Conquering Antibacterial Resistance

Sir Alexander Fleming House, Imperial College of Science, London was chosen by the Society for Medicines Research as a fitting venue for their symposium on "Conquering Antibacterial Resistance", held on 28th September, 2000. The meeting brought together ~62 multidisciplinary scientists, drawn from both the pharmaceutical industry and academia, all with a common interest in understanding the processes involved in antibacterial resistance and ways to overcome it.

Professor Richard Wise (City Hospital, Birmingham) opened the proceedings with an overview of the present situation. Each time an antibiotic is used there is an opportunity for resistance selection. Approximately 100 million kilograms of antibiotics are produced worldwide. Half is for human use (~20% hospital use; 80% in the community) and half is for agricultural use. It is estimated that ~20-50% usage in humans is unnecessary. The figure is even higher in agriculture where about 40-80% of the amount used is highly questionable. Most animal use is for growth promotion thus economics and not health is the overriding factor. In Spain, where drug monitoring is practiced statistics show that where antibiotics are used heavily resistance increases.

Although the emergence of resistance is inevitable certain measures, if adhered to, would considerably reduce the rate at which resistance occurs, Surveillance to give early warning of emerging problems would allow more time to evaluate prevention and control. Better education of practitioners both in the community and in hospitals and phasing out the use of antibiotics in animal husbandry and in agriculture would be important steps towards limiting resistance.

Professor Sebastian Aymes (Department of Medical Microbiology, University of Edinburgh) focused on prescribing practices particularly in hospitals and on the options for minimising resistance. Development of resistance may arise not only from over usage of antibiotics but also from under dosing which can provide a very efficient selection environment. This is particularly true in the developing world. Cross-infection by resistant bacteria is also a major concern. Problem bacteria include: Gram-positive species such as glycopeptide resistant *Staphylococcus aureus* and *Enterococcus faecium* and *E. faecalis;* Gram negative Enterobacteriaecae, particularly Klebsiella spp that have been infiltrated by extended spectrum β-lactamases (ESBLs). *Acinetobacter baumannii* and *Pseudomonas aeruginosa,* which are the most resistant Gram -negative organisms associated with nosocomial infections, are controlled by carbapenems but resistance is emerging to these agents. Infections with multiresistant *Mycobacterium tuberculosis* and penicillin resistant *Streptococcus pneumoniae* are also becoming major league problems. Simple common sense procedures such as: washing hands, avoiding moving the patient, adequate disaffection policy (solutions made up daily), good waste disposal (bed pan washers and incinerators), checking autoclaves (temperature and cycle times), and shorter stays for in-patients are practiced in hospitals where the incidence of resistant infections is low.

Professor Aymes recommended a number of approaches towards the control of outbreaks of methicillin resistant *S. aureus (*MRSA). Firstly, an infection control team should be established. Everyone should have access to an infection control manual. Isolation facilities should be available and gloves and aprons worn by all those in contact with the patient. Access to reference laboratories is essential to identify strain types and resistance patterns (e.g. fluoroquinolone sensitive or multiresistant). Use of dual therapy is recommended especially if vancomycin is not being used. Vancomycin is first line therapy for MRSA infections and should not be used prophylactically or topically. It should not be used to treat pseudomembranous colitis or infections due to *Clostridium difficile, which* can be treated with metronidazole.

Dr Peter Bennett (Department of Pathology and Microbiology, University of Bristol) described the various ways in which resistance genes are donated by one bacterium to another and how resistance genes are integrated into the bacterial genome. He also discussed how these processes have contributed to the current state of antibacterial resistance and what impact they are likely to have on the emergence of resistance in the future. There are several ways by which DNA is transferred between cells namely, transformation, transduction and conjugation. Only about 50 species can undergo natural transformation i.e. DNA release, capture and recombination. Resistance to β-lactam antibiotics has arisen by interspecies recombinatorial events that have generated hybrid penicillin binding proteins (gene mosaics) with reduced affinity for the antibiotics. This type of resistance is of particular concern in pneumococci in

which it is increasing worldwide. The emergence of penicillin resistance in *Neisseria meningitides,* arising from the introduction and recombination of regions of the penicillin-binding protein2 genes (penA) from commensal Neisseria species, is another example of interspecies recombination.

Transduction involves bacteriophage (bacterial virus) transfer of resistance genes. This process is not common. It is responsible for *S.aureus resistance* to erythromycin, neomycin, penicillin and tetracycline. Conjugation *via* plasmids (auxiliary chromosomes) is the most common DNA transfer system. An example is the plasmid pUB2380, which confers resistance to aminoglycosides. Plasmid transfer is a replication mechanism i.e. a single strand of DNA is taken up by the recipient whilst the other strand is retained by the donor. This replication mechanism increases the load of resistance genes. Plasmids can confer resistance to more than one antibiotic and, more importantly, to more than one class of antibiotic. Furthermore, resistance can arise from non-pathogenic bacteria.

In Gram-negative bacteria resistance genes can be located on integrons which act as insertion sites for resistance genes They contain gene cassettes that can be mobilised to other integrons and to secondary sites on the bacterial genome. Up to five or more cassettes can be present on an integron, which leads to multiresistance. Integrons can be located on the chromosome or in transposons (piece of DNA) which have the ability to relocate themselves, or, transposing onto the bacterial chromosome and subsequently onto a plasmid. In fact, the widespread distribution of integrons is thought to be due to their association with transposon plasmids or conjugative plasmids or both. Thus integrons are an important source for the spread of antibiotic resistance particularly in Gram-negative bacteria.

We cannot stop these transfer events which occur by chance. They are an integral part of the evolutionary process which allows bacteria to tolerate or resist changes in the environment. However, it may be possible to influence the spread. Even low frequency events become common place under relentless selection pressure but the frequency of the rate of DNA transfer could be reduced by judicious use of antibiotics i.e. only administer them when necessary.

Professor Jeremy Hamilton-Miller (Department of Medical Microbiology, Royal Free Hospital, London) suggested that ideas on how to prevent or modify the resistance process might be gained if we had the ability to "think like a bacterium". There are a number of possibilities for plotting an antimicrobial strategy: (i) look at old targets such as the cell wall, protein synthesis and nucleic acids using new weapons e.g. linezolid, daptomycin and various vancomycin analogues; (ii) seek new targets: signal transduction systems, lipid A, mycolic acid, aromatic biosynthesis, membranes: (iii) use the chaperone principle where the chaperone has no antibacterial activity, but interferes in such a way that is advantageous to the host antibiotic e.g. combinations including inhibitors of tetracycline efflux pump and β-lactamases; (iv) modulate host defences such as phagocyte function and cytokine response using muramyl peptides and monoclonal antibodies; (v) maintain and replace essential normal flora by with pre-and probiotics; (vi) "smarter" use of existing compounds by specific targeting or giving delayed release formulations: (vii) block receptor sites e.g. using oligosaccharides and probiotics; (viii) prevent the synthesis or function of fimbriae by immunisation; (ix) interfere with cell division e.g. murosomes, autolysins; (x) inhibit quorum sensing signals which switch on virulence factors; (xi) prevent bacteria becoming streetwise by eliminating R-factors e.g. mepacrine and fluoroquinolones; (xii) remove protective layers e.g. endosialidase for *Echerichia coli* KI and type B meningococci; (xiii) moderate toxins and their production e.g. "starfish" inhibitor of cholera toxin and inhibition of staphylococus α- toxin induction. In short, a better understanding of bacterial physiology allows for a more subtle approach to bacterial resistance which is less damaging to the host i.e. adopt a seek and destroy policy.

Dr Kenneth Coleman (SmithKline Beecham Pharmaceuticals, USA) described the rapid advances that had been made during the last decade in bacterial genomics/proteomics, high throughput screening, chemical array synthesis, chemi- and bioinformatics and robotic engineering.

More than 24 complete gene sequences and 54 partial sequences have been determined representing 20 and 36 species, retrospectively. This has opened up the possibility of a large number of novel targets in a broad range of bacteria. Criteria for target selection include: novelty, spectrum, selectivity, essentiality and expression *in vivo.*

In 1970, mupirocin was discovered, its mechanism of action was to inhibit isoleucine tRNA synthetase. This is only 1 of 20 amino acid tRNA synthetases, 19 others were identified in 1995 from gene sequencing. Certain genes may be broad spectrum e.g. fabH which codes for the enzyme β-ketcoacyl-

ACP synthase III, part of the fatty acid biosynthetic pathway, is present in a large number of clinical organims: Gram-positives, Gram-negatives, chlamydia, anaerobes, mycobacteria and a few protozoa. For selectivity one can compare bacterial genes with mammalian genes to measure % homology and ensure that there will be no major interactions. Essentiality, can be determined using gene knockouts (e.g.fabH, GTPases, Mur A1/A2) by for example, looking at growth/no growth in the presence of erythomycin. It is essential to ensure that a new target is expressed *in vivo* i.e. in infection some genes are only expressed *in vitro*, some only *in vivo* and others in both. There are about 100 new targets in staphylococcus and haemophilus whose functions are unknown. Twenty have been identified as being essential.

Generating focussed chemical arrays of 10 -1,000 from a single active compound leads to more rapid SAR generation. In the 1980s, it was possible to screen >30,000 compounds at a rate of 100 compounds/day using ~20 assays/year. Now these figures have increased to 0.5 – 2million, 100,000/day and 70-75 assays/year, respectively. Fast and accurate readers are required to handle these large numbers. This productivity leads to bottlenecks in development. The main issues being solubility, cytotoxicity, serum binding and pharmacokinetics. There is no doubt that new leads, multiple targets and novel structures are desirable but the question remains as to whether bacterial resistance will still gain pole position in the future

Professor Jean-Marie Frère (Centre d' Ingénerie des Protéines et Laboratorie d' Enzymology, Université de Liège, Belgium) discussed the processes involved in antibiotic inactivation by enzymatic modification. Resistance to β-lactam antibiotics is most often the result of production of β-lactamases, which share certain conserved structural elements with the D, D-peptidases, the penicillin binding proteins (PBPs). Resistance to PBPs can arise *via*: (I) the acquisition of a new gene e.g. MRSA; (ii) point mutations e.g. *E.coli* PBP3, *S.pneumoniae* PBP2x; (iii) mosaic genes e.g. *S.pneumoniae* and *N. gonorrhoea* and by (iv) the over expression of a minor resistant PBP e.g. *E.hirae* (PBP5). PBPs exhibit various sensitivities to different compounds. On the other hand, β-lactamases exhibit very diverse sensitivities and the encoding genes are easily transferred between different species. The introduction of new β-lactams into the clinic has repeatedly been followed by the emergence of new enzymes, for example NMC-A and Zn++ metallo-β-lactamases, which can hydrolyse them. Other β-lactamases are variants which differ from the parent by a few point mutations as in the ESBLs (e.g. TEM, SHV) or simply by over expression as with the Class C β-lactamases. In Gram-negative bacteria, β-lactamases are secreted into the periplasmic space. The antibiotic must diffuse into the periplasm by traversing the outer membrane, which constitutes a permeability barrier, and then it must escape the activity of the β-lactamase. The MICs for various antibiotics will be high in the case of β-lactamase overproducers because higher external concentrations will be required to kill the bacteria. When the periplasmic concentration is sufficient i.e. reaches the lethal level; it is equal to the external concentration. By measuring the permeability factor, as well as V_{max} and Km for the β-lactamase and assuming the lethal concentration to be equal to the MIC of the strain which does not produce the β-lactamase, it is possible to predict the MIC. For many antibiotics the efflux phenomenon also comes into play which can replace the effect of the β-lactamase or may be simply additive. If we have a very efficient efflux system with a low Km value we end up with the situation which prevailed with the β-lactamase.

A more conventional approach to bacterial resistance was described by **Dr Axel Dalhoff** (Bayer, Germany). During the last decade new quinolones have been prepared which have a much improved safety profile over some of the earlier members of this class. These new agents have been optimised to obtain maximal activity and optimal pharmacokinetic/pharmacodynamic parameters.

Moxifloxacin

Commonly the antibacterial activity is expressed as the MIC_{90} (the concentration of an antibacterial agent at which ~90% of the bacteria being studied are inhibited). However, it may be more informative to plot the Gaussian distribution of the MICs and also the Gaussian distribution of the pharmacokinetic factors. If the overlap is in the region of ~90% then there is a high probability that a given drug, at a given dose, will inhibit a defined pathogen. During the development of moxifloxacin (Fig 1; Bayer) they looked carefully at tissue distribution (serum, bronchial mucosa, epithelial lining fluid and alveolar macrophages) and found that the levels of the antibiotic were high, being as high as 100x the corresponding serum concentrations. They found that by exposing the bacteria to varying C_{max} doses the bactericidal efficacy was increased. The faster bacteria are killed decreases the time of exposure thus minimising the likelihood that resistance will emerge. The spontaneous mutation frequency of moxifloxacin was compared to gatifloxacin (Kyorin), which also carries a methoxy group at the C8 position and grepofloxacin (Otsuka), which has a hydrogen at C8, and found that moxifloxacin had a lower propensity for the selection of resistant mutants and showed good activity against resistant strains. Dr Dalhoff proposed that pharmaceutical scientists should implement PK/PD studies during Phase III trials to ensure that the right dose is used from the beginning. If, for example the bacterial outcome at 200mg and 400mg is the same choose the higher dose to minimise resistance.
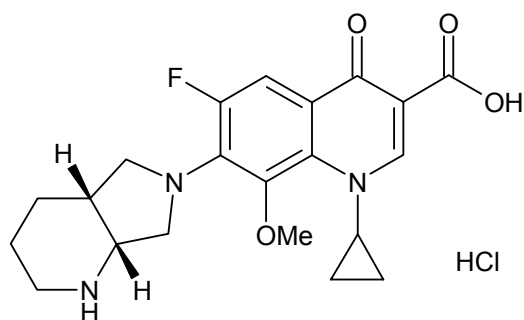

Figure 1 Moxifloxacin

The final speaker **Mrs Pamela Hunter** (Freelance consultant, Surrey) questioned the future of antibacterial research in the pharmaceutical industry. Penicillins and cephalosporins are still among the top ten best selling drugs word wide but the growth rate for antibacterial agents has slowed in recent years. The two main factors contributing to this decline are prescribing and cost. Monitoring the consumption of restricted antibiotics such as amikacin, aztreonam, ceftazidime, imipenem, meropenem, vancomycin, teicoplanin and the combination therapies; cefoperazone/sulbactam, piperacillin/tazobactam and ticarcillin/clavulanate did not compromise patient care. This is true also for partially restricted compounds including quinolones, third generation cephalosporins and aminoglycosides. Injectable antimicrobial agents are generally costly and broad spectrum.
 FDA labeling requirements propose that all systemic antimicrobial drugs for humans contain additional labeling information about the emergence of drug-resistant bacterial strains. It should also state that "antibacterial drugs should only be used in situations where a bacterial infection is either proven or strongly suspected". AMA (American Microbiological Association) have lobbied the FDA to expedite the regulation of the inclusion of antimicrobial agents in consumer products because such use may be a potential source of antibiotic resistant organisms. The Word Health Organisation concur with the view that products such as antibacterial soaps, toothpastes and kitchen cleaners contribute to resistance.
There are a number of approaches aimed at curtailing the transmission of drug resistant organisms, all of which result in a reduction in the sales of antibiotics. What does this mean for the industry?
Although there have been significant developments in bacterial genomics and proteomics, the generation of large combinatorial compound libraries and high throughput screening there is a downside and that is they all carry a high cost. Therefore , while it recognises the need for new strategies towards the discovery of novel antibiotics, with novel modes of action aimed at combating resistance, can the pharmaceutical industry afford to develop them? This dilemma poses new challenges for executives and

researchers alike. Meanwhile it may be prudent to consider, in parallel, the application of modern approaches to the SAR optimisation of existing classes of antibiotics.