Report on the special meeting of the Science Division of the Royal Netherlands Academy of Arts and Sciences (KNAW) held on Monday, 21 June 2004.

Royal Netherlands Academy of Arts and Sciences

The bleak future of antibiotics

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Foreword

Twice a year the Royal Netherlands Academy of Arts and Sciences (KNAW) organises a special meeting to consider a current topic from a number of disciplines. The purpose of the special meeting on 'The bleak future of antibiotics', which was held on 21 June 2004, was to discuss what can be done about the increasing rate of antibiotic resistance.

The resistance of bacteria to antibiotics, mainly caused by the selection pressure effected by these medicines, is not a new problem. The history of antibiotics began seventy-six years ago with the discovery of penicillin by Alexander Fleming. The sulphonamides were introduced in 1936. Throughout the 1950s and 1960s in particular, many new classes of antibiotics were developed and put on the market. Examples include the glycopeptides (1958) and the quinolones (1962). Vancomycine is a well-known glycopeptide which is currently used as the last line of defence against the resistant staphylococcus, MRSA (methicillin-resistant *Staphylococcus aureus)*. The development of these antimicrobial drugs fostered the idea that bacteria had been defeated. However, no-one had reckoned on their adaptability.

Increasingly, pathogenic bacteria which could previously be treated with antibiotics are becoming resistant, and even multiresistant, to antibiotics. The injudicious use of antibiotics on humans and animals and failure to comply with hospital hygiene guidelines properly are the main contributory factors to increasing resistance. Currently, all the antibiotics on the market are meeting a greater or lesser degree of resistance. And, although new drugs are urgently needed, the development of new antimicrobial drugs has been stagnating for some years now. The relatively short treatment period that bacterial infections usually require does not make antibiotics an attractive area of research for the pharmaceutical industry, which does nothing to assist the development of these drugs. The solution to the resistance problem will therefore have to involve encouraging the development of new antibiotics, changing the way antibiotics are prescribed and optimising the use of existing antibiotics.

The special meeting was informed of the current status of the research being conducted to find new antimicrobial drugs. Government, academia and the pharmaceutical industry also provided a glimpse of what the future held. How is the issue of increasing antimicrobial resistance to be tackled? Does the solution lie in changing our behaviour or in antimicrobial innovation?

The committee in charge of the preparations for this meeting, made up of Academy members J.W.M. van der Meer and B. de Kruijff, found a number of prominent researchers who were willing to give a paper on their field of research. This publication is a collection of these papers. On the basis of these papers, the preparatory committee has formulated a number of conclusions and recommendations, which are also included in this collection. It is hoped that these papers will be a stimulus for

everyone involved to give the problem of antibiotic resistance the multidisciplinary attention it deserves.

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Chairperson, Science Division

J.W.M. VAN DER MEER AND B. DE KRUIJFF

Conclusions and recommendations

The fact that pathogenic bacteria are becoming resistant to all the antibiotics in current use is an alarming development, which constitutes a global threat to public health (see, for example, *Nature* of 21 October 2004).

In the papers given at the special meeting on 'The bleak future of antibiotics' the speakers discussed different aspects of the antibiotic resistance problem and, either implicitly or explicitly, contributed their ideas for a solution. Two main observations can be made. Firstly, there is a need to restrict the use of antibiotics wherever possible. Secondly, there is a need to continue to develop new antibiotics.

It is clear that the principal mechanism driving the development of resistance is to be found in the selection pressure exerted by antibiotics, which are often injudiciously and inappropriately prescribed. The quality of the prescribing doctor and the wishes and expectations of patients play an important role in this; these are important potential action points for intervention, although the effect of targeted interventions has been limited thus far.

With the power to introduce legislation, the government is an important partner in efforts to tackle the resistance problem. The support given by the Dutch government to initiatives aimed at improving antibiotic prescriptions and promoting hygiene in hospitals and nursing homes (by supporting the Working Group on Antibiotics Policy (*Stichting Werkgroep Antibioticabeleid*, SWAB) and the Working Group on Infection Prevention (*Werkgroep Infectiepreventie*, WIP) respectively) should be emulated internationally. The Netherlands (together with the Scandinavian countries with a relatively low level of resistance similar to that of the Netherlands) should work hard to ensure that this issue figures prominently on the European agenda. Some initial signs of this are already apparent with initiatives such as ESGAP (European Study Group on Antimicrobial Policy); the European research programme, ARPAC, which is being implemented as part of the EU's 5th Framework Programme; and the European Antimicrobial Resistance Surveillance System, EARSS, which is coordinated from the Netherlands.

The most obvious solution, which has worked well over the past fifty years, is to bring new antibiotics on to the market. Attention was once more focused on the need to develop new antibiotics in an insistent speech by Dutch health minister Hans Hoogervorst at the opening of the EU conference on 'Priority medicines for the citizens of Europe and the world' on 18 November 2004. Regrettably, the development of new antibiotics has been stagnating, mainly as a result of the declining interest of the pharmaceutical industry in this area. The main reasons for this are the high development costs and relatively low return on investment involved. Neverthe-

less, the Netherlands is in a position to make a substantial contribution to solving the resistance problem because it is home to a pharmaceutical industry that produces a range of antibiotics, a food industry that is investing in bactericidal preservatives and excellent researchers, universities and institutes that are making innovative contributions to the development of new medicines, including antibiotics. What is lacking, however, is a targeted, government-sponsored public-private partnership in this area.

This research should involve identifying new targets and gaining insight into the mechanisms of natural resistance.

To summarise, we make the following recommendations to government, policymakers, researchers, financiers, industry, academia and practising physicians:

1. Minimise the injudicious use of antibiotics by:
   a. Making consumers aware of the problem of antibiotic resistance.
   b. Enhancing the quality of prescribing at national and international level by training or retraining current and future prescribing doctors. Interactive teaching programmes such as the programme being developed by the Working Group on Antibiotics Policy (SWAB) can make a contribution in this area.
   c. Stimulating research into prescribing behaviour and into ways of encouraging compliance with guidelines.
   d. Putting the problem of increasing resistance on to the international agenda. The Netherlands should work closely with the Scandinavian countries in this regard. Research programmes such as ARPAC, in which European hospitals with low and high resistance figures work together, can result in the improved use of antibiotics through awareness and participation.
   e. Promoting hospital hygiene; improvement in this area will result in fewer hospital infections, which are almost always caused by relatively resistant micro-organisms.

2. Encourage the launching of new medicines on to the market by:
   a. Creating and promoting a public-private partnership (PPP) between the industry and academia with the aim of developing new antibiotics by researching new targets and promising new bactericidal drugs. Research into resistance mechanisms should be included within this PPP.
   b. Improving the ratio of development costs to returns from antimicrobial medicines for the pharmaceutical industry, including by introducing faster registration and longer patent protection for this class of medicines.

3. In order to put these recommendations into effect, we propose establishing a working group to draw up a specific action plan. The Council for Medical Sciences and the Committee for Biochemistry and Biophysics of the KNAW should be able to take the lead in this area.

N.C. OUDENDIJK

The view of the Dutch Ministry of Health, Welfare and Sport

The importance of a good antibiotics policy is self-evident. But as the title of this symposium says, the future for antibiotics is bleak. In many of our neighbouring countries, this bleak future has already become reality to some extent, with high antibiotic resistance in a number of pathogens. The result of this is that patients cannot be properly treated, if at all, or that they have to be put on to more expensive drugs or drugs with much more harmful side-effects more often than is necessary. It is the task of the Ministry of Health, Welfare and Sport (VWS) to promote public health in the Netherlands and it therefore has an interest in keeping antibiotic resistance in the Netherlands to a minimum.

What can we do?

Prudent prescribing

Prudent prescribing can minimise and even prevent problems. Fortunately, Dutch doctors are well aware of this and are playing an important part in minimising resistance development. However, doctors are confronted with ever more articulate patients who will ask for a medicine more readily than used to be the case. Pressure on prescribing doctors is therefore increasing. Moreover, the antimicrobial drugs to be prescribed have to be matched to the resistance and sensitivities found. The results of clinical microbiology will have to be used as guidance in this regard. It is therefore of great importance for each hospital or health region to develop and maintain an antibiotic formulary.

Good infection prevention in institutions

In addition to prudent prescribing behaviour it is important to have a good infection prevention policy in health care institutions as this prevents bacteria and resistance from spreading. If, however, resistant micro-organisms have spread, the Dutch policy of '*search and destroy*' is an extremely effective way of tackling the most important resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA). It is important to keep a close eye on the situation, otherwise we will lose control of it and we will be facing an irreversible problem. Compare, for example, the situation in the UK, where the percentage of MRSA infections has now risen to an average of 30% of all infections involving *Staphylococcus aureus*.

The serious impact of operating this policy (staff capacity, closure of wards) does raise the question of whether it is actually necessary. But the cost of a permanent, large-scale introduction of such a bacterium will ultimately be much higher due to the cost of treating infection and the need to use more expensive and more toxic antibiotics. The Health Council will issue advice this year on the usefulness and effectiveness of this Dutch MRSA policy. The UK's experiences will be expressly referred to in the advice.

What does the Ministry of Health do?

National surveillance

First, it is important for the Ministry of Health to have reliable information on the extent of the problem and to be able to spot any changes at an early stage. In this way, it is possible to take action quickly if the situation becomes critical. For this reason, the Ministry of Health, acting on the advice of the Advisory Council on Health Research (RGO), has invested a lot of money over the last few years in the establishment of a surveillance system to monitor antibiotic resistance and consumption, both intramurally and extramurally. The Working Group on Antibiotics Policy (SWAB) coordinates this data collection process in close cooperation with the National Institute of Public Health and the Environment (RIVM). Once a year a report (Nethmap) is published that describes the trends, which are, where possible, interpreted and translated into policy.

Guidelines

In addition, the Ministry of Health promotes the drawing up of guidelines, both for effective infection prevention (Working Group on Infection Prevention, WIP) and for the responsible use of antibiotics (SWAB). The Ministry of Health also supports in-service training and retraining programmes in the appropriate use of antibiotics, as developed by the SWAB.

Supervision

The Dutch Health Care Inspectorate (IGZ) oversees the implementation and application of these guidelines. Recently, the IGZ conducted a thematic study into the policy of hospitals as regards infection prevention and antibiotic resistance. The IGZ concluded, among other things, that although many hospitals are familiar with the guidelines their implementation and application of them could be better. The IGZ expects hospitals to make improvements in this regard and will continue to oversee this aspect in future.

Cooperation with the Ministry of Agriculture, Nature and Food Quality

Even if everything in the human domain was in good shape, there is a risk that antibiotic resistance could be introduced from the veterinary domain. Antimicrobial growth promoters were banned in the veterinary sector in 1998. Yet the overall use of antibiotics in the veterinary sector in the Netherlands has increased since then. By way of comparison, four times as many antibiotics are used in the veterinary sector

as in the human sector in the Netherlands. Compared with the UK, twice as many antibiotics are used in the veterinary sector in the Netherlands. The health and agriculture ministries have jointly set up the Antibiotic Resistance Platform. From this platform initiatives are being developed to drive down antibiotic use in the veterinary sector.

International

Resistance problems do not respect national borders. The importation of resistant bacteria from other countries can play an important part in creating resistance problems, even if a country has the problem well under control itself. It is therefore in the Netherlands' interest to ensure that resistance development is kept to a minimum in other countries. There are a number of European initiatives aimed at highlighting the subject in the member states and promoting an effective policy. In 1999 a Community strategy was developed and the European Council issued a recommendation on the prudent prescribing of antibiotics. The European Union also supports international monitoring and surveillance networks, including the European Surveillance of Antimicrobial Consumption (ESAC, in which data on the extent of antibiotic use are collected) and the European Antibiotic Resistance Surveillance System (EARSS, which collects data on antibiotic resistance). The National Institute for Public Health and the Environment (RIVM) is the lead instigator of the successful EARSS.

The enforcement of guidelines is a crucial factor in the fight against antibiotic resistance. Despite incentives introduced by the Dutch government, the individual doctor and the individual hospital continue to be the crucial factor in prevention policy. If they are not constantly focusing on quality, including a good infection prevention and antibiotic policy, government measures can do little to help.

M.J.M. BONTEN

Mathematic modelling of the spread of antibiotic resistance

Mathematic modelling is an aid to gaining a better insight into the complex epidemiology of antibiotic resistance. It can help:
a. to ascertain and quantify the relative importance of different variables;
b. to ascertain the relative share of prevention measures and
c. to predict the expected effect of interventions.
In addition, modelling is indispensable for situations in which a prospective and, preferably randomised, study is not possible.

Colonisation by bacteria

In hospitals, most problems with antibiotic resistance arise in intensive care (IC) wards. This is where the sickest patients are to be found, who have the greatest sensitivity to acquiring a hospital infection. As a result of this, it is in these wards that the average amount of antibiotics prescribed per patient is greatest. It is generally assumed that acquiring hospital infections further reduces the chances of survival of these seriously ill patients (which have already been reduced as a result of the underlying illness). Resistance to antibiotics intensifies this effect because of the increased likelihood of an incorrect choice of antibiotics or the need to use less effective antibiotics.

IC patients have almost always been colonised before acquiring an infection: they are carriers of the micro-organism but do not (yet) have an infection. Only some of the colonised patients do actually go on to acquire an infection as well. The extent of the resistance problem is therefore clearest when the number of colonised patients is considered. The number of infected patients is just the tip of the iceberg. The number of patients in a ward who have been colonised by a particular resistant bacterium may increase because:
a. patients are admitted who are already carriers on arrival or;
b. sensitive bacteria become resistant or;
c. resistant bacteria are passed from patient to patient.
This last scenario usually happens because bacteria hitch a ride on the hands of doctors and nurses. This is known as cross-infection. Strict infection prevention measures, of which one of the purposes is to prevent such cross-infection, apply in all hospital wards.

Hospital epidemiology

With these three different ways of acquiring resistance, the epidemiology within a hospital ward seems reasonably clear. However, the process is much more compli-

cated. Interaction between individuals is a characteristic of infectious diseases, as is the case in IC wards. The number of patients already colonised also determines the risk of patients who have not yet been colonised becoming colonised. This principle is known as colonisation pressure and distinguishes infectious diseases from many other conditions. For example, the risk of someone having a heart attack is hardly likely to be determined by other people but the likelihood of someone acquiring an infection (e.g. influenza) depends to a great extent on the occurrence of this infection in the people with whom an individual comes into contact. This has major consequences for the interpretation of results, such as those of intervention studies. Most of the statistical tests used are based on the independence of the observations made. This is correct, for example, when the ages of two patient populations are being compared but not when the likelihood of colonisation is being compared. In addition, hospital wards are usually relatively small (ten to twenty patients). Because of the rapid turnover of patients, there are enormous fluctuations in the number of colonised patients. However, these natural fluctuations and patient-dependency make changes difficult to interpret. Are these changes down to coincidence or have they actually been caused by the intervention? Where the prevalence is high, the likelihood of spread is much greater than during a period of low prevalence and it is the mere balance of probabilities that a period of high prevalence is usually followed by a period of lower prevalence (autoregression).

Unlike cross-transmission, the occurrence of mutations, which cause sensitive bacteria to become resistant, is not dependent on other patients. This usually happens during antibiotic use, when the resistant subpopulation has a survival advantage (antibiotic pressure).

In an IC ward, several processes are therefore relevant which have an opposite dynamic: patient-dependency and no patient-dependency. Recently, mathematical models based on Markov chain technology have been developed to study and analyse the epidemiology of antibiotic resistance in hospitals, in accordance with these principles.

Modelling resistance

One of the first applications for mathematical modelling in the study of antibiotic resistance in an IC ward was to a large extent based on an old theoretical framework, published at the beginning of the twentieth century, which described the spread of malaria. The model describes the transfer of bacteria from patient to patient via vectors, in this case doctors' and nurses' hands. The reproductive value (R_o) is defined as the average number of secondary infections that occurs in a population which is fully susceptible to the infection. If R_o is >1 this means that an epidemic is likely to occur, whereas an epidemic will 'die out' when R_o is <1. As a situation in which all the patients are still susceptible is rare (in endemic situations there are always a number of patients who have been colonised), it is better in this context to refer to an effective reproductive value (R_e). The value of this R_e is the determining factor for the eventual extent of the resistance problem on the ward. The aim of infection prevention measures is to make the R_e value <1. Although cross-transmission often arises

in hospitals, it requires a number of events to take place in succession: For transmission of a bacterium from patient to patient to be possible, there first has to be contact between a member of hospital staff and a colonised patient. This contact has to result in temporary contamination of the staff member's hands. Then, while the hands are still contaminated, they have to come into contact with another uncolonised patient and this contact has to result in colonisation. Reduction of cross-transmission can therefore be achieved by:

a. reducing the number of occasions when contact takes place;
b. reducing the number of consecutive contacts with different patients;
c. minimising the time for which hands are contaminated and;
d. reducing the likelihood of contact with a colonised patient.

The number of occasions when contact takes place depends on staff workload. The number of contacts with two different patients can be expressed in the 'cohort value of nurses', which is defined as the likelihood that any subsequent contact will be with the same patient. If this likelihood is 1, the nurse only has contact with one patient. Disinfecting the hands ensures that any contamination quickly disappears again, thereby reducing the likelihood of transmission. Finally, a lower prevalence will lessen the likelihood of contact with a colonised patient and will also therefore lessen the likelihood of transmission (= reduced colonisation pressure). The variables are also subject to interaction: a staff shortage will mean that, with the same number of patients to be cared for, the contact rate will increase, the cohort level per nurse will decrease and compliance with hand disinfection procedures will decline.

Use of this model suggested that in a ward in which colonisation by vancomycin-resistant enterococci (VRE) was endemic, infection prevention had achieved a reduction in the endemic prevalence from an expected 76% to an observed 36% and that the R_e of VRE was ± 3. Relatively speaking, the degree of cohorting had the greatest share in infection prevention. The continuous admission of already colonised patients ensured that endemicity was maintained.

Spread outside the hospital

In addition to the spread of antibiotic resistance *within* the hospital, it is possible to study the role of the hospital as a source of antibiotic resistance in society by means of mathematical modelling. Resistance is acquired in hospital and then, when the patient is discharged, introduced into society, from where it can be re-introduced into the hospital if the patient is readmitted before losing the colonisation. This model can also be used to describe and study the Dutch '*search and destroy*' system for methicillin-resistant *Staphylococcus aureus*.

Mathematical modelling is an aid that can be used to study the spread of antibiotic resistance inside and outside hospitals in quantitative terms. The need to describe in detail all the processes is, in my view, highly instructive and has made clear the significance of a number of fundamental principles of population biology, which have not been taken into account until now. This has important consequences for the organisation and interpretation of intervention studies. In addition, modelling can be used to study issues which do not lend themselves to a prospective randomised study set-up and which used to be decided on the basis of intuition and 'expert opinions'.

R.P.T.M. GROL*

The unbearable lightness of antibiotic prescribing and how to change it

Introduction

Antibiotics are an extremely important weapon in the fight against infections. However, resistance is a growing problem. That is why the appropriate and selective prescribing of antibiotics (based on indication, the right drug, the drug of first choice, the right dosage, changing or stopping on time) is of great importance and one of the priorities in today's health care system. There is no lack of scientific information describing the best way of prescribing antibiotics. A number of organisations including, in the Netherlands, the Dutch Institute for Healthcare Improvement (*Kwaliteitsinstituut voor de Gezondheidszorg*, CBO), the Dutch College of General Practitioners (*Nederlands Huisartsen Genootschap*, NHG) and the Working Group on Antibiotics Policy (*Stichting Werkgroep Antibioticabeleid*, SWAB), have drawn up guidelines which describe in precise detail the part antibiotics can play in specific health problems. There are, however, many indications that these guidelines are not being followed closely enough and that as a consequence the resistance problem is becoming even more acute. Here we are entering the territory of research into the quality and implementation of care, a new field of study which has been growing strongly in scientific terms over the last ten to fifteen years.

To be able to successfully take the step from evidence to guidelines we need to gain more insight into the problem of suboptimal antibiotic prescribing and the effectiveness of measures taken to do something about it.[1] This article therefore asks the following questions:

– To what extent are scientific opinions and guidelines on optimal antibiotic use followed; for which problems and situations are they followed and which not?
– If they are not being applied properly, what are the reasons for this?
– What measures are effective in terms of improving the use of antibiotics?

The unbearable lightness of antibiotic prescribing

To be able to tackle the problem of unnecessary antibiotic use effectively, it is necessary first of all to establish, by means of objective statistics, the precise details of the problem. Antibiotics are prescribed both in primary health care, i.e. by general practitioners, and in hospitals. In 2000, 5.7 million prescriptions were written for an antibiotic in the Netherlands, of which 86% were issued by general practitioners.[2]

**In collaboration with Prof. P. de Smet, UMC St Radboud Nijmegen and WINAP*

Moreover, there are considerable regional differences in the prescribing of antibiotics: from 0.36 per patient per year in the Dutch provinces North Holland and Utrecht to 0.45 in Limburg, Groningen and Drenthe. In the United States (US) 23% of all incidents and iatrogenic problems in hospitals are associated with antibiotic use.[3] The additional costs related to antibiotic-resistant infections contracted in hospitals are estimated to be running at 1.3 billion dollars per annum.

General practitioners

General practitioners mainly prescribe antibiotics for bronchial infections and conditions and for urinary tract infections. In the last ten years there is reported to have been a downward trend in the prescribing of antibiotics by general practitioners. However, this only applies to the older, narrow-spectrum antibiotics: -29% between 1992 and 2001.[4] At the same time there has also been an increase in the newer broad-spectrum antibiotics: (macrolides +110% and quinolones +86%). The downward trend is also apparent in other countries (the US and the United Kingdom (UK))[5, 6, 7] and, there too, is an increase in the use of broad-spectrum antibiotics. For example, in the US there has been an increase in recent years in the use of broad-spectrum antibiotics for viral infections (i.e. ineffective) from 24% to 48% in adults and from 23% to 40% in children.[8] Fleming (2003) ascribes the downward trend in the UK mostly to a reduction in the incidence of bronchial infections in the population and not to a change in prescribing behaviour. On the basis of a major study in the Netherlands (*Nationale Studie*) the authors conclude that in the Netherlands too there has been a lower incidence of bronchial infections, but that this is caused by patients with bronchial infections consulting their general practitioner less often than before.[9]

If we look in more detail at the quality of prescribing, figures from research by the Centre for Quality of Care Research in the *Nationale Studie* into the behaviour of 195 general practitioners in 104 representative practices in the period 2002-2003 show a variable picture.[10] For example, prescribing on the correct indication varied from 94% for children with asthma and 93% for children under 6 with a fever to 63% for acute sore throat and 33% for sinusitis.[11] As far as the prescribing of drugs of first choice (usually narrow spectrum) is concerned, this happened in over 70% of cases of acute sore throat and sinusitis, but in only 42% of patients with urinary tract infections. There was no correlation between prescribing on indication and prescribing drugs of first choice. In cities more prescriptions were based on indication than in rural areas (50% as opposed to 27%), while in solo practices fewer drugs of first choice were used than in group practices (72% as opposed to 89%).

Hospitals

In hospitals too, improvements are needed in the way antibiotics are prescribed. First we see a gradual rise in antibiotic use over the years, at least in the 1990s.[12] A file study in eight hospitals that looked into the prescribing of antibiotics to 436 pneumonia patients showed that 38% of the prescriptions were in accordance with national guidelines.[13] A timely switch from intravenous to oral administration took place in

67% of cases and a timely change from broad- to narrow-spectrum antibiotics in 52% of patients. A study conducted in thirteen hospitals to ascertain whether guidelines for antimicrobial prophylaxis were being followed in surgical interventions showed that all the recommendations in the national guidelines had been put into practice for only 28% of the 1,763 patients.[14] Recommendations on the dosage interval and the timing of the medication were found to be particularly difficult to follow, mainly as a result of logistical barriers on the wards.

International

Overall, antibiotic use in the Netherlands compares favourably with that in most of the countries around us. If we compare the antibiotic dosage per day per 1,000 inhabitants (1997 figures[15]) it is four times higher for France, three times higher for Belgium and Italy and 1.5 times higher for Germany and Sweden than in the Netherlands. If we look at the broad-spectrum antibiotics, the differences are even greater. With that, antibiotic use in the Netherlands is the lowest in Europe. This is mainly attributed to the guidelines for general practitioners drawn up by the Dutch College of General Practitioners and to the cooperation between general practitioners and pharmacists under the Pharmacotherapeutic Consultations (*Farmacotherapeutisch Overleg*),[2] but there are also other explanations to be found in cultural differences between countries; more on this below.

Causes and solutions

In order to achieve an improvement in the prescribing of antibiotics, we first need to understand properly the causes, the determinants of optimal and suboptimal prescribing.[1] Much research has already been conducted into what determines whether the quality of care will be better or worse and into methods of improving care. For example, systematic analyses of hundreds of controlled studies into strategies for improving the behaviour of medical professionals and implementing guidelines show that there is no superior method which can be used to tackle all the problems effectively.[16] Clinical behaviour is often difficult to change and most measures or programmes in this area produce only modest improvements (5-10%). Educating professionals, giving them feedback about their prescribing behaviour, financial incentives or sanctions, organisational and logistical measures, regulations, etc. can all result in improved medical behaviour provided that they are well attuned to the problems, the target group and the setting in which the change is to take place.

A proper analysis of relevant factors is therefore at the heart of an effective improvement programme. The literature concerned with relevant factors and effective measures for improving antibiotic use is very comprehensive but is not very easy to digest. Determinants of optimal and suboptimal prescribing are usually to be found not only in the way medical professionals think and act, in patient knowledge and behaviour and in the way in which patient care is organised but also in the wider, socio-cultural environment of doctors and their patients. We present a few relevant factors at each of these four levels and possible measures which could be an effective response to them.

Patient knowledge and behaviour

Lack of knowledge of the difference between viral and bacterial infections, lack of knowledge of the resistance problem, specific notions on the effectiveness of antibiotics, expectations in terms of being given a prescription and compliance with regard to the medication are all factors associated with undesirable antibiotic use. [e.g. 17, 18] For example, 83% of the Canadians were unfamiliar with the concept of antibiotic resistance; this was particularly true of poorly educated young people.[19] A survey of American patients[20] revealed that 27% of people with a cold thought that it would clear up faster with an antibiotic, 58% were not aware of potential risks and 48% expected to be given a prescription. Another study, conducted in the UK[21] among patients with bronchial infections, showed that 87% thought they had an infection, 72% wanted antibiotics and also expected to be given a prescription for it, but only 19% also explicitly asked for one. A Dutch study has shown that if patients with bronchial infections expected an antibiotic, there was a 66% probability that they would be given one, whereas if they did not have this expectation, there was only a 34% probability.[22] For many patients, being prescribed antibiotics also has a great symbolic value.[3] It means that the doctor has made a diagnosis, that treatment is possible and that the patient can assume the role dictated by the illness.

'The desire to digest medicines is one of the principal features which distinguish men from animals' (Sir William Osler).

At all events, programmes designed to improve antibiotic use will therefore have to be aimed at the wider public, both patients and parents and carers of young children, and will have to try to exert influence on their knowledge of, and their ideas and expectations with regard to antibiotic use.[19] This can be done in different ways, such as by educating patients, parents, day nursery staff, teachers, etc. However, it is not clear what effect this has. There are high expectations, in particular, of large-scale programmes in which the mass media are used to provide public information. In this information, different messages have to be presented in a consistent and powerful manner:

a. antibiotic resistance is a major problem with serious risks;
b. antibiotics do not work against viruses;
c. people can avoid infections by washing and disinfecting their hands.

Large-scale programmes of this kind have now been implemented in Canada, Belgium, Australia, the UK and the US with messages such as: 'Do bugs need drugs?', 'Common colds need common sense', 'Save antibiotics, they may save your life', 'Antibiotics: get smart' and 'She is only 5 years old and already has a drug problem: it's called antibiotic resistance'.[19] The programmes include leaflets, posters in public spaces, advertisements in newspapers and information on the Internet. A major information campaign in the US aimed at educating both doctors and parents of young children achieved an 11% reduction in antibiotic use.[23] The Canadian programme 'Do Bugs need Drugs?' showed a reduction in antibiotics and an increase in drugs of first choice. The Belgian national programme (2000-2) showed a significant reduction in the use of antibiotics (-26%). The most cost-effective element of this is probably the broadcasting of television commercials during prime time in January (low cost and a lot of colds). The use of public figures in these commercials can be useful.

Knowledge, opinions and behaviour of medical professionals

If we look at medical professionals and factors that influence the suboptimal prescribing of antibiotics, it clearly has something to do with imperfect knowledge, diagnostic uncertainty, fear of complications, fear of disciplinary cases and communicative aspects, but most importantly with the perceived expectations of patients. Sometimes financial interests are involved. Many doctors find it difficult to tell the difference between viral and bacterial infections and are unsure about the diagnosis and the best way forward, especially if the symptoms have not cleared up after a few days.[19] They prefer to take the certain route rather than the uncertain one[24] and may have quite unrealistic expectations of what antibiotics can do. They are often afraid of complications if they do not treat the patient,[25, 26] while being insufficiently aware of the risks posed by antibiotic resistance.[19] In an interview study,[21] doctors said that probably only 20% of patients with bronchial infections needed antibiotics and that it was mainly non-clinical factors that determined whether patients received them or not. For example, they were prescribed more readily to patients from deprived areas and female patients. Most studies show one of the main factors to be pressure exerted on doctors by patients or patients' perceived expectations. In a study by Mangione-Smith[27] it was found that doctors who thought that patients expected an antibiotic would diagnose a bacterial infection more often and prescribe antibiotics more often. Studies by Britten[28] and by Cockburn[29] found that if patients expect an antibiotic they are three times more likely to be prescribed one than patients who do not expect an antibiotic. However, if the doctor thought that the patient wanted this drug, they were seven to ten times more likely to be given it. The same findings as regards the influence of (perceived) expectations are encountered in a number of cultures, including Korea[26]. After all, providing medication is also frequently a symbolic act towards the patient: a prescription marks the end of the consultation, the doctor has finished and is suggesting to the patient that the consultation is over.[3]

It should be clear that, in view of this wide range of problems and influencing factors, the measures and programmes undertaken to influence prescribing behaviour have to be equally diverse. This is also evident from the systematic and non-systematic literature analyses which have been performed to date.[22, 3, 19] Welschen and colleagues[22] carried out a systematic literature study to ascertain the effect of different strategies and measures put in place to influence the prescribing of antibiotics for bronchial infections in general practice. Eight studies were found, in which all kinds of measures had been evaluated: group education, feedback, information for patients and individual education in the form of a visit to the practice. Although most of the measures did produce some effect (average reduction of 6%), even this overview still provided too little information to make it possible to determine what the most successful methods are. In all cases, traditional education produces too little improvement. *Decision support* by computer, in which the computer displays a message with regard to proper or improper antibiotic use, is found to be an effective aid, especially in hospitals.[30] There have now been a number of effective studies with regard to the deployment of *outreach visitors*, people with special training or experts (e.g. a pharmacist), who give explanations and provide support on a one-to-one basis

e.g. [31, 32] Serious reductions in prescribing are being reported as a result (10-30%). A study by Gonzales [33] used an intervention involving several elements and strategies (education of patients, feedback to doctors and *outreach visits*), which resulted in a significant reduction in antibiotics being prescribed for acute bronchitis. Finally, a few more studies have recently been published in which, as an intervention, patients did receive a prescription but with the instruction only to collect and take the drug if they really thought they needed it. A study by Edwards[34] found that only 53% of patients had actually taken the antibiotic. An overview of five studies looking into this approach also showed substantial reductions in use (reduced by 25-54%).[35]

Organisation of care

A third category of factors influencing antibiotic use is the organisation of care: aspects relating to coordination, collaboration between professionals, agreement on and the transfer of the information required, the logistics of the care process, the control and monitoring systems in place, etc.[17] Information from scientific research into these factors is still lacking to a great extent and further research in this area is required. In antibiotic projects for general practice, all the emphasis is usually placed on the doctor's decision whether or not to prescribe on indication and which drug to use. However, the organisation of care also offers opportunities to improve antibiotic use. Computerised monitoring systems,[15, 37] improved use of pharmacists[31, 38, 39, 40] and the deferment of antibiotic use[35] seem likely to be able to contribute to more responsible antibiotic use.

There may be more organisational options in hospitals. Often antibiotic use is a longer-lasting process there, with different kinds of decisions and actions being taken by different disciplines at different times. A number of disciplines are involved in this process (doctors, nurses, pharmacists, microbiologists, infection control specialists). Problems can arise because of a lack of standardisation and protocolling, a lack of cooperation, coordination and harmonisation, imperfect transfer of information, imperfect monitoring of antibiotic use and a lack of monitoring mechanisms built into the prescription process. Measures to prevent antibiotic resistance must fit in with this. The literature suggests the following main measures and interventions[3, 41, 42]:

- Antibiotic formulary: this describes precisely which antibiotics are to be prescribed in which situation.
- Antibiotic order form: a pharmacist or microbiologist must give permission, either in writing or by computer, before an antibiotic is issued. For example, in an Australian study in which a web-based approval system was used, compliance with antibiotic guidelines for hospitals increased from 25% to 51% within five months[43]
- Automatic stop orders: certain prescriptions are stopped automatically after a number of days because they fall outside the formulary.
- Telephone advice: the doctor wishing to prescribe first discusses the advisability of the prescription with the pharmacist or microbiologist.
- Improving the logistics: improving the teamwork between those involved, reducing the time between requesting laboratory diagnostics and prescribing antibiotics.

– Improving the collaboration between doctor and pharmacist/microbiologist: the pharmacist or microbiologist visits the ward, looks at the indications for each patient on the basis of the record and gives advice and information. In a Spanish study, this was found to result in considerable reductions in areas such as antibiotic prophylaxis.[44]

A recent study in 64 Dutch hospitals[42] that looked at current measures to optimise antibiotic use showed that almost all the hospitals were using an antibiotic formulary (97%) and that in 79% of the hospitals the microbiologist visited the wards regularly, especially the intensive care ward. In 55% of the hospitals the microbiologist or pharmacist had to give permission for certain specified antibiotics to be issued. An automatic stop order was only used in six hospitals and an antibiotic order form in two hospitals. About half of them had, in the last five years, completed some kind of project to improve the use of antibiotics. Having an antibiotics committee was found to improve compliance with local agreements but not with national or international guidelines or evidence from the literature.

Currently, there is, to a large extent, a lack of good scientific information on how effective all these kinds of organisational and structuring interventions are. A Cochrane review of 69 studies, of which 34 concern the effect of organisational and restrictive measures on antibiotic use in hospitals, is being conducted at the present time;[45, 46] however, many of the current studies are of poor quality from a methodological point of view.

Cultural and socio-economic context

Antibiotic use does not stop at the general practice or hospital door. With increasing mobility and globalisation (tourism, trade contacts, immigration), problems with antibiotic resistance no longer stop at our national borders either. Numerous factors in the wider cultural and socio-economic context may influence the prescribing and use of antibiotics. We mention only a few of them here.

The pharmaceutical industry has long exerted a strong influence on the prescribing of medicines. This is also evident from the increase in the new, usually broad-spectrum antibiotics in recent years which have been launched with some aggressive marketing. The increased ability of people to order medicines on the Internet will make the use of antibiotics even more difficult to control. The pharmaceutical industry is increasingly addressing consumers direct (in a concealed way). In some countries antibiotics are freely available, without prescription, at the pharmacy or chemist's shop.

The way in which health care is funded may also explain the differences in the prescribing of antibiotics. Harbath et al[47] compared the situation in France and Germany and attributed the substantial differences in antibiotic use to the low prices for medicines in France (where there is ultra-aggressive marketing of medicines by the pharmaceutical industry to compensate for low revenues), to the reimbursement system used by French pharmacies (higher compensation for relatively expensive medicines, such as certain broad-spectrum antibiotics) and to the low use of generic drugs in France (the trend is to use new antibiotics).

Earlier in this paper, reference was made to the 'cultural' factor in explaining differences in antibiotic use. Culture relates to the ideas that people in a certain society have about the causes of all kinds of ailments and health problems (do people attribute them to external causes, e.g. infections, or to internal causes, such as low resistance?), the way in which people label illness and the coping strategies they adopt (should a cold run its course or should the doctor provide a solution?). In anthropological research conducted in Belgium and the Netherlands, these differences were investigated by asking people to keep a diary for three months[48]. In the Netherlands people label a bronchial infection as a cold for which they usually take an aspirin at first or just let it run its course. In Belgium, people refer to this as bronchitis and do not decide how to deal with it themselves as they consider this to be the doctor's responsibility. In the Netherlands, people do not generally look up to their general practitioner, whereas in Belgium they do; there is little discussion, the doctor makes a decision and informs the patient. In Germany, patients usually adopt a wait-and-see attitude to bronchial infections. People have difficulty with antibiotics and prefer to resort to homeopathic medicines.[47] In France, on the other hand, people visit the doctor precisely to obtain an antibiotic and doctors are put under great pressure.

Deschepper et al[48] relate the use of antibiotics in a country to a number of cultural characteristics of that country, as found in a major anthropological study conducted among IBM employees in 50 countries.[49] Hofstede found big differences between countries, especially with regard to the 'power distance' (the extent to which those with less power in a society expect and accept that power is unequally distributed: i.e. low power distance means an egalitarian society; high power distance means a hierarchical society) and the degree of 'uncertainty avoidance' (extent to which members of a culture feel threatened by uncertain or unknown situations; willingness to accept uncertainty and risks, tendency to avoid any lack of clarity). For example, our own research[50] found a clear difference between the ways in which Belgian and Dutch general practitioners deal with uncertainties and risks. Deschepper et al found a correlation of 0.83 between the level of power distance and antibiotic use (more power distance means more antibiotics) and a correlation of 0.70 between the level of uncertainty avoidance and antibiotic use (more uncertainty avoidance means more antibiotic use). Antibiotics have a clear defensive function: taking the certain rather than the uncertain, wanting to have everything under control, avoiding complications, hedging your bets (the doctor) and inability to accept that there is no clear diagnosis for bronchial infections (the patient).

In an analysis by the Social and Cultural Planning Office of the Netherlands (Sociaal en Cultureel Planbureau) of medicine use in Europe in the widest sense reference is also made to this cultural dimension.[51] Countries with a more egalitarian society (the Netherlands, the UK, Scandinavia) have a much lower level of medicine use than countries with a hierarchical society (France, Italy, Spain, Portugal, etc.). In the researchers' opinion, this difference follows a religious dividing line between countries with a predominantly Protestant population and countries with a predominantly Catholic (and Muslim) population. The response to illness and the attitude to medicines are closely related to people's religious background. The powerful cultural

factor in explaining antibiotic use and the big differences between countries make it essential to intensify international cooperation in the area of antibiotic use and resistance: common guidelines, monitoring of use and resistance and feedback, agreements and implementation programmes are needed, such as those currently established for other health problems (e.g. heart and vascular diseases).

Conclusions

The Netherlands is not doing badly in terms of antibiotic use; the question is how much more can be achieved in this area and therefore in efforts to keep down antibiotic resistance. However, in view of actual prescribing behaviour and the public's knowledge and expectations, there is still a lot of room for improvement. This certainly applies now that our society is becoming more open to people from other cultures who have different expectations and experience of antibiotics and doctors.

The reasons why antibiotic use is less than optimal are, as we have seen, complex. They have to do with patients' knowledge and behaviour (i.e. not being aware of risks and having explicit expectations with regard to medication), with professionals' knowledge and routines (i.e. imperfect knowledge and perceived expectations from patients), with the organisation of care (i.e. imperfect control, coordination and cooperation) and with the cultural and socio-economic context (the influence of the pharmaceutical industry and more general (cultural) notions in society of illness, health and contact with doctors). This means that any programme to rationalise antibiotic use – if it is to be effective – will have to be established on several levels. A national programme for 'rational antibiotic use' could be considered, involving a joint effort by government, professional organisations, patient organisations and insurance companies (similar to the Partnership against Smoking). The following activities could be included:

– public information in the mass media, including television
– patient-oriented information on risks and sensible use
– intensive education and training of professionals, to teach them how to deal with patient expectations and pressure
– computerised monitoring of prescribing
– intensifying and rewarding collaboration between doctors and pharmacists
– measures with regard to the pharmaceutical industry

We have seen that the risks do not stop at our national borders. Because of the current phenomenon of globalisation (tourism, immigration, trade contacts) other ways of dealing with antibiotics will have an even greater effect on our country and on resistance patterns here [52, 53]. We can no longer confine ourselves to Western Europe alone. Evidence can be found in the appearance of resistant tuberculosis strains and MRSA and VRE infections in our country, not only in hospitals but also in society. This requires much closer international cooperation involving international guidelines, agreements, monitoring and feedback of information and implementation programmes.[54]

References

1. Grol R. Beliefs and evidence in changing clinical practice. *BMJ* 1997; 315: 418-21.
2. Baart F, de Neeling A. Antibioticagebruik buiten het ziekenhuis. *Pharmaceutisch Weekblad* 2001; 136(22): 786-90.
3. Avorn J, Solomon D. Cultural and economic factors that (mis)shape antibiotic use: the nonpharmalogical basis of therapeutics. *Ann Intern Med* 2000; 133: 128-35.
4. Kuyvershoven M, Balen F van, Verhey T. Outpatient antibiotic prescriptions from 1992 to 2001 in the Netherlands. *J Antimicrobial Chemotherapy* 2003; 52: 675-8.
5. Steinman M, Gonzalez R, Lindner J, Landefelt S. Changing use of antibiotics in community-based outpatient practice 1991-1999. *Ann Intern Med* 2003; 138: 525-33.
6. Fleming D, Ross A, Cross K, Kendall H. The reducing incidence of respiratory tract infections and its relation to antibiotic prescribing. *Br J Gen Pract* 2003; 53: 778-83.
7. Mc Caig L, Besser R, Hughes J. Trends in antimicrobial prescribing rates for children and adolescents. *JAMA* 2002; 287: 3096-102.
8. Steinman M, Landefelt S, Gonzales R. Predictors of broad-spectrum antibiotic prescribing for acute respiratory tract infections in adult primary care. *JAMA* 2003; 289: 719-25.
9. Otters H, Van der Wouden H, Schellevis F. Respiratory infection and antibiotic prescription rates. *Br J Gen Pract* 2004; 54:132.
10. Braspenning J, Schellevis F, Grol R, red. *Kwaliteit Huisartsenzorg Belicht*: Utrecht NIVEL/Nijmegen, WOK 2004.
11. Dijk L van, Schiere AM, Braspenning J. Gericht voorschrijven van antibiotica. In: Braspenning J, Schellevis F, Grol R, red. *Kwaliteit Huisartsenzorg Belicht*: Utrecht NIVEL/Nijmegen, WOK 2004.
12. Janknegt R. Antibiotica surveillance. *Pharmaceutisch Weekblad* 2001; 136: 816-9.
13. Schouten J, Hulscher M, Kullberg B, Gijssens I, Van de Meer J, Grol R. Adherence to key indicators for antibiotic use in CAP: a multicentre audit in Dutch hospitals 2004a: internal report WOK.
14. Kasteren M, Kullberg B, de Boer A, Mintjes-de Groot J, Gijssens I. Adherence to local hospital guidelines for surgical antimicrobial prophylaxis: a multicentre audit in Dutch hospitals. *J Antimicrobial Chemotherapy* 2003; 51: 1389-96.
15. Cars O, Mokstad S, Melander A. Variation in antibiotic use in the European Union. *Lancet* 2001; 357: 1851-53.
16. Grol R, Grimshaw J. From best evidence to best practice: effective implementation of change in patients' care. *Lancet* 2003; 362: 1225-30.
17. Fine JM, Fine MJ, Galusha D, Petrillo M, Meehan TP. Patient and hospital characteristics associated with recommended processes of care for elderly patients hospitalized with pneumonia: results from the medicare quality indicator system pneumonia module. *Arch Intern Med*. 2002; 162: 827-33.
18. Belongia E, Naimi T, Gale C, Besser R. Antibiotic use and upper respiratory infection: a survey of knowledge, attitudes, and experiences in Wisconsin and Minnesota. *Prevent Med* 2002; 34(3): 346-52.

19. Finch R, Metlay J, Davey P, Baker L. on behalf of the International Forum on Antibiotic Resistance. Educational interventions to improve antibiotic use in the community: report from the International Forum on Antibiotic Resistance (IFAR) colloquium 2002. *The Lancet Infectious Diseases* 2004; 4: 44-53.
20. Vanden Eng J, Marcus R, Hadler J, Imkoff B, Virgia D, et al. Consumer attitudes and use of antibiotics. *Emerging Infectious Diseases* 2003; 9: 1128-35.
21. MacFarlane J, Holmes W, Mac Farlane R, Britten N. Influence of patients expectations on antibiotic management of acute lower respiratory tract illness in general practice: questionnaire study. *BMJ* 1997; 315: 1211-4.
22 Welschen I. *Prescribing antibiotics for acute respiratory tract infections in primary care*. Utrecht University PhD thesis 2004.
23. Perz J, Craij A, Coffey C, Jorgensen D, Mitchel E, Hall S, Schaffner W, Griffin M. Changes in antibiotic prescribing for children after a community-wide campaign. *JAMA* 2002; 287: 3103-9.
24. Broek P.J. van den. Plussen en minnen. *Pharmaceutisch Weekblad* 2000; 135: 1386-9.
25. Kunmar S, Little P, Britten N. Why do general practitioners prescribe antibiotics for sore throat? Grounded theory interview study. *BMJ* 2003; 326: 138-143.
26. Cho H, Hong S, Park S. Knowledge and beliefs of primary care physicians, pharmacists, and parents on antibiotic use for the pediatric common cold. *Soc Sci Med* 2004; 58: 623-9.
27. Mangione-Smith R, McGlynn EA, Elliott MN, Krogstad P, Brook RH. The relationship between perceived parental expectations and pediatrician antimicrobial prescribing behavior. *Pediatrics* 1999; 103: 711-8.
28. Britten N, Ukoumunne OC. The influence of patients' hopes of receiving a prescription on doctors' perceptions and the decision to prescribe: a questionnaire survey. *BMJ* 1997; 315: 1506-10.
29. Cockburn J, Pitt S. Prescribing patterns in clinical practice: patients' expectations and doctors' perception of patient expectations: a questionnaire study. *BMJ* 1997; 315: 520-3.
30. Pestotnik SL, Classen DC, Evans RS, Burke JP. Implementing antibiotic practice guidelines through computer-assisted decision support: clinical and financial outcomes. *Ann Intern Med*. 1996 May 15; 124(10): 884-90.
31. Soumerai SB, McLaughlin TJ, Avorn J. Improving drug prescribing in primary care: a critical analysis of the experimental literature. *Milbank Q.* 1989; 67(2): 268-317.
32. Thomson M, Oxman A, Davis D, et al. *Outreach visits to improve health professionals practice and health care outcomes*. The Cochrane Library, Issue 3. Oxford: Update Software 1998.
33. Gonzales R, Stemer J, Lum A, Baren P. Decreasing antibiotic use in ambulatory practice: impact of a multi-dimensional intervention in the treatment of uncomplicated acute bronchitis in adults. *JAMA* 1999; 281: 1512-9.
34. Edwards M, Dennison J, Sedgwick P. Patients' responses to delayed antibiotic prescription for acute upper respiratory tract infections. *Br J Gen Pract*. 2003; 53: 845-50.

35. Arroll B, Kenealy T, Kerse N. Do delayed prescriptions reduce antibiotic use in respiratory tract infections? A systematic review. *Br J Gen Pract* 2003; 53: 871-7.
36. Sullivan F, Mitchell E. Has general practitioner computing made a difference to patient care? A systematic review of published reports. *BMJ* 1995; 311: 848-52.
37. Walton R, Dovey S, Harvey E, Freemantle N. Computer support for determining drug dose: systematic review and meta-analysis. *BMJ* 1999; 318: 984-90.
38. Pippalla RS, Riley DA, Chinburapa V. Influencing the prescribing behaviour of physicians: a metaevaluation. *J Clin Pharm Ther.* 1995 Aug; 20(4): 189-98.
39. Beney J, Bero LA, Bond C. *Expanding the roles of outpatient pharmacists: effects on health services utilisation, costs, and patient outcomes*. Cochrane Library. 2000.
40. Kaushal R, Shojania KG, Bates DW. Effects of computerized physician order entry and clinical decision support systems on medication safety: a systematic review. *Arch Intern Med*. 2003 Jun 23; 163(12): 1409-16.
41. Schiff G, Wisnieuwski M, Bult J, Parada J, Aggarwal H, Schwartz D. Improving inpatient antibiotic prescribing. *J Comm J Qual Improvement* 2001; 27, 387-402.
42. Schouten J, Hulscher M, Natsch S, Jellema R, Grol R, van de Meer J. *Hospital antibiotic control measures in Dutch secondary care hospitals*. 2004b: internal report WOK.
43. Richards MJ, Robertson MB, Dartnell JG, Duarte MM, Jones NR, et al. Impact of a web-based antimicrobial approval system on broad-spectrum cephalosporin use at a teaching hospital. *Med J Aust*. 2003 Apr 21; 178(8): 386-90.
44. Galindo C, Olivé M, Lacasa C, Martinez J, Roure C, et al. Pharmaceutical care: pharmacy involvement in prescribing in an acute-care hospital. *Pharm World Sci* 2003; 25(2): 56-64.
45. Ramsay C, Brown E, Hartman G, Davey P. Room for improvement: a systematic review of the quality of evaluations of interventions to improve hospital antibiotic prescribing. *J Antimicrob Chemother.* 2003 Nov; 52(5): 764-71.
46. Davey P, Brown E, Hartman G. *Systematic review of interventions to change antibiotic prescribing to hospital inpatients*. Protocol Cochrane Review EPOC 2004.
47. Harbarth H, Albrick W, Brun-Buisson C. Outpatient antibiotic use and prevalence of antibiotic resistent pneumococci in France and Germany: a sociocultural perspective. *Emerging Infectious Diseases* 2002; 8: 1460-6.
48. Deschepper R, Van der Stickele R. De rol van culturele aspecten. *Pharmaceutisch Weekblad* 2001: 136: 794-7.
49. Hofstede G. *Allemaal andersdenkenden: omgaan met cultuurverschillen*. Amsterdam: Contact 1992.
50. Grol R, Whitfield M, De Maeseneer J, Mokkink H. Attitudes to risk taking in medical decision making among British, Dutch and Belgian general practitioners. *Br J Gen Pract* 1990: 40: 134-6.
51. Kooiker S, van der Wijst L. *Europeans and their medicines*. Social and Cultural Planning Office of the Netherlands. 2003.
52. Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim SJ, Reniero A, Hoffner S, Rieder HL, Binkin N, Dye C, Williams R, Raviglione MC. Global trends in resistance to antituberculosis drugs. World Health Organization-International Union

against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. *N Engl J Med*. 2001; 344: 1294-303.
53. Ayliffe GA. The progressive intercontinental spread of methicillin-resistant Staphylococcus aureus. *Clin Infect Dis* 1997; 24 Suppl 1: S74-9.
54. Le Grand A, Hogerzeil H, Haaijer-Ruskamp F. Intervention research in rational use of drugs: a review. *Health Policy and Planning* 1999; 14(2): 89-102.

D.G.A. VENTE

Is the pharmaceutical industry resistant to bacterial resistance?

Research and development (R&D) is a high-risk financial undertaking for a pharmaceutical company. Ninety-three percent of all pharmaceutical research is carried out in the private sector. The entire research and development process for a drug takes from eleven to fifteen years. One hundred research projects, in which 7,000,000 compounds are screened, result in the identification of at most one or two drugs at the end of these eleven to fifteen years. After progressing through a series of phases from idea or concept via preclinical pharmacology, preclinical safety and then clinical pharmacology and safety, we finally reach a situation where one medicine can be considered for registration and reimbursement.

Why do most substances not eventually become drugs? The reasons are partly pharmaceutical (unstable product, technically difficult production), partly pharmacokinetic (poor absorption, half-life in the body too short or too long, which leads to concentrations in the blood being too low or lasting too long), partly pharmacodynamic (insufficiently effective, insufficiently selective, attack mechanisms too complex) and partly toxicological (side effects and secondary infections, therefore unsafe). Aspects such as fierce competition between companies can also have an effect.

Development costs

The development costs for a single drug rose from € 187 million in 1991 to € 895 million in 2001 and this has even doubled since two years ago to € 1.7 billion. The main reason for this is that the regulators are increasingly imposing stricter requirements on research. R&D is therefore risky and expensive: only three out of every ten drugs earn back the research and development costs that were invested during the R&D process. A number of other factors also affect the cost of drugs:

- Increased regulation and quality requirements for research result in increased costs for research and development.
- The patent life is an important factor in keeping prices down.
- Increasing government interference and rising cost of health insurance.
- Use, abuse and accountability.

In most European countries, the government determines the price of drugs. Because of this, they are cheaper here than in the United States where there is a free market for drugs. This costs the sector billions in turnover and therefore profit margin in Europe. European governments are not doing patients or themselves any favours

with this system. Because the American market is much more attractive, European pharmaceutical companies are increasingly moving their research and development departments to the United States, at the expense of high-quality jobs in Europe. This also puts at risk the availability of new medicines (and therefore new antibiotics) to European patients. The introduction of new drugs is a slow process because of the prolonged negotiations on price. These negotiations can also break down. Pfizer predicts that, if this trend continues, the pricing policy will eventually chase the pharmaceutical sector out of Europe. In 1988 there were forty-two innovative pharmaceutical companies in Europe, now there are only sixteen left. This shows that R&D is not without risk and that a concentration of knowledge and finance is required. Governments have discovered that reducing their expenditure on drugs allows them to cut their budget deficits. This certainly affects the growth figures for pharmaceutical companies and lower growth is therefore predicted. In recent years the total sales of the pharmaceutical sector have grown by an average of 9% per annum. This year, growth will fall to 6% and, consequently, the percentage invested in R&D will be put under pressure. For Pfizer, this is 15% of sales this year, which amounts to $7.9 billion, or $152 million per week.

Pfizer is not alone in its criticism of Europe's pricing policy and the associated regulation. Almost all the board chairmen of pharmaceutical companies refer to this issue when presenting their annual figures. Research has to stay close to the market and the market is becoming increasingly lucrative in the United States. In May 2004, during a meeting of the OECD, the Organisation for Economic Cooperation and Development, which is also concerned with the cost of health care, the chairman of the Pfizer board gave a lecture in which he stated that the cut-backs on medicines are incomprehensible, more especially because they put at risk investment in research into new drugs, including antibiotics. In the total health care budget, drugs are a relatively small item: in the Netherlands it is 3.6%. One could therefore be forgiven for asking what we are talking about.

In the Netherlands, the number of antibiotic treatments have fallen by 2% since 2001. However, cutting back on medicines creates the risk that the number of hospital admissions will rise and this item is much costlier. Studies show that if we can reduce heart and vascular disease, infections and cancer by 10%, this will produce a saving of $10 billion in Europe. We at Pfizer believe that this kind of saving is achievable, so long as pharmaceutical companies are given the leeway to develop new drugs (including antibiotics) to combat the above diseases and are allowed to charge a reasonable price for existing drugs.

The pharmaceutical industry is often depicted in negative terms as only being interested in making as much money as possible out of the health problem. This is not true, as the pharmaceutical industry is not the cause of the problem, it is actually a major part of the solution.

New developments

The pharmaceutical industry is often accused, as recently in *The Lancet,* of stimulating antibiotic use on spurious grounds and therefore being partly responsible for

causing resistance. In addition, the pharmaceutical industry is reproached for concentrating more on profitable therapeutic areas than on the fight against infectious diseases. Pfizer has given a commitment to continue research into new antibiotics, despite the fact that this involves scientific and financial risks. At the present time, the emphasis at R&D is on finding an antimicrobial drug to combat tuberculosis.

The increase in bacterial resistance is not the only cause of the current problems surrounding infectious diseases. The high number of people travelling and what is known as the problem of reserved drugs also play a part. Reserving drugs means only using certain drugs (e.g. new antibiotics) in extreme cases because of their specific properties or their price (reserve drug). However, the priority when choosing an antibiotic should be its effectiveness and not its price, otherwise drugs with new sites of action, for example, will be sidelined. In this way, new developments in the pharmaceutical industry of new antibacterial drugs are frustrated.

Currently, Pfizer is investigating a phenomenon described in an article by Biggins et al in Science (2003, 301; 1537-1541). Some bacteria have 'bodyguards', or self-resistance proteins. These are proteins which are capable of rendering certain antibiotics (which, in this case, they produce themselves) ineffective. Besides the resistance mechanisms generally known to date – change in chemistry, removal of antibiotic molecules and enzymatic breakdown of antibiotics – which bacteria use to defend themselves against antibiotics, Biggins et al discovered a fourth mechanism. It takes the form of protection against a class of 'anti-tumour antibiotics', known as enediynes, which pulverize the DNA, thereby making it impossible for the bacterial cell to function and divide. These enediynes are currently being studied by Pfizer and are potentially among the most powerful antibiotics. But the 'bodyguard proteins' and their defence mechanism could mean that even these potentially powerful antibiotics will meet their Waterloo when faced with certain bacteria.

In addition, Pfizer is currently carrying out research into bacteriophages as an alternative to regular antibiotics. One advantage of these viruses is that they are very specific. For example, a bacteriophage which infects *Escherichia coli* is incapable of infecting other kinds of bacteria. It is of vital importance to clarify how the mechanism works in order to develop a new class of antibacterial drugs in due course.

The future

To summarise, Pfizer has no intention of stopping research in the area of antibiotics. However, the investment period is long and delays the introduction of new antibiotics to help resolve the issue of resistance in the short term. Despite the fact that the increase in resistance makes the market less attractive, Pfizer has demonstrated, with the development of linezolid, the first new class of antibiotics in a decade, that it intends to continue investing in antibacterial drugs. It is no coincidence that the slogan in our research laboratories is 'The patient is waiting'.

As far as the resistance problem is concerned, a joint approach by academia, the government and the pharmaceutical industry will be the preferred option for devising a substantive, uniform strategy. The pharmaceutical industry should therefore be involved in the health minister's plans to set up a Centre for Combating Infectious

Diseases. There will also have to be cooperation at international level as this problem does not stop at national borders.

As a pharmaceutical company, we have to strive to offer new antibiotics on the market at a reasonable price. We must tackle bacterial resistance from its roots by coordinating effective collaboration and initiatives in this area, thereby influencing each other's expertise. Here too the pharmaceutical industry is able and willing to invest in these drugs, yet with the knowledge that it is precisely the use of these new antibiotics that provides the financial resources and expertise required to continue to invest.

E. BREUKINK

Is antimicrobial innovation still possible?

Since the development of penicillin seventy-six years ago, many new antibiotics have been discovered and/or developed and come on to the market. However, this trend slowed considerably after 1962, since when only one significant new antibiotic has been introduced: linezolid. The last period of antimicrobial innovation can be divided into two streams. The first stream derives from the development of penicillin and has mainly focused on seeking natural products with an antimicrobial action. These products are often complex structures, which means that the only way of obtaining large quantities is by means of fermentation. Recently, interest in natural antibiotics has declined, mainly because the idea is gaining ground that after fifty years of searching the potential for new antibiotics has been exhausted. However, chemically modified variants of the natural antibiotics are still coming on to the market. The second stream of antimicrobial innovation derives from chemistry. Antibiotics used to be (and still are) generated from structures which do not arise in nature. The most recently introduced antibiotic, linezolid, is a successful example of this. But what does the future hold? Is antimicrobial innovation still possible?

The action of antibiotics

New antibiotics have to meet certain requirements. For example, the target of the new antibiotic must not occur in humans, i.e. it must be unique to bacteria. It must also be essential to the bacterium. A good example of a target is the bacterial cell wall synthesis, the target of many natural antibiotics. To develop a new antibiotic it is essential to have information on the target's structure. Another important requirement is that it is impossible or very difficult to acquire resistance to the new antibiotic. An understanding of how bacteria become resistant is therefore essential. To put it very simply, resistance can generally be acquired via two mechanisms: the bacterium ensures that the antibiotic cannot reach the target (by switching on pumps or shielding the target) or the bacterium switches off the antibiotic directly or alters the target so that the antibiotic no longer recognises it. An example of the latter mechanism is the *VanA*-type resistance of bacteria. Resistance is achieved by making a relatively minor change to the vancomycin target, Lipid II. The Lipid II performs a key function in bacterial cell wall synthesis: it transports building blocks for the cell wall across the cytoplasmatic membrane in bacteria. Normally, vancomycin prevents the building blocks from eventually being built into the cell wall. However, if a small change is made to the peptide side chain of Lipid II, vancomycin loses its affinity for Lipid II and thus loses its action. This type of resistance gives the bacterium substantial protection from vancomycin and is currently on the increase.

It is therefore necessary to choose the target in such a way that it is difficult to change. This is a very laborious process that could be speeded up by making use of the bacteriological warfare that has been going on for millions of years. Bacteria inhabit a niche that contains a lot of competition. In order to survive, it is essential to have a good defence against all attacks. It is therefore hardly surprising that resistance has occurred. But the weapons that the bacteria use have also evolved with them. A thorough search for the right bacterium with the right weapons can be very rewarding. There is a lot of activity in this area. Large numbers of bacteria are screened to ascertain whether they may be able to produce antibiotics. Of the approximately 300,000 bacteria which are screened annually, only 500-750 actually produce a bacteria-blocking substance. At most, one or two of these substances (but often not even a single one) eventually undergo the final tests required to determine their suitability as an antibiotic.

Nisin

One particular family of antibiotics often comes to the fore in this type of screening programme, the lantibiotics. They are relatively short peptides characterised by the way in which they are arranged 'in knots'. They have special ring structures, known as lanthionine-rings, after which they have been named. The best known family member, nisin, is produced by the lactic acid bacterium *Lactococcus lactis* and is used in the dairy industry as a preservative (E234). Nisin kills the bacterium by forming pores in the plasma membrane. Using Lipid II as the target makes nisin's pore formation very efficient. It can therefore be regarded as an example of a classic 'magic bullet'. Unlike the case of vancomycin, it is not so easy, if not impossible, for the bacterium to change the Lipid II so much that nisin can no longer bind to it. This is because it has recently been found that nisin specifically binds the pyrophosphate of Lipid II. This pyrophosphate group cannot be changed without serious consequences for the bacterium. Resistance to nisin is only possible if the cell wall is modified. However, this is so detrimental to the bacterium that it is reversed again as soon as nisin is no longer present. The concept adopted by nisin, targeted pore formation, has now been reproduced in the literature and may have a bright future in wider applications. In other words, antimicrobial innovation is still possible and we have a lot to learn from nature in this regard.

P. COURVALIN

Antibiotic resistance: dealing with this emerging disease

Over the last sixty years bacteria, and in particular those pathogenic for humans, have evolved towards antibiotic resistance. There are two steps in this evolution that one should distinguish: emergence and dissemination of resistance.

There is nothing *Homo sapiens* can do about emergence since it occurs by chance and represents a particular aspect of bacterial evolution. It can result from mutations in house-keeping structural or regulatory genes or from acquisition of foreign genetic information. On the contrary, much can be done to delay the subsequent spread of resistance. Dissemination can occur at the level of the bacteria (clonal spread), replicons (plasmid epidemics), or of the genes (transposons). These three levels of dissemination which co-exist in nature are not only infectious but also exponential since all are associated with DNA duplication. Clonal dissemination is associated with chromosome replication, plasmid conjugation with replicative transfer, and gene migration with replicative transposition.[1] Spread of resistance has repeatedly been shown to be associated with antibiotic use[2] which stresses the importance of the prudent use of these drugs; a notion reinforced by the observation that resistance is slowly reversible.[3,4]

It is therefore conceptually challenging and, maybe, potentially useful to try to predict the future of the relationship between antibiotics and bacteria. For the sake of convenience the examples will be taken mainly from the work carried out in the author's laboratory, although numerous other examples can be found in the literature

The clinically relevant predictable resistance mechanisms are listed in Table 1. Although the fact that they have not yet been reported does not mean that they do not exist in nature, their apparent absence is, at least for some of them, rather surprising. For example, streptococci, including pneumococci and groups A, C, and G, can easily acquire *in vitro*, stably maintain, and phenotypically express conjugative plasmids from enterococci.[5] It is therefore all the more surprising that genes commonly found on plasmids in the latter bacterial genus such as *bla* for penicillinase production, *aac6'-aph2"* for resistance to nearly all commercially available aminoglycosides, and the *van* alphabet conferring resistance to the glycopeptides have not yet emerged in streptococci. The situation is even more odd for *Listeria* which remain susceptible to the majority of antibiotics despite the fact that they can acquire plasmids from both enterococci and staphylococci.[6] On the contrary, the obligate intracellular life style of *Chlamydia* spp. is likely to protect them from contact with foreign DNA and accounts for their retained susceptibility to antibiotics.

Table 1. Predictable resistance (mechanisms)

Organism	Resistance phenotype or mechanism
Streptococcus pneumoniae	Penicillinase, gentamicin, glycopeptides,
Streptococcus groups A, C, G	Penicillins
Listeria monocytogenes	Penicillins, gentamicin
Legionella pneumophila	Macrolides, fluoroquinolones
Salmonella typhi	Third-generation cephalosporins
Haemophilus influenzae	Third-generation cephalosporins
Neisseria meningitidis	Third-generation cephalosporins
Brucella spp.	Tetracycline, rifampicin, streptomycin
Clostridium difficile	Glycopeptides
Clostridium perfringens	Penicillinase
Chlamydia spp.	Tetracycline

How to anticipate resistance?

One should distinguish 'natural' antibiotics (e.g., kanamycin) produced by microorganisms from the environment from semi-synthetic (e.g., amikacin), and entirely synthetic compounds (e.g., quinolones) produced, at least in part, by *Homo sapiens*. In the case of natural antibiotics, the producing microorganisms have to protect themselves from the products of their own secondary metabolism. In order to avoid suicide, the antibiotic producers have developed self-protection mechanisms that are similar to those found in resistant human pathogens,[7] an observation which led to the notion that the producers constitute the pool of origin of certain resistance genes.[8] Therefore, the study of resistance in the strain used for the industrial production of an antibiotic could well allow a strong prediction as to the mechanism that will be found later in bacteria pathogenic for humans. For example, study of glycopeptide producers would have allowed elucidation, long before it actually occurred, of the mechanism by which enterococci and, more recently, staphylococci could become resistant to these drugs.

As already mentioned, bacteria are resistant to antibiotics following horizontal DNA transfer or mutations. Thus, another prediction that one can make is to anticipate transfer to susceptible species of resistance determinants already known in other bacterial genera; such as the recent acquisition of glycopeptide resistance by *Staphylococcus aureus* from *Enterococcus* [9] However, this is a rather limited prediction since it is confined to already elucidated mechanisms. In addition to antibiotic producers, commensal bacteria of mammals, particularly those in the gut, could also represent a pool of origin for resistance genes. This is due to the fact that when treating a patient one does not only address the small minority of bacteria responsible for the infection but also the entire flora of the mammalian host. This could result, in particular in children who are administered oral antibiotics too frequently, in the selection of resistant commensals. These resident bacteria are present in huge

numbers in the digestive tract where they are often in transient, but intimate, contact with exogenous microorganisms that are in various developmental states, including competence. These conditions favor transfer of genes by transformation and by conjugation. Use of antibiotics in animal feed also leads to the selection of a pool of resistance genes that can be transferred to human digestive commensals and, thus, ultimately to human pathogens even in the absence of selective pressure.[10]

In the case of mutations, predictions can be supported by two types of experimental approaches: *in vivo* with intact bacteria or *in vitro* using DNA. Mutations resulting in resistance can be obtained in an accelerated fashion using hypermutators, that is bacteria deficient in the DNA repair system.[11] They are also accumulated using continuous cultures, preferably in chemostats, under suitable selective pressure. A similar enhanced rate of evolution can be obtained by (saturated) DNA mutagenesis followed by transformation into an appropriate host. This technique, for example, was used successfully to study the extent of variations in penicillinase genes that generate extended spectrum ß-lactamases.[12]

Pathways to resistance

In all bacterial species and for all resistance mechanisms one should consider the following pathways to resistance.

Modulation of gene expression

In addition to mutations in structural genes for the drug targets, bacteria can become resistant following mutational events in motifs for gene expression, such as promoters,[13] in regulatory modules, such as two component regulatory systems,[14] or positioning upstream from a gene of a mobile[15, 16] or stable[17] promoter. Enhanced expression of genetic information can also be due to alterations in translation attenuation.[18] The DNA regions involved in gene regulation are not always adjacent to the target gene. This complicates the finding of regulatory mutations and makes detection of resistance by this mechanism generally impossible by genotypic techniques.[19]

Dissemination by transformation

This is more likely to occur in spontaneously transformable bacterial species such as S. pneumoniae, Acinetobacter spp. and Neisseria spp. These bacteria can easily acquire, integrate, and express stretches of DNA. Since the latter can include portions of foreign chromosomes, this process renders chromosomal mutations infectious.[20]

Combination of mechanisms

Because of increased activity of the expanded spectrum of certain drug classes (e.g., ß-lactams and fluoroquinolones) or of local therapy (e.g., extremely high concentrations in the gut following oral administration of glycopeptides that do not cross the digestive barrier) bacteria have to combine mechanisms that confer resistance to the same class of molecules. This is in order to achieve high-resistance levels[21] or expand the substrate range provided by a single resistance mechanism.[22] An example is provided by Gram-negative bacteria and the ß-lactams. Extended spectrum ß-lactamases

are point mutants of 'old' penicillinases.[23] Generally, the biological price to pay for the extension of the substrate range of this enzyme is hypersusceptibility to ß-lactamase inhibitors. However, the presence in certain enterobacteria of the gene for a penicillinase on a small size multicopy plasmid resulting in production of large amounts of the enzyme, confers resistance to ß-lactamase inhibitors by trapping.[24] The net result of this combinatorial approach is the occurrence of Gram-negative bacteria resistant to all ß-lactams except carbapenems and cephamycins that are not substrates for the enzymes.

Two mechanisms are involved in resistance with an increasing frequency

Impermeability

No antibiotic is active against all bacteria. In fact, the intrinsic (natural) resistance of bacteria, better designated insensitivity, defines the spectrum of activity of a drug. This is usually due to the fact that the antibiotic does not penetrate the bacteria. However, microorganisms can become resistant to nearly all drug classes, surprisingly including those that act at the surface of the bacteria (e.g., ß-lactams, bacitracin), by impermeability. This can be secondary to two distinct pathways; one, passive, involves alterations of outer membrane proteins, the porins, which decrease the rate of entry of antibiotics into the bacteria by diminution of the pore size;[25] the other active, involves overexpression of an indigenous efflux pump that exports the antibiotic outside the cell following a regulatory mutation.[26]

Trapping

This mechanism, already mentioned in the case of resistance to ß-lactams by a combination of ß-lactamases, allows titration of the drugs, an alternative to impermeability, for lowering the intracellular concentrations of the antibiotics. This is also the case for aminoglycosides in bacteria which overproduce an enzyme that has affinity for a drug they cannot inactivate since it lacks the modification site (Fig. 2).[27, 28] This mechanism has also been proposed to account for low-level resistance to glycopeptides in staphylococci by overproduction of target sites in the outer layers of the peptidoglycan with the result that the antibiotic does not reach the important target sites where the wall is assembled on the outer surface of the cytoplasmic membrane.[29]

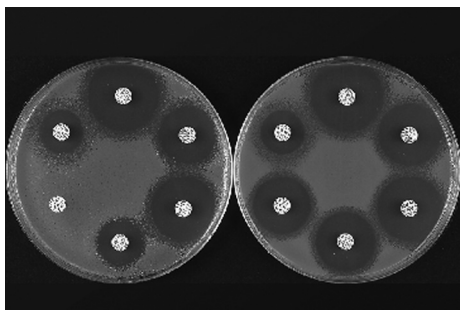

Fig. 1: Disk susceptibility test of *Escherichia coli* BM694 (left) and of strain BM694 harboring plasmid pAT346 conferring tobramycin resistance by trapping (right).[27]

Prediction at the genetic level

Genes from Gram-positive cocci can be transferred by conjugation (of plasmids or transposons) not only among these microorganisms, but also to Gram-negative bacteria.[30] The reverse is not true due to limitations in heterologous gene expression. Consequently one can confidently predict further dissemination of the resistance gene pool of Gram-positive to Gram-negative bacteria.

We have been aware for a very long period of time that 'Everything that exists in the universe is the result of chance and necessity' (Democritus, 460-370 B.C.) which, as we have seen, holds true for antibiotic resistance. Most unfortunately, and for various reasons, it is extremely difficult to think like a bacterium. In other words, prediction of emergence of resistance to a drug class by a precise molecular mechanism is nearly impossible (e.g., glycopeptide resistance in enterococci or plasmid-mediated resistance to fluoroquinolones). It is also difficult to anticipate, among all the conceivable mechanisms of resistance,[31] which will emerge first under natural conditions. However, based on the understanding during recent decades of the physiology (genetics and biochemistry) of bacterial resistance to antibiotics, impressive progress has been made in the techniques for *in vitro* detection and for elucidation of resistance. This should, in turn, prove to be helpful in delaying the second step of resistance: dissemination.

References

1. Courvalin P, Trieu-Cuot P. Minimizing potential resistance: The molecular view. *Clin Infect Dis*. 2001; 33: S138-46.
2. Seppala H, Klaukka T, Lehtonen R, Nenonen E, Huovinen P. Out-patient use of erythromycin: link to increased erythromycin resistance in group A streptococci. *Clin Infect Dis*. 1995; 21: 1378-85.
3. Andersson DI. Persistence of antibiotic resistant bacteria. *Curr Opin Microbiol*. 2003; 6: 452-6.
4. Chiew YF, Yeo SF, Hall LM, Livermore DM. Can susceptibility to an antimicrobial be restored by halting its use? The case of streptomycin versus *Enterobacteriaceae*. *J Antimicrob Chemother*. 1998; 41: 247-51.
5. Macrina FL, Archer GL. Conjugation and broad host range plasmids in streptococci and staphylococci. In: Clewell DB, editor. *Bacterial conjugation*. New York, London: Plenun Press; 1993. p. 313-29.
6. Charpentier E, Courvalin P. Antibiotic resistance in *Listeria* spp. *Antimicrob Agents Chemother*. 1999; 43: 2103-8.
7. Mazodier P, Davies J. Gene transfer between distantly related bacteria. *Annu Rev Genet*. 1991; 25: 147-74.
8. Walker MS, Walker JB. Streptomycin biosynthesis and metabolism. *J Biol Chem*. 1970; 245: 6683-9.
9. Weigel LM, Clewell DB, Gill SR, Clark NC, McDougal LK, Flannagan SE, et al. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science* 2003; 302: 1569-71.
10. Moubareck C, Bourgeois N, Courvalin P, Doucet-Populaire F. Multiple antibiotic resistance gene transfer from animal to human enterococci in the digestive tract of gnotobiotic mice. *Antimicrob Agents Chemother*. 2003; 47: 2993-6.

11. Taddei F, Matic I, Godelle B, Radman M. To be a mutator, or how pathogenic and commensal bacteria can evolve rapidy. *Trends Microbiol*. 1997; 5: 427-8.
12. Petrosino J, Cantu III C, Palzkill T. ß-lactamases: protein evolution in real time. *Trends Microbiol*. 1998; 6: 323-7.
13. Chen ST, Clowes RC. Variations between the nucleotide sequences of Tn*1*, Tn*2*, and Tn*3* and expression of ß-lactamase in *Pseudomonas aeruginosa* and *Escherichia coli*. *J Bacteriol*. 1987; 169: 913-6.
14. Baptista M., Depardieu F, Reynolds P, Courvalin P, Arthur M. Mutations leading to increased levels of resistance to glycopeptide antibiotics in VanB-type enterococci. *Mol Microbiol*. 1997; 25: 93-105.
15. Goussard S, Sougakoff W, Mabilat C, Bauernfeind A, Courvalin P. An IS*1*-like element is responsible for high-level synthesis of extended-spectrum ß-lactamase TEM-6 in *Enterobacteriaceae*. J. Gen. Microbiol. 1991; 137: 2681-7.
16. Rudant E, Courvalin P, Lambert T. Characterization of IS*18*, an element capable of activating the silent *aac(6')-Ij* gene of *Acinetobacter* sp. 13 strain BM2716 by transposition. *Antimicrob Agents Chemother.* 1998; 42: 2759-61.
17. Magnet S, Courvalin P, Lambert T. Activation of the cryptic *aac(6')-Iy* aminoglycoside resistance gene of *Salmonella* by a chromosomal deletion generating a transcriptional fusion. *J Bacteriol*. 1999, 181: 6650-5.
18. Leclercq R, Courvalin P. Resistance to macrolides and related antibiotics in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother.* 2002; 46: 2727-4.
19. Courvalin P. Genotypic approach to the study of bacterial resistance to antibiotics. *Antimicrob Agents Chemother.* 1991; 35: 1019-23.
20. Ferrandiz MJ, Fenoll A, Liñares J, de la Campa AG. Horizontal transfer of *parC* and *gyrA* in fluoroquinolone-resistant clinical isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother.* 2000; 44: 840-7.
21. Arthur M, Courvalin P. Contribution of two different mechanisms to erythromycin resistance in *Escherichia coli*. *Antimicrob Agents Chemother.* 1986; 30: 694-700.
22. Ferretti JJ, Gilmore KS, Courvalin P. Nucleotide sequence analysis of the bifunctional 6'-aminoglycoside acetyltransferase, 2"-aminoglycoside phosphotransferase determinant from *Streptococcus faecalis*: identification and cloning of gene regions specifying the two activities. *J Bacteriol*. 1986; 167: 631-8.
23. Sougakoff W, Goussard S, Gerbaud G, Courvalin P. Plasmid-mediated resistance to third-generation cephalosporins caused by point-mutations in TEM-type penicillinase genes. *Rev Infect Dis*. 1988; 10: 879-84.
24. Mabilat C, Courvalin P. Development of 'oligotyping' for characterization and molecular epidemiology of TEM ß-lactamases in members of the family *Enterobacteriaceae*. *Antimicrob Agents Chemother.* 1990; 34: 2210-6.
25. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev.* 2003; 67: 593-656.
26. Li XZ, Nikaido H. Efflux-mediated drug resistance in bacteria. *Drugs* 2004; 64: 159-204.

27. Ménard R, Molinas C, Arthur M, Duval J, Courvalin P, Leclercq R. Overproduction of 3'-aminoglycoside phosphotransferase type I confers resistance to tobramycin in *Escherichia coli*. *Antimicrob Agents Chemother.* 1993; 37: 78-83.
28. Magnet S, Smith TA, Zheng R, Nordmann P, Blanchard JS. Aminoglycoside resistance resulting from tight drug binding to an altered aminoglycoside acetyltransferase. *Antimicrob Agents Chemother.* 2003; 47: 1577-83.
29. Srinivasan A, Dick JD, Perl TM. Vancomycin resistance in staphylococci. *Clin Microbiol Rev.* 2002; 15: 430-8.
30. Courvalin P. Transfer of antibiotic resistance genes between Gram-positive and Gram-negative bacteria. *Antimicrob Agents Chemother.* 1994; 38: 1447-51.
31. Quintiliani Jr R, Sahm D, Courvalin P. Mechanisms of resistance to antimicrobial agents. In Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. *Manual of Clinical Microbiology,* 7th Edition. American Society for Microbiology, Washington D.C. 1998. p. 1505-25.
32. Pootoocal J, Thomas MG, Marshall CG, Neu JM, Hubbard BK, Walsh CT, et al. Assembling the glycopeptide antibiotic scaffold: The biosynthesis of A47934 from *Streptomyces toyocaensis* NRRL15009. *Proc Natl Acad Sci* USA. 2002; 99: 8962-7.
33. Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn*1546*, a Tn*3*-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol.* 1993; 175: 117-27.

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