could affect the potential geographic range of many infectious diseases', but adds, 'if the climate of some regions

borne disease is simply too complex for scientists to be able to make accurate predictions. 'The potential exists for scientists

not, the fact that biologists are also seeing potentially detrimental effects from rising global temperatures is worrisome enough. It should render additional arguments to the supporters of the Kyoto Protocol, who see it as a necessary first step to curb global warming.

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'The potential exists for scientists one day to be able to predict the impact of global climate change on disease, but that day is not here'

becomes more suitable for transmission of disease agents, human behavioural adaptations and public health interventions could mitigate many adverse impacts'.

However different the interpretations might be, at least all scientists involved agree that the interaction between climate, human behaviour and vector-

one day to be able to predict the impact of global climate change on disease, but that day is not here', Donald Burke, Professor of International Health and Epidemiology at Johns Hopkins School of Public Health in Baltimore, MD, and chair of the NRC panel, remarked. Nevertheless, whether the threat of vector-borne diseases spreading into new areas is realised or

Killing the right bug

Phenotypic and genotypic approaches for identifying infectious agents and antibiotic resistance can help to slow the increasing antibiotic resistance among infectious bacteria

When antibiotics became available after World War II, they were little short of a miracle. Since then, their ability to fight bacterial infections quickly and effectively has literally saved the lives of millions around the globe. But their effi-

health experts therefore urge clinicians and physicians to prescribe more narrow-spectrum antibiotics in order to slow the spread of resistance among bacterial strains. But to do so, clinicians must know as soon as possible which bug they are

bacterial populations provide a perfect breeding ground for multi-resistant super-bugs. Worldwide deaths from methicillin- and the emerging vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA, respectively) amount to 5000 each year. Previously benign organisms such as *Enterococcus faecalis*, *Acinetobacter* and *Xanthomonas* can now be fatal to hospitalised patients with compromised immunity. But also in the community, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* that were once easily treatable with penicillin, are now resistant to most common antibiotics. An analysis of non-hospital antibiotic sales in the 15 European Union member states (Table I), published last June in *The Lancet* by Otto Cars and co-workers from the Department

The over-use of antibiotics in hospitals and private practices has inevitably selected for resistant bacteria that are increasingly able to withstand the very drugs designed to combat them

ciency has also been their greatest weakness, as people now rely on antibiotics to fight any infection whether it makes sense to use them or not. Added to which, most clinicians and physicians, under pressure to save time and money, will prescribe a broad-spectrum one-size-fits-all antibiotic to any patient with a bacterial infection rather than choosing a narrow-spectrum antibiotic targeted to the specific causative pathogen. In the case of life-threatening infections this is certainly justified. But this over-use of antibiotics in hospitals and private practices has inevitably selected for resistant bacteria that are increasingly able to withstand the very drugs designed to combat them. Public

dealing with and to which antibiotic the strain is susceptible, and this can take up to weeks using traditional culture-based methods. Some automated systems are available to determine the infectious strain and its susceptibility to antibiotics within hours, but they come with a price. In the long run, therefore, they will most likely be replaced by DNA-based solutions that will cost a fraction of traditional methods and will be able to identify an infectious agent and its resistance in less than an hour.

The problem of emerging resistance is greatest in hospital environments where the high level of antibiotic use and the propensity for genetic exchange between

There is a prevailing attitude which borders on a sense of complacency that science will provide the answer

of Infectious Diseases at the University Hospital in Uppsala, Sweden, highlights the problem of over-prescribing. In 1997, France and Spain were the highest

Table I. Outpatient antibiotic sales in 1997 in the European Union

	Broad-spectrum penicillins J01CA	Narrow-spectrum penicillins J01CE	Penicillinase resistant penicillins J01CF	Cephalosporins J01D	Tetracyclines J01A	Trimethoprim J01EA	Quinolones J01M	Macrolides and lincosamides J01F	Others ^a	Total
Austria	3.23	1.53	0.01 ^b	1.30	1.81	0.69	1.16	3.65	0.42	13.80
Belgium	10.96	0.21	0.36	2.85	5.09 ^c	0.59	1.95	4.06	0.65	26.72
Denmark	2.39	4.57	0.34	0.02 ^b	0.98	0.30 ^b	0.22 ^b	1.97	0.56	11.35
Finland	3.78	2.30	0.07	2.11	5.50	1.87 ^c	0.52	1.86	1.33 ^c	19.34
France	18.97 ^c	0.31	0.58	3.75	3.38	0.55	1.72	5.98 ^c	1.27	36.51 ^c
Germany	2.67	1.59	0.02	0.89	3.26	0.93	0.75	2.54	0.93	13.58
Greece	7.74	0.37	0.02	4.68 ^c	2.69	0.64	1.04	4.50	1.01	22.69
Ireland	8.03	0.87	0.63	1.23	3.50	0.92	0.38	2.50	0.28	18.34
Italy	11.20	0.02 ^b	0.02	3.21	0.56 ^b	0.87	1.93	5.07	1.11	23.99
Luxembourg	10.58	0.17	0.23	2.86	4.01	0.70	1.61	4.71	0.71	25.58
Portugal	12.08	0.06	0.71	3.28	2.63	1.10	4.04 ^c	3.69	1.24	28.83
Spain	18.01	0.08	0.39	2.48	1.42	0.59	2.48	5.87	1.12	32.44
Sweden	1.36 ^b	4.85 ^c	0.99 ^c	0.59	2.97	0.57	1.01	0.97 ^b	0.20	13.51
The Netherlands	2.90	0.61	0.16	0.12	2.35	0.57	0.70	1.24	0.31	8.96 ^b
UK	6.93	0.78	0.68	0.98	3.66	1.12	0.54	3.22	0.13 ^b	18.04

Numbers are defined daily dose per 1000 inhabitants per day.

^aIncludes sulfonamides, amphenicols, aminoglycosides and glycopeptides.

^bLowest defined daily dose.

^cHighest defined daily dose.

Table taken from Cars, O., Mölstad, S. and Melander, A. (2001) Variation in antibiotic use in the European Union. *Lancet*, **351**, 1851–1853. (Reproduced with permission from The Lancet Publishing Group.)

prescribers with 36.5 and 32.4 doses sold per 1000 inhabitants per day, respectively, compared with, for example, the Netherlands (8.9) and Denmark (11.3). Cars estimates that between 20 and 50% of that use is unnecessary and contributes to the problem of emerging resistance in bacteria. 'To meet the threat to human health posed by resistant micro-organisms it should be in the interest of every country to collect detailed data on antibiotic prescriptions to optimise the use of these drugs', he commented.

There is a prevailing attitude which borders on a sense of complacency that science will provide the answer, that new drugs are constantly being developed to help us tackle this problem. In reality, only one new class of antibacterials has been developed since the 1970s and, even though there has been a recent resurgence of interest in antimicrobial development, any potential new drug could be 10 to 20 years away from the market. In the meantime, further overuse of the existing antibiotics could eventu-

ally render most of them useless. 'If we fail to make wide and wise use of these medicines we have available today, they will likely slip through our grasp', David Heymann, Executive Director for Communicable Diseases of the WHO wrote in a statement last year (<http://www.who.int/infectious-disease-report/dlh-testimony/testmo.pdf>). He went on to conclude that 'The most effective strategy against antimicrobial resistance is to get the job done right first time—to unequivocally destroy microbes—thereby defeat-

The problem, however, is that identifying the pathogen and its resistance often takes too long. Standard diagnosis of systemic bacterial infections usually depends on growth in culture, which requires at least 12–72 hours for detection and can take up to weeks for the notorious *Mycobacterium tuberculosis*, during which time any therapy unavoidably has to depend on broad-spectrum antibiotics. Furthermore, to determine susceptibility to various antibiotics, more phenotypic methods need to be performed. A clinical

Automated 'expert systems' not only lead to patients being treated faster but also make it possible to detect emerging and low-level microbial resistance to antibiotics and thus enable the physicians to devise evasive actions

ing resistance before it starts'. Indeed, antibiotic misuse most often stems from inadequate information concerning the causative pathogen rather than from inappropriate behaviour.

laboratory, therefore, has a great advantage if they have an automated system that can identify the infectious agent and its potential resistance in hours rather than days. 'In seven hours we can provide

nearly 80% of sensitivity testing results', Rafael Canton, Professor at the Servicio de Microbiología, Hospital Ramon y Cajal in Madrid, Spain, said. 'Obviously, if you provide better results, the treatment will be more targeted, the resistance will be less,' he added. The hospital's instrumentation ranges from the semi-automated MicroScan WalkAway system (Dade Behring, West Sacramento, CA) providing optical reading of zones of inhibition to the fully automated VITEK2 system (bioMérieux, France). Such sophisticated systems can also identify the organisms treated and, if coupled to an 'Advanced Expert System', will provide a complete analysis of the bacterial isolate. Not only does this lead to patients being treated faster, but it also makes it possible to detect emerging and low-level microbial resistance to antibiotics and thus enables the physicians to devise evasive action.

While highly prevalent in the United States—up to 80% of laboratories possess such an 'expert system'—in Europe this figure is nearer to 20%, mainly due to the start-up costs of the system. 'My budget has gone through the roof,' Nandini Shetty, a Consultant Microbiologist at the University College Hospital, London, whose laboratory has been using the VITEK2 system for several years now, said. 'There is no doubting that the system does save money in the long run, but the cost benefits are out there in the community and in, for example, saved hospital costs, saved bed costs and this is very difficult to quantify.' It certainly was not reflected in her final laboratory budget, she went on to say and this ultimately prevented the uptake of these systems in the majority of other hospital laboratories in the UK.

But cost is not the only drawback of automated systems. While undoubtedly decreasing the time to identify bacterial isolates, often more than one method needs to be performed to obtain an accurate susceptibility profile. Furthermore, they can lack sensitivity in detecting antibiotic resistance, particularly with MRSA. The other obvious alternative to conventional phenotypic screening is the genotypic approach. Given the amount of data on pathogen sequences now available, it is already possible to identify a bacterium within hours or even less by sequencing, nucleic acid probe hybridisation or PCR. Furthermore, it is also possible to screen a sample for resistance

genes, thus bypassing the associated problems of reduced viability, variable gene expression levels and inducible resistance. 'Genetic testing for the presence of resistance is a welcome advance for us clinical microbiologists,' Shetty said. 'I would say it has a tremendous future and already it is being used in clinical labs for the detection of rifampicin resistance in *M. tuberculosis*, resistance to oxacillin in MRSA and penicillin resistance in *Streptococcus pneumoniae*.'

Indeed, several DNA-based assays for the detection of bacterial resistance are currently being developed. But while identifying the bug itself is relatively straightforward, determining susceptibility to antibiotics requires a thorough understanding of complex resistance mechanisms and the genes involved. Crucially, DNA probe analysis is oriented towards bacterial resistance whereas the basis for treatment is susceptibility. One organism where this does not pose such a problem is *M. tuberculosis*. 'In contrast to any other bacteria or virus I know of, all the [mutations] which correspond to rifampicin resistance are remarkably conserved between susceptible strains,' Barry Kreiswirth, Director of the Public Health Research Institute TB Center in New York, NY, said. 'Whenever you see a mutation in these genes, you can predict this bug will be resistant.'

With costs dropping, laboratories will increasingly be able to capitalise on the specificity, high sensitivity and rapidity of the molecular approach

Given that the actual technology of DNA probe analysis and nucleic acid amplification is not new, it is remarkable that these techniques are not more widespread and have taken so long to reach the clinical microbiology laboratory. Again, cost is still a factor. 'The problem is, it's just too costly to do for every strain. But the good news is that sequencing will get cheaper and cheaper over time,' Kreiswirth said. Moreover, there have been a number of other obstacles to overcome, which has led to the slow acceptance of this approach. The high sensitivity of DNA analysis can be one of its weaknesses as contamination can lead to false positives. 'Sample processing is typically the most troublesome part of these tests,' Craig

Hill, manager for scientific affairs at Gen-Probe Inc. (San Diego, CA) whose latest bacterial identification system is fully automated, commented. 'It has been called the Achilles' heel of first-generation nucleic acid amplification tests.' Indeed, Becton Dickinson Diagnostics Systems (Franklin Lakes, NJ), another manufacturer of genotyping systems, are currently marketing a completely enclosed device that uses pre-dispensed dry reagents to vastly reduce any contamination problems.

With the increasing availability of cost-effective systems, laboratories will be able to capitalise on the specificity, high sensitivity and rapidity of the molecular approach. While still in its infancy, clinical laboratories and physicians are beginning to reap the benefits and this will surely only be heightened by DNA chip technology. Roche Molecular Systems (Basel, Switzerland) and bioMérieux have agreements with Affymetrix (San Diego, CA) to develop the latter's GeneChip technology as the basis of diagnostic kits for bacterial identification and antibiotic resistance analysis. Another collaboration is the mass production capabilities of Motorola Inc. (Schaumburg, IL), the analytical skills of Packard Instrument Company (Meriden, CT) and the intellectual property rights of the Argonne National Laboratory (Argonne, IL) to commercialise and market advanced biochips. Researchers there, together with the Engelhardt Institute of Molecular Biology in Moscow, have developed a novel 'micro-gel' technology whereby specific sets of primers are tethered in an array of gel pads. PCR amplification of the DNA of an isolate followed by hybridization to primers of varying specificity provide a unique pattern of positive signals depending on the genus of the isolate and its spectrum of resistance. 'This is a great opportunity,' stated Andrei Mirzabekov, who leads the team of researchers at the Argonne National Laboratory. 'This pattern will identify the test bacterium in much the same way as fingerprinting identifies human beings.' They have already applied the technology to simultaneously identify several drug-resistant mutations in the *rpoB*, *katG* and *rpsL* genes responsible for the rifampicin, isoniazid and streptomycin resistance of *M. tuberculosis*, which is causing great health problems in Russia and around the globe.

Meanwhile, Raymond Kammer, Director of the National Institute of Standards and Technology envisages another use: 'An anticipated early payoff from the genetic technologies based on DNA chip systems is the development of a rapid low cost test for contagious diseases such as 'strep-throat'. Within around 15 minutes in the doctor's surgery, the chips will be able to assess whether a sick child will require antibiotic treatment. Additionally, the device will be able to identify the particular strain of bacteria from the patient allowing a physician to choose the most effective treatment.' However, this is a

long way from being realised and as Barry Kreiswirth commented on DNA chip technology 'It's overkill—it's like hitting a mosquito with a sledgehammer [...] Technology has advanced the application. I find it ironic that we talk about the Human Genome Project and these pie in the sky approaches when we're not even doing the things we currently have the tools for.'

Antibiotic resistance will always be with us since by their very nature, antibiotics sow the seeds of their own destruction. By implementing today's advanced technology, we can ensure that

we make the most effective use of these powerful medicines before our window of opportunity to do so closes. 'All these [genotypic testing] techniques are getting faster, better and cheaper. If someone gave me a magic wand it would be the way I would deal with drug susceptibility,' concluded Barry Kreiswirth.

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