the antibiotic era and has contributed to an increasing gene pool of resistance in the commensal flora, in hospitals, in the community and in the general environment. Through indiscriminate use, ignorance and complacency, this valuable resource has been squandered and the consequences are now becoming increasingly apparent. Presently, at least 25,000 patients in Europe die per year because their bacterial infections are not treatable with available antibiotics (ECDC/EMEA, 2009). It is a fact that many advanced treatments that we today take for granted (e.g., cancer chemotherapy, care for preterm babies, transplantation and major surgery) cannot take place without the support of effective antibiotics.

The situation in developing and low-income areas is worrisome. Poverty, overcrowding, extremely poor housing, malnutrition, contaminated food and the lack of clean water create a basis for transmission of pathogens. In addition, healthcare systems are weak or non-existing in these environments, and antibiotics are often sold and used without medical consultation. As always, poor people suffer most in all respects, as so amply pointed out by Professor Zulfiqar Bhutta, Aga Khan University, Pakistan. Infections in infants, such as diarrhea and pneumonia, are the cause of 40% of the death toll in this age group in underprivileged areas. Diarrhea that is caused by Shigella, salmonella and cholera takes the lead and the resistance to antibiotics (such as ciprofloxacin) increases rapidly in those pathogens. Resistance data is not complete for the

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whole world, but the available information is cause for alarm and action. The increase of antimicrobial resistance worldwide is then an imperative challenge for those who want to improve health and quality of life for the inhabitants in all parts of the world. This will negatively influence the possibilities to attain the health-related Millennium Development Goals (MDGs).

1.2. Reflections from a global perspective

Dr. Guenaël Rodier, WHO Regional Office for Europe, emphasized that although there is a serious deficiency of global data on antimicrobial resistance, available information shows an alarming increase in resistance affecting all infectious agents. To take multidrug resistant tuberculosis (TB) as an example, globally 440,000 cases are estimated to have occurred in 2008 while a mere 7% of these were actually notified in the official WHO statistics. The malpractice of antibiotics can be characterized as overuse, underuse and wrong-use! Antimicrobial resistance spreads through health care associated infections, usually associated with weak healthcare systems. Dr. Rodier further noted the absence of global momentum, both in the action to improve rational use of antibiotics and infection control and in the development of new antibiotics. However, the main strategic components should be surveillance, prevention, containment, research and innovation. A coordinated global response is sorely needed in which "nobody is exempt from the problem or from playing a part in the solution".

2. Policy challenges to optimizing the use of antibiotics

2.1. Reaching for global access and affordability

The currently biggest problem in low-income countries is the lack of knowledge and presence of misconceptions among the people. Dr. Eva Ombaka, senior consultant and former director of the Ecumenical Pharmaceutical Network, observed that there are some overlooked resources that can be used much more. For example, cell phones are widely available in these environments and there is some limited access to and use of the Internet, especially by the youth. These means can be much more and innovatively used, including using cell phones to pass correct/urgent information to isolated areas. A possible downside is that sick people may source and use unreliable health information from the Internet. A second aspect is the purchase and use of drugs from a "drug shop", where drugs, including antibiotics, are generally freely available. However, the service providers are often not professionally trained and the drugs are of questionable quality-substandard or counterfeit! It remains that the public at large needs to be informed about use of drugs and mobilized to demand and practice better health behavior. For example, prescription drugs should be administered only on prescription, and the right of *any* medical doctor or other health worker to prescribe *any* existing drug should be questioned. Alliances will be necessary to bring about change.

2.2. Rational use: where less is more

Professor Roger Finch, University of Nottingham, UK stated that, for many types of major global infection, there is clearly a therapeutic failure because available treatments are not effective enough. Among these infections are TB, MRSA infections, hepatitis B and C, HIV and most diseases that exist in tropical areas. Better and faster diagnostics would be of great help in selecting most appropriate treatment and in reducing empirical prescribing. To improve disease management electronic support could be employed to improve the quality of prescribing and facilitating linkage of data from diagnostic tests, drug use and outcomes. The drugs presently available should be treated with great caution to preserve their value and thus extend their useful life. Regulatory Authorities have a key role to play in ensuring that generic/off-label drugs have indications appropriate to current clinical needs. Concerted and sustained international collaboration is necessary to effect these changes.

2.3. Perspectives on rational use and access

Dr. Ramanan Laxminarayan, Director of the Global Antibiotic Resistance Partnership, USA, presented an economist's perspective on the actual challenges and found bacterial disease persisting as a major killer. The consumption of antibiotics increases globally in that there is, in addition to much restricted availability because of poverty, an increasing middle class of people who have economic means to buy what they think they need. Dr. Niyada Kiatying-Angsulee, Chulangkorn University, Thailand emphasized perspectives on regional "network of network" from South East Asia involving the establishment of a national alliance, an institute surveillance system and the promotion of rational antibiotic use. Dr. Dana Hanson, World Medical Association, USA, discussed the commitment of physicians as expressed by the World Medical Association to progress based on professional skill and solidarity.

3. Global priority-setting for research and development to manage antibiotic resistance

3.1. Introduction

The session was opened by Professor Zulfiqar Bhutta who stressed the need for an action-based agenda that would take innovation to where it is needed most. The roles of vaccines, diagnostics and antibiotics in relation to bacterial resistance were discussed by Dr. John Clemens, International Vaccine Institute, South Korea, Professor Rosanna Peeling, London School of Hygiene and Tropical Medicine, UK and Dr. Andreas Heddini, ReAct, Sweden. They addressed the state of the current pipeline, identified gaps and included a forward-looking discussion about prioritization of these technologies and their respective and combined potential values.

It has become increasingly clear that antibiotic resistance is a multi-dimensional, complex problem, the roots of which span over many scientific areas and sectors of society. There will not be one magic bullet solution to resolve antibiotic resistance, but rather a variety of counter measures and actions targeting different aspects of the problem. Although antibiotics are by far not the ultimate solution to the problem of bacterial infections, they will be a mainstay in their management. Thus, the question is not whether we need new antibiotics – because we do – but by which mechanisms they should be developed to ensure that any new health technology or product is addressing a global need and that aspects of access and affordability are considered in the process. In addressing antibiotic resistance the targeting several areas is essential:

- Improved rational use (which in principle equals more restrictive use in both human and non-human sectors).
- Improved infection control/hospital hygiene.
- Development of novel antibiotics and complementary technologies (i.e., vaccines and new and/or improved diagnostic methods).

3.2. Pneumococcal vaccines

Strategies to manage antibiotic resistance require combined efforts using several available resources in the health system. Prevention of disease can be achieved through a number of measures, where vaccination stands out as a highly cost-effective intervention. *Streptococcus pneumoniae* is one significant pathogen in which

ongoing efforts to develop more effective vaccines could prove useful in mitigating resistance. *S. pneumoniae* is the most common cause of community-acquired bacterial pneumonia, meningitis and bacteremia in children and adults, with approximately 1.6 million deaths worldwide per annum. Multidrug resistance (≥3 antibiotic classes) is reported worldwide, constituting a majority of *S. pneumoniae* isolates in many countries.

New-generation conjugate vaccines have demonstrated the ability to prevent antibiotic-resistant pneumococcal colonization and disease via direct and herd effects. Employment of these vaccines has also been shown to reduce overall use of antibiotics, which together may synergize to lower the circulation of antibiotic-resistant pneumococci. However, the introduction of pneumococcal conjugate vaccines has induced an increase in circulating "replacement" serotypes, including those resistant to antibiotics. One of these replacement serotypes, 19a, has been documented to have acquired increased levels of antibiotic resistance and to have caused increased rates of invasive pneumococcal disease. Future strategies to contain antibiotic-resistant pneumococcal infections will therefore have to include increasingly broad vaccine serotype-coverage in conjunction with aggressive policies to ensure appropriate use of antimicrobial drugs.

3.3. Development of diagnostics for drug-resistant infections

Increasing access to appropriate treatments for infectious diseases would have a major impact on disease burden, particularly in low-income settings. Because of the lack of rapid diagnostic tests, most common infections have to be managed empirically in accordance with the clinical picture. This causes and drives unnecessary and erroneous antibiotic use, which could have been avoided by the availability of appropriate diagnostic tests. The landscape of diagnostic tests is characterized by a lack of investment in diagnostics research and development (R&D) with little industry interest in diagnostics R&D on diseases prevalent in low-income countries. This low level of interest is due to a perceived lack of return for investment, i.e., pharmaceutical companies are commercial and therefore tend to develop medicines with profit in mind. There is also lack of access to diagnostic services, lack of regulatory transparency and control, as well as inadequate quality standards for test evaluations. Diagnostics are often undervalued. Recent data from the US show that while diagnostics comprise less than 5% of hospital costs and about 1.6% of all Medicare costs, their findings influence as much as 60–70% of decision making in health care.

During recent years, however, several novel diagnostics based on molecular techniques have been developed. These techniques include automated extraction and real-time polymerase chain reaction (PCR) amplification techniques, as well as innovative "lab-on-a-chip" platforms in which thousands of patient samples can be screened for resistance profiles within a matter of hours. Thus, many high quality diagnostics for infectious diseases are available but they are neither affordable nor accessible to most patients in the developing world where disease burden is the greatest. There is an urgent need to increase diagnostic capacity at all levels of the healthcare system to provide accurate, evidence-based management for such major syndromes as fever and lower respiratory tract infections.

Another area requiring better diagnostics is surveillance of antibiotic resistance. Today, surveillance activities are disproportionately geared toward high-income countries and hospital settings. There is a need to establish surveillance networks to cover hot spots where antibiotic resistance will likely emerge, including resource-poor settings. This is also important for the development of new diagnostic tools in which novel biomarkers for early detection of treatment failure could be identified. There is great need for highly sensitive and specific tests in a high throughput format, low technical complexity and for use with non-invasive specimens. Some options for diagnostics at different levels of care include:

- For screening at hospitals

  - Point-of-care (POC) tests for admission
  - Highly sensitive and specific assays for local outbreak investigations and epidemiology studies

- For patient management

  - POC tests to distinguish between viral/bacterial/fungal infections
  - Detection of pathogens within a syndrome and their antimicrobial susceptibility pattern

- For surveillance of resistance

  - Standardized and systematic collections of specimens
  - Highly sensitive and specific tests in high throughput format, low technical complexity

In addition, there is an unmet need for innovative mechanisms to accelerate product development, clinical trials and regulatory approval of new diagnostics as demonstrated in a survey from 2002 carried out by WHO/TDR. The survey showed that more than 50% of countries worldwide lack regulatory oversight for diagnostics but for the WHO/AFRO region, the figure was a staggering 73%.

3.4. The need for antibiotics with novel mechanisms of action

Despite the magnitude of the resistance problem, little progress has been made in R&D for new antibacterial agents effective against resistant strains. For instance, only two new antibiotics had been developed in the past 10 years. Recent data suggests that the biological fitness cost associated with bacterial resistance to antibiotics may quickly be compensated for, i.e., resistance will continue even in the absence of antibiotic selective pressure. This further underscores the need for novel antibiotics.

The past 40 years have seen the emergence of only two new classes of antibiotics: oxazolidinones and cyclic lipopeptides, neither of which is effective against Gram-negative bacteria. The future for antibiotic drug development also appears bleak: among the top 15 pharmaceutical companies, which accounted for 93% of antibiotics placed on the market between 1980 and 2003, only 5 drugs in their R&D pipelines are antibacterials (ECDC/EMEA Technical Report, 2009).

It should be noted, however, that any public investment in drug development should be done in a framework of careful analysis looking at the different options to prioritize among health technologies and research goals to ensure greatest public benefit. Furthermore, should new classes of antibiotics be discovered, measures must be taken to prolong their shelf-life through rational use and a variety of measures related to drug selection, combination and dosage regimens. An exploration of such possibilities is found in this issue (Mouton et al., 2011).

The development of new antibiotics will require a sustained, systematic effort of discovery and development that spans over many years. Further, innovative financing mechanisms for clinical trials, which are very costly to undertake, should be explored. Finally, a mechanism for prioritizing among different antibiotics, diagnostics and other health technologies has been called for. Such a prioritizing framework needs to be based on global surveillance of antibiotic resistance to provide information on prevalence of resistant pathogens. This framework will also allow predictions over time, modeling and analysis of trends. Notably, such a framework cannot be rigid but should provide needs-based information to

underpin scientific agendas and guide public investments in R&D. Elements of such a framework could build in components, such as disease prevalence, mortality & morbidity, deaths averted by a new product, access to antibiotics and their use and risk/likelihood of success.

4. Supply-side bottlenecks: scientific, regulatory and financial challenges

4.1. Challenges in bringing innovation to market

The main reasons for the insufficient availability of novel and effective antibiotics are mainly considered in the scientific, regulatory and financial domains. Professor Anthony So, Duke University, USA reviewed the challenges of bringing innovations to the market. The decline in R&D is caused by a multitude of interplaying factors. The supply-side bottlenecks critically influence the value chain along the life cycle of antibiotics. Among the most difficult scientific and financial challenges in the development program is crossing "the valley of death", i.e., the transition from preclinical to clinical phases. During the market life cycle of a drug, the return of investments (ROIs) must at least be anticipated to exceed the costs for R&D. Currently, the net present value (NPV) for an intravenous (i.v.) antibiotic is considered an order of magnitude less profitable than, e.g., drugs for musculoskeletal diseases. This is due to several factors, among which besides the acknowledged scientific challenges to develop novel antibiotic classes, other factors include the short treatment regimens and the curbs placed by prudent and rational use of effective antibiotics, the therapeutic competition posed by a relatively saturated market and the relatively higher profit margins on other therapeutic drug categories. Different scenarios apply to large pharmaceutical companies compared with small- and medium-sized firms (SMEs). SMEs may face different opportunity costs than research-based, multinational companies. If a start-up firm has a promising antibiotic but little else in its portfolio, it will not have alternative R&D opportunities that a large pharmaceutical firm is likely to have with a diverse R&D portfolio. In looking at the drug R&D landscape for neglected diseases a study of 63 projects found that half of these were being conducted by multinational companies, invariably on a "no profit, no loss" basis, and the rest by small-scale businesses in industrialized countries or developing country firms, with expectations of commercial return (Moran et al., 2005).

4.2. Scientific challenges

The scientific challenges and attrition rates of antibacterial discovery were elucidated by Dr. David Payne, Vice President, GSK, USA. The high expectations following the genomic breakthrough revealing numerous potential bacterial drug targets were followed by disillusionment and a poor success rate. The success rate of obtaining new chemical leads from these screens was only 7%, which is substantially lower than other therapeutic areas. There are several reasons for the difficulties, including the fact that most antibacterial targets are enzymes that are hard to inhibit: compound libraries are biased toward attributes suited for mammalian targets and the safety and spectrum challenges of antibacterial development, especially when it comes to Gram-negative bacteria. More innovative approaches are needed. Some areas currently being investigated include returning to natural product screening, exploring novel chemical space (e.g., boron chemistry) and development of antibiotic potentiators (e.g., efflux-pump inhibitors). Reviewing attrition rates of novel-mechanism antibacterial R&D, it is clear that improving the success rate of Phase 2 starts would improve delivery, but, in turn, this means longer timelines and the requirement of more resources to generate higher quality candidates. In addition, diagnostics could play a very impactful role at enriching clinical trials with the most appropriate patients to prove the attributes of a novel mechanism antibiotic (i.e., enrich for patients with clearly defined bacterial infections and patients with infections that are caused by multiresistant pathogens). Such a plan of action could enable smaller, cheaper clinical trials and improve regulatory outcomes.

Structure-based drug design for discovery of novel antibacterial drugs to circumvent some of the bottlenecks in the search for these antibiotics was suggested by Professor Ian Chopra, University of Leeds, UK. This innovative approach for the identification of new inhibitors of both classical and novel bacterial target proteins was predicted to increase the success rate for discovery of antibacterial drugs in the near future. Indeed, it may be possible to design molecules that simultaneously inhibit two or more functional sites in a target enzyme, which could minimize the potential for the development of resistance. It was stressed that the vast majority of current antibacterial drugs are of natural origin. A return to natural product screening was also desirable as an addition to exploring soil and marine biomass using metagenomic tools. Non-terrestrial opportunities might be considered in the future.

4.3. Regulatory perspectives

The regulatory agencies are often pointed out as having an essential role in the dwindling pipeline of new antibacterial agents. By setting up extensive regulatory barriers for the development program and data needed for approval of new drugs and new indications, the substantial investments needed and risks for a negative outcome will have a significant hampering effect on R&D in this field. Dr. Tomas Salmonson, vice-chair of the Committee for Human Medicinal Products-European Medicines Agency (CHMP-EMA), gave a presentation of the role of the regulatory agencies with special focus on the European situation. In this presentation, Dr. Salmonson shared his views of current and future possible regulatory measures needed to aid the development of antibiotics for which there is a high medical need. Following the GAP analysis (ECDC/EMEA, 2009), jointly published by THE EMA and ECDC (European Centre for Disease Prevention and Control) in 2009 in collaboration with ReAct highlighting the urgent need for novel antibacterial agents especially against multi-drug resistant Gram-negative pathogens, and the outcome of the European Union (EU) conference on this subject held in Stockholm September 2009 (Swedish Government, 2009), specific EU council conclusions were adopted in December 2009 aiming for actions at both the national and European level, including regulatory efforts (Council of the European Union, 2009). Regulatory measures currently taken within the existing legal framework include the ongoing revision of EU regulatory guidelines for antibiotics, where further flexibility to facilitate drug development, such as the possibility of alterative study designs, were discussed. Sponsors are strongly advised to discuss with EU regulators as early as possible in the development program to optimize the path and chances for approval. It was stressed that a decision of approval is always based on a benefit–risk assessment, taking the specific situation for each product and indication into account. Possible regulatory measures inspired by the success of the Orphan drug regulation were discussed. A special designation of antibiotics for which a particular medical need exists coupled with attributed regulatory benefits may be a fruitful way forward. This approach has to be supported by specific EU legislation. It was emphasized that regulators are driven by the goal to enhance the development, availability and adequate use of effective and safe antibacterial agents.

4.4. Financial bottlenecks

The financial hurdles influencing the development of new antibiotics were further clarified by the fact that Big Pharma is currently ruled by the chase for blockbusters. However, it is almost an impossible task to assess the probability of technical success and market potential of a new drug, not to mention prizing 10–15 years ahead, since sales forecasts are wrong 80% of the time. The advice from Dr. Bernard Munos, former advisor in corporate strategy, Eli Lilly, USA, is to accept that forecasting is hopeless and to reposition R&D away from blockbusters and instead focus on breakthrough innovations. Medicines are for people, not for profits; however, profits always follow a true breakthrough drug. Funding should be restricted to breakthrough ideas and clinical development should only be initiated for genuine breakthroughs. The competence for breakthrough innovations and the competence for operational excellence are at crosscurrents in the sense that fixation on one degrades the capacity of the other.

4.5. The role of the pharmaceutical industry in meeting the public health threat of antibiotic resistance

The pharmaceutical industry is of course a major player in the search for new antibiotics and in meeting the public health threat of antimicrobial resistance. Novel ideas on how to combine the realities of commercial entities to the need of the public were proposed, suggesting a completely innovative concept to separate the financial return from the use of a product. This concept, published separately in this issue (Bergström, 2011), was presented by Dr. Richard Bergström, European Federation of Pharmaceutical Industries and Associations.

5. Reengineering the value chain for research and development of antibiotics

5.1. Introduction

This session, introduced by Professor Anthony So (Duke University, USA), focused on lessons drawn from the landscape of neglected diseases that might cross-apply to the R&D of antibiotics. All three initiatives involve public sector funding, two in product development partnerships. Created to help overcome market failures, product development partnerships mobilize both public and private sector resources to develop diagnostics, drugs and vaccines for neglected diseases. Most are disease-specific and even technology-specific in focus. Within the antibacterial space, the challenges of TB drug development may prove particularly instructive though there are both similarities and differences from antibiotic R&D more generally.

However, as these examples illustrate, there is more to filling these R&D gaps than innovative public financing. The Global Alliance for Tuberculosis Drug Development is piloting a new regulatory pathway for testing combination therapies against TB. By testing combinations in parallel rather than serially, years might be shaved off the R&D pipeline. The Drugs for Neglected Diseases Initiative (DNDi) covers more than one disease, but primarily focuses on kinetoplastid diseases. DNDi's experiences in aligning target product profiles with patient needs, building capacity for clinical trial platforms in disease-endemic countries and securing access to proprietary compound libraries may inform efforts for antibacterial drug discovery. Finally, taking a page from information technology, India's Council on Scientific and Industrial Research (CSIR) has sought to apply the approach of open source innovation to TB drug discovery. Supported by public sector monies and tapping a network of Indian universities, this fledgling project has exciting promise.

5.2. The Global Alliance for TB Drug Development

Tuberculosis, although curable, continues to kill someone somewhere in the world about every 15 s – more than 5000 people every day, or two million this year alone. Because of increasing drug resistance, co-infection with HIV and long treatment periods with existing therapeutic regimens, there is a growing demand for new TB drugs.

The principal agenda of The Global Alliance for TB Drug Development (TB Alliance) was explained by its president, Dr. Melvin Spigelman. The Alliance collaborates with research institutions and pharmaceutical companies to share risks, which provides an incentive for partners to collaborate. While retaining management oversight of its drug development projects, the TB Alliance outsources the development of potential drugs to public and private partners, providing funding and scientific guidance. Depending on the project, the TB Alliance either co-invests and co-develops a project, funds and manages it directly, or licenses the technology or intellectual property involved. Project diversity is a stated goal in which potential compounds are selected from a variety of chemical classes, with a wide range of targets within the TB organism, *Mycobacterium tuberculosis*.

Currently, drug sensitive TB requires a regimen of 4 drugs administered for a period of six to nine months. Moreover, there are few available treatments for multidrug resistant TB and tolerability is a problem. Because many patients with TB suffer from HIV/AIDS, there are additional difficulties with anti-retroviral therapy (ART) and anti-TB drug co-administration. There are thus several unmet needs for shorter, simpler therapy, more effective and safer regimens and drugs that can be given together with ART.

The TB Alliance has over 20 projects in its portfolio at different stages of discovery and development, ranging from lead identification to phase III clinical trials. A new paradigm is being developed for rational selection and development of new combinations with the aim of significantly shortening the time of new regimen development.

5.3. Applying lessons from neglected diseases

DNDi is a non-profit drug R&D organization that is developing new treatments for neglected diseases. Dr. Jean-Pierre Paccaud, DNDi, Switzerland, explained the "needs-driven" approach that facilitates basic science, preclinical and clinical research on targeted diseases. The organization's current target diseases include malaria and the three most neglected diseases caused by the prozoan group known as the kinetoplastids: visceral leishmaniasis (VL), sleeping sickness (human African trypanosomiasis, HAT) and Chagas disease.

DNDi's primary objective is to deliver 6–8 new treatments by 2014 for VL, HAT, Chagas disease and malaria, as well as to establish a strong R&D portfolio addressing patient treatment needs. To date, DNDi registered two fixed-dose combination treatments for malaria, a new combination regiment to treat sleeping sickness and a VL combination treatment deployed in Africa. The organization strives to use and strengthen existing capacity in disease-endemic countries via project implementation. A further aim of the model is to mobilize the private sector though incentives/rewards and partnerships built on a strong collaborative basis:

- At early discovery stage:

- Compounds come mainly from Pharma partners

- Biological characterizations are conducted at major parasitology research centers ("reference centers")
- Pre-clinical development with dedicated CROs, etc.

• Clinical trials:

- Collaborating partners include institutions and experts from disease-endemic countries, health authorities, regulatory experts, and frequently, MSF teams

• Registration and manufacturing:

- Pharmaceutical partners provide essential capabilities to ensure sustainability
- Technology transfer for production in Southern countries

The concept of patient-centered Target Product Profiles (TPPs) is central to ensure needs-driven R&D. The TPP details the characteristics of the product to be developed, provides guidance throughout the drug development program and establishes stringent go-no go criteria to insure that the drug developed fully responds to the patient's needs in terms of efficacy, usability in the field and affordability. DNDi's patient-centered TPPs, which are shared between all partners, have been critical for focused and efficient drug development.

Also of great impact on drug accessibility, careful management of intellectual property (IP) and licenses rights is paramount to insuring access.

5.4. India's open source drug discovery initiative

India's open source drug discovery initiative (OSDD) was communicated by Professor Samir Brahmachari, director general, Council of scientific and industrial research, India. OSDD is a novel model for drug discovery based on concepts from open source in information technology (IT) and proposes a new, non-proprietary way of taking leads through the early phases of discovery. Examples include the OSDD where a web-enabled open source platform – both computational and experimental – has been established to make drug discovery cost effective and affordable by using the collective creative potential of students and scientists worldwide. Participants are rewarded with incentives for developing novel algorithms, finding drug targets, leading identification and other contributions. A current project is focused on discovering drugs for TB and making them available to patients at an affordable cost. Although the genome of this pathogen was sequenced 10 years ago, the function of more than 1000 of its 4000 genes remains unknown, opening challenging possibilities in the search for new treatments. To eliminate this problem OSDD recently launched the "Connect-to-Decode" open-source initiative. Within weeks, 830 qualified scientists volunteered to reannotate the entire *M. tuberculosis* genome. The OSDD consortium brings together more than 4300 individuals from 130 countries at the virtual project platform and through sequence based comparison between human genome, human oral and gut flora, more than 50 potential drug targets have been identified excluding leads with potential effects on commensal bacteria. OSDD has been able to create a small molecule open access repository. The virtual platform operates at the modest cost of approximately USD 2 million per annum.

6. Future treatment options – balancing antibiotics with other treatment concepts

Basic research into completely novel antibacterials that do not belong to the established groups of antibiotics has sparked global interest over recent years (Fernebro, 2011). However, interesting as they may be, few, if any, of these compounds have delivered as promised. Antibacterial peptides are the effector molecules of innate immunity and over the past few decades, the search for new drugs and drug targets has prompted an interest in these compounds. Small molecules, on the other hand, can interfere with bacterial virulence factors and secretion pathways that can relieve severe or pathogenic symptoms without killing bacteria. In addition, the normal flora would be left unharmed. Bacteriophages have been used and tested extensively throughout the 20th century, most notably in the countries of Eastern Europe, but many studies have later been deemed to be of unsatisfactory quality and hence the probability of a breakthrough in the near future seems unrealistic.

The prospects of alternative strategies to combat bacterial diseases were further reviewed by Professor Staffan Normark, Karolinska Institutet, Sweden. Professor Normark offered an update of the current scientific platform for alternative strategies (e.g., virulence factor inhibitors, boosting the clearing efficacy in the host, antimicrobial proteins and immunotherapy with human monoclonal antibodies). The approach of using small molecules to inhibit virulence factors of pneumococci and *Staphylococcus aureus* and blocking of type III secretion in Gram-negatives are currently "hot" areas of research. Antimicrobial peptides and phage therapy are still investigated; however, several hurdles in terms of stability and specificity remain in these areas. Existing drugs (such as statins and morphine) can affect the clearing capacity in the host, which should be further investigated. Stimulating the endogenous expression of antimicrobial peptides and the phagocyte function has been shown to be feasible in animal models. However, future prospects were not deemed very optimistic for any of these alternative strategies. At most, these strategies will generally provide add-on therapy to current drugs in a subset of patients, particularly in those with a compromised immune system.

Thus, although new lines of research are looking promising and may produce novel treatment options in the future, these are far from being adequately developed. In a foreseeable future, antibiotics will be the mainstay for treatment of severe bacterial infections, which further emphasizes the pressing need for new classes of antibiotics.

7. Moving toward concerted action

Dr. Bernardus Ganter, adviser, antimicrobial resistance, WHO regional office for Europe, explained the features of antimicrobial resistance that make it "a faceless" disease in that it appears as an abstract threat to treatment and prophylaxis with properties that make it hard to define and grasp. A solution to this communication problem could be to call it "difficult to treat bacteria". It is for certain that there is now an arrival of new strains that are virtually resistant to all existing antibiotic drugs. This is a frightening prospect in light of the decreasing development of new antibiotics. In developing countries there is stagnation in reaching the United Nation's Millennium Development Goals on childhood survival while in emerging economies an increased affordability of antibiotics of varying quality can be seen as well as highly questionable medical rationality.

Steps have been taken by the WHO to approach the problem of increasing bacterial resistance (e.g., the WHO Global Strategy for Containment of Antimicrobial Resistance from 2001). In 2009, a WHO resolution was adopted on the Prevention and Control of Multidrug Resistant TB. This strategy contains the necessary principle of making drugs against TB available only on prescription. We all look forward to the World Health Day in 2011 which will be dedicated to antimicrobial resistance. In Europe, an Antibiotic Awareness Day has been proclaimed for the 18th of November each

year. USA and Canada will follow Europe's initiative with their own awareness days. On this day, a number of meetings will be held that will include discussions about antimicrobial resistance, how it develops, what actions to take, how to broaden the knowledge and how to initiate a call for action among health care professionals.

Mario Nagztaam, policy officer, EU Commission, Belgium, reflected on a number of policy initiatives, recommendations and conclusions within the EU following the Council Conclusions taken by the European Health Council on December 1, 2009. As a response, the European Commission is working on an EU action plan, including, among other issues, possible incentives for the development of novel effective antibiotics. This development was preceded by an expert conference organized by the Swedish Government during its presidency in the EU in the autumn of 2009. In the aftermath of the conference the issue of antimicrobial resistance was discussed at the EU–US summit. The Swedish Prime Minister, Mr. Reinfeldt, and President Obama agreed to form a taskforce, the Transatlantic Task Force on Antimicrobial Resistance (TATFAR). The TATFAR will deliver its final report in March 2011.

The Swedish EU presidency meeting focused on new incentives for the development of novel antibiotics. An extensive review, "Policies and incentives for promoting innovation in antibiotic research", was commissioned from the European Observatory on Health Systems and Policies and the London School of Economics. The essentials of the report were later published (Morel and Mossialos, 2010). The present meeting, "Global need for effective antibiotics – Moving towards concerted action", can be viewed as a follow-up to the Swedish EU presidency meeting.

In the EU there is mandatory, harmonized monitoring of antimicrobial resistance (European Antimicrobial Resistance Surveillance Network, EARS-Net) coordinated by the European Centre for Disease Prevention and Control (ECDC) in Stockholm, Sweden, as described by Dominique Monnet, senior expert and coordinator of ECDC's program on antimicrobial resistance and healthcare-associated infections. Similar activities are taking place in the US where the Centers for Disease Control and Prevention (CDC), Atlanta, GA, collaborate with different states to collect data on prevalence of resistance in health care and other settings. This and other data from patients in Europe, the US and elsewhere show the recent emergence and spread of bacteria that are totally or almost totally resistant to all available antibiotics!

Dr. Anna Lönnroth, acting head of unit, EU Commission, Belgium, expanded on the work of the TATFAR. The objectives of the TATFAR are to (1) increase mutual understanding of US and EU activities and programs on antimicrobial issues, (2) deepen the transatlantic dialogue, (3) provide opportunities to learn from each other and (4) promote information exchange, coordination and cooperation between the US and EU member states. The TATFAR has several working groups dealing with the appropriate therapeutic use of antibacterial drugs, prevention of drug resistant infections and strategies to improve the pipeline of new antibacterial drugs. Its final report is expected in March 2011.

Dr. Dennis Dixon, Chief of the Bacteriology and Mycology Branch at the National Institute of Allergy and Infectious Diseases, USA, asserted that the US National Institutes of Health have a robust research agenda in antimicrobial resistance addressing basic, translational and clinical research. In addition to the standard, investigator-initiated research opportunities there are specific antimicrobial resistance-focused opportunities, including product development partnerships that require academic scientists to collaborate with small and large companies to move promising diagnostics, vaccine and therapeutics through preclinical development. One example of a clinical research initiative, "Targeted Clinical Trials to Reduce the Risk of Antimicrobial Resistance", aims to generate data to guide optimal use of existing antimicrobials, thereby prolonging their lifespan. Ongoing trials funded under this initiative focus on dose, duration, pharmacokinetic and pharmacodynamics (PK/PD) and the absolute need for antimicrobials in disease areas subject to the greatest antimicrobial use. Additionally, a range of preclinical and clinical services, which are free and available to the international research community, have been established to fill gaps along the product development pipeline.

In conclusion, antibiotic resistance is a complex and truly global problem that requires global solutions through concerted action.

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The role of the pharmaceutical industry in meeting the public health threat of antibacterial resistance☆

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ABSTRACT

The established market model for pharmaceutical products, as for most other products, is heavily dependent on sales volumes. Thus, it is a primary interest of the producer to sell large quantities. This may be questionable for medicinal products and probably most questionable for antibacterial remedies. For these products, treatment indications are very complex and encompass both potential patient benefits, possible adverse effects in the actual patient and, which is unique for this therapeutic class, consideration about what effects the drug use will have on the future therapeutic value of the drug. This is because bacteria are sure to develop resistance. The European Federation of Pharmaceutical Industries and Associations (EFPIA) agrees with the general description of the antibacterial resistance problem and wants to participate in measures to counteract antibacterial resistance. Stakeholders should forge an alliance that will address the need for and prudent use of new antibiotics. A variety of incentives probably have to be applied, but having all in common that the financial return has to be separated from the use of the product.

1. Basic conditions

Industry funds its investments in R&D by selling products at a relatively high price during a limited patent life. Patents are nominally 20 years, but once all the studies and paperwork are done, usually 10–12 years remain. Investment decisions are based on the cash-flow that can be generated once the product is on the market. The price is set to reflect the value of the product to society, including affordability and willingness to pay. This we call value-based pricing. However, these things do not work for new antibiotics. First, it is widely held in public policy circles that new antibiotics will be reserved and only gradually released to the market. The desire to focus the usage is understandable, but will reduce sales and hence reduce the ability of the innovator company to recover the costs required to bring that drug to the market. It can also be expected that public support for antibiotic research will be conditional on ensuring equal access to all citizens of the world and hence reduced value from sales in many emerging markets. For this reason, if we want to find a consensus, any public policy solution to the problem must:

- build on private sector commitment, activities and funding;
- anticipate the impact of restricted, or at least agreed, use of new agents; and
- provide for global access to new medicines at a fair price.

Financial incentives for the private sector must be designed to address these challenges and meet these needs.

2. Changing from the past to the future

There is strong support among industry, physicians, pharmacists and others to combat antimicrobial resistance. However, for a number of reasons, it is difficult to expect changes in behavior here and now:

- Local subsidiaries of pharma companies, as well as local companies and generic players, generate cash-flow by selling today's antibiotics.
- Physicians and pharmacists worldwide are reluctant to reduce the use of antibiotics (patient pressure, revenue, risk of litigation, etc.).

Building on the need for change, new solutions should look to the future and aim for a global compact among all stakeholders to ensure that new medicines will not be introduced and used the way they were in the past. Fighting resistance through training of physicians, pharmacists and the general public will be an important priority for individual countries.

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Forging an alliance between all stakeholders will only work if is realistic and addresses the need for, and prudent use of, new antibiotics. Therefore, I pledge to this conference that we need to separate the past from the future. Anything else will not be satisfactory.

A global compact (mirrored on the UN program for good governance and sustainable development) could focus on the agreed and gradual introduction and responsible marketing and use of new agents. This includes the need for differential pricing to ensure equitable access for all patients in need. The experiences from the HIV/AIDS must be harnessed. It is not reasonable that the least developed countries in the world pay as much as those with the highest income. A global compact would require that not only industry but also governments, physicians and pharmacists join forces to preserve the new medicines that our children and grandchildren need.

3. Incentives needed, but there is no general solution

Turning from principles to practicalities: what would work? How do we kick-start antibiotics product development? We cannot force companies to work in this area; rather, we must make them *want* to be active in this area. Before we look at incentives, we need to explore what public authorities can do already today. All medicines must be approved by regulatory agencies. At last year's conference, there was a firm suggestion that regulatory requirements must be revised. We need comparative trials, but I think there should be rational limits set to what has to be established for the requirements of documentation. The relative effectiveness is anyhow best studied in real life. Clearly, the rules must change. And there is scope for a more step-wise approval process for much needed antibiotics.

The second hurdle is payers: pricing and reimbursement bodies that tend to compare prices against the cheapest generic. That may be appropriate for other fields, but not for antibiotics where the aim is to reward follow-on development and a multitude of products.

However, the most important thing is money: what makes small and big pharmaceutical companies hang on. I have a conviction that only private companies can develop new medicines. Financial incentives for private companies can come in many forms. Recent reviews by the London School of Economics (for the Swedish EU Presidency Conference last year) and the Institute of Medicine (for the Countermeasures Workshop) list a great number of incentives. Analyzing these, and reflecting on the dynamics of drug development and the different nature of companies, it is obvious that there has to be a multitude of changes. Some suggestions may be less likely to have success. For example, data protection or extended patents are unlikely to generate any income for products that are kept in reserve. There is no cash-flow on products kept in the drawer. What mechanisms seem most likely to really move the needle? This is a difficult question and the answer that works for one company is often not the same as for another. However, there are some ideas that seem to have the greatest plausibility at a broad level:

- A straightforward push incentive could be created via tax legislation: simple tax credit-based incentives would have the net effect of reducing the cost of development of needed antibiotics. A mechanism to carry forward such credits might be required to permit small companies without active sales to deliver this value, but that is an accounting issue that could be resolved once the principle is accepted. But I am not sure the tax route is feasible for Europe, where tax systems are a national concern. Maybe it would be easier in the US.
- One pull-based mechanism is the idea of transferable rights, i.e. when companies can extend revenue on patented blockbusters as a compensation for developing an antibiotic. Sometimes this is referred to as "vouchers". Such an approach may be criticized by payers, but such approaches do have the intellectual advantage of spreading the costs over large groups of patients who are benefiting broadly from use of modern pharmaceuticals.
- Another possible approach is true value-based pricing, perhaps also combined with advance market commitments. Aligning prices to the value of the new antibiotic and decoupling usage from sales could be powerful tools.
- Advance purchase commitment or prizes are promising. Yet, if they are awarded only for the first product to meet certain standards, they may de-incentivize development of much-needed follow-on drugs (to combat resistance). Unlike other areas, there is a need for a rich and vibrant pipeline of follow-on antibiotics.

4. Conclusion

Most importantly, no single tool will solve the problem. What is really needed is a collection of incentives that addresses the multiple obstacles to success. For instance, and returning to the idea of the prize-based mechanism, it may be warranted to have more general milestone payments up to phase II (that means hypothesis-generation) for all compounds against a certain target, and prizes (that are paid afterwards) for successful phase III trials and registration for a limited number of products in the later phase.

Connecting the aspects I have highlighted, it is clear that incentives that separate the financial return from the use of a product are the only way to change this behavior. Intelligent pull incentives, such as advance commitments and prizes, provide financial rewards to the developer not based on the volume of use of the novel antibiotic. With the right set-up, Pharma companies will have no incentive to drive use: perhaps they will not do any promotion at all. Use would be agreed with public policy makers, purchasers and national health systems.

The mandate by the EU Council of Ministers to the Commission and the joint EU-US Task Force present two unprecedented opportunities to make a difference. The research-based pharmaceutical industry is ready to discuss and wants to be part of the solution.

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A framework for global surveillance of antibiotic resistance☆

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ABSTRACT

The foreseen decline in antibiotic effectiveness explains the needs for data to inform the global public health agenda about the magnitude and evolution of antibiotic resistance as a serious threat to human health and development. Opportunistic bacterial pathogens are the cause of the majority of community and hospital-acquired infections worldwide. We provide an inventory of pre-existing regional surveillance programs in the six WHO regions which should form the underpinning for the consolidation of a global network infrastructure and we outline the structural components such as an international network of reference laboratories that need to be put in place to address the void of these crucial data. In addition we suggest to make use of existing Health and Demographic Surveillance Sites (HDSS) to obtain crucial information from communities in resource limited settings *at household level* in low- and middle-income countries in Asia and Africa. For optimising the use of surveillance data for public health action i.e. priority setting for new drug development, comparative quantification of antibiotic effectiveness at local, national, regional and global level and identification of the action gaps can be helpful.

And indeed, everything that one can discern has numbers,
hence it is impossible to grasp or recognize anything without them.
Philolaos of Kroton, 440 BC

1. Introduction

Trends in antibiotic resistance and their consequences for health, welfare and the economy are rapidly changing (ECDC, 2010a). Antibiotic resistance threatens the success of medical interventions at all levels of health care and creates a set of specific challenges for clinical, therapeutic and public health interventions with local, national, and global dimensions.

Bacteria that belong to the normal flora in humans become indiscriminately exposed to antibiotic compounds every time antibiotics are used. Therefore, the most significant resistance has been emerging among these microorganisms. Since most of them are truly opportunistic pathogens, the most vulnerable segment of societies i.e. the young, elderly and immune-compromised are likely to face infections and the consequences of failing antibiotic effectiveness. Moreover (and this is in contrast to other infectious diseases perceived as threats to public health like zoonoses, bioterrorism and pandemic influenza), the trajectory of antibiotic resistance is rather predictable. Still, no surveillance system exists that would allow measuring the magnitude of antibiotic resistance as a threat to global health.

We argue that inequalities and market forces facilitate and accelerate the critical decline of antibiotic effectiveness and that

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within the next 5–10 years untreatable (or next-to-untreatable) community- as well as hospital-acquired infections will become widespread. This relentless dynamic is caused by often unwanted consequences of the evolving socio-political and physical environment in which we live. Current volume, speed and reach of travel and migration are unprecedented. Increasing civil unrest, food shortages and natural disasters leave vulnerable individuals under crowded conditions. Other economic and behavioural changes also impact on the pattern of antibiotic consumption worldwide. As the result of global medicalisation in the wake of the expansion of the HIV/AIDS pandemic and successful prevention campaigns that followed, recent patient generations around the world have been brought up with the tacit conviction that microbes are causing disease. This has lead to an overwhelmingly changed pattern of health seeking behaviour especially in poorer societies. It explains the growing demand for antibiotic chemotherapy, which, at the same time, is met by the availability of generic compounds produced in emerging market communities. Thus, it will come as no surprise that there will be a massive increase in antibiotic consumption while antibiotics become commodities in unregulated markets. In the following chapter we will describe in more detail how market forces contribute to the decay of antibiotic effectiveness and why a global surveillance effort is urgently needed.

2. Engines of resistance

Antibiotic resistance is driven by the density of antibiotic use, combined with the level of compliance with infection control measures to prevent spread of resistant bacteria. At the population level, several studies have shown a correlation between outpatient use of an antibiotic class and the percentage of bacterial isolates resistant to this class (Albrich et al., 2004; van de Sande-Bruinsma et al., 2008). This relationship between antibiotic use and resistance has also been demonstrated at the hospital level, for example for the carbapenems (Lepper et al., 2002; Lopez-Lozano et al., 2000).

Herrmann developed a dynamic, bio-economic model to better understand the pricing policy of a company which holds the monopoly for an antibacterial compound (Herrmann, 2010). This model revealed three phases: (i) under patent protection when the monopolist endogenously manages the level of antibiotic efficacy (quality) and the infected population (market size); (ii) approaching the end of patent protection when the monopolist behaves more and more short-sightedly, leading to a continuous decrease in the price of the antibiotic; and (iii) after patent expiration when the monopolist behaves competitively in a generic industry, which results in a discontinuous fall of price of the antibacterial (Herrmann, 2010).

These results were recently confirmed by Jensen et al. (2010) who reported on the effects of patent loss and generic entry on ciprofloxacin price, sales and resistance in Denmark. The Danish study showed that, within one year following patent loss, the number of formulations of ciprofloxacin increased from 3 to 10 and the median price per defined daily dose (DDD) decreased by 53%. During the four years following patent loss, outpatient consumption of ciprofloxacin increased by more than 250% and the proportion of *Escherichia coli* from urine samples that were resistant to ciprofloxacin increased by 200% (Jensen et al., 2010). In Europe, outpatient consumption of antibacterials is significantly correlated with the number of antibacterial trade names (Monnet et al., 2005). This relationship was found both in situations where the antibacterials were still protected by a patent and in situations where the market was opened to generic copies of original agents (Monnet et al., 2005).

Consumption of antibacterials varies widely between countries (Goossens et al., 2007) and is significantly correlated with per capita gross domestic product (GDP) (ReAct, unpublished data). Countries with a low per capita GDP also report low average consumption of antibacterials per capita, which is likely due to poor access to these medicines or only access through informal channels because of low individual resources and poor infrastructures. However, the United Nations report that per capita GDP is growing rapidly in many low per capita GDP countries such as China and India but also Brazil, Indonesia, Mexico and Turkey (UN, 2010). In emerging markets, this results in an increase in the sales of pharmaceuticals in general (IMS, 2010) and of antibacterials in particular (LeadDiscovery, 2009).

Newly industrialized countries increasingly contribute to the production of antibacterials and research-based companies and global generic manufacturers have been reported to sign agreements with or invest in generic companies and production facilities in newly industrialized countries (Biospectrum Asia, 2009; Morey, 2010; Singer, 2010). Several last-line, intravenous antibacterials, including the carbapenems imipenem-cilastatin and meropenem, and the penicillin-beta-lactamase inhibitor combination piperacillin–tazobactam, recently lost patent protection and are now available as cheaper, generic presentations from manufacturers in newly industrialized countries. The manufacturers already received approval or have filed applications for their generics in the United States (FDA, 2011; Golikeri, 2010) and in European countries (IMS, 2010).

The data presented above describe a gloomy scenario for antibiotic resistance in the near future: increasing availability of lower priced, generic presentations of last-line, intravenous antibiotics such as the carbapenems will result in increasing use in most countries, which in turn will result in increasing resistance of these antibiotics. In regions of the world with low sanitation coverage and sub-optimal hospital infection control practices, this will promote spread of almost totally resistant bacteria such as carbapenemase-producing *Enterobacteriaceae*. Global travel, global healthcare and medical tourism, will further contribute to global spread of these almost totally resistant bacteria.

This scenario started to unfold in 2010 with the report of cases of New Delhi metallo-beta-lactamase-1 (NDM-1)-producing *Enterobacteriaceae* in patients in the United Kingdom, mainly associated with travel or healthcare contact in the Indian subcontinent (Kumarasamy et al., 2010). NDM-1-producing *Enterobacteriaceae* have since been reported from many countries, in particular in North America, Europe and Asia (CDC, 2010; Chihara et al., 2011; Mulvey et al., 2011; Struelens et al., 2010; Wu et al., 2010). The rapid spread of another carbapenemase, OXA-48, reported in Mediterranean countries, in Europe and recently in Sub-Saharan Africa represents another example of this scenario (Carrer et al., 2010; Moquet et al., 2011). In the latter report from Senegal, five patients died from their infection before antimicrobial susceptibility testing could be completed and proper therapy administered. These recent developments clearly illustrate the urgent need for a global surveillance data that can inform clinicians, public health experts, policymakers and pharmaceutical companies about the dynamic spread of antibiotic-resistant pathogens in a geographical explicit and timely manner.

3. Surveillance of antibiotic resistance: the emerging opportunity for a global network infrastructure

The WHO Global Strategy for Containment of Antibiotic Resistance (UN, 2001) recognized laboratory-based surveillance of antibiotic resistance as a “fundamental priority” for the development of strategies to contain antibiotic resistance and for assessment of the impact of interventions. In face of the above mentioned dimensions of antibiotic resistance as a threat to public health, many countries have established national and regional

Table 1
Estimate of WHONET software use by WHO region.

WHO region	Number of countries	Number of laboratories[a]
AFRO = WHO Regional Office for Africa	13	69
EMRO = WHO Regional Office for the Eastern Mediterranean	15	64
EURO = WHO Regional Office for Europe	39	505
AMRO/PAHO = WHO Regional Office for the Americas/Pan American Health Organization	25	466
SEARO = WHO Regional Office for South-East Asia	6	105
WPRO = WHO Regional Office for the Western Pacific	13	568
Total	111	1777

[a] In some countries, figures reflect the estimated number of laboratories which use the WHONET software, while in others figures reflect the estimated number of laboratories managed with WHONET at the national level.

surveillance collaborations, others have not. Furthermore, there is no formal framework for collaboration among surveillance programs worldwide. This lack of a global framework for collaborative surveillance of antibiotic resistance hobbles efforts to track emerging resistance challenges; to identify, characterize, and contain new threats; and to systematically compare and evaluate the value of national resistance containment activities.

Fortunately, key components of a global surveillance collaboration already exist, and much more has been accomplished worldwide than is generally appreciated. In this chapter, we will highlight current and past surveillance initiatives in the six world regions defined by the World Health Organization. The initial focus of this discussion will be on antibiotic resistance among common community- and healthcare-associated bacterial pathogens including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and others. This will be followed by a survey of WHO-affiliated disease- and pathogen-specific programs, such as those organized for tuberculosis, malaria, HIV/AIDS, and foodborne pathogens.

3.1. Surveillance of antibiotic resistance at national level

A number of early WHO meetings recommended the establishment of local, national, and regional/global surveillance programs (WHO, 1981, 1982, 1994). At national level, priority objectives identified include: monitoring trends in infection and resistance, development of standard treatment guidelines, assessment of resistance containment interventions, early alert for novel resistant strains, and prompt identification and control of outbreaks. A national view permits benchmarking of experiences by facility and geographic distribution, particularly valuable when supplemented by information on pathogen population dynamics, antibiotic use, infection control measures, and patient population demographics. National coordinators also have a critical role in mentoring network participants in quality improvement and use of data to support local action and therapeutic guideline decisions.

To support surveillance at multiple levels, the WHO Collaborating Centre for Surveillance of Antibiotic Resistance in Boston has developed and supported the WHONET software for the management and sharing of microbiology laboratory test results since 1989 (WHO, 1999; www.whonet.org/DNN). At present, WHONET is used in over 110 WHO Member States to support local and/or national surveillance in over 1700 clinical, public health, food, and veterinary laboratories. In most of these countries, the WHONET software is used as a core component of the national surveillance program. Estimates of WHONET use by region are provided in Table 1, and a global map is depicted in Fig. 1.

Data can be entered manually or downloaded into WHONET from existing laboratory information systems, laboratory diagnostic instruments, or desktop applications using the BacLink utility distributed with WHONET. In most laboratories and countries, WHONET is used to manage results for all positive culture results from all specimen types from all microbial species identified by the laboratory. In some instances, data collection is limited to a few so-called indicator pathogens.

The more comprehensive approach to data collection – with information on all species identified, specimens processed, and antibiotics tested – has several advantages over a narrow view of a few priority issues: a broad view of emerging microbial threats, identification of novel strains, detection of hospital and community

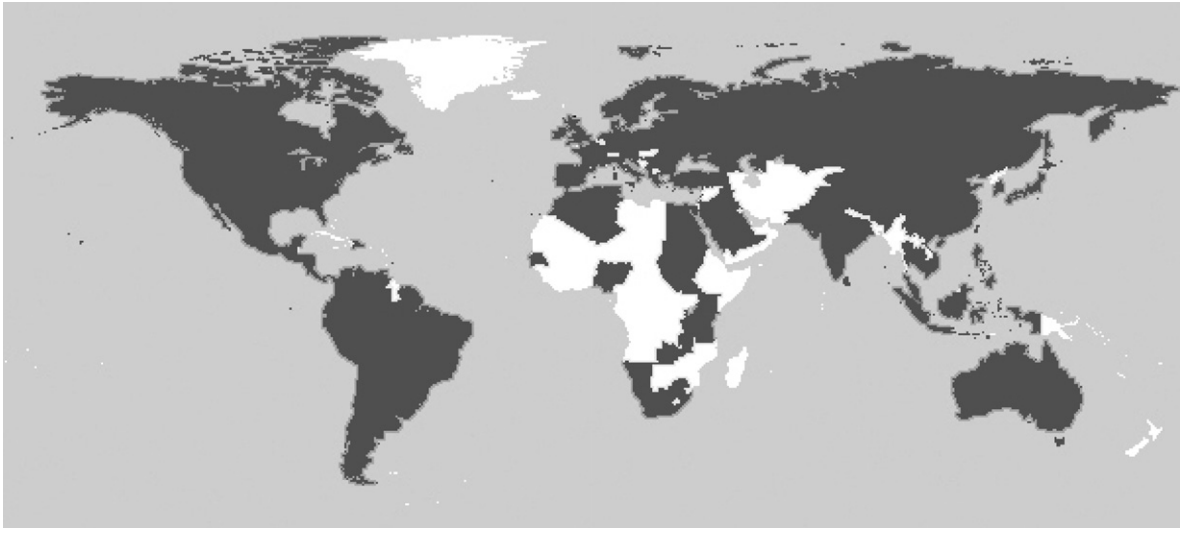

Fig. 1. WHONET use around the world, from http://www.whonet.org/DNN/.

Table 2
Regional programs for surveillance of antibiotic resistance in common bacterial pathogens.

WHO region	Program name	Coordinating institution	Participants	Years of activity	Organisms
AFRO	AFRO Integrated Disease Surveillance and Response (IDSR)	AFRO	43 countries	2002–present	8 epidemic-prone pathogens
PAHO	ReLAVRA = Red Latinoamericana de Vigilancia a las Resistencias Antimicrobianas	AMRO/PAHO	21 countries 519 laboratories	1996–present	16 pathogens All sample types
EMRO	1. ARMed = Antimicrobial resistance in the Mediterranean	St. Luke's Hospital, Malta	9 countries 27 laboratories	2001–2005	7 pathogens, blood and CSF
	2. EMRO Regional Program for surveillance of AMR	EMRO	Proposed	Proposed	28 species All sample types
EURO	1. EARSS = European antimicrobial resistance surveillance system	1. Institute for Public Health and the Environment (RIVM), Netherlands	33 countries 917 laboratories 1578 hospitals	1999–2009	7 pathogens Blood and CSF
	2. EARS-Net = European antimicrobial resistance surveillance network	2. European Centre for Disease Prevention and Control (ECDC), Sweden	28 countries 886 laboratories >1400 hospitals	2010 – Present	7 pathogens Blood and CSF
SEARO	SEARO Regional Program for Surveillance of AMR		Proposed	Proposed	Proposed
WPRO	WPRO Regional Program for Surveillance of AMR	WPRO	13 countries Number of labs is not available	1990–2000	22 species All sample types

outbreaks with any microbial pathogen, and adaptability as new issues are identified. When data can be downloaded from existing microbiology information systems, a comprehensive approach for data acquisition is simpler than a narrower filtered view of certain indicator pathogens.

3.2. Surveillance of antibiotic resistance at regional level

A summary of regional surveillance programs in the six WHO regions is provided in Table 2. Regional activities have been launched in five of the six WHO regions, while in the remaining region (SEARO) the regional strategy for containment of antibiotic resistance published in June 2010 made a commitment to establishing national and regional surveillance over the next few years. Three of the regions (AMRO/PAHO, EURO, and AFRO) continue to be active to this day in resistance surveillance. Two regions (EMRO and WPRO) were active in the past, and WPRO has recently established a new Working Group which has identified surveillance of resistance as a regional priority.

In three of the regional networks (AMRO/PAHO, AFRO, and WPRO), programs were coordinated by WHO staff, while in the other two (EURO and EMRO), activities were led by the initiative of individual institutes and funded by the European Commission (EARSS, Dutch National Institute for Public Health and the Environment, RIVM, until 2009 for EURO and ARMed St. Lukes Hospital, Malta, until 2007 for EMRO). Since January 2010 EARSS, now under the name EARS-Net, has been coordinated by the European Centre for Disease Prevention and Control (ECDC). The EARSS/EARS-Net, ARMed, and AFRO surveillance networks focus on a limited number of pathogens and specimen types of public health importance, while the other regional programs (AMRO/PAHO, WPRO, and proposed EMRO program) have a broader scope for data collection (all organisms, specimens, antibiotics) and targeted data analyses of certain issues of regional importance.

3.3. External quality assurance programs at regional level

Strategies for ensuring and maintaining the quality of laboratory test results are critical to the value of surveillance initiatives. All facilities should have procedures for ongoing assessment of the quality of test reagents and test performance by laboratory technicians. In addition to internal quality control practices, laboratories should also participate in national and/or external quality assurance (EQA) programs. As highlighted in Table 3, all six WHO regions currently have regional EQA initiatives which address routine organism identification and antibiotic susceptibility testing. Three of these (AMRO/PAHO, AFRO, EMRO) are coordinated by WHO offices in collaboration with high-quality microbiology laboratories in the region, while the remaining three (EURO, SEARO, WPRO) are coordinated by independent organizations dedicated to training and/or quality assurance for laboratories in the region.

3.4. Disease- and pathogen-specific networks

The above description has covered surveillance of resistance in a broad range of common, primarily bacterial, pathogens. In addition to these surveillance initiatives, a number of additional WHO-affiliated regional and global surveillance networks have developed over time to support the technical, epidemiological, and strategic needs of specific disease control programs. Details on several dedicated networks coordinated by or affiliated with the World Health Organization are provided in Table 4.

4. Core components for global collaboration

Since most WHO regions are already primed for surveillance for antibiotic resistance, a global collaborative network should be achievable and affordable. However, core tasks and responsibilities need still to be addressed, implemented or harmonized. For operational purposes, we suggest to distinguish between some of these core tasks which include (i) reference work, (ii) quality assurance, and (iii) surveillance. All of these tasks are essential for surveillance and may be accomplished by single but more often separate institutions. Moreover, all three core activities need to be functional at both national and at regional level.

4.1. National level framework

At national level, we envisage that national reference laboratories for antibiotic resistance, national centres for external quality assessment, and a national surveillance centre could coexist in the same institution or consist of different centres that could cater for these functions. Whatever solution fits the country's needs, a close communication and collaboration between the centres would be key. Moreover, it would be the remit of all centres to iden-

Table 3
Regional programs for external quality assurance in common bacterial pathogens.

WHO region	Program name	Coordinating institutions	Participants	Years of activity
AFRO, EMRO, SEARO	WHO AFRO/NICD Microbiology EQA Programme in Africa	WHO/HQ-Lyon and National Institute for Communicable Diseases, South Africa (Routine bacteriology, plague, TB microscopy and malaria)	Total = 50 countries 45 countries in AFRO, 4 in EMRO, 1 in SEARO Number of laboratories Microbiology – 81 TB microscopy – 82 Malaria microscopy – 69 Plague – 18	2002–present
EMRO	1. ARMed = Antimicrobial resistance in the Mediterranean	1. National External Quality Assurance Scheme (NEQAS), United Kingdom	9 countries	2001–2005
	2. EMRO Regional Microbiology External Quality Assessment Scheme	2. WHO/HQ-Lyon and EMRO and	27 laboratorie	
		Central Public Health Laboratory, Oman (Bacteriology)	22 countries 27 laboratories	2004–present
		Health Reference Laboratories, Iran (Serology, mycology, parasitology)	22 countries 27 laboratories	2004–present
EURO	National External Quality Assurance Scheme (NEQAS) In collaboration with	National External Quality Assurance Scheme (NEQAS), United Kingdom In collaboration with		
	1. EARSS = European Antimicrobial Resistance Surveillance System	1. Institute for Public Health and the Environment (RIVM), Netherlands	33 countries 917 laboratories 110 million citizens	1999–2009
	2. EARS-Net = European Antimicrobial Resistance Surveillance Network	2. European Centre for Disease Prevention and Control (ECDC), Sweden	28 countries 886 laboratories	2010–present
AMRO/PAHO	ReLAVRA = red Latinoamericana de Vigilancia a las Resistencias Antimicrobianas	Pan American Health Organization in collaboration with Malbrán Institute, Argentina	18 countries 18 national reference laboratories which provide EQA to 500 sentinel laboratories	1997–present
AFRO, EMRO, SEARO, WPRO	RCPA Quality Assurance Programs Pty Limited	Royal College of Pathologists of Australia (RCPA)	AFRO 4 countries 35 laboratories EMRO 3 countries 51 laboratories SEARO 3 countries 3 laboratories WPRO 12 countries 395 laboratories in Australia and New Zealand 85 laboratories in other WPRO countries	2002–present
WPRO, SEARO	REQA = Regional External Quality Assessment Programme	Pacific Paramedical Training Center, New Zealand – WHO Collaborating Centre for External Quality Assessment in Health Laboratory Services	WPRO 17 countries 22 laboratories SEARO 2 countries 2 laboratories	1991–present

tify and recruit diagnostic microbiological laboratories as reporting laboratories that would report routine antimicrobial susceptibility test results to a national surveillance centre. Until now, laboratory capacity remains critical in many parts of the world, however surprisingly large amounts of quality data are generated but remain underutilized. There are also emerging options to built diagnostic capacity for simple sentinel surveillance around recently established diagnostic centres for HIV, TB and Malaria.

The national reference laboratories should fulfil functions stipulated by a recently published ECDC technical report (ECDC, 2010a,b). This should include species confirmation and the ability to repeat phenotypical antibiotic susceptibility tests, as well as

Table 4
Disease- and pathogen-specific networks.

Program name	Subject	Countries	Coordinator	Years
GFN = Global Foodborne Infections Network (formerly Global Salm. Surv.)	Foodborne pathogens include *Salmonella*, *Campylobactera*, and others	Global 180 countries 1633 laboratories The focus to date has been on capacity-building and quality assurance. As part of the current 5-year strategy, surveillance will be added as an important component	Danish Technical University, Denmark	2000–present
WHO Global HIV Drug Resistance (HIVDR) Surveillance Strategy and WHO HIVResNet	HIV	Global • 60 countries are implementing HIVDR surveillance • 24 laboratories at national, regional and specialized level constitute the Global HIVDR Laboratory Network	World Health Organization	2004–present
WHO Global Malaria Programme	Malaria	Global 72 countries	World Health Organization	1996–present
WHO Global Malaria Programme and WWARN = WorldWide Antimalarial Resistance Network	Malaria	Global 92 countries in literature survey	World Health Organization and WWARN Executive Management Team	2009–Present Literature survey 1975–present WWARN is establishing a network of centres for patient-level resistance studies
Global Project on Anti-tuberculosis Drug Resistance Surveillance	*M. tuberculosis*	119 countries with national laboratories 29 Supranational Reference Laboratories	World Health Organization	1994–present
WHO GASP for WPRO and SEARO GASP = Gonococcal Antimicrobial Surveillance Programme	*N. gonorrhoeae*	23 countries/jurisdictions 28 laboratories	WHO Collaborating Centre for STD, Prince of Wales Hospital, Sydney,Australia	1994–present
WHO GASP for the Americas GASP = Gonococcal Antimicrobial Surveillance Programme	*N. gonorrhoeae*	35 countries 35 laboratories	University of Ottawa, Canada	1990–1999
SIREVA = Sistema Regional de Vacunas	Vaccine-preventable pathogens including *S. pneumoniae*, *H. influenzae*, *and N. meningitidis*	Latin America 20 countries 471 laboratories	Pan American Health Organization	1993–present
LCDC/PAHO Collaborative project on Surveillance of the Antibiotic Resistance in *Salmonella*, *Shigella*, and *Vibrio cholera*	*Salmonella*, *Shigella*, *V. cholera*	Latin America 21 countries 519 laboratories	Laboratory Centre for Disease Control, Canada Pan American Health Organization	1995–present

the determination of minimum inhibitory concentrations (MIC). A repertoire of phenotypic tests indicating the presence of certain resistance mechanisms would be especially useful if molecular characterisation would not yet be available.

External quality assessment (EQA) could be provided in two forms, (i) by supporting a genuine national EQA scheme, whereby highly characterised isolates are distributed to reporting laboratories at regular intervals for species identification and susceptibility testing, or (ii) by assisting of the regional EQA schemes in the regular distribution of isolates provided by regional EQA centres (Bronzwaer et al., 2002; Tenover et al., 2001).

The collection of routine AST results would be the remit of the national surveillance centre. This centre would not need to be a laboratory but could be hosted at national health agency. Important for the surveillance centre however, would be competence to evaluate the collected data for consistency and biological plausibility as well as skills in data management.

For countries where the above mentioned infrastructure is absent, centres at regional level may substitute for any or all of these tasks.

4.2. Regional level framework

Each region should be free to set up its own surveillance framework. The overall structure would be the classical network of network approach as in the EARSS/EARS-net and ReLAVRA of AMRO/PAHO networks (federated structure). In analogy with national level surveillance the three core tasks may be divided between separate centres.

4.2.1. Reference work

Recognizing the global need to strengthen laboratory capacity for the determination of antibiotic resistance, we also propose a network of reference labs at regional level linked electronically among each other and to a roster of international excellence centres. Regional reference laboratories should be equipped to receive a stream of isolates from their national counterparts that fulfil a set of criteria indicating public health importance or probable public health importance (to be defined). They should be able to carry out a repertoire of confirmatory tests incl. molecular identification of genetic resistance determinants to detect emerging resistance

threats and rapidly expedite confirmation tests for novel resistance mechanisms.

As the emergence of novel resistance determinants is a function not only of selection but also of the expansion of clones harbouring these determinants, reference labs also need expertise in methods of molecular epidemiology. Considering molecular typing as essential for the understanding of clonal dissemination, the limitations in portability of conventional molecular epidemiological typing data should be understood and sequence-based typing may be favoured as the preferable option (Grundmann et al., 2010).

Regional centres shall support national reference laboratories and reporting laboratories with protocol implementation, training and capacity building. Standardisation as well as protocol development should be referred to reference laboratory working groups. This approach has successfully worked for the EARSS which profited largely from European expert experience.

To identify the regional reference laboratories, the technical expertise and the willingness to serve the role as a resource for the region should be considered. For each region the key laboratories which meet these criteria would be publicised, thereby giving national reference labs a set of options among which they can select the laboratory which they consider to be most appropriate for their needs and preferences. For each regional reference laboratory, the network would identify the specific technical capacities and tests that individual laboratories have and set about to fill the gaps, for example in PCR, PFGE, and sequencing. This kind of information could be made available through a dedicated network and shared through WHO's GLaDMap initiative – Global Laboratory Directory at www.gladmap.org.

A network of reference labs, proficient in molecular and phenotypic approaches to detecting resistance and clonality in key pathogens is an urgent and affordable goal. These reference laboratories will enable further knowledge and quality improvement in their regions but may or may not be responsible for external quality assessment depending on the region.

4.2.2. External Quality Assessment

Regional EQA programs currently exist in all six WHO regions, and in three regions (AFRO, EURO, and AMRO/PAHO), these EQA activities are integrated with the regional antibiotic resistance surveillance initiatives. Ideally, and for reasons of consistency, a single centre per region should be awarded the task for EQA for routine bacterial identification and susceptibility testing. These programs have demonstrated a track record and capacity for batch production of the required number of specimen to be distributed throughout the regional network. The participation of all reporting laboratories in regular EQA is crucial for antibiotic resistance surveillance for three reasons. (1) EQA assesses the ability of the reporting laboratories to identify antibiotic resistance of clinical and public health importance. (2) It allows the evaluation of qualitative and quantitative susceptibility test results received from reporting laboratories. (3) Results of the EQA decide over comparability of routinely reported test results between different laboratories and countries and thus provide the means for justifying the pooling and comparison of antimicrobial susceptibility test (AST) results across the region.

We would also suggest the implementation a global EQA program to ensure that references laboratories are carrying out the specific confirmatory tests with sufficient accuracy. Global EQA would concentrate on the molecular confirmation tests and for these exercises it would suffice to dispatch DNA extracts. Global EQA should be provided by the roster of international excellence centres which connect to regional reference centres on a routine basis.

4.2.3. Surveillance

Primarily responsible for ongoing routine surveillance would be the Regional Centre for Surveillance, which in practice is often a regional public health authority, not a laboratory, e.g. WHO in the case of AMRO/PAHO (North/South America) and WPRO (Western Pacific) or the ECDC for Europe.

Focusing the surveillance aspect on human infections and clinical isolates is advisable. Possible initial indicator organisms could consist of *S. pneumoniae*, *S. aureus*, *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. The decision on the final selection of organisms may be left to the surveillance working group and network participants. Different ecological, socio-economical and epidemiological circumstances (e.g. Africa vs. China) may require a different set of priority pathogens. If the expertise and capacity of the regional antibiotic resistance reference laboratory allows for the analysis of samples from food, water and veterinary sources, there is no reason to object to their inclusion but care should be taken not to overburden the capacities and one should not make this a remit of the regional reference laboratories. There is a system already in place for global surveillance of food and animal isolates GFN (Global Foodborne Infections Network). So for food/animal/human enteric pathogens issues, we propose to treat them in a way similar to HIV, TB, and malaria. We recognize the significant accomplishments of the global networks dedicated to these specific pathogens and the existing expertise in these areas. The scope of the suggested global framework could be inclusive, but for our present recommendation we focus on the human clinical isolates which consist of the cosmopolitan opportunistic pathogens mentioned in the introduction and are missing from current global collaborative efforts.

5. In-depth surveillance using health and demographic surveillance sites in low- and middle-income countries

The global problem of antibiotic resistance is particularly pressing in low- and middle-income countries (LMICs), where the high infectious disease burden is aggravated by erratic access to antibiotics. Here weak antibiotic policies and a lack of capacity for antibiotic resistance surveillance means that the build-up of antibiotic resistance contributing to global pool of difficult-to-treat-infections is impossible to fathom (Blomberg et al., 2005; Okeke et al., 2005). The prevalence of antibiotic resistance varies greatly between and within countries and between different pathogens. Multidrug-resistant microorganisms, which in developed countries could still be treated by expensive alternative drugs cause infections that become untreatable in resource limited settings. Data from Pakistan indicate that, because of the development of resistance to first line antibiotics, 70% of hospital-acquired neonatal infections could not be successfully treated by using WHO's recommended regimen (Zaidi et al., 2005). Factors described in the first chapter above contribute to the worldwide emergence and spread of antibiotic resistance which is epitomized by the steady stream of pan-resistant hospital infections emerging from Asian, African, and Latin American countries.

Over the past 15 years The International Network for the Demographic Evaluation of Populations and Their Health in Developing Countries (INDEPTH) has developed a series of novel and synergistic tools to measure, map and track the socio-demographic impact of cause-specific morbidity and mortality in difficult to access populations in LMICs (www.indepth-network.org). This has led to a fundamentally new understanding about the magnitude of health events and effectiveness of interventions from drug and vaccine trials to marketing of health products. Using household-based surveys and the totality of the catchment population surrounding central hospitals at so-called Health and Demographic Surveillance Sites

(HDSS), it has been possible to measure the extent of disease in the community against case counts in hospitals offering the means to translate hospital based surveillance information into estimates on the burden of disease in the community. INDEPTH is a network currently comprising 37 Health Demographic Surveillance System Sites in 20 countries. The research sites are in Africa, Asia and Oceania.

Through harnessing the ability of HDSS in these countries, it shall be possible

- to determine the true prevalence of antibiotic resistance
- to reconcile laboratory incidence figures from hospitals (reporting laboratories) with community prevalence
- to ascertain antibiotic use
- to understand major determinants of antibiotic resistance in the community including perceptions and health seeking behaviour
- to assess the burden of disease attributable to antibiotic resistance in LMICs

We suggest to recruit HDSS centres in different countries that could serve as focal points for the training and dissemination of laboratory and surveillance competences in these and surrounding countries as well as providing a reality check by in-depth investigations into the occurrence of antibiotic resistance and antibiotic use.

6. Conclusions

We identified mechanisms that are likely to aggravate the decay of antibiotic effectiveness in the near future. Free market economy is about choice and enhancing the ability of individuals and industry to make these choices. Industry has so far chosen not to invest in innovative antibacterials. Instead, companies recently priced several of the last line antibacterial compounds (such as carbapenems which are loosing patent protection in the near future) to compete on the global generic market. Generic manufacturers in emerging market communities are ready to produce the active pharmaceutical ingredients, often in agreement with parent companies, at low price for world markets. Unregulated access to these drugs – especially in countries where opportunities for transmission and spread are abundant – will lead to the emergence and expansion of antibiotic resistance through migration, travel and trade.

We therefore believe that the need for a global surveillance system for antibiotic resistance is evident, especially as antibiotic resistance fulfils all criteria of health threats that typically warrant surveillance. The threat is emerging, urgent, geographically heterogeneous, transmissible and likely to expand in an epidemic fashion, but also amenable to interventions and effective control efforts. A systematic collection, consolidation and evaluation of the resistance data and their trends will help define the problem, inform national and international control activities and support the monitoring of their effectiveness. Crucially, valid data would provide incentives to invest into anti-infective strategies including novel drug development.

We therefore recommend utilizing and rehabilitating initiatives that have already been developed in the six WHO regions. Existing structures need to be harmonized and core competences need still to be addressed and allocated. For operational purposes, we suggest to separate reference work, quality assurance, and surveillance. All of these tasks are essential for the collection of reliable and consistent surveillance data and may be accomplished by single but more often separate institutions. Moreover, all of these tasks should be addressed by institutions with the appropriate competence at either national and at regional level.

Since the global problem of antibiotic resistance is particularly pressing in low- and middle-income countries (LMICs), where the infectious disease burden is high but access to antibiotics and laboratory service is erratic, we suggest to implement selective sentinel surveillance at research sites that that have access to health and demographic surveillance systems (HDSS). Given that existing networks are able to provide the necessary structures, it will become possible to correlate antibiotic resistance in the community with hospital incidence and contextualize, in these low resource settings, the health care seeking behaviour which determines antibiotic use.

With the improvement of surveillance comes the obligation to communicate the findings in a timely fashion to policy and decision makers. This poses another challenge as long as the available information requires expert knowledge to grasp the medical and epidemiological ramifications. A comparative assessment of the average effectiveness of drugs available for a given infection can assist in comparing trends in antibiotic resistance between countries and regions. An example of this is the Drug Resistance Index that has recently been proposed as a 'Dow Jones index' for drug resistance (Enserink, 2010). This would be a true improvement in the democratic tradition, making the facts available also to a broader public who can hold policy makers accountable for their decisions, and as we have already learned "good surveillance does not necessarily ensure the making of the right decisions, but reduces the chance of wrong ones" (Langmuir, 1962).

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Towards new business models for R&D for novel antibiotics☆

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ABSTRACT

In the face of a growing global burden of resistance to existing antibiotics, a combination of scientific and economic challenges has posed significant barriers to the development of novel antibacterials over the past few decades. Yet the bottlenecks at each stage of the pharmaceutical value chain—from discovery to post-marketing—present opportunities to reengineer an innovation pipeline that has fallen short. The upstream hurdles to lead identification and optimization may be eased with greater multisectoral collaboration, a growing array of alternatives to high-throughput screening, and the application of open source approaches. Product development partnerships and South–South innovation platforms have shown promise in bolstering the R&D efforts to tackle neglected diseases. Strategies that delink product sales from the firms' return on investment can help ensure that the twin goals of innovation and access are met. To effect these changes, both public and private sector stakeholders must show greater commitment to an R&D agenda that will address this problem, not only for industrialized countries but also globally.

1. Introduction

Against a growing burden of drug resistance, the pipeline for novel antibacterials has faltered. The challenges trace to both science and economics and call for the need to consider new business models for bringing novel antibiotics to market.

While there have been some clinically important modifications to existing antibiotics, only two new classes of antibiotics have emerged in the past three decades—oxazolidinones (linezolid) and cyclic lipopeptides (daptomycin). Both drugs are for the treatment of Gram-positive bacterial infections. In the publicly disclosed pipelines of the top 15 pharmaceutical companies, which provided 93% of the new antibacterials from 1980 to 2003, there are only five antibacterials, comprising only 1.6% of the R&D pipeline for these companies. None of these five antibacterials appear to have a novel mechanism of action (Spellberg et al., 2004).

EMEA, ECDC and ReAct conducted a more comprehensive analysis of potential antibiotics, identified from searches of all drug company clinical R&D using two commercial databases and reviewed by an expert scientific committee. The study yielded 90 antibacterial agents with *in vitro* activity in a best-case scenario (based on actual data or assumed based on known class properties or mechanisms of action) against at least one organism in the panel of bacteria selected for their public health importance. This analysis reaffirmed the dismal outlook. Of four with activity against Gram-negative bacteria based on actual data, two acted on new or possibly new targets, and none via novel mechanisms of action (Aronsson et al., 2009).

☆ This paper draws upon presentations held at the workshop, "Towards New Business Models for R&D for Novel Antibiotics," as well as preparatory work for this workshop, conducted by the Duke Program on Global Health and Technology Access. The workshop occurred during the conference, "The Global Need for Effective Antibiotics: Moving Towards Concerted Action" (6–8 September 2010, Uppsala, Sweden).

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2. Bottlenecks in the R&D pipeline

The R&D pipeline for novel antibacterial drugs faces multiple bottlenecks (see Fig. 1):

- *Lead identification*: Upstream in the R&D pipeline, high-throughput screening for antibacterial drug candidates has had a significantly lower yield for antibacterial drug candidates compared to other therapeutic categories.
- *Medicinal chemistry*: The process of transforming these leads into drugs that can enter clinical trials is the stage at which much attrition also occurs.
- *Crossing the valley of death*: The probability of success during lead optimization relies on the size of the medicinal chemistry effort that can be mounted, and this relates to the available financial resources, as well as the opportunity costs, of undertaking this. "Crossing the valley of death" is the term given to the gulf in translational research from basic science to clinical application and the financial chasm in moving from pre-clinical to clinical testing.
- *Regulatory approval*: Recruiting and enrolling adequate numbers of patients in clinical trials can still be challenging and costly. On the other hand, no one wants to cut corners on safety, and antibiotics as a class of drugs already enjoy among the fastest clinical approval times and highest approval rates across therapeutic categories.
- *Reimbursement*: Reimbursement signals have traditionally been mixed—rational use is compromised when high prices place a needed antibiotic out of reach while conserving the use of novel antibiotics also caps the potential for revenue returns to the firm.

In this workshop session, discussions focused on the upstream challenges in the R&D pipeline for novel antibacterial drugs. The value of co-developing diagnostics and drugs was noted, particularly for patient enrollment in clinical trials, but diagnostics development was covered in another workshop.

2.1. Lead identification and optimization

High-throughput screening (HTS) is designed to screen single enzyme targets identified through recent advances, predominantly in genomics. The yield from high-throughput screening has been disappointingly low for antibacterial drug discovery.

Seventy screens conducted by GlaxoSmithKline (GSK) from 1995 to 2001 (67 HTS, three whole-cell) produced five lead compounds, representing a mere 7% success rate. GSK's experience is corroborated by Pfizer's 6.5% success rate in producing lead compounds (personal communication Paul Miller, Pfizer). Even with top-drawer medicinal chemistry resources, lead optimization also proved significantly more challenging for antibacterial R&D than other therapeutic areas. Combining the probability of success of HTS with the success metrics for all the subsequent steps in antibiotic development, it is estimated that it could take 2066 HTS to yield one antibiotic with a novel mechanism of action whereas an average of just 24 screens yielded one drug launch across other therapeutic areas. This is clearly an untenable strategy and illustrates the need for new approaches which some companies are now exploring.

This HTS strategy has not proven particularly well suited for antibiotic discovery (Mullin, 2004; Baltz, 2006). HTS campaigns ordinarily yield multiple leads with target activity. Most antibacterial targets though are enzymes, not receptors, and therefore, hard to inhibit. Though complying with the Lipinski Rule of Five, druggable leads in compound libraries are biased towards mammalian targets which may explain their lack of antibacterial activity (Bleicher et al., 2003). After resources have been expended on drug optimization efforts, safety issues and permeability, explored later in the drug development process, often thwart many of these promising leads (Fernandes, 2006).

In addition to the shortcomings of HTS, the range of compounds explored in these efforts has been limited. Combinatorial chemistry, often used in tandem with HTS, is incapable of generating the molecular complexity and diversity found in the natural products from which many antibiotics have been derived (e.g., vancomycin, daptomycin, cephalosporin C, erythromycin, and rifampicin) (Baltz, 2006). The synthetic compound collections held by firms and most proprietary compound vendors do not represent the range of compound types that might be explored to yield new classes of antibiotics.

Thus interventions at several points in the R&D pipeline might improve the yield of novel antibacterial drugs. First, new approaches to lead generation may help. While compound collections have improved, one cannot rely on conventional high-throughput screening of synthetic compounds. Similarly, improving the probability of transitioning from clinical trial phase 1 to phase 2, through higher quality drug candidates, would also yield greater likelihood of success; however, creating such candidates will likely result in longer timelines and require greater resources.

2.2. Anticipated returns on investment

Investment in antibacterial drug discovery and translational research may also be hampered by relatively less favorable returns. The antibiotics market is less profitable than other, faster-growing therapeutic areas. Antibiotics generated sales of US$42 billion in 2009 globally, representing 46% of sales of anti-infective agents (which also include antiviral drugs and vaccines) and 5% of the global pharmaceutical market. Antibiotics showed an average annual growth of 4% over the past 5 years, compared with a growth of 16.7% and of 16.4% for antiviral drugs and vaccines, respectively (Hamad, 2010). By comparison, global pharmaceutical sales for 2009 are estimated at US$750 billion (Business Wire, 2009).

The metric used to prioritize investments in industry is the risk-adjusted net present value (rNPV): the return in future dollars after adjustment for the investment and any lost income, usually expressed as the number of millions of dollars (Stewart et al., 2001). DiMasi, Vernon and Grabowski estimate (in 2000 US$) the worldwide sales revenue over the product life cycle for a new antibiotic approved in the US during 1990–1994 to be, on average, US$2379 million. This compares to an average of US$4177 million for CNS drugs and US$3668 million for cardiovascular drugs (2004).

Several features inherent to antibiotics contribute to relatively low net present values. Treating an infection may require a short course compared to the lifelong treatment of chronic conditions, and resistance itself limits an antibacterial's lifespan. There is also significant therapeutic competition in a relatively saturated market. Efforts to conserve antibiotics through rational use guidelines also curb the opportunity to expand markets. This tension between conserving antibiotics and generating revenues through increased marketing and sales reflects a major misalignment of economic incentives.

2.3. Regulatory issues

A 1995 study shows that antimicrobial agents have had a higher success rate of U.S. FDA drug approval and a shorter approval time than most other therapeutic classes (DiMasi, 1995). More recently, the picture may be more mixed. Compared to other therapeutic classes, anti-infectives as a class still fare well in the attrition rates from phase I through market approval (50%) and also register among the fastest clinical development times (87 months) of any therapeutic class (Evans et al., 2009). However, four new

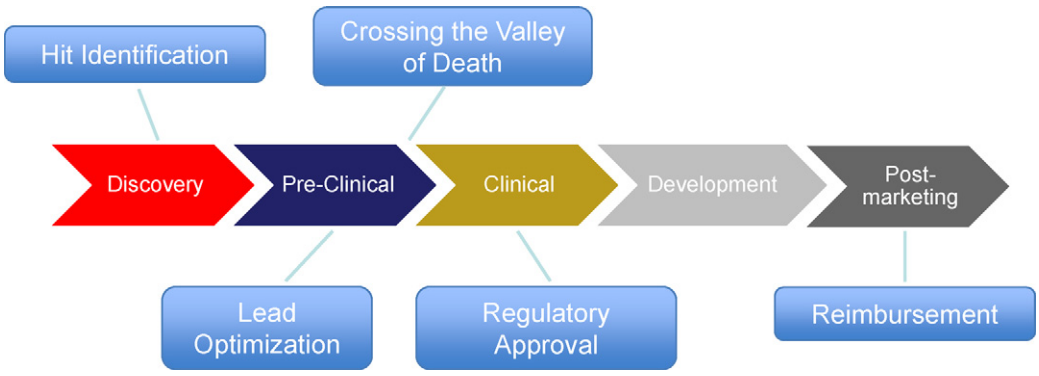

Fig. 1. Defining the bottlenecks in the value chain of pharmaceutical R&D.

MRSA drugs were submitted for registration, and unfortunately, only one progressed to launch, suggesting additional challenges and complexities for the successful registration of new antibiotics.

While antibiotics have enjoyed among the fastest clinical approval periods and highest regulatory success rates, clinical trials for novel antibiotics face several challenges. Guidance for clinical trial requirements has been in flux, leading firms to perceive this process as unpredictably costly. The FDA recently issued draft guidance calling for scientific justification of margins in non-inferiority trials for treatments of acute bacterial skin and skin structure infections (Center for Drug Evaluation and Research, 2010). This guidance may result in tighter margins. The FDA has also required superiority trials for antibiotics used to treat self-resolving non-lethal infections.

Some experts suggest that superiority trials place too high a threshold for regulatory approval. Tight margins on non-inferiority trials may also pose challenges because many antibiotics work well. The FDA has acknowledged that it may be difficult to show that an experimental drug works better than a current one (Tsouderos, 2010). It is critical that new antibiotics show clinical efficacy against infections caused by multi-resistant organisms, and without rapid diagnostics, firms must amass large sample populations in order to capture a sufficient number of patients infected with these drug-resistant pathogens.

Yet efforts to speed drug approval for antibiotics through non-inferiority trials and priority review mechanisms need to ensure that safety is not compromised (Outterson et al., 2010a; Powers, 2007). The U.S. Government Accountability Office found that a quarter of FDA new drug applications (NDAs) from 2002 to 2009 were based on some evidence from non-inferiority trials, and though the number of such NDAs decreased over the period, a majority of these applications received FDA approval. Half of these were for antimicrobial drugs, including tigecycline over which safety warnings were recently issued (U.S. FDA, 2010). Certain biases can creep into non-inferiority trial designs, from poorly defined or unreliable outcome criteria to missing data, and these biases tend to increase false-positive results. Also, more than a third of drugs awarded accelerated approval by FDA since 1992 never had studies done proving efficacy (Harris, 2010). Between 1980 and 2009, over forty percent of systemic antibiotics receiving FDA approval were subsequently withdrawn from the US market. This represents a significantly greater number of discontinuations compared to other therapeutic classes (Outterson et al., 2010b).

3. Towards new business models for antibiotic R&D

The workshop discussed several potential pathways to solving some of the scientific and economic challenges that have contributed to the weak pipeline for antibacterial R&D. While R&D pipelines for treatments of neglected diseases falter for lack of paying patients in developing countries where these diseases are endemic, antibiotics have markets that span both North and South. Nonetheless there are development bottlenecks shared in common for both neglected tropical diseases and for antibiotics. Both share scientific challenges in sourcing compounds and optimizing drug leads as well as financial challenges with insufficient private sector incentives and pricing that may place products out of reach of those in need. While attentive to the differences, lessons in reengineering the value chain of R&D in one area might inform the other. Similarly, among bacterial diseases, the work of groups like the Global Alliance for TB Drug Development has heightened policymaker and funder interest in changing the picture where no new TB drug has been developed in 40 years.

3.1. Setting priorities through target product profiles

The target product profile (TPP) can help signal R&D priorities to funders and researchers. The FDA defines the TPP as a "summary of a drug development program" which provides a "format for discussions between a sponsor and the FDA that can be used throughout the drug development process" (Center for Drug Evaluation and Research, 2007). For the FDA, "beginning with the goal in mind" has helped the agency stay on the same page with firms.

Product development partnerships (PDP) for neglected diseases have adopted the TPP concept to focus priority on developing health technologies that respond to unmet needs in resource-limited settings. TPPs typically lay out a product's desired optimal and minimum-required characteristics, from route of administration to dosing schedule and price. These specifications may be modified as the R&D process yields new information. Specified from the outset, however, TPPs have the potential to align economic incentives to public health priorities, particularly where market-based incentives are wanting.

The Drugs for Neglected Diseases Initiative (DNDi), for example, makes use of TPPs for its portfolios on visceral leishmaniasis, human African trypanosomiasis, and Chagas disease to specify criteria that ensure usefulness and accessibility in resource-poor settings. Such guidance keeps the patients' needs foremost in mind in the R&D process. Overspecifying a target product profile, however, risks missing the unexpected breakthrough in innovation. Striking the right balance between setting parameters that define patient needs and not overspecifying the technological approach is key.

Still the TPP concept may be useful as a policy tool to convey basic criteria of need. The triad of such criteria might include evident public health need, a credible candidate technology, and available resources. The process for setting such priorities in an area like antibiotic resistance is complex. Metrics to demonstrate evident need may include a number of factors, from DALYs

(disability-adjusted life years) to the effect of infections on networks of patients and their families and communities. The process of acquiring such data poses its own challenges. With limited resources and capacity to conduct on-the-ground surveillance, ways to strategically collect local data to construct a global picture will need to be developed. The RAND group applied mathematical modeling to prioritize among potential new diagnostics for several infectious diseases by estimating the number of unnecessary treatments averted with the use of the diagnostic test (RAND Health, 2007). A credible candidate technology might signal low-hanging fruit and a timeline within practical reach. Feasibility might depend on the availability of the underlying platform technology, the commitment of major stakeholders, or available resources—financial and non-financial—already lined up. The commitment of stakeholders includes patients, and shaping the TPP to be patient-centered was considered key.

In assessing technology priorities, there are several ways to consider how to tackle antibiotic resistance. Should priorities for antibiotic resistance focus on syndromic categories (such as upper respiratory infection) or specific pathogens? The priorities for treatment might range from a cure to decreased symptomatology to a means for improving drug adherence. A prioritization strategy that lays out a business plan for bringing the technology to market also might make pricing and accessibility standards explicit from the beginning, perhaps guiding R&D decisions as in the case of DNDi.

3.2. Charting new directions for drug discovery

The workshop explored various approaches to reinvigorating the R&D pipeline for novel antibacterial drugs. These included moving beyond traditional, high-throughput screening to virtual high-throughput screening and structure-based discovery; looking at shelved compounds and existing drugs with new assays and mining sources such as the old journal literature for new leads; and expanding the search beyond small molecules to monoclonal antibodies and synthetic riboswitches, more diverse natural and synthetic compounds, and potentiator approaches such as efflux pump inhibitors. Yet fully exploring these approaches will require greater investment in antibacterial R&D and sizing up which directions to prioritize. The appetite to pursue these various leads will depend, in part, on the success of efforts to reengineer the value chain of antibacterial R&D, by raising the level of public sector commitments, effectively decreasing the costs of R&D, and providing adequate incentives for private firms and public research institutions.

3.2.1. Improving lead identification and medicinal chemistry

While firms have assembled large proprietary compound library collections for drug discovery purposes, these may not yet have been completely mined for novel antibacterial candidates, an area of interest and investment for relatively few firms.

Broader access to these compound collections may aid research efforts focused on finding new antibiotics. Equally important is the often confidential know-how—the biology and medicinal chemistry—behind such compounds in proprietary collections. Resource sharing, particularly of proprietary compound collections and preclinical data, has taken place in other areas of drug discovery. For example, GSK screened its corporate compound library of over 2 million molecules and released 13,500 compounds found to have activity inhibiting the malaria parasite, *Plasmodium falciparum* (Purlain, 2010).

The U.S. NIH Molecular Libraries Initiative hosts at its nine centers its Molecular Library Small Molecule Repository, against which researchers are free to submit assays for testing. Results of such screens, compound structure, and other preclinical data are made available through PubChem, an open access digital repository made available by NIH. Access to preclinical data associated with compounds can prove helpful in predicting downstream success, helping to direct efforts towards the most promising candidates.

While these collections are mainly comprised of small molecules synthesized for drug discovery primarily in other therapeutic categories, 34% of all small molecule new chemical entities approved between 1981 and mid 2006 are either natural products or semi-synthetic derivatives, and the majority of existing antibiotics are derived from natural products (Newman and Cragg, 2007). Compound collections for antibiotic research may need to expand from the contents of existing libraries to reflect better the complex properties of naturally occurring substances that have historically been developed into successful antibiotics (Wright, 2010).

The costly tasks of lead optimization and toxicity testing have also become shared endeavors, supported through both intramural and extramural services provided by programs like NIH's Therapeutics for Rare and Neglected Diseases Program (TRND) and Rapid Access to Interventional Development (RAID) Program. TRND assists with optimizing leads for first-in-man experiments under an Investigational New Drug Application while RAID provides access to NIH intramural or contracted services, from bulk supply and GMP manufacturing to formulation and pharmacokinetic and animal toxicology testing, for outside firms.

In the field of neglected diseases, public-sector R&D institutions such as product development partnerships have worked with experienced pharmaceutical industry chemists to support their medicinal chemistry efforts and frequently engage retired and active industry veterans on their Scientific Advisory Committees. Drawing on such expertise enables these institutions to make more strategic decisions early in the research process. Given the difficulty of lead optimization for novel antibiotics, this suggests another model that public-sector antibacterial discovery efforts might follow to leverage support from the private sector.

Drug discovery efforts have also recognized the shortcomings of HTS and begun looking to new methodological approaches for developing compounds better suited to become antibiotics. This has been the impetus behind the antibacterials unit of GlaxoSmithKline's Infectious Diseases Center for Excellence in Drug Discovery forging alliances with small firms that work on early-stage novel drug discovery projects. For example, GSK in 2007 partnered with Anacor Pharmaceuticals to support use of its boron chemistry platform to search for novel antibiotics. In 2010, the partnering firms announced the alliance had successfully delivered a novel mechanism antibiotic that has achieved clinical proof of concept (GlaxoSmithKline, 2010; Anacor, 2009). Using x-ray crystallography and nuclear magnetic resonance, fragment-based screening has enabled firms to engage in drug design by combining fragments that bind to the identified target (Jones, 2010). Virtual HTS, by which large libraries of compounds may be screened for the structural potential to bind to specific sites on target molecules, has enabled structure-based drug design (Simmons et al., 2010).

3.2.2. Testing drug combinations

The value of combination therapy in countering antibiotic resistance has received close attention in anti-TB treatment. It has been known for decades, since the introduction of the first anti-tuberculosis drug streptomycin, that the use of monotherapy in treating active TB very frequently generates resistance. Currently, many first- and second-line TB drugs have pharmacokinetic profiles poorly suited for use in combination. When co-administered, such drugs with differing half lives might leave gaps in antibiotic coverage from one or the other drug between doses, thereby opening the door to resistance during periods when, in effect,

only monotherapy is achieved. Therefore, optimized novel combinations are needed to advance TB treatment. The current TB drug development approach replaces one drug at a time, and as a consequence, takes decades to introduce a new regimen that consists of even three new agents. Traditional intellectual property barriers also may hamper cooperation to create combination therapies when the component drugs are patented by different firms.

The existence of a global pipeline of new agents in clinical trial for TB coupled with the need for a new paradigm for rational selection and development of novel combination therapy for TB prompted the launch of the Critical Path to TB Drug Regimens initiative. In this partnership among the Bill & Melinda Gates Foundation, the Critical Path Institute, the Global Alliance for TB Drug Development, and various institutions and sponsor companies of potential new TB drugs, efforts to change the traditional R&D approach are underway. Drug combinations would be developed as a unit of therapy without having to change present regimens one drug at a time. With sufficient funding, this alternative development paradigm could shave years off the R&D time required for bringing novel anti-TB drug combinations to market.

3.3. Financing the crossing of the valley of death

Relatively lower anticipated returns on investment have deterred firms with a broad portfolio from investing in the search for novel antibiotics over other therapeutic categories. Of course, these opportunity costs are different for small firms without a broad portfolio of R&D options.

3.3.1. Push incentives

Push incentives that pay for R&D inputs can play a significant role. Notable support for R&D for novel antibiotics has come from both government and philanthropic sources. The US Department of Defense's Defense Threat Reduction Agency is supporting the search for novel antibiotics that align with its bioterrorism threat research (Purlain, 2010). The Wellcome Trust has developed a broad set of projects, primarily through its Seeding Drug Discovery initiative, and provided funding to a number of companies for antibacterial projects. For example, among the Seeding Drug Discovery awards, Prolysis Ltd. received financing to develop a new class of antibiotics to fight hospital-acquired staphylococcal infections, GSK was funded to develop new antibacterials to combat the rise of certain drug-resistant, hospital-acquired infections with a focus on Gram-negative bacteria, and Achaogen received funds for the continued preclinical studies of two antibacterials showing promise against multi-drug resistant *Enterobacteriaceae* and *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Wellcome Trust, 2007, 2009; GlaxoSmithKline, 2007). A further evolution of this model would be to provide funding for a portfolio of programs enabling risk to be spread among more than one project. For small start-up firms, public or philanthropic funding can be an important source of non-diluting cash investment.

3.3.2. Pull incentives

Provided that initial scientific hurdles can be surmounted, the prospect of pull incentives that pay for R&D outputs draws upstream, private capital investment. Incentives, such as tax deductions for R&D, presume the company has revenues to tax, and that may not be the situation of biotech start-ups. Several proposals have been put forth to increase reimbursement to firms for providing much needed antibiotics.

The value-based reimbursement model aims to reward development of novel antibiotics with public health value by using public funds to pay firms for their contribution (Kesselheim and Outterson, 2010). For example, under the proposed Health Impact Fund approach, participating firms would be required to provide a low price globally, pegged to the average cost of manufacturing, and to extend a royalty-free, open license for generic production after 10 years, but in exchange, would receive direct payment from the Health Impact Fund. The Health Impact Fund would offer pharmaceutical firms a share of a fixed fund each year for a period of 10 years following market approval. The payment would be proportional to the share of the health impact of the firm's registered product among all of the registered products. Under the Health Impact Fund proposal, participation would be voluntary, so firms could opt to exercise their monopoly pricing position instead. The fund would have to be sufficiently large, even when divided among participating companies, to provide an adequate financial incentive, particularly to manufacturers of important therapies now protected by patent or data exclusivity. Both valuation of the quality-adjusted life years saved by a specific product and securing long-term financing commitments from partner countries for the Health Impact Fund would be challenging. Others have argued for proposals that require open licensing and generic production as a condition of public financing, rather than after a period of 10 years.

Conservation of valuable antibiotics through rational use and limited marketing is at odds with innovation traditionally financed through sales-based incentives. Conservation goals, while good for public health, undercut drug industry sales and therefore R&D incentives. Proposals have been put forth to compensate firms for capping their sales of novel antibiotics. The Strategic Antibiotic Reserve is a mechanism to pay companies to achieve conservation targets for their drugs (Kesselheim and Outterson, in press). Workshop participants discussed hurdles to the implementation of such a program. It would require global coordination and extended market exclusivity on all relevant drugs to ensure higher reimbursement levels. This coordination would also need to take into account resistance caused by different drugs which belong to the same functional resistance group. Health system incentives and prescribing norms contribute significantly to the way in which antibiotics are used, but the concept of a Strategic Antibiotic Reserve places significant responsibility on the shoulders of drug firms to ensure rational distribution of the limited drug supply.

Through his plenary address, Richard Bergström, Director-General of the trade association for the research-based pharmaceutical industry in Sweden, offered important guidance to this workshop's discussions on pull incentives. Speaking on behalf of industry, he argued that "Incentives that separate the financial return from the use of a product are the only way to change this behavior." Another approach receiving mention was prizes or patent buyouts that are not reliant on the volume of subsequent sales of the product.

3.4. Open innovation approaches

Beyond push and pull incentives, there is a need for new approaches to reinvigorate antibiotic research. R&D efforts for rare and neglected diseases might offer lessons in reengineering the value chain of pharmaceutical R&D. To highlight a few initiatives, these efforts have taken various forms: open access resource sharing, open source innovation, product development partnerships, and South–South innovation platforms.

3.4.1. Open access resource sharing and open source innovation

Some pharmaceutical firms have created avenues for publicly funded scientists to avail themselves of proprietary resources. GlaxoSmithKline's Open Lab initiative is designed to host up to 60 visiting scientists from academia or biotech, providing access to the corporate compound collection. The firm has also provided

seed funding through the Tres Cantos Open Lab Foundation to help support these efforts.

Beyond facilitating greater use of existing proprietary resources, open source infrastructure might be employed to establish new mechanisms for upstream R&D collaboration and resource-sharing. An example is the Open Source Drug Discovery Initiative for TB. An interesting project undertaken by OSDD has been the collective efforts to study the *Mycobacterium tuberculosis* genome in search of novel drug candidate targets. With over 4328 registered participants from 130 countries, the OSDD mustered numerous volunteer contributions needed to complete a remapping and annotation of the genome in just over 4 months. Academia, hospitals, and contract research organizations have signed on to help with *in silico* screening and *in vivo* target validation, identifying lead molecules, and carrying them through preclinical and clinical trials. As of September 2010, the OSDD identified 18 targets, conducted 19 virtual screens, and is currently optimizing two lead novel compounds as potential TB drugs. This initiative, led by India's Council on Scientific and Industrial Research, receives public funding and taps into a network of universities, companies, contract research organizations, and volunteers—all elements that may help make this experiment into open source innovation more feasible. Adding another dimension to its digital platform for scientific collaboration, the OSDD will launch an Open Access Small Molecule Repository comprised of acquisitions from existing libraries, dedicated synthesis efforts, and other contributions. Having disbursed US$12 million from the Indian government, OSDD releases these funds on condition that supported projects are posted on-line and subject to peer review and approval by the community. The open lab notebook of the OSDD facilitates sharing of research results in real time with the community. This type of inclusive, networked approach to R&D demonstrates that while its costs and challenges may be too great for just one firm to bear, platforms that draw on a multitude of collaborators may lower costs, diffuse risks, and recruit a broad array of resources.

Another type of upstream platform from which lessons might be drawn is the Structural Genomics Consortium, which aims to promote drug discovery by creating and placing protein structures in the public domain. Funders nominate protein targets to the "SGC Target List," which is comprised of 2400 proteins. Members of the consortium—over 250 collaborators in 19 countries—contribute to its research activities. While the list and nomination information remains confidential, targets are placed in the public domain upon completion. The SGC contributed 29.6% of the global output of novel human protein target structures in 2009. These research outputs are free from restrictions on use and not covered by intellectual property. Such a model maintains open access to the fruits of its collective labor, while protecting competitive advantage for firms that seek not to disclose the types of targets into which they are investigating. Initiatives like the Structural Genomics Consortium are helping to redefine the line between pre-competitive and competitive research by setting research consortia norms that encourage greater sharing throughout the value chain of R&D.

3.4.2. Product development partnerships

Partnerships forged to bring a specific health technology to market have overcome significant market barriers in the neglected disease space by leveraging strengths and resources from both the public and private sectors. The Drugs for Neglected Diseases Initiative has been a successful pioneer in holding to a specific product profile from discovery to market through collaboration at each stage along the value chain. Its once-daily, fixed-dose combination drug for malaria, ASAQ (artesunate–amodiaquine), for example, was developed in partnership with Sanofi Aventis and is available at cost in the public sector. A second antimalarial combination, ASMQ (artesunate–mefloquine), resulted from South–South collaboration between Brazil's Farmaguinhos and the Indian drug firm, Cipla. For each artemisinin-combination treatment, a host of other partners around the world have also been integral to the process. Clinical trial platforms were developed at the Universiti Sains Malaysia and the Institut de Recherche pour le Developpement in Senegal. In bringing ASAQ to market, DNDi worked with the Indian Council of Medical Research and with the Kenya Medical Research Institute, both of which helped to shape antimalarial policy development through their efforts (ASAQ, 2010). The patient-centered approach DNDi has taken in collaborating with Southern institutions serves as an exemplar that might be emulated in broadening the search for novel antibacterials.

3.4.3. South–South innovation platforms

Indeed, several institutions have taken steps to harness developing country R&D capacity through collaborative infrastructure. Such initiatives may be localized to a specific point on the value chain, such as the European and Developing Countries Clinical Trials Partnership, which facilitates Phase II and III clinical trials for drugs, vaccines and microbicides against HIV/AIDS, TB and malaria in sub-Saharan Africa (European and Developing Countries Clinical Trials Partnership, 2010). Firms have increasingly recognized the advantages of outsourcing clinical trials to Southern countries, where patient samples are readily available, overhead costs are lower, and capacity to uphold clinical research standards is growing (Thiers et al., 2008). But such platforms may also go farther than providing inputs to the existing, industry-dominated R&D value chain.

The African Network for Drugs and Diagnostics Innovation (ANDI) and its sister networks in Asia and the Americas seek to promote regional networks that are locally owned and led to drive innovation for urgently needed therapies. One study found that collaborations more commonly can be found between Northern and Southern institutions (Nwaka et al., 2010). By linking centers of excellence across Africa, ANDI may help build South–South partnerships where few have existed. Their unique strengths, such as access to an underexplored diversity of natural resources and to local patient populations, may propel R&D in novel directions.

4. Conclusions

Facing the global challenge of antibiotic resistance, clearly new business models for bringing novel antibiotics to market will be needed. The workshop discussions laid out key bottlenecks along the value chain of R&D, some scientific and others economic. Some scientific challenges may be surmounted with greater investment, but others will require commitment to new forms of collaboration. Such collaboration will need not only to expand stakeholders' access to compound libraries, but also diversify the compounds available in such repositories. Where there are common challenges, policymakers might draw upon the experience of how product development partnerships for neglected diseases have effectively mobilized public and private resources. This will require a strategy for leveraging public and philanthropic funding to overcome traditional hurdles to antibiotic innovation.

In addition, public sector interventions are needed across the value chain, from improving lead identification and medicinal chemistry to restructuring the reimbursement system. Engaging new and old partners, a platform for antibiotic innovation might benefit from a more open source environment for R&D and from greater South–South exchange. The public sector will also need to take some calculated bets in prioritizing some approaches over others. Without overspecifying the technology approach or compromising the spirit of creative innovation, target product profiles can help signal priorities and anchor public sector commitments to

create products that meet the twin goals of innovation and access. In so doing, some of the proposals put forward may have promise to reengineer the value chain of R&D, to alter the equation of net present value, and thereby, change the way pharmaceutical products are brought to market. The industry's call to delink profit from product sales is no longer business as usual, but an invitation for the scientific, public health and policy communities to consider new business models to meet one of the most pressing global health challenges of our time.

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Diagnostics as essential tools for containing antibacterial resistance

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ABSTRACT

Antibacterial drugs are overused and often inappropriately selected. This exacerbates drug resistance and exacts a high burden from acute respiratory tract, bloodstream, sexually-transmitted, diarrheal and other infections. Appropriate use of existing diagnostic tests, and developing better ones, could avert these costs and would avoid selective pressure from unnecessary antibacterial use. Product profiles of resistance-averting tests would specify WHO 'ASSURED' (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Deliverable) criteria and request susceptibility as well as etiological information. Advances in genomics, nanoscience, microfluidics and bioengineering, as well as innovative funding paradigms can help to overcome research and development barriers for such diagnostics if they are deliberately and forcefully applied. Rapid uptake of new tests requires timely translation of research on cost-benefit analyses into policy, value-based subsidies and reimbursements, as well as behavioral change of health care providers and users.

1. Diagnostics: the "Achilles Heel" of antimicrobial resistance containment

Antibacterials are among the 20th century's greatest innovations and are an invaluable resource for human and animal health today, but their non-indicated use provides needless selective pressure for resistance. Antibacterial stewardship to avert this adverse societal consequence has been described as "the use of the right antibiotic, at the right dose, route and duration, for the right bacterial infection at the right time" (Dryden et al., 2009b). Several inputs, including drug supply, pharmacokinetic and pharmacodynamic information discussed in the accompanying paper by Grundmann et al. (in press) are required for stewardship. An often overlooked but necessary input is objective diagnostic support. Berkelman et al. (2006) have referred to diagnostic oversight as "The "Achilles Heel" of global efforts to combat [infectious diseases] and the antimicrobial resistance that accompanies them".

Recognizing diagnostics as an overlooked tool for containing resistance, the Uppsala conference on "The Global Need for Effective Antibiotics–moving toward concerted action" convened

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a workshop on "mobilizing the development of diagnostics". This diagnostics development workshop was initiated from responses to a questionnaire administered to an expert working group. Expert replies were collated and discussed in two working-group meetings and a workshop including other participants with expertise, interests and stake-holding in the field. The meetings focused on the most important issues relating to diagnostics and drug resistance, identified knowledge-gaps and roadblocks to progress and proposed next steps for spurring the development and use of diagnostics to contain antibacterial resistance. A summary of conclusions was presented to 190 delegates from 45 countries and that included leading stakeholders from civil society, academia, industry, governments, authorities, supranational organizations – at The Global Need for Concerted Antibiotics meeting, inviting further comments. This paper comprises input from all these consultations.

Experts all agree that antibacterials are prescribed in a number of instances when a bacterial infection cannot be assured largely because clinicians cannot make a precise diagnosis soon enough. Overall, it is probable that 50% of human antibacterial use could be avoided without negative consequence (Dryden et al., 2009b). However, without suitable diagnostic support, clinicians will prescribe antibacterials just in case their patients might have a bacterial infection, to protect themselves from litigation or to satisfy patient demands. When patients do require an antibacterial, they may not receive the most cost-effective alternative (Sakoulas et al., 2009; Wise et al., 1998). The overall volume of antibacterial use is correlated with resistance and declines with diagnostic information (Goossens et al., 2005; van de Sande-Bruinsma et al., 2008). The precise contribution that diagnostics could make to resistance containment has not been sufficiently studied but available evidence suggests that diagnostics may be more effective than some other interventions in preventing over-prescribing of antibacterials (Cals et al., 2010), and as discussed later in the paper, better diagnostics will also boost antibacterial development.

1.1. Life-threatening pediatric infections

Over a third of child deaths occur in the first month of life and up to 70% of bacterial isolates from recently cultured neonatal infections in developing countries are non-susceptible to affordable first–line drugs recommended for serious pediatric systemic infections (Bell et al., 2009; Zaidi et al., 2005). Emergence and spread of extended spectrum β-lactamase-producing organisms is compromising more expensive second- and third-line drugs. Child survival depends on adequate laboratory support and on up-to-date surveillance data to inform initial empiric choices. Both are also necessary to prevent the unwarranted antibacterial use that drives resistance but are underused globally and typically absent in the most resource-limited settings (Ishengoma et al., 2009; Okeke, 2011; Opondo et al., 2009; Zaidi et al., 2005). Moreover, precise diagnoses are needed to pinpoint problem areas and roadblocks to reaching Millennium Development Goal #4, which aims to reduce the 1990 under-five mortality by two-thirds (Anonymous, 2007).

1.2. Respiratory tract infections (RTI)

Acute respiratory tract infections were recently identified as one area where diagnostics would have considerable impact for treatment and in preventing antimicrobial overuse (Lim et al., 2006). RTI are the leading reason for seeking medical care and are the most common reasons why antibacterials are prescribed in the community and hospitals in Europe (Amadeo et al., 2010; Ansari et al., 2009; Goossens et al., 2005). In Asia and South America, clinical diagnosis of RTIs by the Integrated Management of Childhood Illness (IMCI) protocols leads to substantial overuse of antibacterials. Diagnostics could reduce this overuse and would annually save

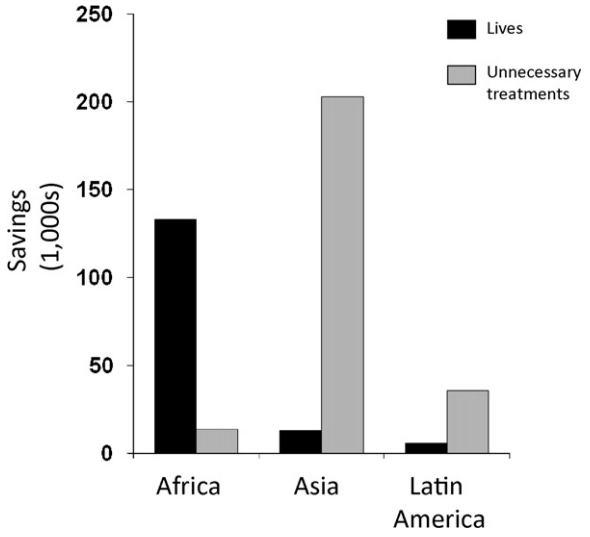

Fig. 1. Benefits of a new test for bacterial pneumonia in developing countries predicted by modeling the benefits of a new test for bacterial pneumonia for children under 5 (Girosi et al., 2006). The model assumes a population of 535 million children (based on 2004 estimates) and that each child has 5–6 acute respiratory tract infections a year. Black bars indicated lives saved from reducing disease burden and grey bars refer to unnecessary treatments saved.

almost 1,50,000 lives in Africa, where access to diagnostics and health professionals is poor (Fig. 1) (Burgess et al., 2007). There is insufficient knowledge on the etiology of RTI and almost no valid rapid diagnostic tests are available on the detection of bacterial infections. These uncertainties have resulted in prescriptive promiscuity, which largely explains the escalating antibiotic resistance of common bacterial respiratory pathogens.

1.3. Hospital-acquired infections

Dissemination of multiply-resistant clones within and among hospitals is a principal reason why resistant nosocomial infections have attained the prominence and accrued the costs they have today (Eber et al., 2010; Enright et al., 2002; Klugman, 2003). New molecular typing methods allow tracking of resistant clones but are limited to hospitals that have access to molecular testing. Rapid screening methods for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) are now also available. Inexpensive tests that can identify and track the etiologic agents of hospital outbreaks due to other bacteria transmitted in hospitals are desirable components of clinical toolkits for containing the most deadly forms of resistant infection and should be achievable with recent genomic advances (Cooke and Holmes, 2007).

1.4. Community-acquired bloodstream infections in malaria endemic areas

Rapid diagnostic tests for malaria that perform well at the point-of-care in resource-limited situations are now being introduced into African health systems and will have application throughout the malaria-endemic world (WHO, 2009). In contrast to the long-standing protocol of treating all fevers as malaria, it is now possible to make precise diagnoses for this disease, illustrating that point-of-care testing for high-burden disease is feasible in the most remote and resource-limited situations, and that it saves on antimalarial costs (Hamer et al., 2007; Shillcutt et al., 2008; Uzochukwu et al., 2009). Studies performed soon after the introduction of malaria rapid diagnostic tests revealed that some community health workers continued to administer antimalarials to patients who tested negative (Bell and Perkins, 2008; Lubell

et al., 2007). More recently, interventions to curb this behavior are producing a decline in antimalarial prescription but patients who test negative for malaria now almost invariably receive one or more prescriptions for antibiotics, shifting the overuse problem from antimalarials to antibacterials (Reyburn et al., 2007). Some of these antibacterial prescriptions will be justified, since bacterial bloodstream infections are an important and overlooked cause of systemic illness in malaria-endemic areas (Berkley et al., 2005; Blomberg et al., 2007; Evans et al., 2004; Kayange et al., 2010; Nadjm et al., 2010; Reddy et al., 2010; Reyburn et al., 2004). Unfortunately, however, in the absence of informative diagnostics, this antibiotic use is poorly targeted, increasing the overall selective pressure toward antibacterial resistance.

1.5. Sexually-transmitted infections

Bacterial sexually transmitted infections are easy to treat in early stages, but have harmful long-term sequelae and are socially stigmatizing, leading the infected to evade care. Stillbirths or debilitation from congenital syphilis, or blindness from gonorrhea or Chlamydia, can arise when children are born to infected mothers. Such problems can be easily avoided by treating infected women before their babies are born. These factors have prompted clinical algorithms but these algorithms have poor specificity, resulting in the overtreatment. As sexual partners also need to be treated, over-diagnosis has important social consequences and amplifies the impact of selective pressure. In resource-limited areas, those who receive a clinical misdiagnosis will commonly be women who are less likely to present with symptoms than men, for whom laboratory detection cannot be achieved through microscopy, and who, as caregivers, are most likely to pass on drug-resistant commensals with the genes they harbor to other individuals (Aledort et al., 2006; Hawkes et al., 1999; Mukenge-Tshibaka et al., 2002; Peeling et al., 2007; Watson-Jones et al., 2005; Zaidi et al., 2003). Time-consuming culture and susceptibility testing is possible in at least some laboratories but because patients with sexually transmitted diseases are commonly lost to follow-up, diagnosis, prescription and dispensing must ideally take place within a single health-center visit. Currently, the repertoire of point-of-care diagnostics for sexually-transmitted diseases is limited and under-utilized. Although it is universally acknowledged that resistance is increasing among *Neisseria gonorrheae* (Tapsall, 2005), there are currently no means for determining drug susceptibility at the point-of-care and most developing countries have little no surveillance data to inform empiric prescribing. Cheaper and more accessible tests could help to curb antibacterial consumption as well as prevent the dissemination of resistant organisms by improperly treated patients.

1.6. Antibacterial development

(So et al., in press) spotlight the slow and slim pipeline for antibacterials and the need for new innovations. Historically, clinically available narrow-spectrum agents have been underutilized and drug development programs have de-prioritized or ignored narrow spectrum hits even though their impact on resistance is lower (Dryden et al., 2009a; Payne et al., 2007). In addition to narrow-spectrum bacteriostatic and bactericidal agents, small molecules targeting bacterial adherence, virulence or signaling (Cegelski et al., 2008; Dryden et al., 2009b; Rasko and Sperandio, 2010) may have chemotherapeutic potential if paired with appropriate and rapid diagnostics. The contributions that diagnostics could make to drug development go beyond enhancing the potential of narrow-spectrum agents. The most pressing need is for antibacterials that show good efficacy against organisms that are resistant to current therapies. Patients pre-selected with appropriate diagnostics can be appropriately targeted to clinical trials of new antibacterials. This will make it possible to enroll fewer patients in clinical trials and to detect improved outcomes more robustly. The reduced clinical trial denominator will make trials cheaper, easier to evaluate and quicker to complete. Such trials will generate antibacterial medicines that require affordable diagnostics for appropriately use. Thus diagnostics have the capability to advance antibacterial development just as they promote evidence-based appropriate use of existing antibacterial drugs.

The examples above illustrate that resistance-promoting drug use, adverse outcomes for patients with resistant and susceptible infections as well as roadblocks to antibacterial development are all exacerbated by inadequate availability and use of appropriate diagnostics. Among equally compelling scenarios in which diagnostic insufficiency is compromising patient care and promoting antibacterial resistance are invasive bacterial diarrheas and preventive therapy for Group B Streptococcus in pregnant women, both of which currently foster antibacterial overuse. Expectedly, diagnostics will not address all interventions that can contain resistance. However, while there are no direct effects of diagnostics on non-prescription use of antibacterials and the dissemination of poor quality antibacterials, to give two pertinent examples, by ensuring that the first prescription is the appropriate one, diagnostics could help to reduce both practices by respectively engendering confidence in sanctioned health providers and detecting drug counterfeits.

Using appropriate diagnostics increases the likelihood that treatment prescribed will cure the patient. Thus diagnostics are a necessary part of quality health care delivery. To optimize the management of bacterial infections and minimize resistance, it would be ideal to have five pieces of diagnostic information relayed promptly, and preferably electronically, to each prescriber at consultation. The information would provide precise answers to the following questions:

1. Does the patient have a bacterial infection, and if not, what is the cause of his/her ill health?
2. In the case of a bacterial infection, what is the causative organism?
3. What is the susceptibility pattern of the organism (or which resistance genes does it carry)?
4. Does the organism have any uncommon or novel mechanism(s) of resistance?
5. If the organism is resistant to one or more 'last resort' agents, what is the minimum inhibitory concentration?

Answers to all the questions are not required for every patient but answers to any or some of the questions will reduce inappropriate antibacterial use. Importantly, information is most useful if it is available before the first prescription must be written.

2. Limitations of present-day diagnostics as relates to drug resistance

Most diagnosis and susceptibility testing for bacterial pathogens performed today depends on culture, biochemical species identification, and diffusion or dilution methods to determine susceptibility. These methods are based on principles that are over 75 years old For rapidly growing bacteria, they work well, allow multiple pathogens to be identified in mixed infections, and allow for follow-on analyses to identify resistance genes and strain-interrelatedness, and require infrastructure and skill sets that are attainable by many laboratories. Unfortunately, because they require the bacterial growth, these methods are slow, typically returning a susceptibility profile in 48 h or longer. For slow-

Table 1
Settings in which diagnostic tests are used (adapted with permission from Girosi et al. (2006)).

Characteristics	No infrastructure	Minimal infrastructure	Moderate infrastructure	Advanced infrastructure	Research level infrastructure
Electricity	Not available	Not reliably available/accessible	Available	Available	Available
Clean Water	Not available	Not reliably available/accessible	Available	Available	Available
Physical Infrastructure	None	Physical space but no actual lab	Poorly or minimally equipped labs	Well equipped labs	State-of-the-art
Staff	No expertise	Minimal expertise available	Nurse, some physicians, poorly or minimally trained technicians	Nurse, physicians, well trained technicians	Clinical scientists, well trained technicians
Examples of actual locations	In the community or home	Health Clinics (Africa); Rural Health Clinics (Asia, Latin America); physician's office (Europe, North America)	Hospitals (Africa); Urban Health Clinics (Asia, Latin America), Primary care clinic (Europe, North America)	Hospitals (Latin America, Asia, Europe, North America)	Reference laboratories, Tertiary care hospitals

growing organisms such as *Mycobacterium tuberculosis*, this period stretches to weeks. Even with automated systems and technologies that can shorten this biological amplification time by up to 50%, culture-based testing does not provide susceptibility information in time to inform the first antibacterial prescription. Thus, although they are valuable accompaniments to clinical care (Cooke and Holmes, 2007), conventional tests cannot promote the most judicious antibacterial use. Outside hospitals and away from clinical microbiology laboratories, although the risk of mortality may be lower, culture and susceptibility testing is difficult to implement because patients would have to return for results and remain ill and infectious in the interim. More rapid diagnostics based on nucleic acid technologies such as PCR, microarrays and sequence based diagnostics, or on advances in protein science–for example MALDI-TOF now have clinical applications and are beginning to permeate clinical diagnostic laboratories in industrialized countries.

The settings in which diagnostic tests need to be used vary widely (Table 1). Many existing tests are currently most needed and least applied in settings with no or minimal infrastructure because they are too expensive and require sophisticated equipment and training. Routine culture and susceptibility testing can be provided in some resource-limited settings (Polage et al., 2006) but in many more, testing is not possible and the need for other technologies is even more pressing. A 2009 assessment of laboratories found that no laboratory in the Tanga region of Tanzania offered bacterial culture and susceptibility testing, even to support diagnosis of life-threatening infections like meningitis and bacteremia (Ishengoma et al., 2009). Similar assessments have come from other developing countries (Okeke, 2011; Tegbaru et al., 2004). In addition to the requirements for technical expertise, aseptic technique and infectious waste handling, many tests require elaborate sample preparation, which is not feasible at the point-of-care and difficult to implement without appropriate laboratory infrastructure.

Culture is a reference standard, but other, faster, options exist. These include immunoassays, nucleic acid detection by standard or real-time PCR, hybridization (including microarrays) as well as methods that identify 16S or other pathogen-specific methods directly in patient specimens, and tests for pathogen antigens or host biomarkers. First-generation immuno assays and nucleic acid tests may require sophisticated equipment and skilled expertise. Additional specialized expertise is needed to routinely use 16S rDNA sequencing, deep sequencing, MALDI-TOF, and other newer technologies for diagnostic purposes. Biomarker tests seek a host factor that is, for example, elevated when a bacterial infection is present, often using simple protocols and materials. Examples include C-reactive protein, a marker of systemic bacterial infection and leucocytes visible by methylene-blue staining or lactoferrin, which are inexpensively targeted markers of inflammatory diarrhea in stool. Biomarker tests have only recently become available and although they show promise in some sub-populations, in others, significant cut offs are not yet known or have only been preliminarily investigated (Carrol et al., 2009; Opintan et al., 2010). Moreover, parasites as well as bacteria can elicit the inflammation on which such tests are based. These newer tests have reduced turnaround time and some have been shown to reduce antibacterial prescription (Cals et al., 2010; Lars et al., 2004). They however stop short of providing susceptibility information, which is needed to inform antibacterial selections when a bacterial infection is present. This deficit is partially ameliorated when these data are available from systematic epidemiological surveillance but rapid tests that could provide susceptibility information would be valuable.

3. Roadblocks associated with developing and using resistance-averting diagnostics

3.1. Roadblocks—research and development

3.1.1. Moving targets

Because microorganisms evolve rapidly, microbiological diagnostics need to evolve as well. This is particularly true for drug resistance, where new mechanisms are constantly emerging. In a hypothetical example for a β-lactamase diagnostic, which would have initially targeted TEM and SHV enzymes, it would have been necessary to adapt the test to detect OXA enzymes and then CTX-M and KPC extended-spectrum β-lactamases. One or more adaptations would be required to incorporate IMP, VIM and other metallo-β-lactamases and most recently, it might have been necessary to tinker with the test again to detect the NDM-1 β-lactamase. This 'moving target' is a disincentive for diagnostic test development, analogous to one of the many disincentives for developing antibacterial drugs, and makes it difficult to ensure that tests are also inexpensive and user-friendly. The moving target conundrum calls for hyperflexible platforms that can be adapted as and when new resistance genes or target microbes evolve. Robust but flexible platforms will also allow for tests to be adapted to the different disease ecologies that occur in different parts of the world.

3.1.2. Sample preparation

Sample preparation is one of the major roadblocks for developing sensitive tests because the microbial load in the sample can vary and in some cases is very low (Yager et al., 2008). Nucleic acid targets are among the easiest to identify and nucleic acid-based platforms are versatile. However these tests depend upon obtaining intact target nucleic acid from complex patient specimens, which inevitably contain nucleases. Similarly, many immunochromatographic tests require some level of antigen purification. Sample preparation is therefore a current bottleneck for converting many promising targets into the miniaturized diagnostics that hold the

greatest potential for use at the point-of-care (Dineva et al., 2007). Emerging technologies, such as sophisticated microfluidics offer some promise in this area, but still require considerable basic research to produce robust and versatile platforms that will perform in the most demanding settings (Yager et al., 2008). In order to overcome the sample preparation challenge, test developers must use specimens from real patients. These are often difficult to come by and could be accessed more easily through the development and use of specimen banks and, in the case of infectious diseases endemic to specific geographic localities, in-country research and appropriate research networks (Mabey et al., 2004; Okeke and Wain, 2008).

3.1.3. Inadequate focus on surveillance

Resistance surveillance is critical to understanding the status and trajectory of antibacterial resistance and containing the problem. There are a number of general and disease-specific surveillance networks but most have little or no coverage in many parts of the world (Grundmann et al., in press). Global surveillance is a "weakest link public good" (Barrett, 2006) and the current uneven landscape means that we have limited capability to detect resistance emergence ahead of dissemination. There is very little focus on developing diagnostics for surveillance, which, in addition to identifying the causative organism and its susceptibility pattern, would need to determine similarities among isolates and resistance genes. Surveillance is also important for determining which diagnostics will be needed as well as when and where. Therefore, just as diagnostics are needed to bolster surveillance, surveillance boosts diagnostics development and use.

3.1.4. Fragmented expertise and the need of increased R&D exchange

Developing diagnostics is often wrongly perceived to be an endeavor with low innovation potential (Pettersson et al., 1987). It will be essential to induce the best scientists to the interdisciplinary enterprise of diagnostics development. In order to develop sensitive, specific and useable point-of-care, we will need significant advances in pathogen and biomarker biology for target-finding, microfluidics for sample processing, target amplification, component design and assembly, detection technology, as well as data collection, handling and dissemination. Multiplex diagnostics capable of detecting the most common pathogens associated with syndromes that cannot be resolved clinically, particularly fever, acute respiratory tract infections and diarrhea, require expertise in parasitology, virology and bacteriology at the front end of the development process. Application of all knowledge bases and earlier discoveries, to diagnostics will also require sophisticated handling of intellectual property challenges associated with multiple innovations, particularly ones that involve biological targets and processes. The Global Strategy of WHO's recently convened Intergovernmental Working Group on Innovation, Intellectual Property and Public Health could provide a way forward in this regard. There is also a better need for policymakers to understand the diagnostic development process, to characterize and document the pipeline and to identify innovation system gaps as well as the points in the process at which candidate tests are most likely to fail. Very few groups involved in test development are addressing all facets of the diagnostic challenge and we need better communication among groups, for example to ensure that optimal detection platforms are paired with optimal sample processing. Currently, there is insufficient exchange between the public and the private sector, or among diagnostic and pharmaceutical industries. Recent public-private initiatives have resulted in product development partnerships, such as those described in Box 1 , may address some of these issues (Hunter, 2008; Mboya-Okeyo et al., 2009). A recent call for proposals from the Bill & Melinda Gates Foundation and Grand Challenges Canada, aims to overcome the problems associated with linking different innovations by allocating funds for component building in phase 1 and then funding a second phase to support integration of the "best-in-class" from each component (Box 1). Clinicians also need to be more tightly connected to the diagnostics development process, to ensure that the most useable tests emerge. As an example, recently introduced rapid molecular tests for sepsis diagnosis were not thoroughly assessed for their 'added clinical value' compared to conventional existing gold standard tests such as blood culture, or clinical diagnosis (Mancini et al., 2010). The developers of nucleic acid tests for sepsis did not incorporate clinician decision-making nor did they estimate the potential impact of different test strategies on appropriate targeting and adequacy of antibacterial therapy for sepsis patients. Diagnostic test developers are also often unfamiliar with the nuances associated with test use in resource-limited settings.

3.1.5. Test evaluation and regulation

A 2004 report observed that 45 of 85 surveyed countries, virtually all of which regulated medicines and health professional practice, do not regulate diagnostics (Mabey et al., 2004). For those that do, there are no universal standards for test evaluation and most do not require clinical trials (Mabey et al., 2004; Peeling et al., 2006b). As such, diagnostic evaluations are often not predictive of in-use conditions. They may use disparate populations, impracticable facilities and small sample sizes (Bachmann et al., 2006; Peeling et al., 2006b; Smidt et al., 2006). The Standards for Reporting Diagnostics Accuracy adopted by about a dozen journals provides a checklist for evaluating diagnostic studies and aims to improve the quality of diagnostic evaluation overall (Bossuyt et al., 2003a,b,c). These Standards have led to a noticeable improvement in the quality of published diagnostic test evaluations but improvements are still needed (Smidt et al., 2006). When evaluations are properly performed, it is often unclear how products should be regulated and registered.

3.1.6. Funding

Funding and perceived return on investment is a primary roadblock to the development of diagnostics, particularly those that would have the most benefit in resource-limited settings. As the same disincentives for developing drugs for poor patients apply to diagnostics, and so will their solutions (So et al., in press; Usdin et al., 2006). Progress made in recent years has allowed some of the best advances for human diagnostics to develop innovative tests for malaria, tuberculosis and HIV in spite of market disincentives (Boehme et al., 2010; Larsen, 2008; Usdin et al., 2006). A pre-market commitment is presently lacking for many other diagnostics, particularly those that could assist in containing bacterial resistance. If the constraints associated with testing in resource limited systems are taken into account during development, a single assay platform should be able to serve both developed and developing country communities. Mechanisms are needed to encourage researchers to produce globally applicable tests where possible. There has been a recent increase in available funding for diagnostic development (Box 1), in part spurred by the rising costs of antimicrobial chemotherapy due to resistance. The parallel publication "explosion" (Yager et al., 2008) demonstrates that increased funding can promote research on diagnostics. However, levels of funding and resources for diagnostics research and development are still far below what is available for drugs and vaccines. Many recent calls that focus on diagnostics have been for short-term projects and do not acknowledge the long-term investment that may be needed to overcome the formidable technical challenges that must be overcome to make point-of-care diagnostics. Funding is also needed for basic microbiology, chemistry and nanoscience research, which could overcome technical roadblocks to diagnos-

Box 1: Examples of diagnostic development initiatives

Funding and Technology

- The European Union has recently funded a number of diagnostic development projects. These include InTopSens ("A highly integrated Optical Sensor for point-of-care label free identification of pathogenic bacteria and their antibiotic resistance"), which aims to develop a tool for detection of sepsis pathogens and relevant antibiotic resistances using label-free biosensors; TheraEDGE, which will develop a viable molecular diagnostic test for respiratory bacterial and viral pathogens and relevant antibiotic resistances using single-molecule detection techniques with a target turnaround time of under an hour and RAPP-ID ("Development of RApid Point-of-Care test Platforms for Infectious Diseases") to develop point-of-care platforms for respiratory infections, sepsis and TB.
- Europe's Innovative Medicines Initiative (IMI), a public private partnership that aims to support more rapid discovery and development of better medicines for patients, has extended its focus and include some diagnostic development.
- Grand Challenges Canada has called for proposals to develop diagnostic technologies and plans a second call to ensure that different innovations are linked to produce workable point-of-care diagnostics.
- The Bill and Melinda Gates Foundation-supported Foundation for (FIND) is using modern technologies to develop diagnostics for resource-limited laboratories. FIND was the key player in a public-private partnership that resulted in the development and field testing of Xpert MTB/FIF, an automated molecular platform for detecting TB infection and identifying rifampicin-resistant (which are often multidrug resistant) strains (Boehme et al., 2010). FIND is also supported by the US National Institutes of Health (NIH) and the UK Department for International Development (DFID), both of which are increasing support for diagnostics development.
- NIH has issued calls to develop point of care diagnostics, including those for nontraditional health care settings that would include resource-limited areas. It has also promoted public-private consortial arrangements and offers contract research services for specific development tasks in which test developers may lack infrastructure or expertise.
- The Program for Appropriate Technology in Health (PATH) and investigators at the University of Washington are developing multiplex diagnostics for diarrheal disease and acute fever. The aim is to produce a microfluidics card or "lab on a chip" that would be suitable for point-of-care use in developing countries http://www.path.org/projects/microfluidics_card.php. PATH's center for point-of-care diagnostics also provides funding and support for field testing of diagnostic test candidates that have promise for resource-limited health care systems.

Networking and Implementation

- The WHO TDR program, the African Development Bank, the EU and other partners inaugurated the African Network for Drugs and Diagnostics Innovation (ANDI) to promote local research and development (Mboya-Okeyo et al., 2009). Asian and South American counterparts of ANDI have also been recently launched.
- To assist developing countries with the challenge of regulating diagnostic products and selecting high-quality products for the public sector, the WHO has established prequalification programs for some diagnostics (WHO, 2009; WHO/TDR, 2008). It is hoped that new diagnostics that are developed to support antimicrobial containment will receive this type of support.
- Recent initiatives by the Global Fund, the Clinical Laboratory Standards Institute, the American Society for Microbiology, the African Society for Laboratory Medicine and other professional organizations to build laboratory capacity in developing countries are welcome and timely and will assist in the effort to boost diagnostic capabilities worldwide.

tics. Currently, most diagnostic research is performed in academic institutions and within small and medium sized enterprises, which are highly grant-dependent. Researchers in these environments, being more distant from markets than are large companies, incur greater risk and have fewer resources for field evaluation. In addition, small and medium sized enterprises are very dependent on venture capital and therefore their capabilities are easily influenced by fluctuations in the financial market.

3.1.7. Time to development

Interest and appreciation in the value for diagnostics has increased exponentially in the last five years. However users will have to wait many more years for research and development challenges to be overcome, and for necessary tools to reach the market because, as shown in Fig. 2, it can take up to 10 years to develop a priority diagnostic. This long-term investment is a roadblock for test development. It is also a barrier to test use because the absence of a needed test entrenches substitute behaviors and practices, which may be difficult to change when a suitable test becomes available.

3.2. Roadblocks—the use of diagnostics

3.2.1. Test cost

Appropriate diagnostics for resistance control will necessitate increased volume and diversity of work for clinical laboratories. In resource-limited areas, this means that new laboratories will have to be built, equipped and staffed. In higher-income countries that already have good laboratory networks, increased volume must be accompanied by increased automation because staffing is costly. Ideally, diagnostics for infectious diseases would be less expensive than antibacterial drugs. However, many recently developed rapid tests are expensive, perhaps rightly so, given their absolute cost and cost of development. Costs could fall with market penetration and increased use but presently, high prices impede introduction of new tests into resource-constrained and budget-conscious health systems. Paradoxically, low uptake in turn reduces the incentive to develop diagnostics and keeps the price of diagnosis high.

3.2.2. Test speed

For diagnostics to impact selective pressure from antibiotic use, speed is critical. Most current tests require culture of the organism as an essential first step. This amplification typically takes 18 h or longer, for fast-growing species even though it is presently feasible to modify current protocols to reduce incubation times without compromising sensitivity or specificity. Thus, there is a pressing need to improve detection speed for culture-based methods and to develop tests that return etiology and susceptibility results without requiring prior organism culture. Nucleic acid amplification tests are rapid but have not, as predicted, replaced culture-based detection as predicted two decades ago. Most nucleic acid amplification tests are complex to operate, and require cumbersome DNA extraction steps which limits their feasibility in standard microbiology laboratories.

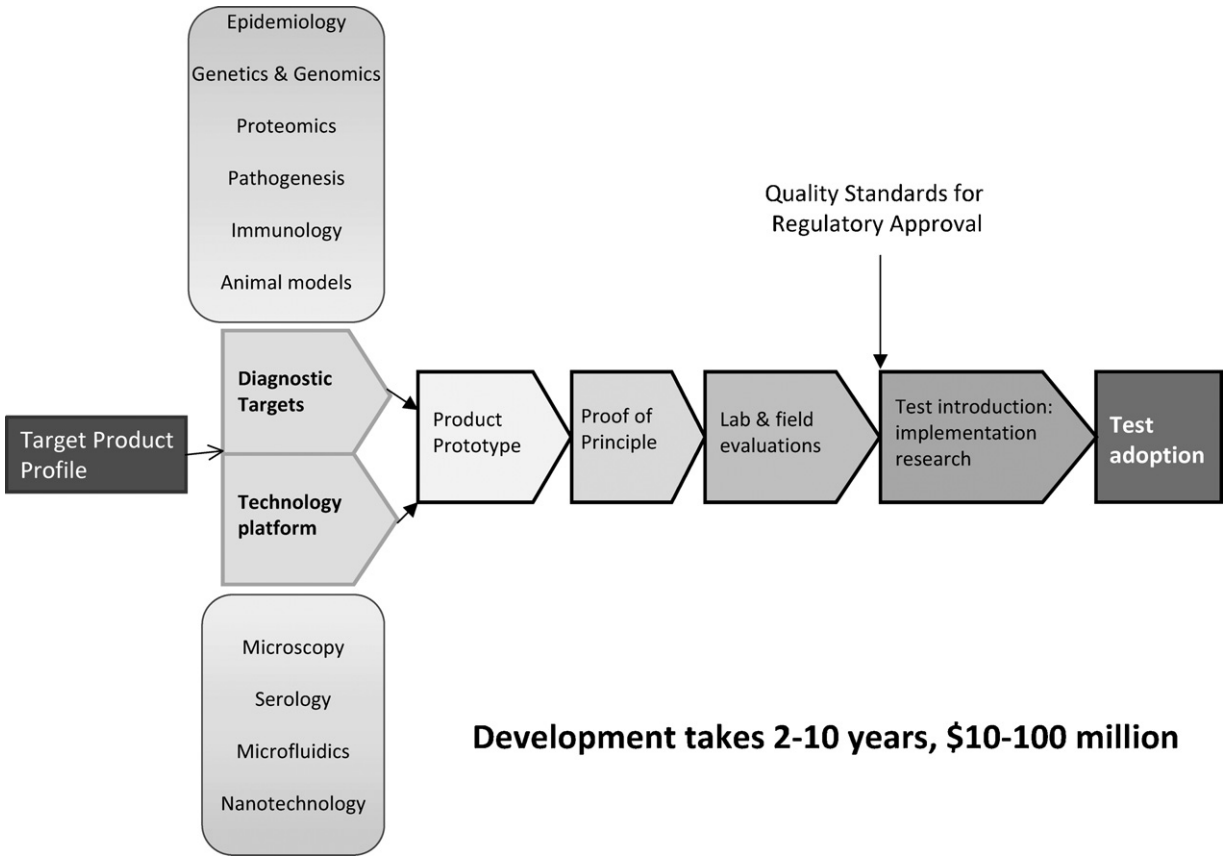

Fig. 2. Innovation pathway for appropriate diagnostics.

3.2.3. Test spectrum

Diagnostics are most useful clinically when they can inform patient care (Wootton, 2006). Many recently developed rapid diagnostics identify only a single pathogen (e.g. rapid malaria and MRSA tests). Single tests are an important first step, particularly for very common pathogens, but they limit the overall diagnostic value. Diagnostic flowcharts or multiplexes may increase the cost-effectiveness of testing and treatment since they offer more patients a precise diagnosis and they reduce the chance that an antibacterial will be prescribed when a single negative test is returned. Only when the indirect but heavy cost from drug resistance is considered will the true value of such tests be visible. In resource-limited settings, the absence of alternate treatments may also deter the use of diagnostics. Multiplex tests however, are even more challenging to develop than single pathogen tests and needed testing panels may vary geographically (Yager et al., 2008). While they may reduce the cost of diagnosis for uncommon infections, they increase the absolute cost for diagnosing more common ones. They may also be more difficult to set up and interpret.

3.2.4. Sample collection and test complexity

Many of the specimens needed for today's tests are difficult to access. In areas where trained physicians or nurses are not available, collecting spinal fluid, blood, vaginal swabs and other invasive or semi-invasive specimens may be impossible. Just as the availability of trained health professionals will dictate specimen accessibility, test accessibility is also determined by the level of training laboratory technicians have received. Tests yielding multiple or quantitative end-points (such as titers) may be particularly hard for semi-trained technicians to perform and clinicians to interpret. Even when optimally trained personnel attend a patient, the sample sent to the lab on occasion lacks diagnostic value because extraneous contamination was not avoided, the sample container was inappropriate or the patient was too ill to provide sufficient sample.

3.2.5. Test limitations

Many rapid and molecular tests can only detect known mechanisms of resistance so that newly emerged mechanisms will be missed. Molecular methods may provide false positives since they detect unexpressed genes. Tests that do not require isolation and identification of a causative organism may reduce the chance that an unusual strain or specimen is sent to a reference laboratory for follow-up, unless specific protocols are put into place to ensure this. In many cases, the true limitations of tests are unknown. Studies evaluating diagnostic tests are often not rigorous enough and in some cases are not performed (Banoo et al., 2006; Peeling et al., 2006a,b). In some cases, evaluations are difficult to perform, particularly in those instances when reference standards are not sensitive or specific and protocols for evaluations do not exist. Tests may perform differently in different parts of the world due to variations in pathogen prevalence, disease severity or host genetics or immune status (Leeflang et al., 2009; Peeling et al., 2006b). Moreover, the nature of clinical expertise paired with a test may influence false-positive and false-negative rates. These factors make it challenging for health systems to select the tests that will optimize patient care and correctly inform prescribing.

3.2.6. Biosafety

Testing exposes individuals other than the patient and his or her caregiver to potentially pathogenic organisms, often in a biologically amplified form. Wherever testing is to be introduced, it is key to provide protection for health workers during sample collection and processing, and to assure safe disposal. These cannot

be guaranteed in resource-limited settings, where health workers have become infected as a result of testing (Mason, 2008; Yager et al., 2008). Thus, biosafe alternative methods, such as molecular testing following sample inactivation, or accessories to ensure safe test use, such as solar disinfection systems, need to be developed and used (Nathavitharana et al., 2007).

3.2.7. Access

Diagnostics are less accessible than medicines, even for disease conditions where they have been prioritized, such as HIV. Recent years have seen some strengthening of global laboratory infrastructure and the development of rapid tests for TB, HIV and malaria that are being put into use in resource-limited settings. Many large programs supporting drug access, particularly those that are donor-driven, tend to be vertical, whereas diagnostic development to contain resistance will have to be a horizontal process. To have optimal impact, tests must be performed and their results used. Worldwide, most outpatients can only afford to see a consulting physician, health-worker, or in some instances unlicensed practitioner, once. Thus, diagnostic information that is not available at the point of care may not influence drug choice or contain resistance.

3.2.8. Supply chain management, technology transfer and local production

Governments and health care aid programs for developing countries that distribute medicines require similar, integrated programs for diagnostics and accessories. Supply chain failures in any area negatively impact evidence-based health care delivery. For example, in a Uganda clinic, stock outs of gloves prevented malaria diagnostic testing when antimalarial drugs and rapid diagnostic tests were in stock (Kyabayinze et al., 2010). Although diagnostics may be introduced through donor-supported programs, their availability needs to be assured irrespective of donor commitment. For resource limited health systems, particularly in the case of tests for which the market elsewhere is small, these objectives may best be achieved by local manufacture. There are notable exceptions in emerging economies but in many of the least affluent countries, a bouquet of roadblocks- ranging from start-up and operating costs to shortage of biomedical and bioengineering expertise and regulatory bottlenecks – will be needed to make local production and distribution possible.

Table 2
Modeling demonstrates that tests requiring less infrastructure produce large health benefits, even with less than perfect performance. Results assume access to testing.

	Lives saved by new test for bacterial pneumonia	
	Good performance	Perfect performance
Minimal infrastructure	405,000	596,000
Advanced infrastructure	142,000	261,000

3.2.9. Testing environment and culture

Hospital laboratories are being downsized and medical and allied health education programs are changing, de-emphasizing microbiology and thereby compromising testing and depreciating the importance of test results in clinical diagnosis. In the US, medical students no longer have to take a practical (wet) microbiology laboratory and in many developing countries, such laboratories have been cut or discontinued due to funding constraints. In high-income countries, diagnostic facilities are increasingly being centralized. This has the advantages of reducing costs and increasing the scope of testing available, particularly for rarely performed tests. It also offers 'out of hours' testing to patients at institutions that cannot offer such a service. Centralization however adds transportation time to the time-to-diagnosis and hampers communication between laboratory personnel and physicians (Raoult et al., 2004). Bacteriology laboratories are uncommon in some countries and new HIV and/or TB laboratory programs often do not improve capacity in basic bacteriology even though such methods are inexpensive and easier to set up. Many developing countries have no accredited laboratories or routes to accreditation (Olmsted et al., 2010). Reassuringly, an African Society for Laboratory Medicine will be launched in March 2011 with the aim of promoting quality of laboratories in Africa. The Society will carry out accreditation for laboratories at different levels of the health care system.

4. Diagnostics as tools for limiting antibacterial resistance

We have identified a number of steps that should be taken to move toward better use of diagnostic technologies in limiting antibacterial resistance. These steps, summarized in Table 3 and described in more detail below, cover a breadth of areas, from pol-

Table 3
Diagnostics as tools for limiting antibacterial resistance: next steps.

Need for action	Next steps
Strengthening the case for diagnostics	In depth situation analysis to better understand the challenges for diagnostic development and use State a compelling case for diagnostics in resistance containment in multiple venues
Product profile and development	Develop target product profiles that incorporate the meet the Affordable, Sensitive, Specific, User-friendly, Robust and Rapid, Equipment-free and Deliverable to areas of need ("ASSURED") criteria concept and will promote maximal antibiotic resistance containment Ensure susceptibility is included in diagnostic product profiles for bacterial infections Research suites of tests for given localities Develop local surveillance systems to ensure appropriate product development
Increased research and development, collaborations and information exchange	New funding programs that promote longer cycles and public-private initiatives Create appropriate networking platforms and resources Apply modern technologies and evaluating diagnostics Joint academia – health care – industry initiatives that include researchers from developing countries Closer collaboration between the pharmaceutical and diagnostics industries
Uptake by health systems	Advocating routine use of existing diagnostics Harmonized regulation for diagnostics between different countries Research to identify behavioral determinants influencing diagnostics use and interventions to promote effective integration of testing into health practices
Making the cost-effectiveness of bacterial diagnostics more visible	Cost-benefit analyses Offering equivalent or greater subsidies and reimbursements for diagnostics, as compared to medicines

icy and economics, to technical and behavioral. Concerted efforts by multiple stakeholders – governments, health care providers, funding agencies, private industries, regulatory authorities, academic researchers, as well as patients and the general public – will be required to complete these actions.

4.1. Strengthening the case for diagnostic development

Diagnostics point to a cure but do not produce one, leading many clinicians, patients and policymakers to undervalue them. There are several, potentially high-impact interventions that could contain resistance by effecting disease and infection control. Diagnostics are often rightly ranked below these strategies in terms of prioritization. However diagnostics are not merely preventive interventions, they are essential components of curative ones and their use should therefore be considered in the context of drug use, as well as for prevention. In the absence of a concerted interest in containing resistance, diagnostics may be perceived as cost-ineffective. Thus diagnostics need to be 'marketed' as part of the effort to conserve medicines because their benefits often accrue to health systems and regions, and not just to individual patients, particularly where they address antibacterial resistance. In addition to making the case for diagnostics, it is necessary to identify the areas that will produce the most gain. This can be done through the convening of experts and through modeling approaches, such as those recently performed by the RAND Corporation, in combination with a regular market analysis for bacterial infections diagnostics (Girosi et al., 2006; Urdea et al., 2006).

4.2. Product profile and development

Developing product profiles (functional requirement specifications) to meet clearly defined needs, including agreed roadmaps for point-of-care test development is a priority for advancing resistance-averting diagnostics. This should be done with a broad range of expertise and include stakeholders from academia, health care, industry and regulatory authorities. At the very least, point-of-care diagnostics, particularly those that will be used in resource limited settings, must be Affordable, Sensitive, Specific, User-friendly (requiring minimal training), Rapid and Robust, (possible to transport, store and use at high ambient temperature and humidity), Equipment-free and Deliverable to areas of need ("ASSURED") (Mabey et al., 2004; Peeling et al., 2006a,b). To impact resistance, they must rapidly – within 30 min – delineate bacterial infections from those that are viral, parasitic, fungal or non-infectious, with high specificity and sensitivity, and at a price that is cheaper than the most commonly used antibacterial treatments. For community-acquired infections in resource limited areas, there is a pressing need for rapid diagnostics that can be used with limited amount of training and ideally no requirement for equipment, electricity, extraneous reagents (including water) and employing patient specimens that can be collected non-invasively. They should have some form of internal quality assurance, and results should be available in less than an hour. In principle, many diagnostics that are used in primary care settings elsewhere could also be of use in resource-poor settings. However, it must also be possible to transport, store and use tests at high ambient temperature and humidity levels. Finally, any target product profile for a diagnostic to be used in resource-limited settings, must contain input from practitioners working at such locations.

Many existing point-of-care tests identify or point to an etiologic agent but do not return a susceptibility test result. Profiles for products that provide susceptibility information, even if they overlook etiology will be important for containing resistance. Point-of-care tests along this line are now foreseeable: it should be possible to develop a point-of-care test that detects extended spectrum β-lactamases in urine or sputum, for example. Affordability is vital in all settings. If laboratories in high-income countries are to increase the volume of specimens handled substantially, automated testing platforms may be necessary. Overall, the profile of an ideal product will be difficult to meet and therefore insisting on all criteria in a single product could stifle development. It is therefore important for experts to weigh criteria and to be willing to compromise on non-essential features when profiles are developed. The FDA Clinical Laboratory Improvement Amendments (1988) propose that lower performance levels may be acceptable for "simple tests", such as automated instrumentation or point-of-care diagnostics. The idea is that a sensitivity as low as 70% may be accepted for tests used in physicians' offices in high-income countries, as long as they minimize the chance of human error (FDA, 2008). In resource-limited countries where point-of-care tests are likely to be performed by partially trained personnel, there is every chance that tests will perform below their stated accuracy. However, even though such tests will be applied to life-threatening infections, modeling has shown that in areas where access to care is limited and laboratory facilities are minimal or non-existent, lower test performance may be tolerable (Table 2) (Burgess et al., 2007).

Suites of essential diagnostic tests, that is, region-specific 'essential tests lists', must be locally tailored to ensure that common endemic diseases are covered. This in turn requires surveillance at levels that currently do not occur in many low-income countries and a requirement for reference laboratories with superior diagnostic facilities. Current and future evidence-based medical practice depends on the quantity and quality of available surveillance data. Diagnostics can improve both. The rapid development and clinical introduction of HIV laboratory diagnostics and point-of-care malaria diagnostics in many parts of Africa demonstrates that both laboratory-based and point-of-care diagnostic tests can be used in resource-limited health systems and that they do improve the quality of care and precision of antimicrobial chemotherapy (Hopkins et al., 2009; Kyabayinze et al., 2010; Larsen, 2008). Tests that can identify patients with bacterial infections would have a similar potential.

4.3. Research funding

One of the most obvious needs is a further augmentation of existing funding initiatives (Box 1), and in particular, initiating long-term support programs that will allow a concerted battle against impeding roadblocks. Many of the market-based mechanisms for research and development highlighted in the accompanying paper by (So et al., in press) could, and therefore should, be applied to diagnostics. Incentives and granting programs that encourage the development of flexible diagnostic platforms, integration of multiple targets per syndrome into a single test, as well as developing diagnostics that provide information on antibacterial susceptibility are especially needed. Existing programs that support drug development should be recast as supporting health innovations that include diagnostics. This will encourage investigators working at the cutting edge to be attracted to diagnostic innovation. We also need new business models to make diagnostic development more attractive to industry. Models that have been successful in promoting antiinfective drug discovery would mostly apply but there is also need for further incentive building, with the ultimate goal of developing diagnostics that are cheaper than medicines.

In addition to supporting applied research directly focused on diagnostics, there is need to invest in basic science projects that will fill knowledge gaps. Examples include microbiology research on the nature and density of pathogen material in infected specimens, microfluidic strategies for processing specimens and amplifying targets at point-of-care, nanoscience and bioengineer-

ing innovations that could make it possible to miniaturize tests and biophysical detection systems that obliterate the need for sophisticated equipment. Finally, very little is known about health-seeking and health practice behaviors that promote or retard the introduction of diagnostic tools into different types of health systems. Programs are needed to support social and behavioral research as well as modeling studies that assess diagnostic needs and cost-effectiveness.

4.4. Applying modern technologies and evaluating diagnostics

A wide range of new technologies are applicable to diagnostics research (Fig. 2). Genomic and proteomic methods increase the efficacy of finding diagnostic targets and other technologies will result in faster, cheaper and more reliable tests. For example, nanotechnology and microfluidics may make it possible to develop molecular tests on small, disposable and cheap platforms that can be used at the point-of-care. Other technologies often perceived as high cost, such as surface plasmon resonance, MALDI-TOF, automated molecular tests, microarray-based methods, become more cost effective if used routinely and intensively. These and other technologies have the potential to decrease the time required for detection of diagnostic targets, such as pathogen-derived proteins and DNA, from hours to minutes and will revolutionize the development of diagnostics in the next few years. A number of in-progress diagnostic initiatives using these technologies are currently in progress (Box 1). The resulting new diagnostics must be rigorously evaluated according to appropriate standards (Banoo et al., 2006; Bossuyt et al., 2003a,b,c; Peeling et al., 2006b).

4.5. Collaboration and information exchange

There is a need for closer collaboration between the pharmaceutical and diagnostics industries and better interactions among all stakeholders. We envision joint academia-industry initiatives recruiting broad diagnostic expertise to develop, evaluate, validate and implement new resistance-averting diagnostics. Therefore, it is essential to create appropriate networking and information-exchange platforms and resources. Special attempts must be made to include developing-country researchers, who work in areas with the greatest burden of disease (Okeke, 2011; Okeke and Wain, 2008; Peeling and Mabey, 2010). A global diagnostics database that includes information on potential and tried targets and technologies, which also offers networking opportunities for investigators, may be the option.

4.6. Uptake by health systems

Where possible, it would be advantageous to develop some tests that apply to different health systems irrespective of resource and location. This will make it possible to introduce differential pricing schemes that could make such tests globally accessible. Regulatory pathways for diagnostics need to become faster, more uniform, more transparent and easier to navigate. Global or regional harmonization of regulatory requirements will make it unnecessary for companies to conduct a clinical trial in every country to obtain approval and the WHO's bulk procurement scheme, which lists tests with acceptable performance, can offer tests to Ministries of Health in developing countries at negotiated pricing.

The potential benefit of optimized diagnostic procedures in current clinical practice should be modeled. Social, ethical, environmental, economical, and political factors, that influence the adoption of new diagnostic technologies and delivery into health systems, should be identified. When available, diagnostic tests and services are typically underutilized (Polage et al., 2006), pointing to a need for input from behavioral scientists and social marketing experts to identify and address barriers for acceptance diagnostics, particularly at the point-of-care, as well as to understand motivational factors which may help overcoming hurdles to effectively use appropriate diagnostics in patient management. These findings must be used to develop and implement better education of policy makers, prescribers and patients. This can be done as part of antibacterial resistance containment initiatives as well as by bolstering existing resources on diagnostics.

More immediately, existing diagnostics have an important but underexploited role in containing antibacterial resistance today. Although bacterial culture followed by diffusion or dilution testing is typically too slow to inform the first empiric prescription, in the current era of multiple resistance, pre-emptive culture of initial specimens can inform a second prescription in the event that one is necessary (Sundqvist and Kahlmeter, 2009). At the point of care, a C-Reactive Protein (CRP) test has been shown to be effective in reducing antibiotic prescribing for acute respiratory tract infections (Andre et al., 2005; Cals et al., 2010; Jakobsen et al., 2010; Takemura et al., 2005), as have streptococcal antigen tests in the US and France. Other existing biomarker, microscopy and pathogen antigen tests can produce rapid results to inform the first prescription and all illustrate that it will be worthwhile to develop tests that return even more information (Charles and Grayson, 2007).

4.7. Costs and cost-effectiveness

Antibacterial drugs are currently often underpriced, in that their sticker price does not include the cost of resistance. Nonetheless, many patients that need these life-saving therapies cannot afford them and they are therefore often further subsidized. Treatment, reimbursement and subsidy costing need to be revised so that diagnostics are cheaper than drugs. This can be done by offering equivalent or greater subsidies and reimbursements for diagnostics, as compared to medicines. Also, the costs and benefits should be studied by performing cost-effectiveness analysis of new diagnostics compared with standard approaches for diagnosis of infectious disease.

5. Conclusion

Antibacterial resistance can only be contained by an integrated approach that includes all stakeholders. Diagnostics are an under-recognized and underexploited tool for resistance containment. In industrialized countries, they represent only 2% health expenses of but influence 60–70% of health decisions and in developing countries, spending on diagnostics ranges from negligible to 6% (Lewin, 2005; Peeling and Mabey, 2010). As antibacterial resistance containment receives the attention it deserves, the message to clinicians, scientists and patients alike needs to shift from recommending "prudent use" of antibacterials to enabling development and appropriate use of antibacterials through diagnostics. Maintaining antibacterial efficacy should be presented as a patient safety concern and diagnostics are an important part of this paradigm.

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Conserving antibiotics for the future: New ways to use old and new drugs from a pharmacokinetic and pharmacodynamic perspective

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ABSTRACT

There is a growing need to optimize the use of old and new antibiotics to treat serious as well as less serious infections. The topic of how to use pharmacokinetic and pharmacodynamic (PK/PD) knowledge to conserve antibiotics for the future was elaborated on in a workshop of the conference (The conference "The Global Need for Effective Antibiotics – moving towards concerted action", ReAct, Uppsala, Sweden, 2010). The optimization of dosing regimens is accomplished by choosing the dose and schedule that results in the antimicrobial exposure that will achieve the microbiological and clinical outcome desired while simultaneously suppressing emergence of resistance. PK/PD of antimicrobial agents describe how the therapeutic drug effect is dependent on the potency of a drug against a microorganism and the exposure (the concentration of antimicrobial available for effect over time). The description and modeling of these relationships quantitatively then allow for a rational approach to dose optimization and several strategies to that purpose are described. These strategies include not only the dosing regimen itself but also the duration of therapy, preventing collateral damage through inappropriate use and the application of PK/PD in drug development. Furthermore, PK/PD relationships of older antibiotics need to be urgently established. The need for global harmonization of breakpoints is also suggested and would add efficacy to antibiotic therapy. For each of the strategies, a number of priority actions are provided.

1. Introduction

In an era of increasing emergence of drug resistance and lack of new antibiotics there is a growing need to optimize the use of old and new antibiotics to treat infections. Although the efficacy of new antimicrobials and dose–response relationships is reasonably described, this is often not the case for older agents. Much progress has been made over the past two decades in elucidating exposure–response relationships of antimicrobials, particularly regarding pharmacokinetic (PK) and pharmacodynamic (PD) principles. Perhaps even more important is the increasing awareness that optimizing therapy not only involves maximizing therapeutic outcome but also includes minimizing the risk of resistance emerging during therapy, both in the infecting pathogen and in the normal flora. However, the exposure–response relationships for efficacy and resistance selection are often distinctly different. Optimizing outcome is directed at the individual patient level whereas emergence of resistance is an ecologic issue and a trade-off between these two objectives is not always easy to achieve. In any event, it is essential that clinical decisions be based on exposure–response relationships. In some instances, this information is readily available but is not implemented; in many more cases, specific research is warranted. The knowledge obtained from further research should provide the tools for pol-

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icy changes but may also facilitate implementation of existing guidelines.

We will first discuss exposure–response relationships in general and provide the background of the PK/PD principles that can be used to optimize antimicrobial therapy (Section 2). These principles will serve as the backbone for a number of topics that are subsequently highlighted and involve the use of PK/PD in optimizing the use of antimicrobial agents: development of antimicrobials (Section 3), emergence of resistance (Sections 4 and 5) and the use of PK/PD in establishing and revising breakpoints for old and new antimicrobials (Section 6). Each of these topics concludes with statements that should improve the use of antimicrobials and indicate where research is needed in that specific area.

2. Background of PK/PD and exposure–response relationships

PK/PD of antimicrobial agents describes the triangular relationship between the potency of a drug against a micro-organism, subject exposure to a drug (the concentration of antimicrobial available for effect over time) and drug effects (Fig. 1). This relationship is somewhat different from that for non-antimicrobial drugs because the receptor of an antimicrobial drug is located within the microorganism instead of on a cell in the human body. Thus, the intended beneficial effects on the host will be secondary to the killing or growth inhibition of the pathogen. In this view, antimicrobial therapy is only one of the factors contributing to curing patients, albeit a significant one for most infections. Dosing regimen optimization is accomplished by choosing the dose and schedule that results in an exposure that will achieve the primary aim of the therapy (i.e. clinical outcome, resistance suppression or a specific degree of microbiological effect).

2.1. Effects of exposure

To determine the optimal exposure it is necessary to have a quantitative measure. The measure most often used is the area under the time-concentration curve (AUC) over 24 h (AUC_{0-24h}) (Fig. 2). The AUC can be regarded as the integration of the concentration over time and thereby represents the 'total' exposure of the antimicrobial during the period indicated and is expressed in (concentration × time) units (Mouton et al., 2005). One of the characteristics of the AUC is that it is, for many drugs, correlated to dose in a linear fashion. Thus, for example, an increase of the dose with a factor of 2 will yield an AUC that is twice as large. Similarly, applying the same dose twice will also result in an AUC that is twice as large, although for drugs with a relatively long half-life, accumulation affects this correlation during the first days of therapy.

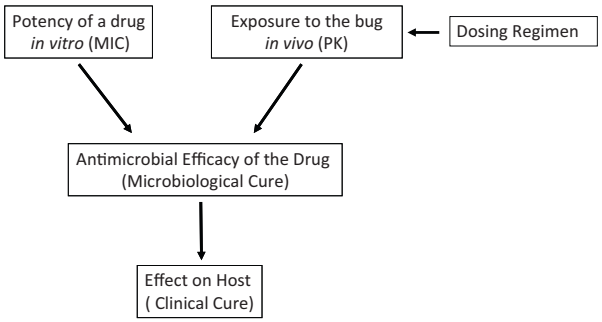

Fig. 1. Triangular relationship between the potency of a drug against a microorganism (usually expressed as a MIC), exposure of an antimicrobial drug (concentration of antimicrobial available for effect over time) and antimicrobial drug effects. The exposure of the drug is dependent on the pharmacokinetic properties of the drug and the dosing regimen.

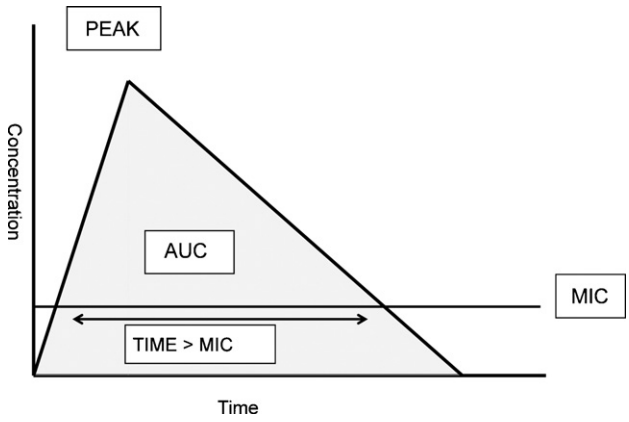

Fig. 2. Concentration–time curve showing the pharmacokinetic parameters peak (or C_{max}) and area under the time–concentration curve (AUC) (shaded area) and the pharmacodynamic index $T_{>MIC}$.

Exposure–response relationships have been studied in various *in vitro* experimental systems as well as in other hosts than humans. The primary purpose of these studies is to determine the exposures resulting in certain effects and subsequently deduce the optimal exposures needed for cure. Typically, this relationship is studied in animal systems where the neutropenic thigh model and pneumonia model in mice are the ones most commonly used. In these models, mice are rendered neutropenic and commonly infected with a specified inoculum of 10^6 bacteria in the thigh or lung. Treatment is then initiated and after 24 or 48 h the total bacterial count is determined for each organ. Using different doses and dosing intervals, ranges of exposure are obtained and subsequently plotted to the number of colony forming units (CFU) yielding exposure–response relationships. An example is depicted in Fig. 3, which shows the effect of different doses of levofloxacin in neutropenic mice with a pneumococcal lung infection (Scaglione et al., 2003). It is apparent that for relatively low AUCs virtually no effect is observed (outgrowth of bacteria to 10^8 CFU), whereas for high values there is a significant effect (decline to 10^2 CFU). The relationship can be described by a sigmoid curve. Notably, since the drug pharmacokinetics in mice differ from those in humans, the dose-response relationships will be markedly different, whereas the exposure–response relationships will be similar. The latter has been demonstrated in a number of studies and summarized recently (Ambrose et al., 2007). Ambrose and colleagues showed that exposures required for certain responses in preclinical models correlated well with exposures required for cure in humans.

However, except for a few early investigators, it was not fully appreciated until two decades ago that it is not only the total daily

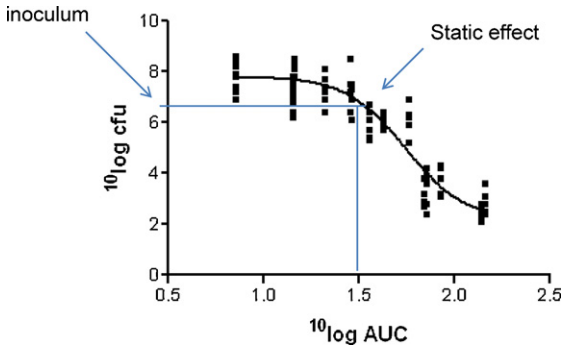

Fig. 3. Exposure (area under the time–concentration curve, AUC)–response (colony-forming units, CFU) relationship of levofloxacin and *S. pneumoniae*. Vertical line indicates the AUC required for a static effect, i.e. no net change in CFU after 24 h of treatment. After data in Scaglione et al. (2003).

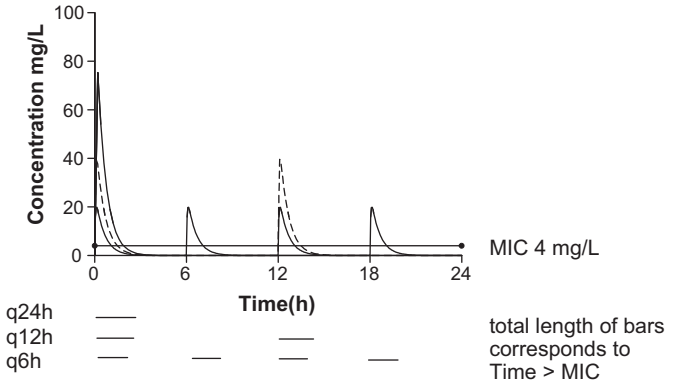

Fig. 4. Diagram showing that the $T_{>MIC}$ increases while the AUC remains the same if daily doses are divided. The length of the bars beneath the figure corresponds to the $T_{>MIC}$. For some antimicrobials (e.g., beta-lactam antibiotics), it is the $T_{>MIC}$ that is primarily correlated to effect.

dose, and thus 24-h exposure that determines outcome, but also the frequency of dosing (Fig. 4) (Eagle et al., 1950; Leggett et al., 1989). Whereas for most classes of drugs the AUC is correlated to effect, it has been shown that the efficacy of beta-lactam antibiotics is more dependent on the time the concentration of the antimicrobial remains above the minimal inhibitory concentration ($T_{>MIC}$) of the microorganism than on the AUC (Craig, 1998). Consequently, it is both exposure itself and the shape of the concentration–time curve, and thereby the frequency of dosing, that determine outcome. A more extended description of these relationships can be found elsewhere (Craig, 1998; Drusano, 2004).

2.2. Effects of the minimal inhibitory concentration (MIC)

As stated above and indicated in Fig. 1, the efficacy of an antimicrobial is dependent on exposure as well as its potency against the microorganism. The potency is usually expressed as a MIC. For antimicrobials in which the effect is AUC-dependent, there is a relationship between exposure, MIC and response in the sense that the response is dependent on the ratio between exposure and potency, or AUC/MIC. Fig. 5 shows the survival of four groups of rats infected with isogenic *Pseudomonas* strains with different MIC values and treated with varying doses of the quinolone lomefloxacin (Drusano, 2004; Drusano et al., 1993). The two groups with different MIC and AUC values (expressed as dose) but the same AUC/MIC ratio display the same response.

This principle is further demonstrated by a patient study illustrated in Fig. 6 (Rodriguez-Tudela et al., 2007). The figure shows the probability of cure in 132 patients with oropharyngeal *candidiasis*. For each patient, an estimate was made of the AUC based on the dose received (doses varied between 50 and 400 mg). The MIC of the *Candida* strain was also determined. The AUC/MIC ratio was determined for each patient, resulting in seven groups of similar AUC/MIC values. The proportion of patients cured was then determined for each AUC/MIC group and plotted against the AUC/MIC ratio. It is apparent that for relatively low AUC/MIC ratios virtually no effect is observed, whereas the probability of cure for high ratios approaches 100%. The shape of the curve follows the typical sigmoid response pattern. Again, it has to be emphasized that there is a range of AUC and MIC values, but it is the ratio between them that determines the outcome. Thus, if a certain exposure required for a certain effect was known or established, the MICs that could be covered by that exposure can be derived from the relationship between AUC/MIC ratio and effect, and vice versa. Optimal dosing then follows from the pharmacokinetics of the drug (which vary from individual to individual)

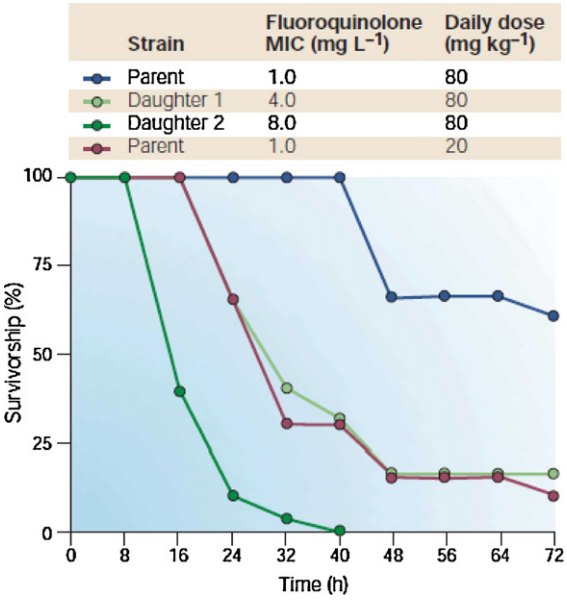

Fig. 5. Survival of four groups ($n = 20$) of neutropenic rats infected with 10^9 colony-forming units (CFU) per ml of three isogenic strains of *P. aeruginosa* treated with a fluoroquinolone (lomefloxacin) showing that survival of the groups (parent and daughter 1) with same AUC/MIC (AUC expressed as dose) ratio are similar. From Drusano (2004). Reproduced with permission from the publisher.

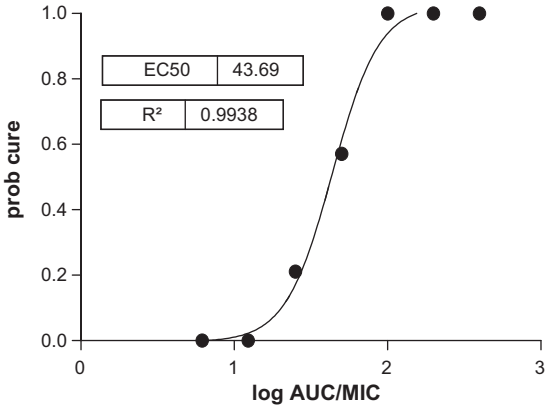

Fig. 6. Exposure–response relationship of fluconazole in patients with *oropharyngeal candidiasis* (Rodriguez-Tudela et al., 2007). Each data point represents the proportion of patients cured within a group representing a certain area under the time-concentration curve (AUC)/minimal inhibitory concentration (MIC) value. Reproduced with permission from the publisher.

and the MICs of the microorganisms (which vary from strain to strain).

3. Dose finding for new and old antibiotics

The classical phases of development of drugs involve phase 1, 2 and 3 studies before registration and phase 4 after registration or marketing authorization. Briefly, phase 1 studies involve escalating doses to establish the pharmacokinetic properties of the drug and detect possible toxic properties and other side effects. Phase 2 mainly focuses on confirming appropriate dosing regimens while in phase 3 the efficacy of the drug is established in comparative clinical trials. Phase 4 includes post-marketing surveillance, mainly to uncover side effects that are relatively uncommon and could therefore not have been detected in the earlier phases involving only a limited number of patients. Antimicrobials are different from other drugs because the final receptor is situated in the microbe and the

effect of the drug, and thereby the exposure–response relationships, involve the microorganism rather than the effect on human physiology. This allows the exposure–response relationships to be studied to optimize the dose for new and old drugs in experimental systems. Translating these relationships to treatment of infections in humans may then provide a tool to change the way antimicrobials are evaluated and approved.

3.1. Dosing regimen determination for new antibiotics

Because PK/PD describe exposure–response relationships, it follows that the response could be predicted and the optimal exposure for cure designed. The pharmacokinetics of the drug allow the derivation of a dosing regimen that should result in the desired exposure and is increasingly being used in antimicrobial drug development. It allows a rational choice to be made between drug candidates and supports determination of doses and exposures in phase 3 studies. This process involves several steps, starting with a description of the exposure–response relationship. As has been argued, this can be done in animals and in *in vitro* studies. From the results of these studies the target exposures needed for the microorganisms in question can be readily derived. The pharmacokinetic characteristics of the drug follow from the phase 1 studies and can be used to determine the required doses to achieve the desired exposure. An important issue here is the variation of pharmacokinetic profiles in the patient population. When a certain PK/PD target index (e.g., AUC/MIC ratio) is desired for every individual within the population, this should be true not only for the population mean but also for the part of the population with a higher elimination rate and thus lower than average exposure. To that end, Drusano et al. suggested an integrated approach of population pharmacokinetics and microbiological susceptibility information by applying Monte Carlo simulations (Drusano et al., 2000, 2001). This method takes the variability of the input variables into account and generates slightly different pharmacokinetic parameter values concordant with the variation in the population (Bonate, 2001). Thus, PK/PD index values are generated for both the population mean and every possible individual in the population. The population distribution of these index values is then used to estimate the doses needed to determine optimal exposures in the population, including those individuals with a high elimination rate. This approach has been used by several authors (Ambrose and Grasela, 2000; Bhavnani et al., 2005; Mouton et al., 2004), including for setting and evaluating clinical breakpoints, as well as establishing doses in phase 2 and 3 trials (Ambrose, 2006; Mouton, 2003). In conclusion, dosing regimens in phase 2 and 3 trials should ideally be based on preclinical PK/PD studies indicating potential pharmacodynamic targets that ascertain a high probability of microbiological cure. The data from phase 1 pharmacokinetic studies indicate the exposure of the antimicrobial after administration of the drug to humans. Thus, the extent of studies in humans to determine dosing regimens (phase 2) and large comparative trials (phase 3) could be reconsidered. Presently, many clinical trials are labeled phase 2/3 and carried out as comparative trials. We should use these studies to confirm the predicted efficacy based on PK/PD while simultaneously getting a reasonable indication of major safety concerns. Side effects that occur at a relatively low frequency need to be uncovered by exposure to (far) more patients than would be possible before market authorization and postmarketing surveillance would be more suitable to that purpose. These comparative trials also need to demonstrate that the antimicrobial effects of treatment by the new agent are not inferior to agents already available and using PK/PD tools may be more suitable to that end. Finally, PK/PD should predict the effect for less susceptible microorganisms. This approach will ultimately pose less risk to patients, increase the probability of effectiveness, determine a dosing regimen optimal for patient care and be less likely to result in resistance development (see below).

3.1.1. Suggested priorities

- During drug development and approval processes
  - Use PK/PD principles and tools when developing dosing regimens for clinical trials and setting breakpoints.
  - Develop methods using PK/PD to increase the power of comparative trials and (thereby) reduce the number of subjects in the studies.
  - Expand post-marketing surveillance (phase 4) to increase detection of adverse effects.

3.2. Dosing regimen determination for old antibiotics

In the past, antimicrobial agents were developed more on a trial and error basis and many were licensed before controlled clinical trials became mandatory (Podolsky, 2010). Accordingly, for these drugs, the information to optimize dosing regimens using exposure–response relationships is not readily available, if at all, and it is unclear whether the current dosing regimens used are optimal or even efficacious. Even if comparative trials were performed in the past to determine whether one antibiotic was non-inferior or superior to another, the dosing regimens are often changed over time. These changes in dosing regimens pose a significant problem because old off-patent antibiotics are increasingly being prescribed to patients now that emerging resistance creates an increasing challenge in antimicrobial treatment of Gram-negative bacteria in particular. In many cases microorganisms are now fully resistant to commonly used drugs and some of these old agents are used as a last resort. Examples include extended-spectrum-beta-lactamase (ESBL) producers, and recently, KPC (*Klebsiella pneumoniae* carbapenemases) or NDM-1 (New Delhi Metallo-beta-lactamase) producers (Hammerum et al., 2010). Old drugs, such as colistin and fosfomycin, must then be used without any certainty that the correct dosing regimens are being applied (Lim et al., 2010). A re-evaluation of these drugs is urgently needed, including establishing PK/PD relationships and the optimal dose.

3.2.1. Suggested priorities

- Obtain exposure–response relationships for old antimicrobials.
- Develop criteria to re-evaluate approval and indication of all antimicrobials presently available, prioritizing those older agents required for the management of multiresistant organisms.
- Establish a mandatory process to re-evaluate indications and dosing regimens of antimicrobials. Market authorization should be awarded for a limited time period (e.g. 5 years) instead of granting unlimited duration.

4. Exposure–response relationships and emergence of resistance

In the previous section a quantitative description was given regarding the relationship between exposure intensity (e.g., the AUC/MIC ratio) and efficacy. An important characteristic of this relationship is that it is sigmoid and monotonic (Figs. 3 and 6). That is, at very low values of exposure intensity, there is no measurable effect, whereas at larger values, the greater the exposure intensity, the greater the bactericidal effect up to a maximal value. For suppression of resistance selection during treatment, this is *absolutely* not the case. Here, the relationship between exposure and resistance selection is distinctly non-monotonic and has the shape of an inverted "U". Tam and colleagues demonstrated this relationship

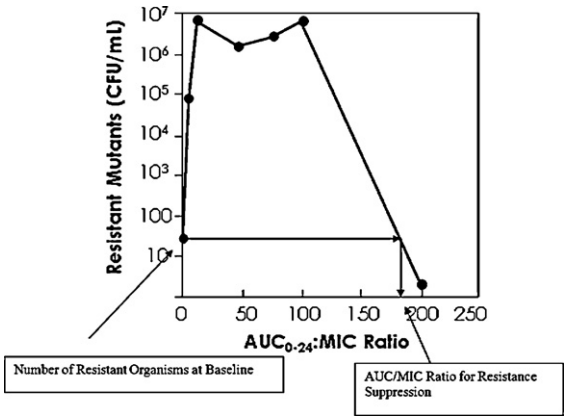

Fig. 7. Changes in a quinolone-less susceptible subpopulation as a function of the area under the time–concentration curve (AUC)/minimal inhibitory concentration (MIC) ratio (Tam et al., 2007b). Reproduced with permission from the publisher.

for several strains of bacteria in a mouse thigh model and also in a hollow fiber infection model (HFIM), where the effect of a quinolone against *P. aeruginosa* was investigated (Tam et al., 2005, 2007b). At the end of the experiment, the size of the resistant subpopulation was plotted against the AUC/MIC ratio. The first data point is the number of resistant colonies at baseline before therapy initiation (Fig. 7). As can be seen in the figure, even small exposures cause considerable amplification of the resistant subpopulation. Ultimately, a maximal value is attained after which increased exposure causes a decline in the number of resistant colonies towards baseline. The horizontal line in Fig. 7 demonstrates the regimen intensity required to return the number of resistant colonies to baseline (AUC/MIC ratio circa 190). Other investigators have found similar relationships (Firsov et al., 2003; Stearne et al., 2007). The markedly increased intensity required for resistance suppression compared with the exposure required for efficacy is important. Until now, most dosing regimens have been optimized for efficacy, but the shape of the curve in Fig. 7 indicates that the values required for efficacy may amplify resistant subpopulations. Thus, it is important to identify an exposure (and thus dose) that suppresses resistance as well as provides a good bactericidal effect.

Whereas the general relationship between exposure and emergence of resistance can be described by an inverted U-shaped pattern, there are three factors that generally have an impact on the value of the maximum and exact shape of the curve: The first is the number of bacteria present or the inoculum size in experimental settings. The second is the duration of therapy and the third is the presence and activity of an immune system.

4.1. Inoculum size

Jumbe et al. (2003) examined the effect of levofloxacin against *P. aeruginosa* in a granulocyte-replete mouse thigh infection model. They first demonstrated (Fig. 8) that there was a relationship between regimen intensity (indexed to AUC/MIC ratio) and the ability to kill microorganisms at the primary infection site. Subsequently, they showed that this relationship was markedly affected by the initial inoculum size (Fig. 8: panel a vs. panel b). In panel a, the challenge was 10^6 bacteria and in panel b 10^7 bacteria. The established mutation frequency was lower than 1 in 10^6 and higher than 1 in 10^7 for the strain used. After a 2-h lag, therapy was initiated. The difference in the size of the inoculum resulted in a 2–5-fold difference in the exposure intensity required to attain the same antibacterial effect. This difference occurs because in panel a there is a single susceptible population, whereas in panel b there are two populations, a susceptible one and a less susceptible one

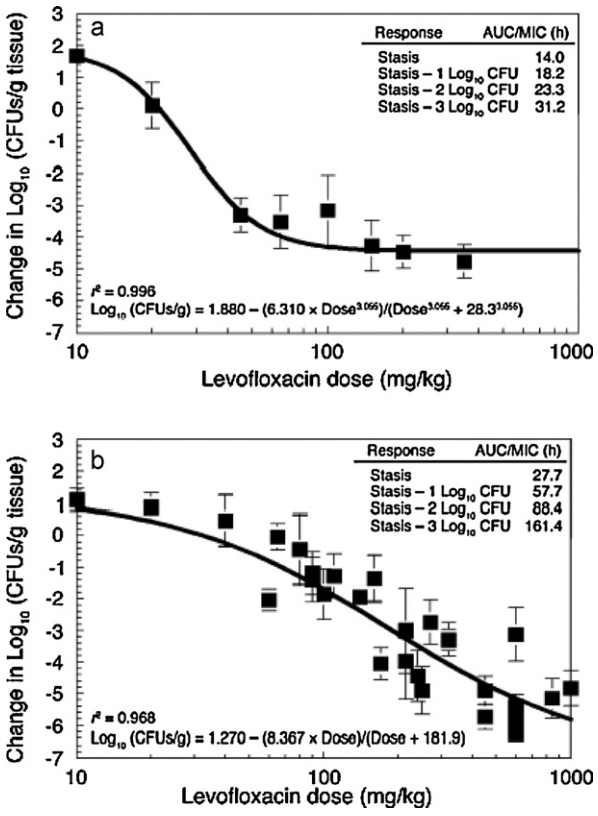

Fig. 8. (a) Exposure–response of *Pseudomonas aeruginosa* bacterial cell kill as a function of levofloxacin exposure. A challenge of 10^6 colony-forming units (CFU) was employed. (b) Exposure–response of *P. aeruginosa* bacterial cell kill as a function of levofloxacin exposure. A challenge of 10^7 CFU was employed.
From Jumbe et al. (2003). Reproduced with permission from the publisher.

(the resistant mutants). The latter population (i.e. the less susceptible one) responds less well to antimicrobial therapy. Jumbe et al. (2003) also employed a complex mathematical model to analyze all the data simultaneously, calculating the exposure necessary to suppress resistance emergence from the model parameters. In a prospective evaluation two regimens were studied: one predicted to amplify resistant subpopulations and one predicted to suppress resistant subpopulations. The total population and resistant subpopulation are displayed in Fig. 9 together with their response to the two regimens (panels a and b). The lines are prospective prediction lines rather than best-fit lines. Clearly, the regimens behaved exactly as predicted and indicate that the degree of exposure – here expressed as an AUC/MIC ratio – is a tool that we may employ to help suppress resistance emergence. It is critical to apply this insight to our currently available drugs to prolong their useful lifespan. It is, perhaps, even more imperative to apply this principle to new drugs currently under development in order to slow down the cycle of drug development/resistance emergence. However, it is important to note that this is just one example; relationships may be different for different classes of drugs and the mutation frequency is variable.

4.2. Duration of therapy

Another simple principle is that the longer therapy continues, the more difficult it is to suppress amplification of a resistant subpopulation. A regimen that only lasts for 4–5 days may provide good bactericidal effect and be adequate to minimize amplification of a resistant mutant subpopulation. However, extending that regimen to 10 days may cause therapy failure by resistance emer-

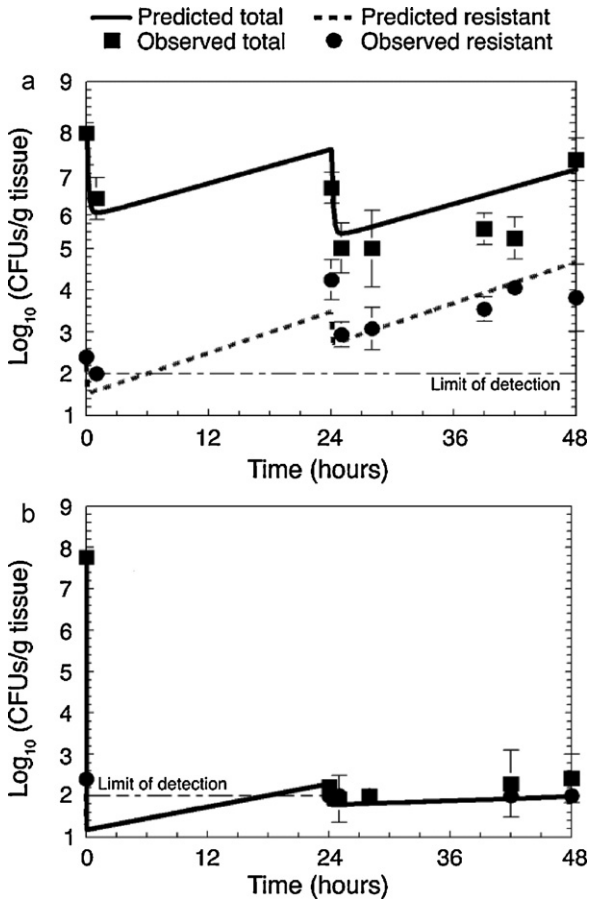

Fig. 9. Prospectively predicted (lines) and observed responses of *Pseudomonas aeruginosa* total population (solid line and squares) and levofloxacin-less susceptible population (dashed line and circles). Panel a: a regimen designed to amplify the less susceptible population (area under the time–concentration curve (AUC)/minimal inhibitory concentration (MIC) ratio = 53). Panel b: a regimen designed to suppress the less susceptible population amplification (AUC/MIC ratio = 157).
From Jumbe et al. (2003). Reproduced with permission from the publisher.

gence if not all bacteria were killed. Tam et al. (2007a) examined the effect of garenoxacin against *S. aureus*. The authors evaluated two regimens: based on extensive mathematical modeling; one regimen with an AUC/MIC ratio of 100 and one with a ratio of 280. The lower intensity regimen was chosen to suppress resistance amplification for 4–5 days while the more intense regimen was chosen to suppress resistance amplification for a full 10 days. Of interest, the regimens were predicted to provide the same maximal kill rate for 4–5 days. The result is shown in Fig. 10, panels a and b. In panel a, the total population is displayed. As prospectively predicted from the mathematical model, both regimens have exactly the same 5-day kill rate. After this period, however, the less intense regimen ceases to be effective. In panel b, we can see that this failure is due to amplification of the resistant subpopulation. If therapy had been ended at day 4 or 5, little or no resistant mutant amplification would have occurred. This point was proven in a subsequent publication by Drusano et al. (2009a) where the behavior of the susceptible and resistant populations was studied after the drug pressure had been withdrawn. Briefly, the susceptible population took over and demonstrated that regimens should be very intense to obtain maximal bactericidal effect and to suppress resistance. In addition, regimens should be as short as possible in order to maximally suppress resistance

4.3. Effect of the immune system

The effect of exposure on emergence of resistance has also been studied in the HFIM. In this model, no immune system exists and microorganisms will re-grow in the absence of antibiotic pressure unless the whole population is eradicated. In contrast, in real clinical life most patients have a functional immune system; in particular, patients have granulocytes that contribute to bacterial kill. Drusano et al. (2010) recently demonstrated that, depending on the species, granulocytes can eradicate microorganisms up to about 10^5–10^6 CFU/g. For *S. aureus* and *P. aeruginosa*, granulocytes can kill up to 50 (CFU/g) per day. Consequently, if the antimicrobial treatment drives the total population of the organism down to around 10^2–10^3 CFU, as was done in Fig. 10, it is highly likely that terminating therapy after 5 days will allow the residual population to be eradicated by the immune system with minimal amplification of resistant mutants. Retaining a functional immune system is consequently instrumental in reducing emergence of resistance.

4.3.1. Suggested priorities

- Promote strategies for early treatment to reduce the increment of the infectious microorganism and maximize the antibacterial effect.
- Prevent underdosing to suppress or decrease the potential amplification of resistant mutant subpopulations.

5. Modifying exposure–response relationships to prevent emergence of resistance

Resistant bacteria may emerge during therapy and from a clinical perspective, despite the doubtless benefits of antimicrobial agents, their intense use over the years has resulted in selection for resistance against these compounds in bacterial populations (Sykes, 2010).

It is widely accepted that once a bacterial population becomes resistant, either by mutation or by acquisition of resistance genes, it tends to persist. Resistance may be spread to or amplified in different bacterial populations, including those in nosocomial and community settings. In addition, resistance genes may be transferred to other susceptible populations (Livermore, 2005). Resistant organisms may accumulate several mechanisms of resistance, creating multi-resistant, extensive resistant or pan-drug resistant organisms for which few or no antimicrobials are currently available (Souli et al., 2008). Some of these organisms have become epidemic even in the community, where selective pressure may theoretically be lower.

From the sections above, it is obvious that a relationship exists between the pattern of exposure and emergence of resistance. Even more important, this relationship has also been described quantitatively and therefore provides the possibility to design dosing regimens that prevent or at least decrease the probability of resistance emergence or spread. These designs are based on hypothetical dosing regimens leading to a decrease in resistance emergence while retaining activity and have also been verified to actually work. Since the relationship between emergence and resistance follows an inverted U-shape as discussed in Section 4, it follows that there are two basic strategies; the first being a decrease and the second an increase in exposure. Unfortunately, neither of these strategies is applied as much as one would wish for, particularly in reducing exposure. Indeed, problems with the irrational use and of antibiotics and thereby unnecessary overexposure have been widely described (Gyssens, 2001; Harbarth and Samore, 2005).

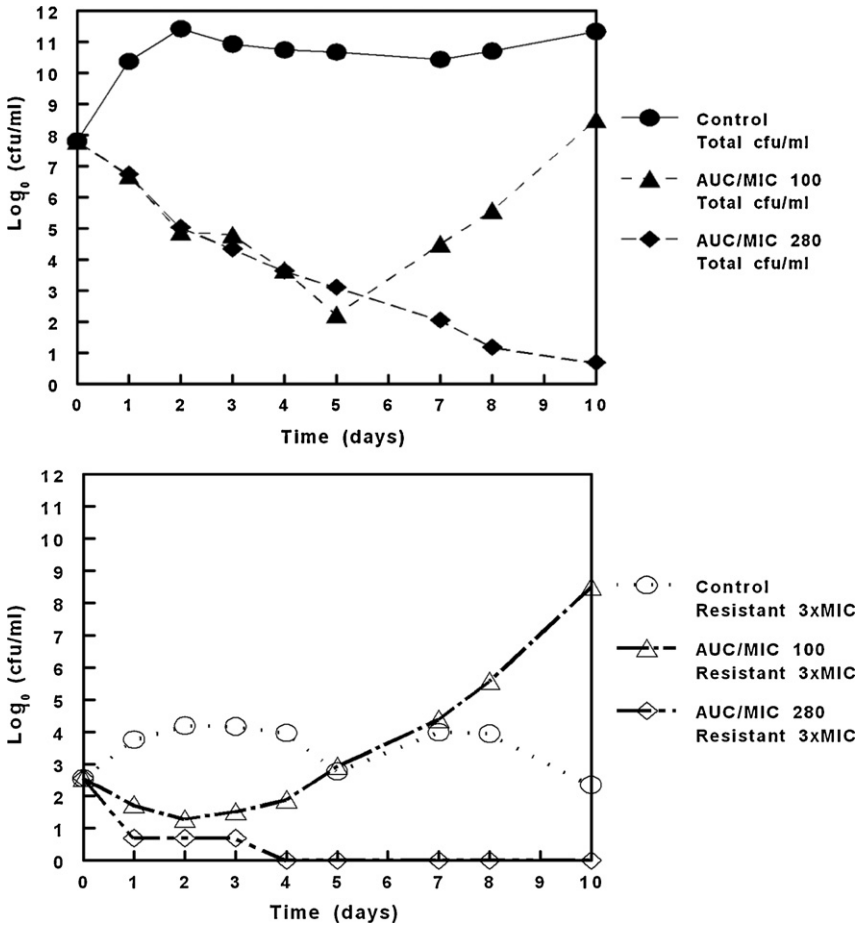

Fig. 10. (a) Impact on the total population of *Staphylococcus aureus* over time by two regimens of garenoxacin. (b) Impact on the less-susceptible population of *S. aureus* over time by two regimens of garenoxacin.
From Tam et al. (2007a). Reproduced with permission from the publisher.

5.1. Reducing exposure by reducing the duration of therapy and prophylaxis

In addition to reducing the unnecessary use of antibiotics, one of the simplest and most effective ways to reduce exposure is to shorten courses of antibiotic treatment and prophylaxis. With rare exceptions (e.g. bacteremia due to *S. aureus,* endocarditis, osteomyelitis), there is no evidence to support most of the traditional 10–14-day courses of antibiotics, which are based more on conventional wisdom than strong evidence. Short-course therapy for urinary tract infection, acute otitis media, tonsillopharyngitis, sinusitis and pneumonia is slowly gaining support (MASTIN study group, 2002; Lutters and Vogt, 2002). The short course paradigm is, in principle, widely extensible to the broad range of antibiotic prescriptions (including perioperative antibiotic prophylaxis) used by surgeons in both hospital and ambulatory settings. However, there is an ongoing problem of unnecessary prolongation of perioperative antibiotic prophylaxis (Bratzler et al., 2005; Dettenkofer et al., 2002; Huskins et al., 2001) despite extensive evidence arguing in favor of short course or even single dose administration (Saxer et al., 2009). A large cohort study demonstrated that extended antibiotic prophylaxis after cardiovascular surgery did not decrease the risk of surgical site infection but instead increased the risk of carriage of antibiotic-resistant pathogens (Harbarth et al., 2000).

An important barrier to rational antibiotic use and decreased treatment duration is the lack of efficient and affordable diagnostic tools with high sensitivity and specificity to distinguish bacterial from viral diseases. Few biomarkers are available to guide antibiotic treatment and duration decisions. Procalcitonin is the best studied of these markers and in several controlled clinical studies, it has shown to be useful in reducing inappropriate use of antibiotics and guiding duration of treatment (Christ-Crain et al., 2006; Harbarth et al., 2009). The need for development of new diagnostic tools is discussed in greater detail in another article in this issue (Okeke et al., 2011).

Among available strategies to decrease antibiotic usage, reductions in duration of antimicrobial treatment are the safest and likely to be the most palatable to practicing clinicians (Rice, 2008). More studies are needed, however, to define minimal lengths and maximal doses of therapy to ensure that efforts at reduced use are safe and effective.

5.1.1. Suggested priorities

- Implementation of short-course therapies based on both preclinical data (Section 4) and available evidence from prospective studies.
- Implementation of perioperative antibiotic prophylaxis guidelines.
- Development and use of diagnostic tools to reduce inappropriate use of antibiotics and length of therapy.
- Randomized controlled studies to define the optimal duration of therapy.

5.2. Reducing exposure by cycling and sequential therapy

Interventions targeted at reducing selection pressure via scheduled repetitive cycling of different classes of antibiotics on wards or in institutions have been pursued in attempts to control the emer-

gence of antibacterial resistance locally. Typical cycling protocols use periods of one to several months. The theoretical benefit of cycling primarily rests on the assumption that resistance affects only single antibiotics or antibiotic classes and that resistant bacteria are less fit and will have a growth disadvantage upon withdrawal of the selective antibiotic pressure. Resistance should then decrease during periods of non-exposure, which would justify cycling protocols. However, reported clinical effects of cycling remain inconclusive for two primary reasons: methodological flaws undermine published intervention trials (Brown and Nathwani, 2005; Nijssen et al., 2006) and, far more troubling, the evolution of bacterial multidrug resistance in health care settings has outpaced our assumptions.

Many resistant bacteria are commonly not less fit with compensation of fitness through additional mutations (Schulz zur Wiesch et al., 2010). Consequently, it should be no surprise that a decline in resistance has not been observed in response to reduced usage through the cycling periods. Furthermore, in locations with a high prevalence of multidrug resistance, unspecific resistance mechanisms (e.g. up-regulation of efflux systems) cause co-selection pressure for different classes of antibiotics affected by the same efflux system (O'Fallon et al., 2009). Any antibiotic classes or other compounds that are substrates of the efflux system (such as triclosan) maintain selection pressure during cycling periods (Chuanchuen et al., 2001). Because *P. aeruginosa* has several efflux pumps, it is a typical example of a pathogen that may not be affected by cycling (Tsukayama et al., 2004). Mobile genetic elements, which carry several unrelated resistance determinants and have been noted with increasing frequency, also contribute to co-resistance. The linkage of ESBL/carbapenemases, aminoglycoside modifying enzymes and quinolone resistance genes on transferable mobile genetic elements in enterobacteria and *Acinetobacter* is specifically relevant and frequent (Mak et al., 2009; Miro et al., 2010; Mooij et al., 2009; Vinue et al., 2010). It has been suggested and confirmed from clinical experience that such Gram-negative bacteria may not respond to cycling strategies (Raineri et al., 2010).

Cycling exposes patients to high homogenous selection pressure against employed antibiotics, potentially extending to other classes with associated co-resistances. Thus, cycling selection pressure possibly promotes the development of resistance within short periods facilitating outbreaks of multidrug resistant bacteria (Damas et al., 2006; Hedrick et al., 2008; Meyer et al., 2009; Nijssen et al., 2010; van Loon et al., 2005). Additionally, mathematical modeling corroborates the limited success reported thus far from clinical trials of antimicrobial cycling (Bergstrom et al., 2004). In summary, there is little evidence, empirical or theoretical, that cycling of homogeneous antibiotic exposure controls the emergence and spread of antibiotic resistance (Kollef, 2006; Sandiumenge et al., 2006; van Loon et al., 2005). Therefore, this intervention should not be implemented as a routine standard protocol (Brown and Nathwani, 2005; Levin and Bonten, 2004).

On a patient level, sequential use of antibiotics and its impact on the emergence of resistance remains poorly described. Because chronic and recurrent infections carry a high risk of emergence of resistance, cycling or sequential usage of different antibiotic classes may influence resistance. With the exception of *Helicobacter pylori* infections (Gisbert et al., 2010), no available clinical studies provide corroborating evidence. In addition, mathematical modeling also fails to support such regimens (D'Agata et al., 2008). IV-oral step-down therapy and de-escalation principles with a change to a reduced-spectrum antibiotic based on microbiological results are widely recommended aspects of antibiotic stewardship. Their effect on control of resistance emergence has yet to be quantified, however.

5.2.1. Suggested priorities

- Discourage the use of cycling schemes
- Perform further studies on the sequential use of antibiotics

5.3. Increasing exposure through combination therapy

The use of combinations of antimicrobial agents is common practice during clinical therapy, most notably for the treatment of severe infections and empirical therapy. The most accepted rationale for a combination antimicrobial therapy approach is an increase in the spectrum of coverage, even though current antimicrobials possess extremely broad activities. Assuming the pathogenic organism is susceptible to one antibiotic, the incremental benefit of combination therapy in the sense of synergistic activity is uncertain as evidenced by two recent meta-analyses (Paul et al., 2004; Safdar et al., 2004). In these studies, no significant difference in outcome was found between patients that received combination therapy vs. those that received monotherapy, except perhaps for infections caused by *P. aeruginosa*. One of the reasons that no significant difference in outcome was observed between the groups receiving monotherapy and combination therapy might have been diversity of patients and indications. Indeed, two other recent studies did demonstrate superiority of combination therapy for specific patient groups. In one meta-analysis, Kumar et al. (2010a) did not find an overall benefit, but when stratified for mortality, the group that showed the highest mortality did significantly better with combination therapy. In another study from the same authors, early combination antibiotic therapy yielded improved survival compared with monotherapy in septic shock (Kumar et al., 2010b). These studies show that for severely ill patients or patients with *P. aeruginosa* infections, combination therapy could be warranted. This observation is in line with studies that have looked at the effect of combinations in *in vitro* pharmacokinetic models and animal studies (den Hollander et al., 1997; Louie et al., 2010; Mouton et al., 1999b). These studies also show that the effect of combination therapy may be dependent on the resistance mechanism, i.e. with a similar phenotype in terms of MIC, the effect of the combination can be beneficial (Drusano et al., 2009b).

A specific topic is the use of combination therapy to minimize the risk of emergence of resistance. This has been demonstrated for the treatment of patients with AIDS and patients with tuberculosis, although the optimal exposures of the individual drugs and combinations have yet to be established (Lienhardt and Davies, 2010). For these disease entities, treatment with monotherapy is regarded as obsolete and even dangerous. If then, for the treatment of 'common' bacterial infections the risk of emergence of resistance is increasing for various reasons, it seems prudent to treat these infections with combinations of antibiotics, not only to increase the probability of cure but more so to retain activity of the antimicrobials. This is particularly true for those microorganisms that are known to become resistant during treatment, such as *P. aeruginosa* and other non-fermenting bacteria, which are ubiquitous in nature. Although it is difficult to show this benefit in clinical trials, there are several preclinical studies that clearly indicate that combination therapy in some instances may prevent the emergence of resistance (Louie et al., 2010; Mouton, 1999a). Resistant mutants usually occur at fixed frequencies (range 10^{-9} to 10^{-10}). However, under certain circumstances, especially during chronic infections such as bronchopulmonary infections in cystic fibrosis or patients with chronic obstructive pulmonary disease (COPD), resistant mutants can emerge at higher frequencies. This means, that even at low numbers, these populations contain bacteria with hypermutator phenotypes. These phenotypes are caused by mutations in DNA repair or error avoidance systems (mainly the mismatch repair system) (Blazquez, 2003; Chopra et al., 2003). Consequently, the probability to accumulate mutations in resistance

genes is higher, an event that leads to the emergence of resistant mutants at higher frequencies. Although hypermutators initially have lower fitness than wild-type strains, compensatory mutations can stabilize these populations and several mutations can accumulate leading to resistance to different antimicrobials (Harrison and Buckling, 2005; Oliver et al., 2000). Different approaches have been proposed to control hypermutators, but it is still an area of basic research. To some extent, their impact can be diminished with combination therapy (Oliver, 2010; Plasencia et al., 2007).

Specific attention must be paid to the use of old antibiotics in combinations. As stated above, old antibiotics (such as colistin) have been reintroduced as last resort therapies. However, they are also used (or are promoted to be used) in combination treatment. However, efficacy of these antibiotics in combinations has not been studied systematically and therefore it remains unclear whether combinations provide a clear benefit in these cases.

5.3.1. Suggested priorities

- Use combination therapy for severely ill patients.
- Use combination therapy for specific indications (e.g., *Pseudomonas* infections).
- Increase research to show benefits for specific indications.

5.4. Speaking the same language – defining clinical susceptibility

One of the most important issues over the past decade in the discussion on appropriate treatment and emergence of resistance is the 'language of resistance'. There are two important concerns related to the same issue: The first pertains to the methods used for susceptibility testing and the second to the interpretation of the test itself. At present, there is no international standard for routine susceptibility testing in either the human or non-human context. Serious progress was made in 2006 when the International Organization for Standardization (ISO) published a reference standard for the susceptibility testing of rapidly growing aerobic bacteria (ISO, 2006). This method sets the benchmark for two widely used methods, namely the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In an accompanying guideline it is described how susceptibility testing methods should be calibrated against the ISO standard. Unfortunately, there are still methods used worldwide that have not undergone a rigorous comparison with the ISO standard. The world will benefit greatly when there is complete harmonization of methods that show good correlation with the reference standard.

Perhaps an even more pressing problem is that the interpretive criteria differ worldwide, which is especially evident between CLSI and EUCAST. There are several reasons for these differences, mainly historical ones, but the fact remains that strains may be called susceptible using CLSI criteria and resistant using EUCAST criteria. A similar situation existed within Europe until a few years ago, where every country applied its own breakpoints; even worse, many laboratories used breakpoints as they saw fit. Whereas a harmonization process is under way in Europe and European harmonized breakpoints are available for use, not all European countries and laboratories have implemented this process. As a consequence, resistance rates still differ in Europe in part because of the interpretation used and not because of real differences. Importantly, many breakpoints still in use are considered too high by present day (EUCAST) standards and detection of resistance is therefore hampered while at the same time strains are categorized as susceptible, although infections caused by these microorganisms cannot be treated adequately. Two prominent examples show that if old breakpoints that are too high are used and strains are classified as susceptible, the probability of a fatal outcome increases (Tam et al., 2008) (Bhat et al., 2007). It has to be emphasized that PK/PD relationships readily predicted the outcome in both these studies and can therefore be taken as a validation for the application of pharmacodynamic principles in setting breakpoints. Whereas dosing and indications use to differ, which account for differences in breakpoints in the past, differences tend to disappear with the globalization and the ready dissemination of medical knowledge. It would therefore seem appropriate in the near future to harmonize breakpoints worldwide. Such an objective will require a long process and careful thought must be given on how best to accomplish this goal. To begin with, a world committee on antimicrobial susceptibility testing would be needed to set up and describe the process to accomplish this objective.

5.4.1. Suggested priorities

- Set up a committee to examine the pathway to harmonize breakpoints worldwide
- Provide expert guidance for clinicians to better understand breakpoints

5.5. Speaking the same language – defining resistance

Clinicians are primarily interested in susceptibility testing regarding treatment, and clinical breakpoints are set with that goal in mind. In contrast, epidemiologists and others involved in early detection of resistance are more interested in emergence of resistance as a mechanism. The presence of a resistance mechanism does not always mean that the microorganism (or rather patient) cannot be treated: if exposures following adequate dosing are high enough with respect to the MIC of the microorganism causing the infection in such a way that a near maximum effect can be reached (see Section 2), there is no reason not to use that agent. Clinical breakpoints are used in clinical laboratories and constitute the basis of their reports because they are primarily focused on guiding therapy. However, clinical breakpoints are clearly not designed for early detection of resistance or detection of resistance mechanisms. This point was recognized by the EUCAST when reassessing breakpoints in Europe (Kahlmeter et al., 2006). The EUCAST has therefore, apart from clinical breakpoints, defined wild-type (WT) distributions of bacteria (and fungi) that delineate the MICs of naturally occurring bacteria. The upper end of the WT distribution is demarcated by the epidemiological cut-off value (ECV). It is specific for each species and thus separates microorganisms without (wild type) and with (non-wild type) acquired resistance mechanisms to the agent in question. A microorganism with a value higher than the ECV is suspected of harboring a resistance determinant and these values can be used to monitor resistance development. However, until now ECVs have not been used on a wide scale for that purpose.

5.5.1. Suggested priority

- Implement the use of the epidemiological cut-off value (ECV) on a wider scale.

6. Concluding remarks

Exposure–response relationships have changed the way we look at the efficacy of antimicrobials and have provided us with a tool to design evidence-based dosing regimens. Although there is still a great deal of exploring to do and discoveries to be made, the present state of knowledge is now such that it can serve as a firm base for policy changes and their implementation. In this review, we have attempted to provide a number of priorities that need and can be acted upon relatively fast. Some of these priorities include the establishment of a committee or other working party to prepare the necessary policy changes. This and the other reports in this issue of Drug Resistance Updates are the result and compilation of presentations and discussions during the ReAct conference "The

Global Need for Effective Antibiotics–moving towards concerted action" in Uppsala 2010. We strongly believe that these reports can serve as an excellent starting point for the work of the proposed committee or working party.

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Critical shortage of new antibiotics in development against multidrug-resistant bacteria—Time to react is now☆

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ABSTRACT

Two commercial databases (Pharmaprojects and Adis Insight R&D) were queried for antibacterial agents in clinical development. Particular attention was given to antibacterial agents for systemic administration. For each agent, reviewers were requested to indicate whether its spectrum of activity covered a set of selected multidrug-resistant bacteria, and whether it had a new mechanism of action or a new target. In addition, PubMed was searched for antibacterial agents in development that appeared in review articles. Out of 90 agents that were considered to fulfil the inclusion criteria for the analysis, 66 were new active substances. Fifteen of these could be systemically administered and were assessed as acting via a new or possibly new mechanism of action or on a new or possibly new target. Out of these, 12 agents were assessed as having documented *in vitro* activity against antibiotic-resistant Gram-positive bacteria and only four had documented *in vitro* activity against antibiotic-resistant Gram-negative bacteria. Of these four, two acted on new or possibly new targets and, crucially, none acted via new mechanisms of action. There is an urgent need to address the lack of effective treatments to meet the increasing public health burden caused by multidrug-resistant bacteria, in particular against Gram-negative bacteria.

1. Introduction

In recent years, several reports from the scientific community have raised concerns that antibacterial drug development will not adequately address the problems posed by antibiotic resistance among important bacterial pathogens (Boucher et al., 2009; Bradley et al., 2007; Cars et al., 2008; IDSA, 2004; Nathan, 2004; Norrby et al., 2005; Spellberg et al., 2004; Tickell, 2005). In its First European Communicable Disease Epidemiological Report, the European Centre for Disease Prevention and Control (ECDC) rated antimicrobial resistance as one of the most important infectious disease threats in Europe because of the increase in infections due multidrug-resistant bacteria in Europe (Amato-Gauci and Ammon, 2007). The recent emergence, in European hospitals and globally, of bacteria that are totally, or almost totally, resistant to currently available antibiotics is even more threatening since treatment options for infected patients are extremely limited (Lepape and Monnet, 2009; Nordmann et al., 2009; Souli et al., 2008). In a recent joint technical report, ECDC and the European Medicines Agency (EMA) in

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collaboration with Action on Antibiotic Resistance (ReAct) estimated that at least 25,000 patients die each year in the EU from an infection due to multidrug resistant bacteria (ECDC/EMEA, 2009). Antibiotic resistance is also a major public health issue in low and middle income countries. One study indicates that 70% of hospital-acquired neonatal infections could not be successfully treated by the regimen recommended by the World Health Organization (WHO) (Zaidi et al., 2005). A recent study in Tanzanian children confirmed that ineffective treatment of bloodstream infections due to antibiotic resistant bacteria predicted fatal outcome independently of underlying diseases (Blomberg et al., 2007). In that hospital-based study, crude mortality from bloodstream infections caused by Gram-negative bacteria was 43%. Reducing the consequences of antibiotic resistance requires a multifaceted approach, including rational use of existing antibacterial agents and control of the spread of resistant micro-organisms in hospitals and the community. Although these measures are essential to preserve the effectiveness of existing antibiotics, implementation has generally been weak and the prevalence of bacterial resistance, including multi-drug resistance, continues to increase. Development of new antibacterial agents with activity against multi-drug resistant bacteria is therefore perceived as a critical public health need.

In 2006, a think-tank group on Innovative Drug Development from the EMA's Committee on Human Medicinal Products (CHMP) was set up to allow EU regulators, industry and academia to discuss different aspects of drug development (EMA, 2007). Arising from this discussion, an ECDC-EMA Working Group was constituted in 2008 to carry this work forward. An important focus of their efforts was to assess the gap between the burden of disease imposed by multi-drug resistant bacteria and the development of new antibacterial agents. The aim of the present study was to provide, as accurately and as comprehensively as possible, an account of the status of the antibacterial drug development pipeline by documenting and characterising the activity of new agents that have entered clinical development. Particular attention was given to antibacterial agents for systemic administration.

2. Methods

2.1. Search strategy and selection criteria

2.1.1. Selection of databases

Identification of agents was a joint undertaking between the EMA and the Strategic Policy Unit of ReAct at Duke University (Durham, NC, USA). Three commercial databases were identified for the analysis of the research and development pipeline: Pharmaprojects (T&F Informa UK Limited, London, UK) (Pharmaprojects, 2008), Adis Insight R&D (Wolters Kluwer Health, Amsterdam, NL) (Adis, 2008) and BioPharm Insight (Infinata, Norwood, MA, USA) (BioPharm, 2008). A preliminary sensitivity analysis showed that using Pharmaprojects and Adis Insight R&D for antibacterial agents that had entered Phase II of clinical development resulted in a 10% yield increase in comparison to the use of one database only. The addition of the database BioPharm Insight did not result in any significant yield increase. As a result, Pharmaprojects and Adis Insight R&D were selected to identify antibacterial agents in clinical development.

2.1.2. Search strategy and selection of antibacterial agents

Pharmaprojects and Adis Insight R&D were searched using a data-lock point of 14 March 2008 for agents that had entered clinical development or for which an application had already been filed to at least one national regulatory agency. Agents that had reached clinical trials but were reported as suspended, i.e., put on hold, in accordance with Pharmaprojects' definition, were considered to still be under active development, and were therefore included in the study. However, agents with a status of "no development reported" or "discontinued" according to the databases' definitions were excluded from the study.

2.1.3. Combined dataset

The results produced by the database searches were matched by compound name, synonyms and originator in order to avoid duplicate entries and to eliminate inconsistencies (e.g., misclassifications) in the combined dataset. If discrepancies in the reported development phase of the agent were found between the databases, the most advanced registered phase was used. Where compounds were marked as "discontinued", "no development reported" or "suspended" in one of the databases, but not in the other, these were considered as still being under active development.

2.1.4. Literature search

PubMed was searched for antibacterial agents in development that appeared in review articles (identified as such by PubMed) published in English between and including January 2006 and January 2009, based on the terms listed in the box.

The search used the following Boolean combinations of Medical Subject Headings (MeSH) terms and also search terms previously described by Talbot et al. (2006):

Anti-Bacterial Agents/therapeutic use [Mesh] AND
Bacteria/drug effects [Mesh] AND
Bacterial Infections/drug therapy [Mesh] AND
Drug Resistance, Bacterial [Mesh]

OR

Anti-Bacterial Agents [Mesh] AND
Drugs, Investigational [Mesh] AND
Humans [Mesh] AND
anti-bacterial agents [Substance Name]

OR

antimicrobial drug development

OR

investigational antimicrobials

OR

novel antimicrobials

If an agent identified through the literature search had not been identified earlier during the database searches, this agent was added to the list for the final analysis, provided that it met the entry criteria.

2.2. Assessment strategy

2.2.1. Inclusion criteria

All chemical or biological agents that were identified by the searches and, to the knowledge of the ECDC-EMA Working Group, were not licensed anywhere in the world, were eligible for assessment if a direct antibacterial effect was documented. Vaccines, monoclonal antibodies and agents which had a mechanism of action involving only immuno-modulation, were excluded.

The selected agents were then assessed for their antibacterial spectrum and included in the analysis if they displayed activity against at least one of the chosen antibiotic-resistant bacteria. These bacteria were chosen because they represent indicators for multidrug resistance in bacteria that are among those most commonly isolated from blood cultures in Europe (Biedenbach et al., 2004):

- Methicillin-resistant *Staphylococcus aureus* (MRSA);
- Vancomycin-intermediate and vancomycin-resistant *S. aureus* (VISA/VRSA);
- Vancomycin-resistant enterococci (VRE);
- Penicillin-resistant *Streptococcus pneumoniae* (PRSP);
- Third-generation cephalosporin-resistant *Enterobacteriaceae*;
- Carbapenem-resistant *Enterobacteriaceae*;
- Carbapenem-resistant non-fermentative Gram-negative bacteria.

Agents that were being developed only to treat other bacteria not included in this list, e.g., agents that appeared to be under development only to treat tuberculosis or infections due to *Helicobacter pylori* or *Chlamydia trachomatis*, were excluded from the assessment.

2.2.2. Assessment procedure

Agents identified by the searches were divided into five batches and each batch was allocated to a team of two reviewers, including one from the ECDC-EMA Working Group and one external reviewer chosen for their experience in the field. Reviewers were unaware of the identity of their team counterparts. Each reviewer independently assessed their allotted list of agents and assigned an antibacterial spectrum of activity and a level of novelty to each agent following the methodology below. As a final step, all assessments were discussed in the ECDC-EMA Working Group in order to resolve possible discrepancies.

The two outcome parameters considered for the assessment were the spectrum of *in vitro* activity and novelty of the agent using the approaches and definitions given below. Reviewers based their assessment on information available in the two databases as well as any information that they could find in the public domain.

In vitro activity of each agent against the selected bacteria was assigned based on the following approaches:

(a) Data on *in vitro* activity was reviewed whenever available. For agents belonging to a known class where actual data on *in vitro* activity was not reported, assumptions on activity were made based on the properties of the known antibiotic class or of the mechanism of action involved.
(b) For agents from known classes, the assessment of *in vitro* activity disregarded any known potential for cross-resistance and co-resistance with other classes.
(c) When assessing *in vitro* activity, individual reviewers made a judgment based on MICs regarding the potential for the agent to be clinically active against the selected bacteria. It was decided not to take into account any pharmacokinetic data or pharmacokinetic/pharmacodynamic (PK/PD) analyses when scoring the potential antibacterial activity of the agents, since the amount of available data was very variable. However, if there was already information on non-clinical or clinical efficacy, these data were factored into the assessment.
(d) For formulations intended for topical administration or inhalation, the assessment took into account the possibility that very high local concentrations of the antibacterial agent might be achieved.

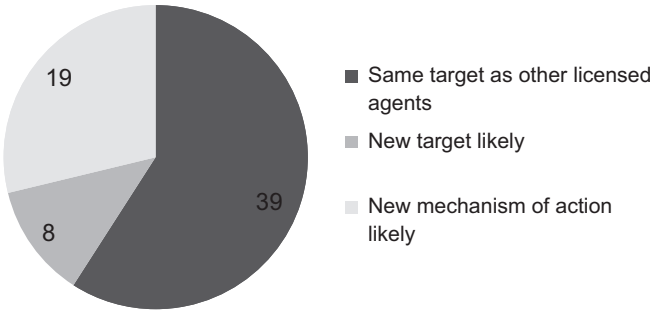


Fig. 1. Novelty of new antibacterial agents.

Novelty was rated according to the following criteria:

(a) Substance with a new mechanism of action known or very likely;
(b) Substance with a known mechanism of action that likely acts on a new target;
(c) Substance that acts on the same target as that of at least one previously licensed antibacterial agent.

3. Database searches

3.1. Overall findings from the database searches

In total, 167 agents were identified through search of the two selected databases and were examined by the reviewers. Only 90 of these agents were considered to fulfil the inclusion criteria for the analysis, of which 24 were new presentations of licensed antibacterial agents and 66 were new active substances.

Fig. 1 displays these 66 new active substances which, in a best-case *in vitro* activity scenario; i.e., based on actual as well as assumed *in vitro* activity based on class properties, could have activity against the selected bacteria.

3.2. Findings from the literature review

The literature search for information on antibacterial agents in development yielded 29 articles (Abbanat et al., 2008; Aliphas et al., 2006; Bishop and Howden, 2007; Boucher et al., 2009; Bush et al., 2007; Drew, 2007; Falagas and Karageorgopoulos, 2008; French, 2008; Korbila and Falagas, 2008; Kwa et al., 2008; Leeds et al., 2006; Lo et al., 2008; Lomovskaya et al., 2007; Mesaros et al., 2007; Moreillon, 2008; O'Neill, 2008; Page, 2007; Pan et al., 2008; Poulakou and Giamarellou, 2007; Projan and Bradford, 2007; Scheinfeld, 2007; Song, 2008; Talbot, 2008; Talbot et al., 2006; Theuretzbacher and Toney, 2006; Van Bambeke et al., 2007; Vergidis and Falagas, 2008; Vicente et al., 2006; Yang and Kerdel, 2006) that were considered relevant to the topic of antibacterial agents in development and were subsequently analysed. From these articles, the single additional agent that potentially fulfilled the study inclusion criteria was a novel efflux-pump inhibitor MP-601,205 (Lomovskaya et al., 2007). However, this agent does not possess any direct intrinsic antibacterial activity and, at the time of the data-lock point, no clinical study involving co-administration with an antibacterial agent had commenced. It was therefore excluded from the analysis.

3.3. Characteristics of the new active substances

Of the 66 new active substances, 30 were in Phase I of clinical development, 16 in Phase II, nine in Phase III, eight had been filed to a regulatory agency and three were reported to have been

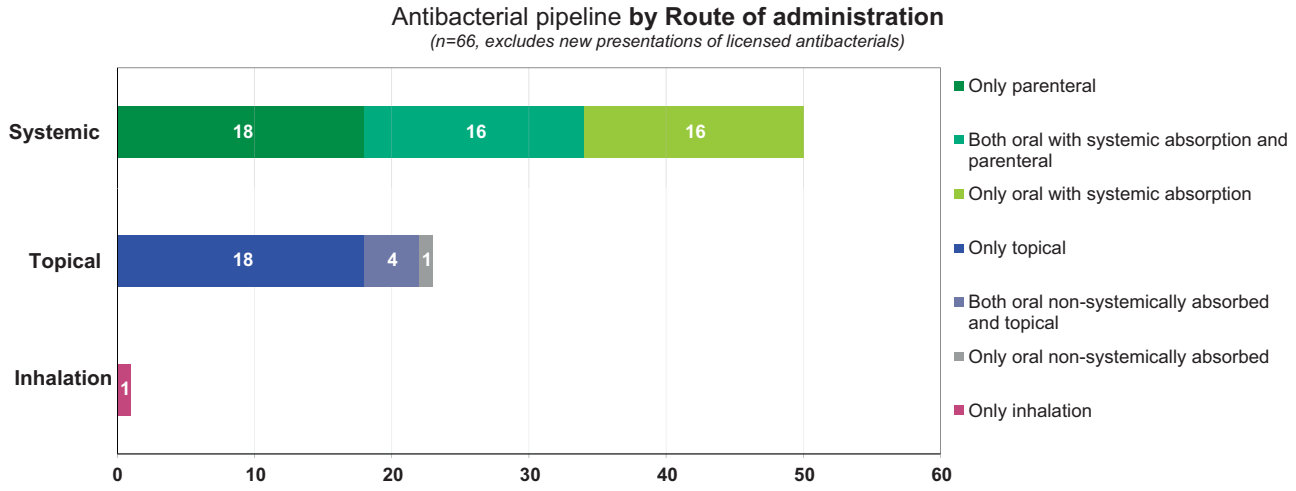


Fig. 2. Routes of administration of new antibacterial agents ($n = 66$, excludes new presentations of licensed antibacterials) Please note that some agents have several possible routes of administration.

suspended from further development. An analysis by route of administration (Fig. 2) showed that, at the time of the search, 50 of these 66 new active substances were formulated for systemic administration (16 for oral, 18 for parenteral, and the remainder for either oral or parenteral administration).

Twenty-seven of these 66 new active substances were assessed as having either a new mechanism of action or a new target. The remaining 39 agents belonged to known antibacterial classes or groups, i.e., quinolone (15), β-lactam (6 agents), oxazolidinone (3), diaminopyrimidine (2), macrolide (2), pleuromutilin (2), aminoglycoside (1), ansamycin (1), FabI inhibitor (1), glycopeptide (1), metallic ion (1), streptogramin (1), tetracycline (1) and hybrid (oxazolidinone/quinolone and rifamycin/fluoroquinolone) (2). They were thus assessed as acting on the same target as that of at least one previously licensed anti-bacterial agent, and hence not considered for the remainder of this analysis.

Of the 27 new active substances assessed as having a new mechanism of action or a new target, there were 15 agents which could be systemically administered (Table 1, Fig. 3) and thus considered potentially useful for the treatment of serious invasive infections. Of these 15 systemically administered agents, 13 were judged to have activity against at least one of the selected antibiotic-resistant Gram-positive bacteria and eight against at least one of the selected antibiotic-resistant Gram-negative bacteria. Among antibiotic-resistant Gram-positive bacteria, MRSA was the most often covered by these agents (13 out of 15) and VRE the least covered (5 out of 15). Of the eight agents with activity against antibiotic-resistant Gram-negative bacteria, four had an activity based on actual data and four had assumed activity based on known class properties or mechanisms of action. Of the four agents with activity against antibiotic-resistant Gram-negative bacteria based on actual data, two acted on new or possibly new targets and none via new mechanisms of action.

Table 1 presents the individual characteristics of all 15 antibacterial agents in Fig. 3. Out of these 15 agents, only seven had a new mechanism of action, of which six were antibacterial peptides or proteins as indicated in Fig. 3.

4. Discussion

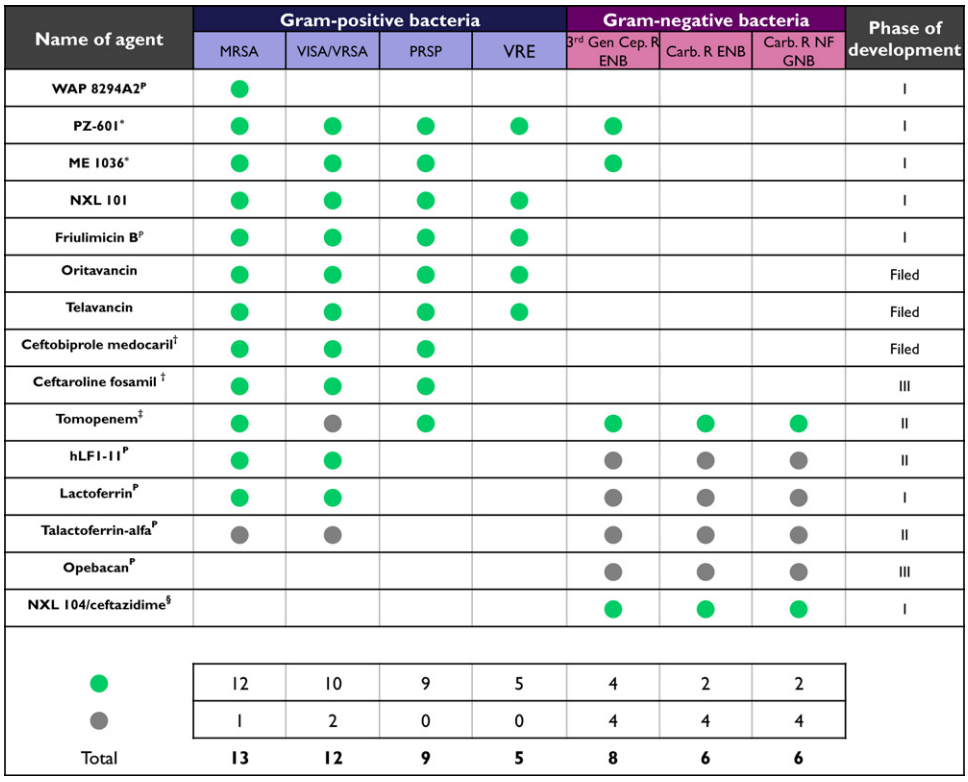
This study is believed to be the first review to compile publicly available information from commercial databases on antibacterial agents in clinical development and evaluate their novelty and potential use against antibiotic-resistant bacteria of public health interest.

We limited our study to agents in clinical development because these agents are the most likely to reach market within the next 5–10 years. A decision was made to take an optimistic approach to

Table 1
New systemic antibacterial agents with new target or new mechanism of action and *in vitro* activity based on actual data or assumed based on known class properties or mechanisms of action against the selected bacteria ($n = 15$, as of 14 March 2008).

Name of agent	Mechanism of action (MoA)	Degree of novelty	Route of administration[a]
WAP 8294A2[b]	Membrane integrity antagonist	New MoA	IV, Top
PZ-601	Cell wall synthesis inhibitor	New target	IV
ME 1036	Cell wall synthesis inhibitor	New target	IV
NXL 101	DNA gyrase inhibitor/DNA topoisomerase inhibitor	New MoA	IV, PO
Friulimicin B[b]	Cell wall synthesis inhibitor	New MoA	IV
Oritavancin	Cell wall synthesis inhibitorMembrane integrity antagonist	New target	IV, PO
Telavancin	Cell wall synthesis inhibitorMembrane integrity antagonist	New target	IV
Ceftobiprole medocaril	Cell wall synthesis inhibitor	New target	IV
Ceftaroline fosamil	Cell wall synthesis inhibitor	New target	IV
Tomopenem	Cell wall synthesis inhibitor	New target	IV
hLF1-11[b]	Chelating agent/immunomodulation	New MoA	IV, PO
Lactoferrin[b]	Chelating agent/immunomodulation	New MoA	IV, PO
Talactoferrin-alfa[b,c]	Chelating agent/immunomodulation	New MoA	PO, Top
Opebacan[b,c]	Membrane permeability enhancer/immunomodulation	New MoA	IV
NXL104/ceftazidime	β-Lactamase inhibitor + cell-wall synthesis inhibitor	New target	IV

[a] Information on routes of administration is uncertain in early drug development. IV, intravenous; PO, oral; Top, topical.
[b] Antibacterial substance of peptidic nature with a new mechanism of action known or very likely.
[c] Agents with only assumed *in vitro* activity.



Name of agent	Gram-positive bacteria				Gram-negative bacteria			Phase of development
	MRSA	VISA/VRSA	PRSP	VRE	3rd Gen Cep. R ENB	Carb. R ENB	Carb. R NF GNB	
WAP 8294A2[P]	●							I
PZ-601*	●	●	●	●	●			I
ME 1036*	●	●	●		●			I
NXL 101	●	●	●	●				I
Friulimicin B[P]	●	●	●	●				I
Oritavancin	●	●	●	●				Filed
Telavancin	●	●	●	●				Filed
Ceftobiprole medocaril[‡]	●	●	●					Filed
Ceftaroline fosamil [†]	●	●	●					III
Tomopenem[‡]	●	◉	●		●	●	●	II
hLF1-11[P]	●	●			◉	◉	◉	II
Lactoferrin[P]	●	●			◉	◉	◉	I
Talactoferrin-alfa[P]	◉	◉			◉	◉	◉	II
Opebacan[P]					◉	◉	◉	III
NXL 104/ceftazidime[§]					●	●	●	I
●	12	10	9	5	4	2	2	
◉	1	2	0	0	4	4	4	
Total	**13**	**12**	**9**	**5**	**8**	**6**	**6**	

Fig. 3. New systemic antibacterial agents with new target or new mechanism of action and *in vitro* activity against selected bacteria based on actual data or assumed *in vitro* activity. ●, Activity based on actual data. ◉, Assumed activity based on known class properties or mechanisms of action. 3rd Gen Cep. R ENB: third-generation cephalosporin-resistant *Enterobacteriaceae*; Carb. R ENB: carbapenem-resistant *Enterobacteriaceae*; Carb. R NF GNB: carbapenem-resistant non-fermentative Gram-negative bacilli. *Are no more active than earlier carbapenems against Gram-negative bacteria. The relative novelty of these agents was based on a better profile of activity against antibiotic-resistant Gram-positive bacteria. †Reported MRSA activity suggests a different binding profile to PBPs than currently licensed cephalosporins. ‡Reported activity against bacteria resistant to earlier carbapenems might not actually represent a different target range but could be due only to evasion of resistance mechanisms by the new agent. §Ceftazidime is a licensed cephalosporin. Only the β-lactamase inhibitor NXL 104 displays additional enzyme inhibition resulting in a broader range of activity than earlier agents. [P]Antibacterial substance of peptidic nature with a new mechanism of action known or very likely. Phase of development refers to the highest phase of development, regardless of indication. Total represents the number of agents active against each of the selected bacteria in a best-case scenario.

the identification of agents potentially active against the selected panel of antibiotic-resistant bacteria. For example, when the combined dataset was built, the possibilities of cross- and co-resistance were not taken into account during the assessment. Furthermore, in the absence of *in vitro* susceptibility data, assumptions on *in vitro* activity based on class properties were made.

Most of the agents identified using this optimistic approach were under development for invasive infections caused by antibiotic-resistant Gram-positive bacteria, especially against MRSA. Only eight agents had potential activity against antibiotic-resistant Gram-negative bacteria and only four based on actual data. Among these four agents, only two acted on new or possibly new targets and none via new mechanisms of action. The lack of novelty among these agents illustrates the current paucity of development of agents active against multi-resistant Gram-negative bacteria. This reflects the difficulties encountered in identifying new bacterial targets and the possibility that the majority of targets amenable to antibacterial activity have already been identified (Payne et al., 2007). Other reports have painted a more optimistic picture of the future availability of new antibacterial agents (Theuretzbacher, 2009; Wong, 2005). However, these reports do not particularly focus on the development of agents against multidrug-resistant bacteria as is the case in this study.

Overall, our findings corroborate earlier reports on the lack of antibacterial drug development to tackle multi-drug resistance (Spellberg et al., 2004; White, 2005), including those from the Infectious Diseases Society of America (IDSA) (Boucher et al., 2009; Talbot et al., 2006). Spellberg et al. (2004) evaluated the research and development programs from the 15 major pharmaceutical companies and the seven major biotechnology companies. The commercial databases used in the present analysis also cover the many firms involved in pharmaceutical research and development that are not among the largest, as well as all the supplementary sources that were used in the IDSA studies. In addition, these databases include information from the specialised literature and information directly available from companies. Furthermore, our study took into account all investigational agents in clinical development, i.e., Phases I–III, or for which an application had already been filed to at least one national regulatory agency, whereas the IDSA studies were limited to Phases II and III.

Our study has some limitations. It could be argued that there are many agents in pre-clinical development that may have an activity against multi-drug resistant bacteria. However, there is little data for assessment of compounds in pre-clinical development and these compounds have a high attrition rate. Moreover, it should be noted that the databases that we used did not include information on agents that were, so far, under development only by academic groups. This study describes the situation at the data-lock point of 14 March 2008. Obviously, new compounds have since entered clinical development and been included in the databases while others have been discontinued. We are also aware that, occasionally, information on compounds is only made available in the public domain at a late stage of development.

Multidrug-resistant Gram-negative bacteria represent a major challenge for the future (Boucher et al., 2009). The lack of agents that could be administered systematically and with activity against Gram-negative bacteria displaying new mechanisms of action as

found in this study is of particular concern, especially if the high attrition rates for agents in early stages of clinical development (Payne et al., 2007) are taken into consideration. In fact, it is unclear if any of the agents identified in this study will ever reach the market. Even if a public health driven approach for research and development of antibacterial agents starts in the near future, the burden of antibiotic resistance is likely to continue to increase. Therefore, a European and global strategy to address this serious problem is urgently needed, and measures that spur new antibacterial drug development need to be put in place.

As early as 2004, a report from the World Health Organization on "Priority Medicines for Europe and the World" identified infections caused by resistant bacteria as the number one therapeutic area requiring priority medicines based on the potential public health impact (Kaplan and Laing, 2004). In 2003 and 2005, two EU conferences addressed the role of research and of actions to promote new technologies to fight antibiotic resistance (Cornaglia et al., 2004; Finch and Hunter, 2006). The need for involvement of the public sector into research and development of new antibiotics has been pointed out by both the international network ReAct – Action on Antibiotic Resistance (Tickell, 2005) and the European Academies Science Advisory Council (EASAC, 2007). Our study sends another clear message that the present antibiotic pipeline will not meet the public health needs. The results of this study were presented at the conference "Innovative incentives for effective antibacterials" held in Stockholm during the Swedish Presidency of the EU on 17 September 2009 (Swedish Government, 2009). At the conference, a review of possible regulatory, financial and other incentives to stimulate research and development of new antibiotics was presented (Morel and Mossialos, 2010). In response to the call for action on the urgent need for novel antibiotics, the European Health Council called upon the EU Commission in December 2009 during the Swedish Presidency, to develop a comprehensive action-plan on antibiotic resistance, including concrete proposals concerning incentives to develop new effective antibiotics. This action-plan is to be presented in November 2011. Moreover, a Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) was established in November 2009. The goal of the TATFAR is to increase the mutual understanding of EU and US activities and programs on antimicrobial issues, deepen the transatlantic dialogue, provide opportunities to learn from each other, and promote information exchange, coordination and co-operation between the EU and the US. One of the focus areas for the TATFAR is to identify strategies for improving the pipeline of new antimicrobial drugs, diagnostic procedures and techniques, and maintaining existing drugs on the market. The TATFAR aims to conclude its work by March 2011. Incentives to stimulate research and development of novel antibiotics were further discussed at the Conference "the Global Need for Effective Antibiotics-moving towards concerted action" held in Uppsala Sweden, September 2010 (So et al., 2011).

Conflict of interest

All authors, members of the working group and reviewers of agents in the database have declared potential conflict of interest to the EMA. Declarations of interests can be obtained upon request.

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Fighting bacterial infections—Future treatment options☆

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ABSTRACT

This review summarizes ongoing research aimed at finding novel drugs as alternatives to traditional antibiotics. Anti-virulence approaches, phage therapy and therapeutic antibodies are strategies that may yield drugs with high specificity and narrow spectra. Several candidates are currently being evaluated in clinical trials, mostly for topical applications, but so far, none have been approved for market authorization. Candidates based on antimicrobial peptides (natural, semisynthetic and synthetic) are also being tested in clinical trials, mostly for the topical treatment of chronic infections. An alternative to the development of new antibiotics is to find potentiators of traditional antibiotics; in this respect, beta-lactamase inhibitors are already in clinical use. Novel variants are under investigation as well as efflux pump inhibitors.

1. Introduction

Because of the development of antibiotic resistance in virtually all clinically important pathogens, alternatives to conventional antibiotics are urgently needed. The majority of the antibiotics used today originate from Actinomycetales, mostly *Streptomyces*, and were isolated during the "golden age" of antibiotics discovery, the period from the 1940s to the 1970s. Natural products have proven to be highly efficient for the treatment of bacterial infections and, not surprisingly, the variety of drugs based on natural products is enormous. There are drugs with broad and narrow spectra for oral, topical or parenteral administration and with activities against almost all known pathogens. Most natural product-based candidates currently under development are new, improved versions of old drugs, exemplified by the recently FDA-approved glycopeptide *telavancin* (Guskey and Tsuji, 2010). The chemical modification of existing drugs has proven to be the most efficient way to develop novel drugs active against resistant strains. However, these new agents are doomed to suffer from resistance development as well. Therefore, the development of antibacterial drugs with completely new modes of action is much needed. In 1995, the complete genome of *Haemophilus influenzae* was published, marking the beginning of the genomic era. Hopes were high that novel targets would be identified, leading to new drug candidates. The results, however, have been poor. Employees at GlaxoSmithKline published a review in 2007 summarizing the efforts made by the company in target-based drug research (Payne et al., 2007). Seventy screenings of libraries containing between 260,000 and 530,000 molecules resulted in five candidates, none of which have subsequently passed clinical trials to become licensed. The study reveals the complexity in finding novel antibacterial drugs. Still, alternatives to conventional antibiotics are needed; research to find those alternatives is ongoing. This report attempts to review the efforts made within seven fields: antimicrobial peptides (AMPs), antivirulence strategies, phage therapy, therapeutic antibodies, vaccines, potentiators of currently used antibiotics and antibacterial biomaterials. Characteristics of antibacterial drugs that may result from these research fields can be found in Table 1.

2. Antimicrobial peptides

2.1. Background

Antimicrobial peptides (AMPs), which are present in all animals, are evolutionary conserved components of the innate immune defense (Zasloff, 2002). Eukaryotic AMPs are small (10–50 amino acids), cationic and contain both hydrophobic and hydrophilic parts (Hancock and Sahl, 2006). Traditionally, they have been described as "antimicrobial", with most having the ability to disrupt bacterial membranes, killing the bacteria. The exact mechanism of action is

☆ From the ReAct Conference "The Global Need for Effective Antibiotics—Moving Toward Concerted Action, ReAct", Uppsala University, Uppsala, Sweden, 2010.

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Table 1
Characteristics of antibacterial drugs that may result from different research strategies.

	Risk of resistance development	Spectrum of specificity	Spectrum of potential target bacteria	Routes of administration
Antivirulence strategies	Low	Narrow	Broad	Mainly topical
Antimicrobial peptides	Low	Broad	Broad	Mainly topical
Bacteriophages/lysins	High/low	Narrow	Broad	Topical and systemic
Therapeutic antibodies	Low	Narrow	Broad	Systemic
Vaccines	Low	Narrow	Broad	Mainly systemic
Potentiators	High	Broad and narrow	Broad	Topical and systemic
Antibacterial biomaterials	Uncertain	Broad	Broad	–

unknown. It has recently been discovered that some AMPs are not directly bactericidal, but rather exert their effects by immunomodulation (Bowdish et al., 2005). Generally, AMPs are broad-spectrum antibiotics active against not only bacteria but also certain viruses and fungi. At higher concentrations, many may exhibit toxicity to eukaryotic cells. Magainins, which are AMPs from frogs, are among the best-studied AMPs (Berkowitz et al., 1990). AMPs of prokaryotic origin are called "bacteriocins" and often have a narrower spectrum (Riley and Wertz, 2002). Nisin, a bacteriocin, is the commercially most important AMP. It has been used extensively for food preservation (Riley and Wertz, 2002). The polymyxins, bacterial lipopeptides, were introduced into the clinic in the 1960s, but owing to their toxicity, they were replaced by other antibiotics (Falagas and Kasiakou, 2005). They are now commonly used only in topical therapy and considered a last-resort treatment of severe infections caused by multidrug resistant Gram-negatives such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

2.2. Clinical potential

The broad-spectrum activity and rapid mode of action of AMPs make them promising drug candidates. The level of induced resistance against AMPs is also anticipated to be low (Zasloff, 2002). Most AMPs in preclinical and clinical trials today have been developed for topical applications (Hancock and Sahl, 2006). Examples of indications are catheter site infections, cystic fibrosis, acne and wound healing. The development of AMPs into drugs, however, has encountered several difficulties. Not only may some AMPs be toxic but also the production cost for synthetic peptides is high and their *in vivo* stability, especially toward proteases, is an issue (Hancock and Sahl, 2006). A theoretical concern for the pharmacological use of AMPs closely related to human ones is that selection for bacterial resistance could generate organisms of higher virulence potential. The fact that AMP function does not depend on specific amino acid sequences, but rather on biochemical properties, has opened the opportunity to develop synthetic peptide mimics for therapeutic use.

2.3. Ongoing research

Two AMPs (*omiganan* and *pexiganan*) have shown efficacy in Phase III clinical trials, but neither of them has been approved for clinical use (Hancock and Sahl, 2006). *Pexiganan*, a synthetic analogue to the magainins, was developed for the topical treatment of diabetic foot ulcers, whereas *omiganan* was investigated for the prevention of catheter-related infections. Development programs for these two drug candidates are still running (www.dipexiumpharmaceuticals.com, www.migenix.com). Several synthetic peptide mimics have shown efficacy in animal models (Choi et al., 2009; Livne et al., 2009; Radzishevsky et al., 2007; Rotem et al., 2008; Sarig et al., 2010). Oligomers of acylated lysines (OAKs) (Livne et al., 2009; Radzishevsky et al., 2007; Rotem et al., 2008; Sarig et al., 2010), arylamide foldamers (Choi et al., 2009) and lipohexapeptides (Zhang and Falla, 2010) are all classes of broad-spectrum peptide mimetics that have been used successfully *in vivo*. Moreover, candidates from the groups of OAKs and arylamide foldamers have shown efficacy against staphylococcal infection in mice when delivered systemically (Choi et al., 2009; Livne et al., 2009; Sarig et al., 2010). Some synthetic peptide mimics are also in clinical trials. Promising results were recently reported from a Phase Ib trial of the intravenously delivered defensin-mimetic *PMX-30063*, active against *Staphylococcus* spp. Phase II trials for skin and soft tissue infections are underway (www.polymedix.com). *LTX-109*, a broad-spectrum synthetic peptidomimetic, is currently being evaluated for nasal decolonization of MRSA in a Phase I/IIa trial (www.lytixbiopharma.com). Peptides that are more closely related to endogenous ones are also being investigated. The human-derived peptide *DPK-060* has successfully gone through Phase I/IIa clinical trials for topical use against atopic dermatitis (www.dermagen.se, Schmidtchen et al., 2009). Two other investigational drugs (drugs under study but not yet approved for clinical use), *hLF1-11* and *talactoferrin*, both based on human lactoferrin, have been tested in clinical trials (Velden et al., 2009, www.agennix.com). These peptides appear to have both immunomodulatory and antibacterial properties. Talactoferrin has been shown to stimulate wound healing and was recently evaluated as oral therapy against severe sepsis in a Phase II trial with promising results (www.agennix.com, Engelmayer et al., 2008). Prophylactic oral administration of bovine lactoferrin has been reported to reduce the incidence of early onset sepsis in very-low birth weight premature infants (Manzoni et al., 2009). *IMX942*, another peptide-based candidate with immunomodulatory properties, has shown efficacy in animal studies and recently went through a Phase I clinical trial (delivered intravenously) (www.inimexpharma.com, Scott et al., 2007). A lantibiotic (bacterial AMP), *NAI-107*, is also under preclinical development (Jabes and Donadio, 2010). Finally, the first fungal AMP, *plectasin*, was identified a few years ago (Mygind et al., 2005). It is highly effective against *Streptococcus pneumoniae*, non-toxic to eukaryotic cells, stable, has the potential for systemic delivery and can be produced by recombinant expression (Mygind et al., 2005). Promising preclinical data have recently been published on plectasin and an optimized, more broad-spectrum version of it, *NZ2114* (Andes et al., 2009; Ostergaard et al., 2009; Brinch et al., 2010). The mode of action of these peptides, targeting the bacterial cell wall precursor Lipid II, has also been elucidated (Schneider et al., 2010).

3. Antivirulence strategies

3.1. Background

Most traditionally used antibiotics kill bacteria by interfering with essential cellular processes. An alternative to this approach

is to disarm the pathogens, making it easier for the host innate immune system to clear the infection. Virulence is defined as the ability of a pathogen to cause disease. Some of the major targets of antivirulence research are toxins, quorum-sensing, biofilm production, type III secretion and adhesion. Toxins are produced by numerous pathogenic bacteria and in many cases the immune defense can manage the infection well if the toxins are taken out of the equation. A large number of Gram-negative bacteria release their toxins by type III secretion, making the machinery used in this process another potential target. Type III secretion involves the formation of a needle-like structure that delivers toxins and other effector molecules directly into host cells (Galan and Wolf-Watz, 2006). Quorum-sensing – another potential target – can be described as the process by which bacteria "talk" to each other. Bacteria can sense each other by taking up small molecules secreted by other bacteria nearby; in this way, bacteria can act as a population instead of as individuals (Kaufmann et al., 2008). Quorum-sensing enables bacteria to form biofilms, an effective approach to becoming more resistant toward both antibiotics and host immune responses. Finally, host cell adhesion is a critical initial step in bacterial colonization and thus a promising target for antibacterial drugs.

3.2. Clinical potential

Targeting bacterial virulence is attractive since it is specific toward pathogenic bacteria and spares the commensal flora. Since bacterial viability is not directly targeted, it is also unlikely that inhibitors of virulence would show cross-resistance with existing antibiotics or even evoke novel modes of resistance. Antivirulence drugs could be used both systemically and locally. The indications would vary depending on the specific virulence mechanism targeted. One application could be prophylactic use under certain circumstances, such as travelling, a bioterrorism threat or during an epidemic. Developing drugs based on antivirulence has several issues that need to be addressed. The evaluation of new drugs relies on robust *in vitro* assays for pharmacological studies. Since many antivirulence drugs do not have a phenotypic effect that can be assayed *in vitro*, novel assays must be set up that mimic the *in vivo* setting. Others, such as inhibitors of toxins, may be easier to monitor. The narrow specificity of virulence inhibitors requires rapid, precise diagnostic methods, as well as novel assays for susceptibility testing.

3.3. Ongoing research

The development of anti-toxin antibodies is the antivirulence strategy that is closest to clinical application (described below in the section about therapeutic antibodies). Several other alternatives, however, are under investigation (Table 2). Because of the bioterrorism threat, inhibitors of anthrax toxins have been identified (Laine et al., 2010; Min et al., 2004; Tonello et al., 2002; Karginov et al., 2005; Moayeri et al., 2006; Mourez et al., 2001; Shoop et al., 2005; Xiong et al., 2006; Panchal et al., 2004; Turk et al., 2004), with several of these having shown potency in animal models (Karginov et al., 2005; Moayeri et al., 2006; Mourez et al., 2001; Shoop et al., 2005; Xiong et al., 2006). At least one is under commercial preclinical development (Xiong et al., 2006). Inhibition of toxins produced by *Clostridium botulinum* (Boldt et al., 2006; Burnett et al., 2003; Eubanks et al., 2007; Li et al., 2010; Pang et al., 2010; Roxas-Duncan et al., 2009; Schmidt and Stafford, 2002; Silhar et al., 2010), *P. aeruginosa* (Arnoldo et al., 2008), Shiga toxin-producing *Escherichia coli* (Armstrong et al., 1991; Kitov et al., 2008; Nishikawa et al., 2002; Paton et al., 2000; Watanabe-Takahashi et al., 2010; Kitov et al., 2000), *Staphylococcus aureus* (Ragle et al., 2010) and *Vibrio cholerae* (Hung et al., 2005) has also been reported. The mode of action of these inhibitors includes direct binding to the toxin, binding to the toxin receptor and manipulation of gene expression. One of these toxin inhibitors, *Synsorb-Pk*, was tested in clinical trials, but failed to show efficacy (to my knowledge the only non-antibody virulence inhibitor tested in clinical trials) (Trachtman et al., 2003). An elegant example of toxin inhibition is the use of a cholesterol biosynthesis inhibitor to block staphylococcal virulence (Liu et al., 2008). Similarities between crystal structures in human and bacterial biosynthetic pathways led the researchers to screen squalene synthase inhibitors for activity toward staphylococci, resulting in the identification of an inhibitor with potency *in vivo*. Several studies have identified type III secretion inhibitors active against important Gram-negatives such as *Yersinia*, *E. coli*, *Chlamydia*, *Shigella*, *Pseudomonas* and *Salmonella* (Aiello et al., 2010; Felise et al., 2008; Gauthier et al., 2005; Hudson et al., 2007; Kauppi et al., 2003; Larzabal et al., 2010; Muschiol et al., 2006; Negrea et al., 2007; Pan et al., 2009; Veenendaal et al., 2009; Wolf et al., 2006). Many of these inhibitors are effective toward more than one bacterial species and some are currently being investigated for commercial development (www.microbiotix.com, www.creativeantibiotics.com). In the search for inhibitors of adhesion, a group of compounds designated *pilicides* has been identified. These compounds inhibit *E. coli* pilus synthesis, thereby making the bacteria less adhesive (Pinkner et al., 2006; Svensson et al., 2001). Recently, modifications of a pilicide were shown to render it effective toward curli production as well, enhancing its anti-adhesive properties (Cegelski et al., 2009). Many companies and researchers are also investigating methods to prevent biofilm formation. *RNAIII-inhibiting peptide* (*RIP*) targets quorum sensing in *S. aureus* and has been shown to prevent biofilm formation *in vivo* (Balaban et al., 1998; Giacometti et al., 2003). Furanone-based compounds (Hentzer et al., 2003) and acyl-homoserine lactones (Geske et al., 2005) have been shown to prevent *Pseudomonas* biofilm formation via quorum sensing inhibition. Recently, novel inhibitors for quorum sensing in *E. coli* and *V. cholerae* were also identified (Gutierrez et al., 2009). Other targets for antivirulence drugs are Gram-positive sortases (Chenna et al., 2010; Kudryavtsev et al., 2009; Maresso et al., 2007; Oh et al., 2004; Suree et al., 2009), the heptose biosynthesis in Gram-negatives (De Leon et al., 2006; Desroy et al., 2009; Moreau et al., 2008), FimH in *E. coli* (Larsson et al., 2005; Wellens et al., 2008) and iron acquisition systems (Banin et al., 2008; Ferreras et al., 2005; Kaneko et al., 2007). Some of these are under commercial development (Desroy et al., 2009; Moreau et al., 2008). Furthermore, an inhibitor of QseC signaling, *LED209*, was recently discovered (Rasko et al., 2008). QseC is a sensor histidine kinase required for regulation of virulence factors in many important Gram-negatives. LED209 was shown to attenuate virulence in a mouse model of *Salmonella* infection.

4. Bacteriophages and lysins

4.1. Background

Bacteriophages (phages) are bacteria-specific viruses present in nearly all environmental niches. Most known phages are "lytic" phages, which ultimately lyse and kill the host bacterial cells to release their progeny (O'flaherty et al., 2009). Other phages are "lysogenic" and do not kill the host, being incorporated into the bacterial genome as prophages and only attack and lyse the host organism on rare events (O'flaherty et al., 2009). Phage therapy, investigated worldwide during the first half of the 20th century, has a long and controversial history. It was abandoned by the Western world after the introduction of antibiotics, but continued to be used in the former Soviet Union. The Eliava Institute in Georgia, which has treated thousands of people for almost a

Table 2
Examples of inhibitors of virulence that have shown efficacy in animal models.

Target	Inhibitor	Mode of action
Adhesion	Pilicides/curlicides (Cegelski et al., 2009)	Inhibit *E. coli* pilus and/or curli assembly
Biofilm production/Quorum-sensing	RNAIII-inhibiting peptide (Balaban et al., 1998)	Inhibits *S. aureus* quorum sensing
	Furanone derivates (Hentzer et al., 2003)	Inhibit *P. aeruginosa* quorum sensing
Toxins	Hydroxamate lethal factor inhibitor (Shoop et al., 2005)	Inhibits *B. anthracis* lethal factor protease activity
	Cisplatin (Moayeri et al., 2006)	Inhibits *B. anthracis* protective antigen
	Polyvalent inhibitors of anthrax toxin (Mourez et al., 2001)	Inhibit *B. anthracis* toxin assembly
	β-cyclodextrin derivatives (Karginov et al., 2005)	Block the *B. anthracis* protective antigen pore
	BPH-652 (Liu et al., 2008)	Inhibits *S. aureus* production of staphyloxanthin
	Ac-PPP-tet (Watanabe-Takahashi et al., 2010)	Alters the intracellular transport of *E. coli* Stx2
Type III secretion	INP0007 and INP0403 (Hudson et al., 2007)	Block type III secretion in *Y. pestis* and *Salmonella enterica* serovar Typhimurium
Virulence gene regulation	LED209 (Rasko et al., 2008)	Inhibits QseC signaling in several Gram-negative pathogens
	Virstatin (Hung et al., 2005)	Inhibits *V. cholera* production of cholera toxin and the toxin coregulated pilus

century, is an example of an institute entirely devoted to phage research and phage therapy (Kutateladze and Adamia, 2008). However, the documentation of these clinical applications has been poor and much has been published in Russian. The food industry is another potential area of use for phages. In 2006, the US Food and Drug Administration (FDA) approved the use of bacteriophages in the prevention of *Listeria monocytogenes* contamination of meat and poultry (www.intralytix.com).

4.2. Clinical potential

Because phages are the natural predators of bacteria, using them as antibiotics may seem quite straightforward. Properties that make phages good drug candidates are that they are cheap to produce and very specific (Hanlon, 2007). However, the risk of resistance development is high and there may be clinical problems with neutralization of phages by the host immune response (Merril et al., 2003). Theoretically, the safety profile of phages should be excellent because of their specific action; however, little formal clinical data are available. Another major concern is the lack of data regarding efficacy and pharmacokinetics. Phages have very narrow specificity and thus phage therapy will require good diagnostics to identify precisely the infecting agent. The indications for phage therapy currently being evaluated include topical use on wound infections caused by, for example, *P. aeruginosa* or *S. aureus*, as well as other chronic infections, such as *P. aeruginosa* infection in cystic fibrosis patients. Another potential area of use is oral treatment for enteric infections. It may also be possible to use phages in a prophylactic fashion as decolonizers, eradicating the nasopharyngeal carriage of, for example, *S. aureus* in high-risk groups. An alternative to using whole phage particles is to use phage lysins. These are highly potent enzymes degrading the bacterial cell wall (Fischetti, 2010). Resistance development toward lysins has not yet been reported (Fischetti, 2010).

4.3. Ongoing research

In recent years a handful of clinical trials with phage therapy in humans have been reported. *BioPhage-PA* has been tested in Phase I/II trials as topical treatment against *P. aeruginosa* ear infections and Phase III trials are underway (Wright et al., 2009, www.biocontrol-ltd.com). Trials are also considered for an aerosol variant for use in patients with cystic fibrosis (www.biocontrol-ltd.com). A phage cocktail, *BFC-1*, containing phages specific for *P. aeruginosa* and *S. aureus* strains is currently being evaluated as treatment on burn wounds (Merabishvili et al., 2009). Another phage preparation, *WPP-201*, also topical, was tested against venous leg ulcers in a Phase I safety trial (Rhoads et al., 2009, www.novolytics.co.uk). Nestle, the Swiss food company, conducted a Phase I safety trial of T4 phage given to healthy volunteers and recently followed that up by initiating a clinical trial for the use of phages in an oral rehydration solution given to diarrheal children (NCT00937274, Bruttin and Brussow, 2005). Recent case reports describing phage therapy in humans include treatment of bacterial sepsis, chronic bacterial prostatitis, burn wounds and other ulcers, as well as eradication of a colonizing MRSA strain (Jikia et al., 2005; Leszczynski et al., 2006; Letkiewicz et al., 2009; Markoishvili et al., 2002; Marza et al., 2006; Weber-Dabrowska et al., 2003). Many of these reports originate from the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences. Moreover, this establishment has an ongoing clinical trial with experimental phage treatment of infections with drug-resistant bacteria (NCT00945087). On the pre-clinical stage, a number of companies are trying to develop phage-based products for decolonization of nasal staphylococci (www.gangagen.com, www.intralytix.com, www.novolytics.co.uk). Phages have been used successfully in many animal models. This work includes experimental infections with *Burkholderia cenocepacia* (Carmody et al., 2010), *Enterococcus faecium* (Biswas et al., 2002), *Enterococcus faecalis* (Uchiyama et al., 2008), *E. coli* (Bull et al., 2002; Capparelli et al., 2006; Nishikawa et al., 2008; Tanji et al., 2005; Wang et al., 2006b), *Klebsiella pneumoniae* (Chhibber et al., 2008; Vinodkumar et al., 2005; Kumari et al., 2010), *P. aeruginosa* (Debarbieux et al., 2010; Heo et al., 2009; Mcvay et al., 2007; Wang et al., 2006a; Watanabe et al., 2007; Vinodkumar et al., 2008), *Salmonella enterica* (Capparelli et al., 2010), *S. aureus* (Capparelli et al., 2007; Gupta and Prasad, 2011; Hoshiba et al., 2010; Matsuzaki et al., 2003; Wills et al., 2005; Zimecki et al., 2009, 2010) and *Vibrio vulnificus* (Cerveny et al., 2002). An alternative to the use of whole phage particles is to use lysins. So far, lysins have only been found that are potent against Gram-positives. Promising *in vivo* data have been reported on lysins active against *Bacillus anthracis* (Schuch et al., 2002; Yoong et al., 2006), *Enterococcus* spp (Yoong et al., 2004), *S. aureus* (Daniel et al., 2010; Rashel et al., 2007), *Streptococcus agalactiae* (Cheng and Fischetti, 2007; Cheng et al., 2005), *S. pneumoniae* (Entenza et al., 2005; Grandgirard et al., 2008; Jado et al., 2003; Loeffler et al., 2003, 2001; Mccullers et al., 2007; Witzenrath et al., 2009) and *Streptococcus pyogenes* (Nelson et al., 2001). Bioengineered phages are also a possibility. An example

of this is currently in clinical trials, evaluated for the decolonization of *S. aureus* (Fairhead, 2009, www.phicotherapeutics.co.uk). Finally, veterinary applications of phages are under investigation, especially in poultry (Johnson et al., 2008).

5. Therapeutic antibodies

5.1. Background

Therapeutic antibodies are already on the market, the majority of them for the treatment of cancer (Reichert et al., 2005). None of these antibodies have been approved for the treatment of bacterial infections, but several are undergoing preclinical and clinical trials. The concept of passive immunization is not a new one: before the antibiotic era, patients were regularly given antisera prepared from inoculated horses. Anti-bacterial antibodies can be divided into two categories: those that bind directly to the pathogen and those that aim to neutralize toxins or other virulence factors (Bebbington and Yarranton, 2008). Antibodies that bind directly to the bacteria usually work by opsonizing the bacteria for phagocytosis. Those that bind to virulence factors disarm the bacteria, thereby giving the host a chance to clear the infection immunologically.

5.2. Clinical potential

Therapeutic antibodies have the advantage of being very specific, which means that they do not affect the commensal flora. Intended for systemic use only, they can be produced toward any pathogen. Indications for antibodies currently in clinical trials include the prevention of *S. aureus* infection in high-risk groups, as well as prophylaxis and treatment of anthrax. Antibodies are widely used for cancer treatment and thus many questions regarding safety and pharmacokinetics may already have been addressed. However, as with antivirulence strategies and phage therapy, the narrow specificity increases the requirements for rapid diagnosis. Therapeutic antibodies are also relatively costly to produce and are usually intended for small markets, strongly suggesting that the final product may be expensive.

5.3. Ongoing research

Several antibody-based antibacterial drugs are currently being evaluated in clinical trials (Table 3). However, a number of promising candidates – *Veronate*, *Altastaph* and *Aurograb* – directed toward *S. aureus* recently failed Phase II/Phase III studies because of lack of efficacy (Bebbington and Yarranton, 2008). Many other staphylococci-specific antibodies have been identified (Brown et al., 2009; Hall et al., 2003; Ragle and Bubeck Wardenburg, 2009; Tilahun et al., 2010; Walsh et al., 2004; Park et al., 2007) and one of these, *Pagibaximab*, is currently being evaluated for prevention of infection in low-birth-weight infants in clinical trials (www.biosynexus.com, Weisman et al., 2009). Several studies also report antibodies that target *B. anthracis* toxins (Zhou et al., 2008; Albrecht et al., 2007; Mohamed et al., 2005; Peterson et al., 2006; Staats et al., 2007; Vitale et al., 2006; Cui et al., 2005; Maynard et al., 2002; Wild et al., 2003; Chen et al., 2009; Herrmann et al., 2006; Hull et al., 2005; Zhao et al., 2003). *Anthim*, *Raxibacumab* and *Valortim* are all anthrax-specific antibodies currently undergoing clinical trials (www.elusys.com, www.pharmathene.com, Subramanian et al., 2005). Another common target for therapeutic antibodies is *P. aeruginosa*. *KBPA101*, a monoclonal directed toward LPS O polysaccharide of *P. aeruginosa* serotype O11, is currently undergoing clinical trials (Horn et al., 2010; Lazar et al., 2009). *KB001*, also a *Pseudomonas*-specific antibody inhibiting type III secretion, is being evaluated in cystic fibrosis patients in clinical trials (Baer et al., 2009, www.kalobios.com). In addition, antibodies targeting *Pseudomonas* quorum sensing (Kaufmann et al., 2006), alginate (Pier et al., 2004, www.aridispharma.com) and flagellin (Neville et al., 2005) have been reported. Polyclonal *Pseudomonas*-specific antibodies produced in hens and transported to the egg yolk are in clinical use for the treatment of cystic fibrosis patients in Sweden (Nilsson et al., 2008). These antibodies are not approved by the European Medicines Agency (EMA) or the FDA, but Phase III clinical trials are underway (www.immunsystem.se). Antibodies directed to shiga toxin-producing *E. coli* are also in development and two (*ShigamAbs* and *Urtoxazumab*) are currently undergoing clinical trials (Bitzan et al., 2009; Kimura et al., 2002; Lopez et al., 2010; Mukherjee et al., 2002). Further, the promising results of a Phase II clinical trial evaluating monoclonal antibodies directed toward *Clostridium difficile* were recently published (Babcock et al., 2006; Lowy et al., 2010). Most of the antibodies described thus far are directed toward toxins and are very specific to the bacterial species producing those toxins. A more broad-spectrum antibody has also been reported targeting the poly-N-acetylglucosamine (PNAG) of bacterial polysaccharide. This antibody, currently under preclinical development, has shown *in vivo* protection toward both *S. aureus* and *E. coli* (Cerca et al., 2007; Pier et al., 2004, www.alopexx.com).

6. Vaccines

6.1. Background

The oldest vaccines are based on attenuated or killed whole cells (e.g. BCG against tuberculosis). New techniques have made it possible to produce vaccines based on modified toxins and protein-conjugated polysaccharides. Suitable antigens have traditionally been found by the immunization of animals and the identification of immunoreactive proteins. The breakthrough of genomics has provided novel methods for selecting antigens. Bioinformatics has made it possible to identify specific groups of proteins, such as surface-exposed proteins. This way of finding novel antigens has been called “reverse vaccinology” (Rappuoli, 2000). An alternative method, the ANTIGENome technology, has also been described (Meinke et al., 2005). Peptide libraries covering the whole genome of a pathogen are screened for immunogenicity by the addition of serum from humans previously exposed to the pathogen.

6.2. Clinical potential

A functional vaccine is probably the most cost-effective antibacterial drug possible. It would be hard to question the usefulness of the vaccine strategy—after all, it has been used to eradicate smallpox. However, vaccines are used prophylactically, often offered to general patient populations, i.e. the safety issue is extremely important.

6.3. Ongoing research

Research into vaccines is so extensive that it is hard to grasp the full picture. This section will focus on vaccine candidates currently undergoing clinical trials or in advanced preclinical development. Efforts to develop a *P. aeruginosa* vaccine have been ongoing for a long time, but though several have seemed promising in early clinical trials, none have been approved (Doring and Pier, 2008). However, *IC43* has recently completed Phase II clinical trials (NCT00876252). This candidate vaccine is a recombinant fusion protein of OprF and OprI, two *P. aeruginosa* outer membrane proteins (www.intercell.com). Meanwhile, at least three staphylococcal vaccines are currently being evaluated in clinical trials. *V710* is, based on the IsdB protein, involved in iron acquisition and identified using the ANTIGENome technology (Etz et al., 2002).

Table 3
Selected antibacterial therapeutic antibodies in clinical development.

Antibody	Target organism	Company	Reference
Anthim (ETI-204)	*B. anthracis*	Elusys Therapeutics	www.elusys.com
Raxibacumab	*B. anthracis*	Human Genome Sciences	Subramanian et al. (2005)
Valortim (MDX-1303)	*B. anthracis*	PharmAthene/Medarex	www.pharmathene.com
CDA1/CDB1	*C. difficile*	Medarex/MassBiologics/Merck	Lowy et al. (2010)
ShigamAbs	Shiga toxin-producing *E. coli*	Thallion Pharmaceuticals	Bitzan et al. (2009)
Urtoxazumab	Shiga toxin-producing *E. coli*	Teijin	Lopez et al. (2010)
Anti-Pseudomonas IgY	*P. aeruginosa*	Immunsystem	Nilsson et al. (2008)
KB001	*P. aeruginosa*	KaloBios Pharmaceuticals/Sanofi Pasteur	www.kalobios.com
Panobacumab (KBPA101)	*P. aeruginosa*	Kenta Biotech	Lazar et al. (2009)
Pagibaximab	*S. aureus*	Biosynexus	Weisman et al. (2009)

The other two candidates, *GSK2392105A* and *SA3Ag*, are multivalent vaccines containing three and four antigens, respectively (NCT01160172, NCT01018641). A previous attempt to develop a staphylococcal vaccine focused on polysaccharides, but this vaccine (*StaphVAX*) failed to show efficacy in Phase III trials (Schaffer and Lee, 2008). The polysaccharide strategy has otherwise been very successful, exemplified by the novel conjugate vaccines that have been developed toward *S. pneumoniae* and *Neisseria meningitidis*. These vaccines show good efficacy but do not cover all clinically important strains. Therefore, efforts are made to develop vaccines with better coverage. At least one pneumococcal non-polysaccharide vaccine, *IC47*, is currently being evaluated in clinical trials. It is a multivalent vaccine containing three broadly conserved protein antigens (Giefing et al., 2008, www.intercell.com). Another interesting candidate, killed whole cell vaccine (WCV), has shown potency in animal models and clinical trials are in preparation (Malley, 2010; Lu et al., 2010). For meningococci, serogroup B has been especially difficult to target because its polysaccharide is identical to a human one. A couple of vaccines based on outer membrane vesicles (OMVs) have been used locally, but no universal one has been approved. Several are now in clinical trials (Granoff, 2010), including variants based on multiple bioengineered strains (Van Den Dobbelsteen et al., 2007; Zollinger et al., 2010). Two promising protein-based vaccines are also undergoing clinical trials (Gorringe and Van Alphen, 2009), one containing two variants of a lipoprotein (*rLP2086*) (Fletcher et al., 2004) and one containing five antigens engineered into three recombinant proteins (Giuliani et al., 2006; Pizza et al., 2000). Efficient vaccines are also lacking against Group A streptococci (GAS) and Group B streptococci (GBS). *StreptAvax*, a 26-valent GAS vaccine candidate based on the M protein antigen, was recently evaluated in a Phase I clinical trial (Kotloff et al., 2004). Other GAS vaccines are in preclinical development, including *Pep-Vac StreptInCor*, which is based on the conserved parts of the M protein (Brandt et al., 2000; Guilherme et al., 2009), as well as two protein-based candidates with novel antigens identified via reverse vaccinology (Rodriguez-Ortega et al., 2006) and the ANTIGENome technology (Fritzer et al., 2010). For GBS, the most common strategy has been to use polysaccharide conjugates and several monovalent or divalent candidates have been tested in clinical trials (Edwards, 2008). One 4-valent candidate is currently undergoing clinical trials (NCT01150123). Protein-based candidates, however, are under development (Maione et al., 2005; Doro et al., 2009). When it comes to vaccines toward gastrointestinal pathogens, several candidates are under evaluation for prevention of enterotoxigenic *E. coli* (ETEC) disease (NCT01060748, NCT00993681). One *C. difficile* candidate, based on toxoids, is being evaluated in clinical trials (Kotloff et al., 2001). Other *E. coli* variants in addition to ETEC have been targeted in vaccine research. Several promising candidates have failed in late stage clinical trials, and are no longer in clinical evaluation. Two recent publications, however, identify novel antigens for future vaccine development (Alteri et al., 2009; Moriel et al., 2010). Finally, even though there is a licensed vaccine for protection against tuberculosis, efforts are being made to develop one with better efficacy. Several candidates are being evaluated in clinical trials. *Hybride-1* is based on a fusion protein of Ag85B and ESAT-6 (Weinrich Olsen et al., 2001) and *Mtb72F/AS02A* contains two antigens selected for their ability to boost pre-existing immunity induced by BCG or TB infection (Von Eschen et al., 2009). *AERAS-402/Crucell Ad35* (Abel et al., 2010) and *MVA85A/AERAS 485* (Mcshane et al., 2004) are two additional candidates, both based on virus vectors.

7. Potentiators of currently used antibiotics

7.1. Background

An alternative to the development of novel antibiotics is to find potentiators of the already existing ones. These potentiators could function either by reversing resistance mechanisms in naturally sensitive pathogens or by sensitizing naturally resistant strains. The most common resistance mechanism toward clinically important beta-lactams is the production of beta-lactamases or alternative penicillin-binding proteins (PBPs). Treatment with a beta-lactam in combination with a beta-lactamase inhibitor is already used clinically, and three inhibitors have been registered: clavulanic acid, tazobactam and sulbactam (Drawz and Bonomo, 2010). Another resistance mechanism common among Gram-negatives is the overexpression of efflux pumps. This is also a possible target for potentiators, although no therapeutic efflux pump inhibitors are currently available on the market.

7.2. Clinical potential

The good news about potentiators is that they work, i.e. we already have them in the clinic. The bad news is that resistance to beta-lactamase inhibitors has been reported (Buynak, 2006). Most beta-lactamase inhibitors under preclinical investigation have been developed for use against Gram-negatives such as *P. aeruginosa* and *E. coli*. These bacteria, and *S. aureus*, are typical targets also for the novel efflux pump inhibitors.

7.3. Ongoing research

Several novel beta-lactamase inhibitors are currently being investigated. *NXL104* is active against class A and C beta-lactamases, with shown potency in several Gram-negatives, including *E. coli* and *K. pneumoniae* (Stachyra et al., 2009). It is being evaluated in Phase II clinical trials in combination with ceftazidime. *ME1071* (*CP3242*), currently undergoing clinical trials in Japan (www.meiji.com, Ishii et al., 2010), has shown efficacy in Gram-negatives such as *P. aeruginosa*, *A. baumannii*, *E. coli* and *K. pneumoniae*. *BLI-489* is a penem-based inhibitor with potency in combination with piperacillin in experimental infections with piperacillin-resistant *E. coli* and *E. cloacae* (Petersen et al., 2009). Several other agents with beta-lactamase inhibitory properties have been reported, including the 6-alkylidenepenem sulfones (Pattanaik et al., 2009), the oxapenem analogues (Simpson et al., 2003), *LK-157* (Paukner

et al., 2009) and *BAL30376* (a combination of three inhibitors) (www.basilea.com). The other major area of research within this field is the development of efflux pump inhibitors. Examples of compounds reported for Gram-negatives include pyridopyrimidine derivatives active in *P. aeruginosa* (Yoshida et al., 2007; Lomovskaya et al., 2001), aryl-piperazines for use in *E. coli*, *Acinetobacter*, *Klebsiella* and *Enterobacter* (Schumacher et al., 2006; Pannek et al., 2006; Kern et al., 2006), quinolines for *Enterobacter* (Chevalier et al., 2001) and more recently quinazolines active in *Enterobacter*, *Klebsiella* and *P. aeruginosa* (Chevalier et al., 2010). Inhibitors with activity in Gram-positives have also been identified, especially for *S. aureus* (Sangwan et al., 2008; Vidaillac et al., 2007; German et al., 2008). As yet, no efflux pump inhibitor has been approved for clinical use. One candidate, *MP-601,205*, with intended use in combination with fluoroquinolone antibiotics, was tested in clinical trials but these trials were not pursued because of tolerability issues (Lomovskaya et al., 2007). Efflux pump inhibitors, however, are still included in the developmental programs of Mpex, the company that identified *MP-601,205* (www.mpexpharma.com). Other creative examples of potentiating currently used antibiotics include the use of bioengineered phages (Lu and Collins, 2009) as well as activating a drug toward a pathogen that is naturally resistant to it, as exemplified by the combined use of meropenem and clavulanate to treat successfully tuberculosis in mice (Hugonnet et al., 2009). Another approach was reported last year when it was shown that targeting a regulator of stress response (AmgRS) in *P. aeruginosa* led to increased tobramycin sensitivity (Lee et al., 2009). A similar strategy, targeting the bacterial SOS response, is under preclinical development (www.achaogen.com).

8. Antibacterial biomaterials

8.1. Background

Bacterial infections at the sites of implanted medical devices are conditions of immense clinical importance. Both permanent implants and short-term biomedical devices such as catheters and endotracheal tubes may be colonized. Urinary tract infections related to catheters are the most prevalent form of nosocomial infections (Klevens et al., 2007). Bacterial biofilm formation on the medical device is the main reason for the high prevalence of infections. For devices used in urology, especially urinary catheters and ureteral stents, growth of bacteria may not only lead to infection but also to a phenomenon known as encrustation. Basically, urease produced by the infecting agent hydrolyzes urea present in the urine, resulting in an elevation of pH, ultimately leading to precipitation of salts and deposition of crystals on the surface (Morris and Stickler, 1998). Encrustation may lead to blockage of the catheter and severe complications. The bacteria most commonly associated with infections on medical devices are staphylococcal species, except for the ones on urinary devices, where Gram-negatives (e.g., *E. coli*, *P. aeruginosa*, *Enterobacter aerogenes*, *Acinetobacter acinus*, *Klebsiella* spp and *Proteus* spp) are most common (Leone et al., 2003). *Proteus mirabilis* is the pathogen most commonly associated with encrustation (Morris and Stickler, 1998). Attempts made to reduce the medical implant-related infections include systemic antibiotic prophylaxis as well as local administration of antimicrobial agents. The severity of device-related infections ranges between relatively mild infections to life-threatening conditions, with consequences of health risks for the patients as well as higher costs because of replacements of infected implants and prolonged hospitalizations.

8.2. Clinical potential

Efforts to develop medical devices with antibacterial properties have been ongoing for a long time. Most polymeric materials commonly used for medical devices such as catheters are easily colonized by bacteria. Therefore, efforts have been exerted to add antibacterial surface coatings onto the devices. These coatings may release antibacterial compounds, have antibacterials covalently bound to them or in themselves be resistant to bacterial colonization. Central venous catheters (CVCs) and urinary catheters with antibacterial coating are on the market (Hockenhull et al., 2009). The same strategy is under investigation for implants for orthopedic joint replacements most commonly made of titanium, stainless steel or other metal alloys. These materials show excellent biocompatibility as well as high-quality mechanical properties, but are not resistant to bacterial colonization. The most common antibacterial coatings in use today are those containing silver. Silver is known for its potent broad-spectrum antibacterial activity toward both Gram-positives and Gram-negatives, which is believed to be attributed to the release of silver ions (Monteiro et al., 2009). Coatings releasing antibiotics are also available. Concerns regarding these kinds of devices include silver toxicity as well as resistance development (Monteiro et al., 2009; Hamill et al., 2007).

8.3. Ongoing research

Within the field of urology, research on antibacterial devices mainly focuses on urinary catheters and ureteral stents. Several catheters with antibacterial coating are available, including variants with silver alloys and antibiotics (Schumm and Lam, 2008). Numerous clinical trials have been undertaken to evaluate the efficacy of these coatings. Recently, a meta-analysis of all these trials was performed (Schumm and Lam, 2008) showing that silver alloy catheters reduced the incidence of asymptomatic bacteriuria during short-term urinary catheterization. Catheters coated with minocycline/rifampicin or nitrofurazone reduced the incidence after a week, but when measured later the reduction was not significant (Schumm and Lam, 2008). The clinical implications of these results have been questioned, however (Stickler, 2008). Novel, investigational antibacterial coatings are under development. The antiseptic triclosan has shown good efficacy in several *in vitro* and *in vivo* studies (Chew et al., 2006; Cadieux et al., 2006). A triclosan-eluting stent is currently being evaluated in a Phase II clinical trial (Cadieux et al., 2009). Gendine-coated catheters have also been investigated in animal studies with promising results (Hachem et al., 2009). Several other antibacterials have shown efficacy in animal models with catheters, including chloroxylenol/thymol (Mansouri and Darouiche, 2008) and chlorhexidine/protamine sulphate (Darouiche et al., 2008). An innovative strategy that has been tested in clinical trials is "bacterial interference" (Trautner et al., 2007; Prasad et al., 2009). The catheters are precolonized with an avirulent *E. coli* strain before insertion, potentially preventing adherence of virulent strains. Just as for urinary catheters, CVCs with antibacterial properties are commercially available, including variants impregnated with silver, silver/platinum/carbon, chlorhexidine/silver sulphadiazine, minocycline/rifampicin and heparin (Hockenhull et al., 2009). Their usefulness has been debated, but recent meta-analyses concluded that antibacterial coatings do have a beneficial effect on the rate of device-derived infections, especially minocycline/rifampicin (Hockenhull et al., 2009; Wang et al., 2010). Other alternative coatings for CVCs that have been investigated include *N*-acetylcysteine (Mansouri and Darouiche, 2007), triclosan/DispersinB (Darouiche et al., 2009) and gendine (Hanna et al., 2006). A novel, 5-fluorouracil-coated variant has recently completed Phase III clinical trials with encouraging results (Walz et al., 2010). For permanent implants based on metal alloys, coating strategies are also under investigation. Antibiotics such as gentamicin have traditionally been used in bone cement for prophylactic local delivery.

Presently, cementless bone implants are more routinely used, increasing the need for other antibacterial strategies. The task is somewhat more challenging than for non-permanent devices because osseointegration has to be taken into account. Calcium phosphate-based coatings have shown good biocompatibility and may be used as carriers for antibacterials (Radin et al., 1997). Promising *in vivo* data have been reported for calcium hydroxyapatite in combination with tobramycin (Moojen et al., 2009) and gentamicin (Alt et al., 2006). Other strategies include polymerization of vancomycin onto the implant (Lawson et al., 2007), direct spraying of the implant with antibiotics in methanol (Aykut et al., 2010; Darouiche et al., 2007), as well as using poly(D,L-lactide) (PDLLA) (Kalicke et al., 2006; Vester et al., 2010) or sol-gel as carriers. A recent publication describes the successful use of silver-coated megaprostheses in a clinical trial of patients with bone sarcoma (Hardes et al., 2010). Silver coating resulted in a reduction in infections as well as fewer amputations (Hardes et al., 2010). Finally, the concepts of the other fields of this report have also been applied to prevent bacterial colonization of medical devices. This includes directly targeting biofilm formation on biomaterials using quorum-sensing inhibitors (Cirioni et al., 2007; Lovetri and Madhyastha, 2010, Anguita-Alonso et al., 2007, Cirioni et al., 2006; Christensen et al., 2007), as well as attempting to eradicate the bacteria using bacteriophages (Fu et al., 2010; Curtin and Donlan, 2006; Carson et al., 2010) or AMPs (Trautner et al., 2005; Minardi et al., 2007).

9. Discussion

Several publications have reported that the big pharmaceutical companies are cutting down on research aimed at finding new therapeutics for the treatment of bacterial infections, citing a less favorable economic incentive compared with medications for chronic medical conditions (Boucher et al., 2009). Novel strategies, however, are under investigation, both within the academic community and in biotech/pharmaceutical companies. Many of the fields examined in this report have not yet generated products approved by the EMA or the FDA, but candidates are in clinical trials. Most of these candidates have narrow specificity spectra and would probably not have been considered during the "golden age" of antibiotics. Yet, in times of emerging resistance, all additional antibacterial drugs are welcome. The more diverse our arsenal against bacterial pathogens becomes, the better will be the range of tailored treatment alternatives that can be offered. Broad-spectrum antibiotics are not always the best choice, especially not when considering the commensal flora and the risk for opportunistic infections. However, to take advantage fully of the alternative antibiotics under development, new diagnostics are needed that are more precise and faster than those in use today. The importance of better diagnostics for these novel antibacterial approaches to become successful cannot be stressed enough in today's complex medical environment.

Anti-virulence approaches, phage therapy and therapeutic antibodies are fields that will yield drugs with high specificity and hence narrow spectra. They are all still awaiting their first drug approved for market authorization. To this date, only one antivirulence non-antibody candidate, *Synsorb-Pk*, has entered clinical trials (Trachtman et al., 2003). In theory, antivirulence strategies are tempting both because of the specificity of the resulting drugs and because of the low selective pressure for resistance development. These drugs, however, are at least a decade away and it is questionable whether they will ever be used as first-line drugs for life-threatening conditions (e.g. sepsis) when time is limited. Because of their narrow specificity, the same holds for therapeutic antibodies though these are a bit further in development. Although the regulatory authorities have turned down several candidates, it is still a promising field. Antibody-based drugs are common within oncology and inflammatory disease and recently the first virus-specific antibody, *palivizumb,* for treatment of RSV infection, was approved. With several antibacterial antibodies in late-stage clinical development, it is probably just a question of time before the first one will be in the clinics.

It has to be acknowledged that phage therapy is already in clinical use, even though parts of the Western world are not aware of it. Although the available literature within this field is largely limited to case studies and uncontrolled trials, phage therapy appears to be an alternative for certain indications. For complicated wounds and ulcers caused by multidrug-resistant bacteria, phage therapy could be an option. In the EU, it has been proposed that specific sections concerning phage therapy should be included in the Advanced Therapy Medicinal Product Regulation to make it easier to get approval for clinical trials involving such therapy (Verbeken et al., 2007). It may be easier, though, to get approval for lysins than for full phage particles. Interestingly, thus far, resistance development toward lysins has not been observed. However, most lysins are only stable *in vivo* for less than 30 min (Loeffler et al., 2003), making stability one of the issues that has to be solved before clinical use.

Just as for therapeutic antibodies, the field of AMPs has had several candidates in late-stage clinical trials that failed to be approved for clinical use. Some of these disapprovals have been questioned and it is possible that candidates such as pexiganan would have been approved if trials had been designed differently (Moore, 2003). Most AMPs have quite broad spectra, but because of stability and toxicity problems, they are mainly evaluated for topical use. Just as bacteriophages, they have the potential to fill the niche as topical therapeutics toward complicated wounds and ulcers. Another possible application for AMPs is as decolonizers. It will be interesting to follow the development of plectasin and the improved version of it in that these candidates seem to have the potential to be used systemically. Further, AMPs with immunomodulatory properties have gained recent interest and may find future use as anti-inflammatory agents.

Improvement of existing drugs has become the most successful way of generating novel antibiotics. It works well and several candidates of traditional classes are in late-stage clinical development, such as the cephalosporin *ceftaroline* (Corey et al., 2010), the tetracycline *amadacycline* (*PTK0796*), the streptogramin *NXL-103* (Politano and Sawyer, 2010) and the macrolide *CEM-101* (Woosley et al., 2010). However, all these candidates have a limited lifespan in that eventually resistance will develop. This situation may be what we have to get used to, i.e. the continued development of new drugs that can replace those already in clinical practice. An alternative would be successful vaccination strategies. It will be of special interest to follow the new vaccine candidates that have been identified through genomics and proteomics. These candidates represent genuinely novel approaches and the first one to be approved may well be the beginning of a new era. Moreover, the development of medical devices more resistant to bacterial adherence may reduce the rate of infections and hence limit the need for antibiotics. Even though many of the coatings that are evaluated and in use today involve traditional antibiotics, several take advantage of different alternatives such as silver and antiseptics.

Again, most of these alternative drugs could only replace the currently used antibiotics if efficient diagnostics are developed in parallel. This strategy is also attempted by the company developing the *Pseudomonas* monoclonal antibody KBPA101 (www.kentabiotech.com). Still, even if these novel drugs cannot replace antibiotics, they may become a much-needed complement to the drugs in use today. The overuse of antibiotics has left us in our current predicament. We need to learn from that mistake and take better care of the drugs that are still viable.

10. Methods

The PubMed database was searched using the search terms "antimicrobial peptides", "antivirulence", "phage therapy", therapeutic antibodies", "efflux pump inhibitor", "beta-lactamase inhibitor","biomaterial", "implant" and "vaccine" in combination with the terms "novel" or "antibacterial", or both. For the vaccine field, "vaccine" was also combined with the names of bacterial species considered especially important. Further searches on PubMed were performed based on the authors/companies/substances that were received in the first round. Only publications written in English and published in the past ten years were included, unless an earlier publication was regarded as particularly interesting. Company web pages and ClinicalTrials.gov were subsequently searched for updates on the developmental stages.

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