

Report from the European Conference on the Role of Research in Combating Antibiotic Resistance, 2003

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CONFERENCE FACULTY

Plenary lectures

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Working Group 1

Chairpersons: H. Goossens (Antwerp, Belgium), S. Stefani (Catania, Italy). Rapporteur: R. Cantón (Madrid, Spain). Presenters: S. Stefani, H. Grundmann, R. Cantón, G. Huys (Ghent, Belgium), J. Swings (Ghent, Belgium).

Working Group 2

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Working Group 3

Chairpersons: R. Norrby (Solna, Sweden), G. M. Rossolini (Siena, Italy). Rapporteur: J. Vila (Barcelona, Spain). Presenters: A. Wennberg (Brussels, Belgium), J.-M. Frère (Liege, Belgium), J. Goka (London, UK), A. White (Harlow, UK).

Working Group 4

Chairpersons: R. Cauda (Rome, Italy), R. G. Finch (Nottingham, UK). Rapporteur: P. Gastmeier (Hanover, Germany). Presenters: P. Davey, E. Tacconelli (Rome, Italy), R. Cauda (Rome, Italy), R. G. Finch, B. Cookson (London, UK), S. Stone (London, UK).

Working Group 5

Chairpersons: D. Greco (Rome, Italy), A. Voss (Nijmegen, The Netherlands). Rapporteur: A. Pantosti (Rome, Italy). Presenters: S. Bronzwaer (Luxembourg), A. Cassone (Rome, Italy), F. Daschner (Freiburg, Germany), K. Kristinsson (Reykjavik, Iceland), D. Monnet (Copenhagen, Denmark).

Working Group 6

Chairpersons: G. Cornaglia (Verona, Italy), P. Courvalin (Paris, France). Rapporteur: R. Kozlov (Smolensk, Russia). Presenters: P. Huovinen (Turku, Finland), L. J. V. Piddock (Birmingham, UK), A. Lönnroth, H. Labischinski (Wuppertal, Germany).

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ABSTRACT

Europe has been at the forefront of efforts to control antibiotic resistance, and this globally important health care problem has prompted numerous recommendations for action at both the national and international levels. Starting in 2002, research on antimicrobial resistance has been considered to be one of the specific objectives of the Sixth Framework Programme (FP6) within the European Union. This report summarises the plenary presentations, as well as the findings of six Working Groups covering specific areas of antibiotic resistance, given at a conference in November 2003 entitled 'The Role of Research in Combating Antibiotic Resistance', co-organised by the European Union and the European Society for Clinical Microbiology and Infectious Diseases, and held in Rome under the patronage of the Italian government.

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INTRODUCTION

Infectious diseases are the second leading cause of mortality in the world, resulting in *c.* 15 million deaths annually [1]. The burden of bacterial infections continues to rise because of changing patterns of microbial aetiology, an increase in the number of hosts with impaired immunity, the ageing of Western populations, and the spread of disease through globalisation and urbanisation. In addition, the alarming emergence and spread of antibiotic resistance among common pathogenic bacteria threatens the effectiveness of therapy for many infections. The spread of resistance to multiple antibiotics among *Streptococcus pneumoniae*, *Staphylococcus aureus*, enterococci, Enterobacteriaceae and *Pseudomonas aeruginosa* is of particular concern.

Europe has been at the forefront of efforts to control antimicrobial resistance, and this globally important health care problem has prompted numerous recommendations for action at both the national and international levels [2,3]. The European Union (EU) has convened several invitational conferences concerning resistance, and published the *Community Strategy Against Antimicrobial Resistance* [4] in 2001. This document called for further research to improve our understanding of the molecular mechanisms of resistance, to develop new vaccines, antimicrobial agents and diagnostic tests, and to evaluate strategies for resistance control.

Starting in 2002, research on antimicrobial resistance was considered to be one of the specific objectives of the Sixth Framework Programme (FP6) within the EU. Accordingly, the DG

Research of the European Commission and the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) co-organised a conference entitled 'The Role of Research in Combating Antibiotic Resistance' (28–30 November 2003). This conference, held in Rome under the Patronage of the Italian government, had the following aims:

- To clarify the state-of-the-art and research priorities in fields where information is particularly needed, namely microbial ecology, mechanisms of resistance, genomics and new molecular targets for antibiotics.
- To identify the resistance topics most suitable for European research and to contribute to the establishment of the European Research Area in this field.
- To stimulate partnerships across public, private and governmental bodies for future research initiatives.
- To identify ways of overcoming bottlenecks in European research, such as those associated with decreasing industrial incentives for research and development of new antibiotics.
- To increase the visibility of antibiotic research among scientists, clinicians, politicians and the general public.
- To contribute to the education of researchers and public health officers in the specific field of antibiotic resistance, and the means for its control, through concerted research.

This report summarises the plenary presentations given at the Conference and the findings of six Working Groups covering specific aspects of antibiotic resistance.

PLENARY PRESENTATIONS

Introduction

The plenary presentations introduced many of the main topics for consideration during the Working Group discussions, namely the epidemiology of resistance, the impact of resistance on human health, the evaluation of interventions to control resistance, the role of the pharmaceutical industry, and the means by which European research can be stimulated and co-ordinated.

Epidemiology of resistance

An improved understanding of the molecular epidemiology of antibiotic-resistant bacterial strains is central to efforts aimed at controlling resistance. Research has been facilitated in recent years by advances in genetic analysis, such as multilocus sequence typing (MLST; <http://www.mlst.net>), an unambiguous and highly discriminatory method of characterising (or 'genotyping') isolates according to specific DNA sequences [5]. As MLST data are portable between laboratories, the method is particularly suited to multicentre epidemiological studies, e.g., those performed by the European Antimicrobial Resistance Surveillance System (EARSS) [6]. However, the relatively high cost of MLST analysis means that the use of this technique should be confined to the sequencing of highly variable genotypes.

It is hoped that the networking of MLST systems will allow countries with low rates of resistance to monitor prospectively the evolution of resistant organisms, such as methicillin-resistant *S. aureus* (MRSA). The international spread of MRSA in hospitals is a major concern [7]. According to EARSS data, 22% of all *S. aureus* isolates ($n = 18\,726$) from invasive infections between 1999 and 2002 in Europe were MRSA (Fig. 1) [6]. An increasing number of MRSA strains are susceptible only to vancomycin and other glycopeptides, but decreased vancomycin susceptibility has now emerged in every MRSA lineage [8]. Eleven major clones of MRSA have been identified by MLST [9], and evolutionary relationships between these clones have been inferred from MLST and other genomic data by means of the novel BURST (Based Upon Related Sequence Types) program [9,10]. Further data on the spread



Fig. 1. Data from the European Antimicrobial Resistance Surveillance System showing the proportion of invasive *Staphylococcus aureus* isolates ($n = 18\,726$) collected between 1999 and 2002 that were methicillin-resistant [6].

and evolution of MRSA will be gained from the proposed Genetic Analysis of MRSA in Europe (GAME) study. This collaborative study is intended to combine phenotypic and genotypic analysis of MRSA isolates with information on antibiotic prescribing guidelines, antibiotic use and infection control practices, to allow prospective measurement of the effect of these activities on MRSA epidemiology.

Recently, considerable epidemiological research has been directed at the spread of MRSA in the community setting, particularly in the USA [11]. MRSA strains in the community tend to be more virulent than hospital clones. In particular, strains that produce the Panton-Valentine leukocidin cytotoxin are associated with severe skin infections and pneumonia [12]. MLST and other data suggest that community-acquired MRSA clones arise independently of local nosocomial strains [13], although further research in this area is required.

The evolution of resistance in Gram-negative bacteria is also the subject of important research. *Escherichia coli* is a species that evolves predominantly in a clonal manner. Consequently, this organism shows low genetic diversity, genetic persistence and international clonal distribution. *E. coli* acquires resistance primarily through the spread of mobile genetic elements, i.e., integrons, transposons and—most importantly—plasmids. Plasmids carry genes coding for, for example, various β -lactamase enzymes (e.g., TEM, SHV, CTX-M and AmpC cephalosporinases). Further

research is required to investigate the origin of these elements and, in particular, the possibility of their transfer from poultry and livestock. Once acquired, plasmids maintain their continued existence within cells through a variety of 'addiction' systems, toxin-antitoxin mechanisms, post-segregational cell killing and restriction modification systems. In view of these systems, and the co-selection of multidrug resistance on plasmids, research is required to determine the extent to which resistance in *E. coli* can be reversed by proper antimicrobial use. Also, there is a need for more data relating resistance and virulence in this species.

Other species, such as *P. aeruginosa*, evolve in a predominantly panmictic manner, whereby DNA exchange (or 'recombination') occurs between cells. These species show high levels of diversity, short-lived clonality and local or regional distribution of clones. This ecological heterogeneity has important implications for the evolution of resistance and the assessment of measures to control resistance. In addition to being intrinsically resistant to many antibiotics, *P. aeruginosa* acquires resistance through mutational up-regulation of efflux pumps, derepression of AmpC β -lactamase, and a reduction in the number of porin proteins. Exposure to antibiotics favours the selection of strains with a high mutational frequency (so called 'hyper-mutable' strains). Oliver *et al.* [14] showed that 36% of patients with cystic fibrosis ($n = 30$) were colonised by hyper-mutable strains of *P. aeruginosa*. However, data from Germany indicate that levels of fluoroquinolone usage and resistance in *P. aeruginosa* can be correlated only in strains with low genetic diversity (H. Grundmann, personal communication). Although these data over-simplify the matter, they suggest that ecological studies of the relationship between antibiotic usage and resistance must be stratified according to the genetic diversity of target pathogens.

Resistance and human health

Despite decades of research into the effects of antibiotics, the risk posed to human health by antibiotic resistance is poorly defined. Consequently, current antibiotic policies are based largely on precautionary principles. Clear quantification of the risks of resistance would provide a firmer basis for such policies and would aid

compliance among patients, physicians and other stakeholders.

Research to quantify the clinical impact of resistance is challenging. One theoretical approach would involve a long-term ecological experiment to assess the risk-benefit relationship of antibiotic consumption. This study could be based on longitudinal epidemiological studies, such as the Framingham [15] and MONItoring Cardiovascular disease (MONICA) [16] studies. It would involve continuous monitoring of antibiotic usage, health outcomes and confounding factors in appropriate population cohorts. A conceptual framework for such a study has been proposed under the title 'Risk Evaluation and Benefit Evaluation of Consumption of Chemotherapeutic Agents' (REBECCA) (F. Baquero, personal communication).

Interventions to control resistance

Little is known about the effectiveness of the many types of interventions aimed at controlling antibiotic resistance [17]. Recently, researchers in the UK have evaluated systematically the evidence supporting interventions to improve antibiotic prescribing in hospitals and isolation policies in the hospital management of MRSA.

The analysis of hospital antibiotic prescribing practice was conducted by members of the British Society for Antimicrobial Chemotherapy, the UK Hospital Infection Society and the Cochrane Centre [18]. Of 360 intervention studies published since 1980, 215 (70%) were excluded immediately from the analysis because of an uncontrolled 'before and after' design or the collection of an inadequate time series. A further 30 (10%) studies were excluded subsequently because of various methodological flaws (e.g., flawed control data or randomisation procedures), leaving 61 (20%) studies with eligible data. Although the quality of published studies has improved in recent years, this high exclusion rate indicates a considerable waste of research resources and a need for improved methodological training among researchers.

The interventions evaluated by eligible studies included 'restrictive' measures (e.g., automatic antibiotic substitution or cessation orders) and educational measures. Most were applied to the selection of the antibiotic regimen; very few studies have assessed interventions aimed at the

initiation or cessation of antibiotics. Many studies were unplanned analyses of measures instituted to control resistance epidemics, and various flaws were identified in the statistical methods used. Antibiotic usage was the most common outcome measure, with $\leq 30\%$ of studies assessing health care costs, resistance patterns or clinical outcomes. Only eight studies recorded data on both resistance and antibiotic usage, thereby allowing correlations between these factors to be evaluated. These data provide good evidence that reductions in antibiotic usage can reduce resistance in multiply-resistant Gram-negative bacilli. Two studies suggested that reductions in resistance could be achieved in *Clostridium difficile*. However, there is little evidence for the effectiveness of this kind of measure in controlling MRSA or vancomycin-resistant enterococci (VRE). A corresponding analysis of interventions to control antibiotic prescribing in ambulatory care is in preparation (P. Davey, personal communication).

The review of isolation policies for the management of MRSA was conducted by researchers at various UK centres [19]. Of 254 publications screened, only 46 were included in the review. Few of these were planned, prospective studies, and only six provided interpretable data. The results provided limited evidence for the effectiveness of single-room isolation and cohorting measures, but conflicting evidence for isolation wards.

There are several encouraging examples in which interventions have been associated with a reduction in resistance rates, although there is a lack of good-quality data indicating that widespread effects can be achieved or maintained [20]. The aforementioned systematic reviews provide more information concerning ways to improve research methods than on the effectiveness of specific interventions. Changes in patterns of microbial aetiology, resistance and medical practice have occurred since many of the primary studies were performed. Hence, there is a need for ongoing research, including prospective, well-controlled studies comparing single and combined interventions and the associated costs. Standardised effect measures and reporting procedures for antibiotic prescribing interventions are required to allow data to be compared and pooled. This may be an objective that is well suited for EU-supported research, as may be the standardisation of routine infection control data.

Non-antibiotic approaches

Research into non-antibiotic approaches to anti-infective therapy and prophylaxis declined substantially when antibiotics were introduced in the 1940s. Interest in these therapies increased with the development of organ transplantation, the occurrence of the HIV epidemic and, most recently, the rise in antibiotic resistance. Non-antibiotic approaches comprise pathogen-specific methods (e.g., preventative and therapeutic vaccines, antibody therapies and adoptive cell transfer) and non-pathogen-specific methods (e.g., biological response modifiers, replacement therapy, augmentative therapy and immunoadjuvants).

Prophylactic vaccines have had a limited effect in reducing the disease burden from certain bacteria, e.g., *Strep. pneumoniae*. However, no vaccines exist for the pathogens in which bacterial resistance is most threatening, i.e., Gram-negative bacilli, *P. aeruginosa*, MRSA and VRE. The most promising approach to novel therapeutic vaccines is the development of DNA vaccines. For example, adjuvant immunisation with a DNA vaccine in mice infected with tuberculosis improved immune responses and tissue eradication [21].

Antibody therapies have a potential role in the prevention of disease (e.g., pneumonia) caused by encapsulated pathogens, in the prevention and treatment of sepsis (e.g., endotoxin neutralisation), in antitoxin treatment (e.g., botulism), in post-exposure prophylaxis (e.g., tetanus, hepatitis), and in rapid protection against agents of bioterrorism (e.g., anthrax toxins). The search for pure antibody preparations has led to the development of active peptide fragments and complement D regions. For example, Italian researchers have produced an antibody peptide active against a wide variety of bacterial and fungal pathogens [22].

Biological response modifiers include cytokines (e.g., colony-stimulating factors, interferons and interleukins) and other natural and synthetic compounds (e.g., thymosin- $\alpha 1$). Combination therapy with thymosin- $\alpha 1$, a peptide responsible for immune reconstitution in thymectomised animal models, improved survival when combined with interferon and amantidine in a mouse model of influenza [23]. Drotrecogin- α (recombinant human activated protein C) reduced 28-day all-cause mortality and appeared to be cost-effective

in patients with severe sepsis [24,25]. Certain antibiotics (e.g., macrolides) have shown cytokine-modifying effects that suggest a potential role in chronic degenerative and atherosclerotic diseases. However, routine use of antibiotics for such conditions would add greatly to the selection pressure for antibiotic resistance in bacteria, suggesting a need for research to isolate the anti-inflammatory properties of these agents from their antibiotic effects.

Role of the pharmaceutical industry

Despite the pressing need for new antibiotics, industrial research in this area is declining. Of c. 400 candidate drugs currently under development by pharmaceutical companies, only ten are antibiotics (H. Labischinski, personal communication). The development of new antibiotics is challenging and expensive. Typically, following the identification of a new molecular target, it takes >2 years to produce a candidate antibiotic. It takes a further 6 years and €800 million to develop a compound for the market. Market success rates are lower for new antibiotics than for other drugs because of obstacles associated with the chemistry and pharmacology of antibiotics, trends in clinical practice, regulatory requirements, and the market within the bacterial infection field.

The need for new antibiotics will be fulfilled only through a partnership between health care authorities, academia and the pharmaceutical industry. It may be worthwhile for industry to work with health authorities to contribute to high-quality research into the optimisation of current antibiotic usage and infection control. Both measures may help to control resistance and, in the long term, extend the commercial life of antibiotics. Other measures needed to reverse the industrial decline in research and development include the prioritisation of research and the development of incentives.

European research: 6th Framework Programme (FP6)

Europe is a leading region for the biological and pharmacological research upon which drug development is based. However, there is insufficient investment allocated to the critical development stages between basic research and the

pharmaceutical market. The European Research Area is a joint effort by the EU and Member States to address structural deficits in European research. The FP6 is the first venture to be initiated under this scheme.

The FP6 has launched several new instruments to support European research. 'Networks of Excellence' are designed to promote excellence within a particular research area and to address the fragmentation of research by linking resources and expertise around a joint programme of activity. 'Integrated Projects' generate the knowledge required to implement defined priorities by integrating resources to achieve clearly defined scientific and technological objectives. Both types of initiative are selected through open calls for proposals and peer review. Networks of Excellence are funded for ≥5 years, while Integrated Projects are funded typically for 3–5 years. Additional supportive instruments include 'Specific Targeted Research Projects', which fund research activities of a more limited scope, 'Coordination Actions' promoting networking, and 'Specific Support Actions', which fund information and communication activities (e.g., conferences).

The FP6 has a total budget of €17.5 billion. Of this, €2.255 billion is allocated to 'Life Sciences, Genomics and Biotechnology', the first of seven thematic priorities. Within this priority, 'Application-orientated genomic approaches to medical knowledge and technologies' includes research on combating resistance to antibiotics and other drugs. There is also scope for research on the public health aspects of drug resistance under the heading 'Supporting policies and anticipating scientific and technological needs', within a cross-cutting priority entitled 'Specific activities covering a wider field of research'.

The first call for proposals under the FP6, launched in 2002, resulted in the funding of four projects concerning antibiotic resistance (Table 1). The second call, which closed in November 2003, invited proposals for new integrated projects in two topics relevant to antimicrobial resistance: 'Functional genomics of antibiotic-producing organisms' and 'New molecular targets for the development of drugs against pathogens causing severe resistance problems'. In addition, there was a call for specific targeted research projects or concerted actions under the topic 'Novel approaches to address antimicrobial resistance through non-antimicrobial based therapies'. Proposals

Table 1. Research proposals on antimicrobial resistance selected from the first call for proposals under the European Commission 6th Framework Programme (FP6) in 2003

FP6 research topic	Project title	Project type	Project scope	FP6 contribution (€)	Start date
Testing antiviral drug resistance and understanding resistance development	European Vigilance Network for the Management of Antiviral Drug Resistance ('viRgil') ^a	NoE	Network system to predict and monitor resistance in HBV, HCV and influenza	9 million	Spring 2004
Basic mechanisms behind resistance	Molecular mechanisms behind resistance to inhibitors of cell-wall synthesis ('COBRA')	STREP	Modification of cell-wall synthesis in <i>Staphylococcus aureus</i> , <i>Streptococcus pneumoniae</i> and enterococci, and production of β -lactamases in Gram-negative organisms	3 million	February 2004
Basic mechanisms behind resistance	Molecular mechanisms of resistance, virulence and epidemicity in <i>Strep. pneumoniae</i> ('PREVIS')	STREP	Survival and growth of <i>Strep. pneumoniae</i> in antibiotic-rich milieu and inter-species competition for colonisation and spread	3 million	January 2004
Workshop on how to address antimicrobial resistance by exploiting microbial genomics	'Micro-MATRIX' meeting (Spain)	SSA	Strategies to address antimicrobial resistance through the exploitation of microbial genomics	34 000	17–20 April 2004

^aContract still under negotiation.

HBV, hepatitis B virus; HCV, hepatitis C virus; NoE, Network of Excellence; SSA, specific support actions; STREP, Specific Targeted Research Projects. See text for explanation of project types.

submitted under this call are being assessed currently.

Proposed topics for the two remaining calls for proposals (the third and fourth calls) are currently open to discussion and suggestions from the scientific community. Topics that may be considered in one or the other of these calls include: management of respiratory tract infections; nosocomial infections; functional microbial genomics; fungal resistance; and the transmission of resistance between animals and humans.

WORKING GROUP 1: MICROBIAL POPULATION BIOLOGY AND ECOLOGY OF RESISTANCE

Introduction

Extensive release of antimicrobial agents in all environments (i.e., both in hospitals and in the community) during recent decades has had an important impact on microbial populations. Patients, healthy persons, animals and the general environment have become important reservoirs for resistant bacterial populations and resistance genes. Bacteria have developed resistance mechanisms through mutations of pre-existing genes or

have gained additional genes through horizontal gene transfer (HGT). Both evolutionary processes have contributed to the enrichment of the resistance gene pool, thereby enhancing the opportunities for the persistence and transmission of antimicrobial-resistant bacteria and resistance determinants. As a result, resistance is now documented in bacteria that, traditionally, were fully susceptible. In addition, multidrug-resistant bacteria have risen to prominence, and new 'opportunistic', and often multidrug-resistant, organisms are recognised increasingly as important pathogens in both the nosocomial and community settings.

The assembly of resistance-related genetic elements probably originated from heterologous sources in various organisms, including pathogens and other bacteria belonging to the normal microbiota. These elements have acted as extremely efficient amplifiers for the evolution of resistance. It has been demonstrated, both in Gram-positive and in Gram-negative bacteria, that resistance genes may persist in well-adapted clones in a particular niche, and that they may spread to other clones of the same or different species as a consequence of gene mobilisation. These new clones may share the same niche, but may also explore and

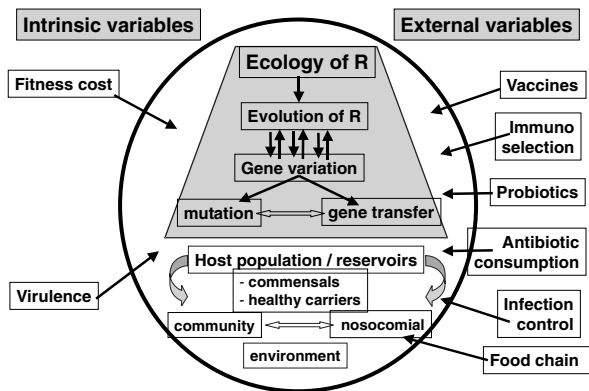


Fig. 2. Intrinsic and external factors affecting the ecology of bacterial resistance and the evolution of resistant populations.

colonise new environments, increasing the likelihood that the resistance genes will become fixed in the bacterial population, and that bacterial evolution will progress towards resistance [26].

An accelerated evolution of bacterial resistance is being observed currently. Some of the processes involved in this progression are starting to be understood, but various factors affecting the ecology of resistance and the evolution of resistant populations need to be addressed. These factors can be clustered into two groups:

- Intrinsic factors of bacterial populations—including the bacterial fitness cost associated with resistance, as well as the mechanisms leading to the assembly of genetic elements involved in resistance evolution, including the superimposition of virulence determinants.
- External factors influencing the bacterial population—including vaccination, immunoselection, antibiotic use and other interventions, both in patients and in the food chain (Fig. 2).

Intrinsic variables

Cost of resistance and the influence of mutators

Resistance has an important biological cost for bacteria, in terms of reduced fitness. This is a key parameter in understanding the stability and potential reversibility of resistance [27]. The advantage associated with resistance, whether originating from mutation or recombination, may be limited by the cost associated with the presence of mutated genes or newly acquired genes. The cost of resistance is negligible compared with the advantages of resistance in the presence of antibiotics. In the absence of antibiot-

ics, resistant bacterial populations may be superseded by susceptible populations because of the fitness cost associated with resistance. Resistant bacteria might also retain resistance in the absence of antibiotics by compensating for the biological cost of resistance via new compensatory mutations or gene exchange. Some of these compensatory advantages may correspond to virulence determinants or other functions permitting resistant bacteria to adapt to different habitats or environments, including those conditions generated by the immunological system.

Studies are needed to determine the importance of the biological cost of resistance and associated compensation mechanisms in resistant populations, and to understand clearly the role exerted by antibiotics in these processes. In-vitro models are required to identify environmental conditions that influence the emergence and maintenance of secondary mutations and/or the acquisition of new genes compensating for the fitness cost. Moreover, factors eliminating ancestral, susceptible sub-populations within resistant populations must be researched. These factors should be investigated in different conditions and environments with the assistance of mathematical modelling. Furthermore, no data exist on the interplay of the immunological system with these processes.

Under long-term sequential antibiotic selective pressure, bacterial populations may accumulate multiple mutations. This effect has been described clearly in stable mutator Gram-negative bacilli and, to a lesser extent, in Gram-positive bacteria. Mutators may achieve a mutation rate up to 200-fold higher than that in normal bacterial populations, frequently by alterations in the DNA mismatch repair system. The transition from 'normo-mutable' to mutator status may be accelerated under environmental pressures, including those exerted by antibiotics. As a consequence, cumulative mutations yield resistant bacteria with multidrug-resistant phenotypes. Mutator genes are more prevalent in 'chronic coloniser' pathogens, but their presence has not yet been explored in efficient epidemic strains or endemic populations. Mutator genes are likely to induce deleterious mutations, presenting an indirect selective disadvantage for the bacteria. However, periodic selection and genetic compensation processes may contribute to the stabilisation of mutator strains. Moreover, certain mutators are more proficient than normal strains at HGT and

recombination, which may contribute to the persistence of resistance genes and resistant strains. The use of mutators in fitness cost experiments may help to accelerate an understanding of this parameter and its influence on the irreversibility of resistance.

The potential benefits of an increased mutation rate for adaptation—without the continuous costs of deleterious mutations—have been reviewed recently [28]. This effect arises only under stress conditions in so-called ‘transient’ or ‘inducible’ mutators. In these populations, the hyper-mutator phenotype is recognised only in the presence of antibiotics. However, it may be reversed by the depletion of mechanisms involved in mutagenesis systems or by recombination with wild-type mismatch repair alleles in the absence of stress conditions. Further studies are needed to elucidate the importance of mutators in the development and maintenance of resistant populations.

Mechanisms of genetic assembly

Traditionally, plasmid exchange was considered to be the most important mechanism for the mobilisation and spread of resistance determinants. Sequence analysis of genomes has now revealed the importance of other genetic elements in the spread of newly detected determinants, such as those carrying genes encoding carbapenemases, non-classic extended-spectrum β -lactamases (e.g., CTX-M enzymes), plasmid-mediated AmpC enzymes, plasmid-mediated fluoroquinolone resistance in Gram-negative bacteria, and resistance to tetracycline, macrolides and vancomycin in Gram-positive species. Most genes encoding these determinants are inserted in complex structures resulting from the assembly of different genetic pieces, often from different ancestors [29]. Active research to identify and describe these structures and their integrative elements is underway. This research aims also to scrutinise the epidemiology of these factors in different bacterial hosts and their distribution in different environments. However, there have been fewer attempts to understand the specific mechanisms leading to the assembly of these components into complex constructions, and the way in which these constructions are preserved within bacterial populations.

Virulence determinants and resistance

The interplay between virulence and resistance phenotypes in the maintenance of single bacterial

cells and bacterial populations should influence future networks of research. Virulence mechanisms may be acquired in non-virulent populations as a consequence of evolutionary processes. Such processes include mutations and the construction of pathogenicity islands by genetic recombination [30]. In common with resistance, virulence may also have an important biological fitness cost for bacteria.

Continuous antimicrobial challenges to well-adapted, virulent bacterial populations may facilitate the persistence of resistance. Conversely, the acquisition or expression of virulence determinants by resistant bacteria may contribute to persistence of virulence. In both cases, the resulting virulent and resistant clones are capable of spreading to different environments and hosts. Virulence and resistance determinants may be linked in efficient transmissible elements. In this case, a polyclonal structure of virulent and resistant populations can be expected to arise. Different examples have been shown among Gram-positive organisms, including community-acquired Panton–Valentine leukocidin-producing MRSA strains, and enterococcal surface protein-producing multiresistant strains of *Enterococcus faecalis* and *Enterococcus faecium*.

Similar approaches are used by other pathogenic bacteria [31], but these require further research. The balance between virulence and resistance features, with regard to the different levels at which bacterial populations adapt to various environments or hosts, may lead to future interventions to control resistance.

External variables

Vaccination and immunoselection

Vaccination has an important impact on bacterial populations, leading to a potential decrease in the number of pathogenic bacteria. Moreover, vaccination programmes benefit not only the vaccinated human population, but also individuals who are not vaccinated. In the case of typical respiratory tract pathogens able to produce invasive diseases, such as *Strep. pneumoniae* and *Haemophilus influenzae*, vaccination has influenced the distribution of bacterial serotypes. Drastic reductions in certain serotypes have been associated with decreases in resistance [32]. However, this modification has also led to the emergence of other rare serotypes, and it may be responsible for the increase in

certain resistance mechanism variants that were previously in a minority. Thus, the influence of vaccination on microbial population biology and the ecology of resistance, particularly on specific (multidrug) resistant clones, should be a priority in research on combating antibiotic resistance.

The importance of immunoselection of bacterial populations by hosts, and the influence of this process on the presentation of disease, is beginning to be understood. The effect of immunoselection on antimicrobial resistance still requires study. Some of the factors involved are certainly related to bacterial colonisation and/or persistence abilities, and also to the limitations of the immune system in eliminating these bacteria. This has been shown with specific populations of *Neisseria meningitidis* and *Helicobacter pylori*. In situations of immunological tolerance, the persistence of bacteria during antibiotic challenge influences development of resistance. Interestingly, the effect of immunoselection on organisms belonging to normal microflora has not been studied to the same extent, and there has been little research on the distribution of resistant populations and the net balance of resistance determinants and gene structures for gene transfer within these populations.

Antibiotic consumption and infection control

The incremental relationship between increased antibiotic consumption, in both humans and animals, and the selection of resistant organisms has been documented many times, and has been the subject of previous European conferences. Conversely, a decline in antibiotic use is expected to decrease the prevalence of resistant bacterial populations. However, in some cases, the presence or fixation of the resistance genes in specific structures has rendered attempts to decrease resistance unsuccessful [33].

There are well-documented differences between European countries in terms of resistance patterns and antibiotic consumption. In addition to resistance surveillance, studies of interventions designed to control resistance are required. Attempts have been made to control the spread of extended-spectrum β -lactamase (ESBL)-producing organisms, AmpC β -lactamase-hyper-producing *Enterobacter* spp. and *P. aeruginosa*, MRSA, and macrolide and/or β -lactam-resistant *Strep. pneumoniae* and *Strep. pyogenes*. However, more specific study designs should be used to

demonstrate the modification of resistant bacteria population structures by these interventions. Moreover, the influence of reducing individual exposure to antibiotics on the availability of the resistance gene pool, particularly those genes and vectors participating in HGT, needs to be investigated in man, in both the nosocomial and community settings, and in animals. Furthermore, the impact should be evaluated on both pathogenic organisms and the normal microbiota. Again, the use of mathematical models may benefit the design of such studies [34].

A closely related area of interest concerns the impact of new antibiotics on bacterial populations. This effect should be monitored in surveillance studies to ascertain the emergence of bacteria resistant to these new antibiotics, and to assess the effect of new antibiotics on the ecology of resistance determinants. Active surveillance procedures for sentinel studies should be designed to research these relationships.

Infection control measures may reduce the transmission of resistant organisms, and thereby decrease the absolute pool of resistant bacteria and the gene pool of resistance determinants. This has been investigated recently by tracing integron structures, and further research should investigate other gene-capture units participating in HGT, such as phage-like structures.

The identification of co-selection factors for resistant populations and the maintenance of resistance determinants should also be studied. These factors include non-antibiotic challenges, such as exposure to heavy metals, toxic chemicals, organic solvents, household disinfectants and non-antimicrobial drugs. Some of these challenges may occur in the environment, and may be important in favouring the assembly and evolution of different mobile elements.

Probiotics, prebiotics, synbiotics and food chain modifications

There is renewed interest in Europe in the use of probiotics, prebiotics and synbiotics, in the hope that they may benefit mucosal immunoresponse and prevent bacterial infections. Prebiotics are chemicals used mainly to manipulate the composition of colonic microbiota, and improve health by stimulating colonisation with probiotic bacteria. Probiotics are non-pathogenic bacteria that, when ingested or applied to mucosal surfaces, are expected to benefit host health or physiology. The

combination of prebiotics and probiotics is defined as 'synbiotics', which represents a new field of investigation. These strategies are used to: prevent bacterial infections, mainly in the gastrointestinal tract (including those produced by *H. pylori*); regulate the mucosal immune response; avoid intestinal inflammation; and prevent allergic reactions. Prebiotics and probiotics are also used in animals, particularly since the use of antimicrobial agents as growth promoters has now been limited.

Prebiotics and/or probiotics should be introduced only after a risk assessment of their effects on normal bacterial populations in humans, animals and the environment. The potential production of toxins and virulence factors affecting bacteria and hosts should be investigated carefully. In addition, standardised studies are needed to elucidate their clinical usefulness and the mechanisms responsible for any beneficial effects. The direct influence of probiotics and prebiotics on natural mucosal bacterial populations, and the interactions between these agents and the immune system, should also be researched [35].

A potential risk associated with the use of probiotics is the transfer of resistance determinants from these bacteria to normal microbiota. This effect should be monitored carefully in humans and animals. Moreover, the potential transfer to humans, through the food chain, of probiotic organisms used in animal husbandry and food technology should be evaluated. The potential resistance gene transfer processes should be evaluated, as well as the resulting impact on bacterial populations. Gaps in knowledge in this area include an absence of microbiological breakpoints and few quantitative and qualitative data on HGT of antibiotic resistance genes to organisms used in food technology.

Tools for research and endpoints

Future studies into microbial population biology and the ecology of resistance should be stratified into descriptive, predictive and intervention studies. Descriptive research should use quantitative ecological methods. In addition, new technologies for comparing population genomics should be introduced to search for resistance determinants and to identify genomic structures involved in HGT. These studies should be performed using

time-series comparisons to elucidate evolving phylogenies. Moreover, predictable, fitter strains able to spread readily in the population should be included as indicators within descriptive studies. Predictive studies should use mathematical models to predict the emergence and persistence of individual resistant bacteria within populations, and the evolution of these strains. The use of mathematical modelling to predict modifications of genetic determinants, including mutation and recombination processes, will also contribute to an understanding of the ecology of resistance. Observational studies are important to improve overall knowledge of the problem of resistance and to develop control strategies, but intervention studies are mandatory for ascertaining the impact of these strategies. The emergence, persistence and modification of individual bacteria, bacterial clones, resistance gene carriers and resistance determinants should be the endpoints for each of these three types of research.

Conclusions

The application of microbial population biology to the study of the ecology of antibiotic resistance is a newly introduced strategy which should be understood in terms of the evolution of bacterial populations and the genetic determinants responsible for resistance. The targets for this research comprise individual bacteria, bacterial clones, bacterial population communities in different hosts and environments, individual resistance genes, and genetic structures carrying resistance genes that participate in HGT. The investigation of internal and external factors affecting these targets requires differential research strategies to allow identification of the mechanisms involved in the emergence of resistance, together with their means for dissemination and persistence within bacterial populations. Descriptive, predictive and intervention studies in this area will help to combat antibiotic resistance.

WORKING GROUP 2: THE CLONAL SPREAD OF RESISTANT BACTERIA IN EUROPE

Introduction

The key issues discussed in this Working Group were the clone concept, the geographical expan-

sion of resistant bacterial clones in Europe, the role of mobile elements in the development and spread of drug-resistant clones, the technology and concepts applicable to the molecular epidemiology of resistant clones as a guide to infection control, and the role of laboratory support for international communicable disease surveillance.

The Working Group developed five statements, each presented below with supporting commentary.

Evolution and fitness of resistant clones at the bacterial cell level

Bacterial population genetics indicate that many human pathogens are composed of a limited number of successful clonal lineages which share conserved genetic elements involved in pathogenicity and resistance to antibiotics. These bacterial lineages, and the associated stable genetic elements, can be viewed as mosaics of clonal selection and evolution. Certain clinically significant multidrug resistance problems arising worldwide (e.g., β -lactam-resistant *Strep. pneumoniae* and MRSA) are related, in part, to the international dissemination of epidemic clones that have accumulated several resistance traits. These clones have been shaped within the bacterial species through an evolutionary process that includes: (1) the selection of prevalent strains more proficient than others in colonisation ability or virulence; (2) the integration in these strains, by HGT, of resistance gene elements or whole resistance islands; (3) mutations conferring additional resistance traits; and (4) mutations that compensate for the energetic cost of these genetic alterations, and thereby restore the biological fitness of the cell.

Dissemination by HGT of mobile elements is itself a major driving force in the evolution of bacterial populations towards resistance. Plasmids and non-replicative elements, such as conjugative transposons, are exchanged efficiently between bacteria. Transduction and natural transformation are also efficient means of resistance acquisition in some species. Finally, integrons favour the accumulation and stabilisation of resistance genes in a single bacterial host, and thereby contribute to the regulation of their expression. Together, these elements constitute a powerful 'toolbox' that makes possible

virtually unlimited dissemination of almost any resistance gene throughout the bacterial world. Studies that include investigations on the molecular epidemiology of mobile genetic elements contribute significantly to a comprehension of the clonal spread of antibiotic resistance. This approach has been used successfully to study the dissemination of ESBLs in different phylogenetic groups of *E. coli* and in different species of enterobacteria (e.g., TEM-4, TEM-24, CTX-M-9). Researchers have been able to differentiate between several distinct paths of evolution, i.e., those characterised by few clones with a single resistance gene-carrying plasmid (international clones), by many clones with a single resistance gene-carrying plasmid, or by many clones with a single resistance gene-carrying transposon integrated in different plasmids (e.g., Canton *et al.* [36]).

Statement 1

The integrated study of all elements of bacterial evolution, including the core genome, pathogenicity islands, resistance islands, resistance genes, resistance mutations and compensatory mutations, together with an assessment of fitness by in-vitro (competition assays) and in-vivo (animal model) studies, will improve greatly our understanding of the successful adaptation of prevalent clones. Specific attention should be paid to the study of mobile genetic elements involved in the evolution of resistant clones (i.e., their origin, diversity, mode of assembly, mechanisms of transfer and classification) in order to yield a comprehensive picture of the spread of antibiotic resistance.

Methods for clonal detection and characterisation

Numerous molecular epidemiological surveys have delineated the widespread expansion in Europe of antibiotic-resistant clones of Gram-positive organisms such as penicillin-resistant pneumococci, MRSA (including specific clones susceptible to gentamicin or producing Pantone-Valentine leukocidin), glycopeptide-resistant *Ent. faecium*, *Mycobacterium tuberculosis* resistant to rifampicin and isoniazid, as well as Gram-negative organisms such as ESBL-producing enterobacteria, *P. aeruginosa*, and *Salmonella enteritidis* (e.g., Lelievre *et al.* [37]). However, these surveys

relied on methods with variable discriminatory power and ability to characterise resistant clones.

In practice, the results of phenotypic methods, such as antibiotic susceptibility testing, could be used to detect particular resistant clones, provided that these methods are designed adequately (i.e., in terms of the drugs to be tested, mode of expression of the results, definition of the patterns, etc.) and that their discriminatory power is assessed. Genotyping by pulsed-field gel electrophoresis or single-loci sequencing can be extremely effective in studying outbreaks at a local level, whereas PCR-based systems that rely on observations of genomic sequence polymorphisms at multiple loci can be used at a later stage to inform surveillance programmes. Multiplex PCR, binary probe typing and DNA microarrays constitute high-throughput technologies suitable for characterising resistant clones at this macro-epidemiological level [38]. It would be useful to build databases of international epidemic clones of major resistant pathogens. If such data bases were updated regularly, DNA microarrays could be designed and applied to screen patients for these clones upon hospital admission.

Statement 2

Progress should continue to be made in the harmonisation of genotyping methods and nomenclature. The markers obtained by these methods should be integrated into European surveillance programmes on communicable diseases and bacterial resistance. The most cost-effective and polyvalent techniques should be favoured and validated by pilot studies. Databases of genetic markers of major international resistant bacterial clones should be established. It should be recognised that such schemes require investment in many countries to make first-screen and reference typing services available more widely to hospital resistance surveillance and infection control programmes.

New tools to study the dynamics and expansion of resistant clones

New combined typing systems based on genomic sequencing can provide comprehensive and phylogenetically relevant information on multiresistant clones, such as MRSA. These

systems allow investigation of the evolutionary history of successful clones at national or international level, e.g., community-acquired MRSA clones carrying the Panton–Valentine leukocidin gene that have evolved from ST30 methicillin-susceptible *S. aureus* [9]. The integration of results of molecular epidemiological research into mobile elements and resistance determinants, based on genome typing systems involving analysis of amplified fragment-length polymorphisms, single-nucleotide polymorphisms, variable number of tandem repeats or microarray profiling, will be of great help in elucidating the dynamics of major successful clonal complexes such as B2 *E. coli* (EXPEC), CC30 MRSA and CC1 *Ent. faecium*. This type of integrated approach can also be used to investigate the accumulation of resistance elements in particular clones (e.g., *M. tuberculosis* resistant to rifampicin and isoniazid).

Many factors interact to determine the spread and persistence of resistant clones in humans, animals and the environment. These factors relate to the host (e.g., previous hospitalisation, antibiotic therapy, the number of persons at home), the pathogen (e.g., virulence, resistance traits) and antibiotic use. Mathematical models are being developed to study and quantify in a predictive manner the respective roles of these factors in the dynamics of resistance at the population level.

Statement 3

New genome-based tools should be used to study the dynamics and expansion of resistant bacterial clones. Integrated and combined typing systems based on genomic sequencing will help to elucidate the evolution of major successful clonal complexes. Again, mathematical models will help to assess the respective roles of the factors (e.g., host, pathogen, antibiotic pressure) that co-determine the spread and persistence of resistant clones, and to predict the dynamics of resistance at the population level.

Statement 4

Well-defined communities in which host movements and interrelations between hosts are limited sufficiently to allow accurate investigations—such as hospitals or hospital units (e.g., intensive care units), nursing homes, schools or

small rural areas—provide opportunities for measuring the prevalence of successful clones at the population level and for studying the factors modulating their spread, such as environmental factors (e.g., antibiotic use), host factors, bacterial factors and genetic elements.

Epidemiology of resistant clones at the local, regional, national and global levels

Numerous surveillance systems based on local, regional, national or international networks (or networks of networks) are devoted to, or at least involved in, antibiotic resistance monitoring. When properly organised, such surveillance can estimate the prevalence or incidence of resistance, with the surveyed population as a denominator (i.e., through 'enhanced surveillance', as recommended by the World Health Organisation (WHO)). The EARSS is developing this approach at a European level. These systems constitute a potential basis for organising epidemiological studies that target successful clones or clonal complexes. Such studies can be used to assess parameters that modulate clonal spread, thereby allowing these data to be integrated within predictive models. A well-defined sentinel scheme for sampling representative isolates at a local laboratory level needs to be designed. Two-stage genotyping (as described above) would be a cost-effective means to identify important clones and to compare their distribution over time and place in the population surveyed. National reference centres that conduct multicentre antibiotic resistance surveillance and alert programmes can play a pivotal role in this process, and should be linked in European networks, where available.

Statement 5

Efforts should be made to integrate data regarding clonal type frequency distribution for major bacterial pathogens more efficiently by means of existing surveillance systems dedicated to antimicrobial resistance. This integrated European system should be developed by involving and networking the available national reference centres. It should enable early detection and warning of emerging epidemic multidrug-resistant clones that warrant immediate control.

WORKING GROUP 3: ANTIMICROBIAL DRUG DISCOVERY—EXPLOITING MICROBIAL GENOMICS AND COMBINATORIAL CHEMISTRY TO FIND NEW MOLECULAR TARGETS

Introduction

The development of new antimicrobial agents during the last 10 years has been characterised by a high degree of activity within the fields of antiviral agents, antifungals and antibiotics active against Gram-positive bacteria. The main reasons for this are the HIV/AIDS epidemic, the increasing number of immunodeficient patients, and the rapid increase of infections caused by multidrug-resistant organisms such as MRSA, penicillin-resistant *Strep. pneumoniae* and VRE. However, during the same period, there has also been a marked increase in clinically important antibiotic resistance in Gram-negative bacteria, e.g., ESBL-producing Enterobacteriaceae and carbapenem-resistant, non-fermenting organisms such as *Acinetobacter* spp., *Pseudomonas* spp. and *Burkholderia* spp. Despite this, there are no new antibiotics in clinical development that have satisfactory activity against such organisms, and very few compounds in preclinical evaluation. Other microorganisms of major clinical importance for which there is a lack of new and improved treatments are *M. tuberculosis* strains resistant to rifampicin and isoniazid. There is also a lack of agents with documented activity against many microbes that may be used for bioterrorist purposes, in particular the smallpox virus.

Obvious reasons for this worrying situation include the extremely high cost of developing new antimicrobial drugs, and the tendency for clinicians to keep new antibiotics in reserve in order to reduce the risk of worsening resistance. In the case of *M. tuberculosis*, a major problem is that resistant strains are most common in developing countries, which cannot afford the costs of newly developed drugs. These factors have resulted in a limited economic incentive for pharmaceutical companies to initiate research aimed at finding new treatment modalities for infections.

Obstacles to be overcome during the development of a new antimicrobial agent are the requirement for activity against multiple targets

(especially for antibiotics), large microbial populations that give rise to a high likelihood of resistance mutations, and the possibility of horizontal transfer of resistance genes between microorganisms. Approaches to the identification of new agents include the screening of natural products, revisiting old chemical structures, and microbial genomics.

Screening of natural products

β -Lactams and many other antibiotics are the result of screening for naturally occurring products with antibacterial activity. Although such methods proved highly successful in the past, they are now being abandoned because they are cumbersome and expensive. Moreover, it is likely that the most common natural products with antimicrobial (or at least antibacterial) activity have been identified already.

Revisiting old chemical structures

Many candidate antimicrobial compounds have been withdrawn from full clinical development because they showed unfavourable animal toxicity profiles, resistance problems or pharmacokinetic disadvantages, such as poor solubility of parenteral drugs and insufficient oral absorption. It is likely that these problems could be overcome for some products. β -Lactamase inhibitors, such as clavulanic acid and sulbactam, have overcome some of the problems associated with the degradation of penicillins and cephalosporins by β -lactamase enzymes. Examples of chemical alterations increasing the gastrointestinal absorption of antimicrobials include esterification (e.g., piv-mecillinam, cefpodoxime proxetil and valacyclivir) and various types of enterocoating to prevent the inactivation of erythromycin by gastric acid. Another example of the positive effects of chemical modification is imipenem, or *N*-formimidoyl thienamycin, a considerably more stable and soluble molecule than thienamycin itself. This antibiotic also exemplifies the chemical modification of an unfavourable pharmacokinetic profile. Renal degradation of imipenem results in low active drug concentrations in the urine and a marked risk of nephrotoxicity. By combining imipenem in a 1:1 ratio with cilastatin, an inhibitor of the relevant renal enzyme, this metabolism is blocked and nephrotoxicity is avoided.

Microbial genomics

During the last 30 years, only one new class of antibacterial agents with a novel mode of action has been developed: the oxazolidinones, of which linezolid was the first member. Rapidly increasing knowledge of microbial genetics offers obvious possibilities to search for new targets and overcome the previous stagnation. However, the initial optimism and hopes for immediate therapeutic developments resulting from genomic mapping have been dampened somewhat by the fact that the products and/or functions of many genes are unknown. Identification of gene products and their function requires access to bioinformatic systems including and combining information on genomics and proteomics. Different bioinformatic tools have been designed to search for similarities among different genomes. However, it may be the case that a large number of the existing targets for antibacterial activity have been identified already by other methods. Another problem in microbial genomics results from errors in the nucleotide sequences obtained by many of the early sequencing projects, together with limited annotation. This emphasises the urgent need for systematic and continuous annotation of genomic information. Preferably, such annotation—as well as information about gene products and their functions—should be collected centrally in an easily accessible data base.

Fig. 3 shows the steps involved in the exploitation of genomic information to provide a potential target for antimicrobial drug intervention, and

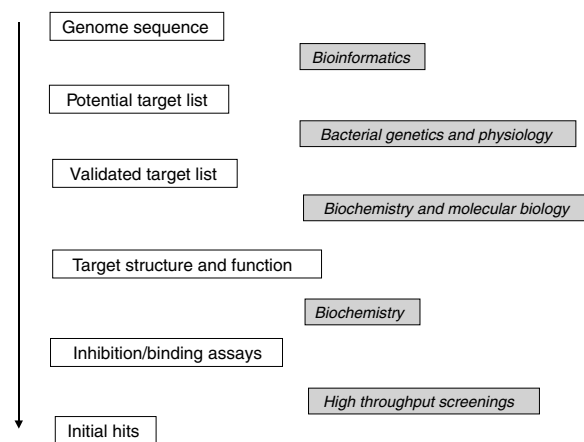


Fig. 3. Summary of the process by which new molecular targets for antimicrobial agents are identified and exploited using genomic information.

the subsequent identification of drug candidates. Clearly, a multidisciplinary approach is required. It could be argued whether research efforts should be orientated more horizontally or vertically, but probably the differences in approach will have little significance.

In general, an antimicrobial target should: (1) provide specific or highly selective activity against the microbe with respect to the human host, together with activity against the desired spectrum of pathogens; (2) be essential for the growth or viability of the pathogen; and (3) have a known function so that assays and high-throughput screens can be created. An absolute prerequisite for this research is an improved knowledge of microbial physiology and pathogenesis. This knowledge may also provide opportunities for novel approaches directed against the pathogenetic mechanisms of infections, rather than targeting the microorganisms themselves directly.

Gene disruption is a time-consuming process that, nevertheless, provides valuable in-vivo information about gene function. This technique is often required to demonstrate that a gene product is essential for bacterial growth or viability, and therefore that it constitutes a likely candidate molecular target. A variety of genetic tools to test whether gene products are essential have been developed. These include:

- random approaches, such as transposon mutagenesis and conditional lethal mutations;
- sequence-directed approaches, such as plasmid insertion mutagenesis and allelic exchange mutagenesis;
- target modulation analysis, such as antisense RNA and microarray technology.

Approximately half of the genes in bacterial genomes either lack sufficiently significant sequence similarity to permit putative functional assignment, or have likely homologues whose function is unknown. In neither case can a function be predicted for the gene product. However, several genetic, biochemical and computational methods may provide insights into the function of these proteins. Among these is the prediction of molecular function from high-resolution X-ray or nuclear magnetic resonance imaging of the molecule.

Once an essential target gene has been identified, it can be cloned and expressed, and the protein purified and crystallised to solve the structure. Knowledge of the three-dimensional

structure of a target may help in understanding its function, and will also provide the opportunity for a rational approach to the design of new antibiotics and inhibitors. Availability of a purified protein also allows production of monoclonal antibodies against the target, which are powerful tools in further studies of its functions.

Conclusions

Finding new molecular targets is merely the first step in drug discovery. The discovery of a new antibacterial agent that interacts with these targets has been empowered by new methodologies such as molecular modelling, combinatorial chemistry to synthesise a high diversity and variety of compounds, and high-throughput methods that allow the rapid screening of hundreds of compounds.

An important issue is allocation of responsibilities and resources between academia and industry. As a general rule, it seems prudent to suggest that academia should concentrate mostly on microbial physiology, mechanisms of pathogenesis, and target identification and characterisation. The main role of industry lies in the identification and screening of potential drug candidates. Industry has advantages in terms of its access to excellent bioinformatics units, and extensive computer systems for screening for modes of activity and potential toxicity.

In summary, antibacterial drug discovery has, traditionally, relied upon random screening or modification of known antibacterial agents, often identified as a result of screening for natural products with antimicrobial activities. These strategies have failed to deliver sufficient molecular diversity to counteract the constant increase in antimicrobial resistance. Hopefully, the technologies mentioned above should permit a variety of novel agents with new mechanisms of action to be developed.

WORKING GROUP 4: CLINICAL AND EPIDEMIOLOGICAL RESEARCH TO EVALUATE THE OUTCOME OF ANTIMICROBIAL-RESISTANT INFECTIONS

Introduction

There is a lack of reliable data in Europe concerning the outcomes resulting from antimicrobial resistance, including attributable mortality, pro-

longation of hospital care, and the economic impact on individuals and health care systems. This information is a prerequisite for estimating the burden of resistance, and is essential to enable health system administrators, policy-makers and health care workers to prioritise, develop and implement solutions to the problem. The lack of data concerning these outcomes results, in part, from problems with the study designs and methods used in their determination [39,40]. For example, methods for measuring the economic impact of resistance are in their infancy, and the studies available leave many questions unanswered [41,42]. Moreover, the impact of antimicrobial resistance should be considered from several viewpoints, namely those of prescribing physicians, patients, health care service managers and providers, the pharmaceutical industry, the general public and governments. Thus, studies to measure the various defined outcomes must vary in design according to these viewpoints.

This Working Group addressed the research objectives and methodological issues relevant to studies of the clinical and economic outcomes of resistance.

Study designs

The Working Group identified two types of study design that are potentially useful for evaluating interventions to reduce antimicrobial resistance. First, some participants favoured the use of cohort studies and case control studies to determine the burden of infections caused by antimicrobial-resistant pathogens. The most important problem with these studies is the difficulty in controlling for potential confounders. Second, other participants favoured the use of randomised, controlled trials to demonstrate the potential benefit of introducing appropriate intervention measures, such as decision-support systems (e.g., TREAT), guidelines and other methods for optimising antimicrobial treatment and infection control measures.

Outcomes

Most published studies on the impact of resistance have limited their assessment to endpoints such as mortality and length of hospital stay. Costs and further outcomes after discharge, or after transfer to other facilities (e.g., rehabilitation units), are calculated rarely, but should be con-

sidered when evaluating the impact of antimicrobial resistance from a public perspective.

The direct medical costs of resistance include the costs of more expensive antimicrobial agents, labour and laboratory consumables, the costs of hospitalisation for extra days necessitated by the failure of initial therapies, and the cost of isolation and other infection control measures. To date, very few studies have provided information about the costs of care for infections caused by a given antimicrobial-resistant pathogen in comparison to the antimicrobial-sensitive variant of the same pathogen. Furthermore, because of the wide variability of health systems in Europe, it is difficult to compare such information from different countries. Therefore, it is essential to develop validated, internationally accepted outcome measurements that assess the clinical and economic impact of antimicrobial-resistant infections and, in turn, provide a means of assessing the impact of a particular intervention.

Types of infection

Studies are required to investigate the consequences of infections caused by antibiotic-resistant organisms in the community setting, as well as in the hospital. Research into patient outcomes associated with nosocomial infection is facilitated by the availability of better microbiological information in hospitals compared with the community. Moreover, nosocomial pathogens include resistant species of high priority, such as MRSA and Gram-negative multiresistant bacteria. Nevertheless, most antimicrobial prescribing takes place in the community, and it is also important to investigate the consequences of infections caused by relevant resistant pathogens in this setting (i.e., meningitis, urinary tract and lower respiratory tract infections). This research will require computer linkage systems to provide comprehensive information about resistance in the community and patterns of antimicrobial prescribing.

Multinational and multidisciplinary approach

The variability between European countries in antimicrobial prescribing and infection control measures complicates research in this area. Researchers should exploit available international data from hospitals and the community, since these correspond to the minimum data set

required. Also, the WHO should include appropriate coding for antibiotic-resistant infections in the ICD-10 system. There is a clear need for close cooperation between clinicians, microbiologists, epidemiologists and health care economists to measure the impact of antimicrobial resistance.

Research infrastructure

Overall, this Working Group reached the following consensus with regard to the research infrastructure required for studying the impact of antimicrobial resistance:

- multinational and multidisciplinary networks that investigate variations in practice against defined outcomes among different countries;
- validated support systems that inform an appropriate prescribing infrastructure in relation to local microbial ecology (e.g., TREAT);
- development of validated international, national and local guidelines and policies;
- collaboration with the WHO to ensure that the ICD-10 codes address antimicrobial resistance 'fields'.

Conclusions

The following priorities were formulated for clinical and epidemiological research to evaluate the outcome of antibiotic-resistant infections:

- identification of validated outcomes (biological, clinical and economic) to support the measurement of the antimicrobial resistance burden, and to assess the benefit and outcomes of interventions;
- ascertainment of the clinical and socio-economic burden of antimicrobial resistance associated with community- and hospital-acquired (health care-associated) infection;
- mathematical modelling and rigorous systematic reviews of the existing evidence linking antimicrobial resistance to intervention and outcomes;
- development and implementation of high-quality methodologies for the design and analysis of antimicrobial resistance and outcome studies;
- development of educational, behavioural and organisational strategies to improve the management of infections and reduce the burden of antimicrobial resistance;
- pharmacokinetic and pharmacodynamic outcome-based studies that inform prescribing of generic antimicrobial agents;

- identification of factors that support the sustainability of effective strategies to control antimicrobial resistance (i.e., sustainable and continuous quality improvement systems).

WORKING GROUP 5: MEASURES TO CONTROL RESISTANCE BY INTEGRATING MICROBIOLOGICAL, EPIDEMIOLOGICAL AND ECOLOGICAL RESEARCH

Introduction

Ultimately, research into antibiotic resistance aims to prevent resistance development and spread of resistance through interventions directed principally at reducing the overall use of antibiotics and limiting the transmission of antibiotic-resistant infections. The question of how such interventions should be planned and evaluated through research with a sound scientific basis is critical. This Working Group attempted to identify the gaps in the present evidence base, both in the identification of the risk factors for the development of antibiotic resistance and in the design of countermeasures.

Burden of disease

A number of critical issues should be clarified in order to set the stage for subsequent research. Importantly, it is necessary to establish the burden of disease attributable to antibiotic resistance. Although resistance is recognised as a major public health threat, data are still needed to demonstrate the associated costs in terms of excess morbidity and mortality and economic outcomes. With the exception of multidrug-resistant tuberculosis, for which the evidence is compelling [43], published data concerning the impact of resistance remain scarce and incomplete. Data on the burden of disease are necessary to increase awareness in medical professionals and in the general public, and to support requests to policy-makers for research funds and intervention campaigns.

Is 'prudent' use of antibiotics practicable?

The increased use of antibiotics has been blamed for the increase in antibiotic resistance. Although 'prudent' antibiotic use, a term often interpreted as 'restricted' use, has been advocated to coun-

teract the threat of resistance, its practicability has not been explored. Health care providers and prescribers must be convinced that the benefit to the global community offered by an appropriate use of antibiotics does not translate into increased risks for individual patients. It is obvious that 'prudent' antibiotic use excludes inappropriate use, e.g., for the management of viral infections, or for extended periods in the case of routine surgical prophylaxis. However, whenever there is uncertainty in the clinical diagnosis and/or in the aetiology of the infection, 'prudent' antibiotic use turns out to be an ill-defined 'grey area', and a matter of personal experience rather than a clear-cut concept. For some infections, e.g., otitis media, the early use of antibiotics is still controversial, and different countries have different medical practices [44]. Studies to evaluate the outcomes of these infections according to the therapeutic strategy employed, including the withholding of antibiotics, are necessary.

Surveillance programmes

Monitoring changes in the susceptibility of microorganisms, and the emergence and spread of antimicrobial resistance, is a priority for research. Many local and international antibiotic resistance surveillance systems have been implemented in recent years. These systems have diverse aims and means of funding, e.g., by national governments, international organisations or pharmaceutical companies [45]. Surveillance should aim to establish the need for interventions and to evaluate the effect of interventions. Accordingly, the results of surveillance should be disseminated rapidly to all interested parties. Studies should contribute to an improvement in the quality of existing surveillance programmes, and to the design of new and more focused surveillance programmes.

Surveillance programmes should also integrate studies in different areas, including molecular epidemiology and clinical outcomes as well as descriptive epidemiology. Molecular techniques should be used to confirm and validate phenotypic susceptibility data (e.g., detection of the *mecA* gene in MRSA) and to demonstrate the genetic basis for the emergence and spread of resistance. The detection of resistance genes, and of the genetic elements on which they are carried, is necessary for a deeper insight into the transmission of resistance traits, especially in multire-

sistant isolates. In addition, molecular typing methods should be used to recognise clusters and to establish clonal relationships among isolates in order to clearly understand the local and global spread of resistance. The study of virulence factors and the detection of virulence markers in resistant isolates would contribute further to an understanding of resistance transmission dynamics. The collection of outcome data is also necessary, as these can provide important information for decision-makers. Outcomes to be considered include not only clinical endpoints (e.g., morbidity, mortality and length of hospital stay), but also economic endpoints, such as the costs of newer, more expensive antibiotics, additional tests, and prolongations of hospital stay associated with infections by antibiotic-resistant bacteria.

Integration of information systems

Another priority in the struggle against antibiotic resistance is research in the field of information technology aimed at integrating health care and clinical information systems. Health care system data bases, such as those maintained by laboratories, pharmacies, hospitals and general practitioners, contain a wealth of information relevant to the susceptibility of microorganisms and antibiotic use, both in the hospital and in the community. Although these data are produced routinely, they are often not available for an integrated analysis. Research should be directed towards developing information technology instruments to integrate these systems and collate data for epidemiological studies on antibiotic resistance. These data bases could be exploited, through the development of data mining systems, for the early detection of novel resistance or new trends in resistance development. In addition, decision-support systems should be developed and evaluated.

Alternatives to classical epidemiology

Methods of classical epidemiology remain central to research into antibiotic resistance. These methods have been used widely to monitor resistance trends and to design and evaluate intervention measures, such as vaccination campaigns in the community and infection control measures in the hospital. However, in the future, alternative methodologies may provide a better understanding of resistance and the means for its prediction

and control. Although antibiotic use is a primary factor determining the emergence of resistance, other risk factors must also be evaluated. Risk-assessment models that combine classical epidemiology and mathematical models should be developed. These would help to identify gaps in knowledge necessary to evaluate risk factors in a defined setting.

New mathematical models should be generated to predict the evolution of resistance [46] and to evaluate the impact of different control measures without the need for empirical testing. Importantly, the health economic impact of intervention measures should be amenable to analysis by mathematical models. These models may allow prediction of the cost of the intervention and the reduction in expenses associated with a decrease (or a stabilisation) of resistance. Finally, investigations aimed at developing neural networks and artificial intelligence systems should be implemented with the aim of building decision-support systems for diagnostic and therapeutic purposes. In particular, these may be targeted at the management of infections caused by antibiotic-resistant microorganisms.

Conclusions

The priority areas mentioned above are interconnected fields in which research on antibiotic resistance should concentrate. As all medical and public health activities should be based on robust scientific evidence, research should aim to fill existing gaps in the evidence base concerning the identification of risk factors for antibiotic resistance development and the evaluation of control measures. In this regard, epidemiological studies—integrated with new molecular disciplines and mathematical modelling—remain central.

WORKING GROUP 6: RESEARCH POLICY—HOW TO ADVANCE ANTIMICROBIAL RESISTANCE RESEARCH AND TRANSLATE KNOWLEDGE INTO NOVEL SOLUTIONS

Introduction

This Working Group discussed the current bottlenecks in European research, the dramatic reduction in industrial investment into the research and

development of new antibiotics, the need for partnerships between industry, public health authorities, academic bodies and regulatory authorities to support research, the role of scientific societies in training scientists and in promoting and co-ordinating research and associated information, and the issue of research funding.

The need for new antimicrobials

It must be reaffirmed strongly that there is an urgent need for new antibacterial agents. Many new and important challenges are evident already within current medical practice (e.g., VRE, methicillin- and vancomycin-resistant staphylococci, multidrug-resistant *P. aeruginosa* and other glucose-non-fermenting Gram-negative bacilli), and these are likely to become increasingly pressing in the near future. These challenges should be defined clearly and communicated at all levels, ranging from health care policy-makers to the customers/patients, as part of an effort to emphasise the burden of bacterial infections and their enduring importance, particularly in the context of the globalised culture of mega-cities and mass tourism. The specific contribution of antimicrobial resistance to the infection problem must be emphasised, since a greater awareness of this problem is still needed at the level of health care providers and the general public, as well as at the political level.

Education and training of students, physicians and clinical microbiologists must include the principles of rational antimicrobial therapy and the main resistance problems. This represents a joint task for schools and professional societies, and important contributions can come from industry.

Public awareness of the microbial threat and of antimicrobial resistance should be increased through educational initiatives that promote an appropriate physician-driven use of antibiotics. In this regard, the adjective 'appropriate' seems endowed with a more positive and proactive meaning. As such, this term might carry the right message more effectively than the classical term 'prudent', which seems often to simply advocate an abstention from something. A strictly economics-based view of the problem of antimicrobial resistance seems short-sighted and should not substitute for a comprehensive clinical and public health viewpoint. Strategies

aimed only at limiting pharmacy costs may save money, but more often than not, will decrease both appropriate and inappropriate antibiotic use [47].

Many novel antimicrobial agents are likely to be niche products, endowed with narrow antibacterial spectra and/or targeted to specific clinical problems. Therefore, an important educational goal will be to change the current attitudes of physicians and customers towards large-spectrum, multi-purpose and user-friendly compounds. Scientific societies must play a leading role in this process, as they are well-suited to serve as forums for discussing, harmonising and monitoring interventions, mostly at the international level. It is necessary also to reinforce the role of these societies in the development of better clinical and microbiological practices, and in lobbying specialists in disciplines related to infectious diseases.

The basic directions for research

The problem of antimicrobial resistance, and the inability of current antibiotics to cover all clinical needs, suggest two basic directions for research in this field, namely:

- research on the development of new antimicrobial drugs;
- research on the appropriate use of existing drugs.

These two research lines must be explored in parallel, since a sufficient development of novel compounds is unlikely to occur in the next few years, resulting in a dramatic lag-phase in the therapeutic possibilities for an increasing number of bacterial infections, and prompting a more appropriate use of existing antimicrobials.

Both basic and clinical microbiology appear to be essential for research on new antimicrobial compounds, and for better definition of the appropriate use of existing drugs.

A better understanding is needed of bacterial physiology and population dynamics, of the role played by the commensal microflora as a resistance gene pool, and of intrinsic resistance in opportunistic pathogens. A better understanding is also needed of the impact of antibiotics on microbial population dynamics, and of the impact of biocides on the selection of multiply-resistant bacterial clones. To this aim, the exchange of biological materials (strains, genes, etc.) between

researchers should be facilitated, while bearing in mind the necessity to prevent opportunities for the acquisition of hazardous organisms for bio-terrorist purposes. Once again, it must be emphasised that scientific societies can play an important role in this matter by issuing guidelines for these exchanges, asking national authorities for less restrictive rules, and playing an active role in certifying the nature of the exchanges and the goals of people involved.

The basis for resistance monitoring and appropriate use of antibiotics is microbiological diagnostics. Faster and more cost-effective detection of resistance through rapid diagnostic systems is a prerequisite for the use of better-targeted antibiotics.

The need for partnership

There is an urgent need for interdisciplinary and public-private partnerships to support research in this area. Exchanges between industry, public health bodies and academic bodies will entail not only sharing of costs, but also co-ordination of the respective research activities.

Unfortunately, and in spite of the urgent need for new antibiotics, while some pharmaceutical companies (including Abbott, Bayer, Johnson & Johnson, GlaxoSmithKline, Novartis and Pfizer) remain committed to this field of research, many other companies (such as Aventis, Bristol Myers Squibb, Lilly, Roche and Wyeth) have terminated or reduced their research and development activities in this area drastically. The reasons for this decline include:

- high failure rates during research and the lack of developmental compounds;
- preference for research into treatments for chronic rather than acute conditions;
- increasing generic competition and high regulatory hurdles;
- high development costs (e.g., because of the need to secure a license for multiple indications for new antibiotics);
- a view that the promises of genomics, high-throughput screening and combinatorial chemistry have not delivered sufficiently.

Other companies (e.g., Cubist and Essential Therapeutics) have changed their strategies to focus on the development of a limited number of compounds rather than invest in research. This move has taken place because partnerships with

major companies concerning targets and screening technologies have proved difficult to achieve.

It must be emphasised that, although research success rates—in terms of newly discovered molecules—are low, development success rates are high. Antibacterial agents have short clinical development timelines and high success rates, even though securing a license for multiple indications usually proves quite costly. This last point, together with the increased burden of resistance, means that many novel antibiotics are likely to be niche products, tailored to specific clinical needs.

Academic institutions conduct very little research aimed at developing novel antibiotics, since they usually lack compound chemistry resources and knowledge about industrial research processes. The strengths of academic research lie in studies on basic bacterial processes (e.g., antibiotic resistance mechanisms, new targets for treatments, mutation dissemination and persistence, pathogenicity and eradication). Moreover, academia has the potential to establish networks of researchers from disparate fields to investigate key issues with state-of-the-art techniques, influencing treatment strategies (e.g., through the recognition of novel β -lactamases), and proposing new strategies for the prevention and treatment of infections (e.g., MRSA). Given the importance of individual creativity within academic research, small-scale activities can also play a fundamental role, either alone or within the framework of larger forms of co-operation and multicentre research.

A collaborative effort is needed to address structural deficits in European research by fostering a culture of co-operation between all stakeholders (including pharmaceutical companies, academic researchers and regulatory authorities), and advancing mutual knowledge and collaboration between public and private research institutions. The key issues in antibiotic resistance (rather than individual priorities) must be identified jointly, and the research priorities and funding measures defined clearly.

Funding antibiotic research

Funding antibiotic research and interventions for the appropriate use of antibiotics should be a combined responsibility of governments, the pharmaceutical industry, academic bodies and

the EU. Once the key issues in antibiotic resistance have been identified, a clear definition of priority research goals must take into account the possibility of public funding (at a sufficient level) and of complementary private interventions.

In spite of many solemn declarations, European countries have been investing less and less in research activities during the last few decades. In the European Council's Lisbon declaration of March 2000, all EU member states agreed on a common strategy for economic growth. Two years later, the Barcelona European Council reviewed progress towards the Lisbon goal, and, in a new declaration, reinforced by further communications of the European Commission [48,49], the EU member states agreed that future EU spending in this field must be increased, with the aim of approaching 3% of gross domestic product by 2010. This target matches roughly the research and development investments by the other major players. Thus, in 1999, the total expenditure on research and development, expressed as a percentage of gross domestic product, was 1.8% in Europe, compared with 2.7% in the USA and 3.1% in Japan. Investment levels in several European countries are already close to or beyond these levels, but the profound diversities existing in Europe make this objective quite ambitious. The gap between Europe and its major competitors is also significant in terms of employment, since researchers account for only 5.7/1000 of the industrial workforce in Europe, as against 8.1/1000 in the USA and 9.1/1000 in Japan [50].

In addition to being under-resourced, European research is fragmented. The European Research Area represents a joint effort by the EU and member states to address structural deficits in European research, and create a favourable environment for research and innovation [51]. By focusing on a limited number of priorities, it is intended to increase the efficiency and impact of research.

Academic researchers wishing to secure funding for research on antibiotic resistance face several obstacles. Academic research is usually very expensive, while budgets are limited and competition is intense. Moreover, antibiotic resistance does not rank high on the lists of priorities for funding, perhaps partly because of the absence of strong patient organisations in this field. Finally, application procedures for public

funding are often complicated, and notification of the outcome of applications is often slow. It would be useful to establish on-line facilities for the submission, review and ranking of applications.

The dramatic reduction in industrial investment in research and development of new antibiotics should prompt appropriate incentives for industry, particularly in areas of high medical need. Potential incentives that could be provided by regulatory authorities include clear and dependable definitions of the attributes of antibiotics regarded as useful and sufficient for registration, and prolonged patent lives. The international competitiveness of industry should be encouraged by the stimulation of multidisciplinary research and development, durable partnerships, and integration of different stakeholders representing all the required competencies.

Otherwise, Europe will continue to witness—for a long time to come—the humiliating trend of European companies ‘investing across the ocean’, following the consolidated ‘brain drain’ trend in favour of a workplace endowed with a competitive, but more science-friendly spirit, and fewer ‘over-regulated and over-complicated bureaucracies’ [52].

CONCLUSIONS

The measures currently underway within Europe to prevent and control resistance to antimicrobial agents are far from sufficient. The consequences for public health threaten to become acute within a short time, and ongoing actions should not be delayed while existing deficiencies in knowledge are filled. At the same time, further research is essential, and this Conference has highlighted areas where resources should be concentrated.

Much research remains necessary at the level of basic microbiological science, including molecular epidemiology, mechanisms of resistance and their transmission, dynamics of clonal spread, interplay between resistance and virulence determinants, and microbial genomics. Advances in these areas will provide valuable data to develop interventions to prevent and control resistance, including more efficient forms of rational antibiotic design. Microbiological research must encompass the area of ‘resistance ecology’, including population biology, host population genetics, and the ecological relationships between resistance in

hospitals, the community, agriculture and the general environment. Progress has been made in Europe to improve and co-ordinate laboratory surveillance of resistance. Nevertheless, further improvements are essential and, in particular, there is a need for surveillance focused upon specific settings and key organisms.

Ultimately, efforts to control resistance aim to improve the quality of clinical care. The lack of good-quality, standardised data defining the impact of resistance on clinical outcomes of infections, and the associated economic burden, is an important deficiency in present knowledge. Well-designed studies covering this field, in both the community and hospital settings, are necessary to provide data robust enough to convince health care authorities, governments and the general public to invest in interventions to control resistance, and to support the necessary changes in behaviour, as well as to aid the evaluation of these interventions.

Although numerous approaches to reducing resistance through better and more targeted use of antibiotics have been proposed, research is required before many of these can be implemented. For example, further work is required to develop and evaluate computerised prescriber decision-support systems and diagnostic tools. The array of additional interventions requiring study includes infection control measures, probiotics, vaccination and various non-antibiotic approaches to the treatment of infection. Studies to evaluate the comparative effectiveness of disease management strategies and other resistance control interventions, in terms of clinical and economic outcomes, as well as antibiotic use and resistance patterns, are essential. In designing future studies, researchers should take account of lessons learned from critical analysis of the studies performed to date.

The urgent need for new antibiotics with targeted antibacterial spectra and activity against multiresistant pathogens is accepted widely. Thus, the movement of pharmaceutical industry resources away from this pursuit constitutes a crisis. The reasons behind the decline in industrial antibiotic development are complex. Consequently, strategies to reverse it must be multifactorial and must include consideration of public-private partnerships, regulatory approval policies, prescribing practices and diagnostic paradigms. Ultimately, the interests of industry and

society converge within the long-term aim of extending the effectiveness of new antibiotics.

All of these efforts hinge on a greater appreciation on the part of health care providers, governments and the public of the threat posed by existing and future levels of antimicrobial resistance. As such, the institution of well-designed educational programmes continues to be a priority. In this regard, scientific and professional societies offer a most valuable resource. It is regrettable that this field lacks the vocal support of organised patient groups. Persuasive data from studies designed to link antibiotic consumption and long-term health status in individuals (as in the proposed REBECCA study) may be required to raise both public and political concern over antibiotic resistance.

The current fragmentation of European research resources makes it difficult to gather adequate competence and complementary skills to achieve the scientific objectives proposed by this Conference. The FP6 offers excellent opportunities to address this situation, although much groundwork remains to be done to mobilise the research field and to increase the critical mass of resources. The discussions at this Conference will be of central importance to the formulation of topics for the remaining calls for proposals within the Priority 1 sub-area, 'Combating resistance to antibiotics and other drugs'.

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