IDENTIFYING RESISTANCE™
INTERNATIONAL NEWSLETTER
AUGUST 2001
no 1

Let's fight together !

Since its creation in 1963, with manual API® strips followed by VITEK & VITEK 2 systems, bioMérieux has long been committed to providing laboratories all over the world with the most reliable and cost-effective solutions to identify bacteria and their antibiotic resistance.

Today, more than 11,000 labs worldwide are using a bioMérieux ID/AST system - by far the largest installed base. Our research & development teams are working constantly to keep our systems, reagents and expert data bases up-to-date, improving accuracy the time to result and financial outcomes for the laboratory.

But, because we are all faced with the increasing health issue of bacterial resistance and nosocomial infections, we now want to go a step further : this newsletter, IDENTIFYING RESISTANCE, with information ranging from the best specialists to the most practical lab solutions, should become a quarterly rendez-vous between us. Feel free to send us your comments or suggestions. Combining our areas of expertise, we are sure we will contribute to improving the fight against bacterial resistance and sowing the seeds better health.

Thierry Bernard,
Director of Clinical Marketing

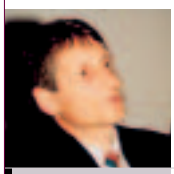
BIOMÉRIEUX

STATE-OF-THE-ART

Why Identify Resistance?

Clinical Needs

The Importance of Determining Resistance Phenotypes for Successful Antibiotic Therapy



Philippe Moreillon CHUV, Lausanne, Switzerland

Medical microbiologists know that prescribing the best possible treatment is more complicated than recognizing a particular microbe's susceptibility or resistance to a given antibiotic. For each patient, it is important to understand the factors of resistance and know something about the person for whom the antibiotic is being prescribed. Resistance genes may, at times, mask unsuspected dangers, and the risk of treatment failure may be more substantial than what the culture dish tells us. There are a number of examples of the pitfalls of various bacterial species.

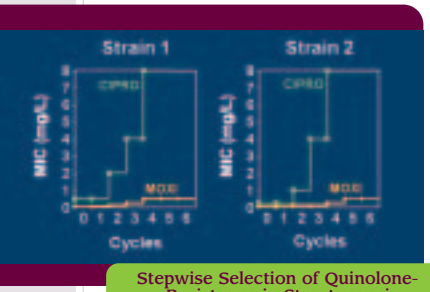
Penicillin-Resistant (MIC > 2mg/l) Pneumococci in Europe (1998-Alexander Project)

Pneumococci are responsible for acute otitis media, acute sinusitis, pneumonia, bacteremia and meningitis. These bacteria, which in the past responded to practically all antibiotics, have developed resistance to nearly all the drugs used to treat them, especially penicillins. With the notorious exception of meningitis, the therapeutic consequences of such resistance are limited. Indeed, pharmacokinetic data and clinical experience indicate that, except for meningitis, one can safely treat infections due to intermediate resistant pneumococci (MIC > 0.1 and < 2 mg/l) and even higher resistance levels (MIC > 2 mg/l) by increasing the doses of penicillins. In the case of meningitis, on the other hand, penicillin-resistant *Streptococcus pneumoniae* (PRSP) should never be treated with penicillin, regardless of the resistance phenotype. This is due to the fact that appropriate penicillin concentrations cannot be reached in the cerebrospinal fluid. The situation could become worse tomorrow if pneumococci develop high levels of resistance, as they appear to be doing in the United States. Be on the lookout for more news on this subject.

Staphylococci are problematic insofar as they may express resistance to methicillin or glycopeptide heterogeneously. How can one be sure that the most resistant bacteria have been tested for? What's more, highly resistant populations represent only a small proportion (10^2-10^5 CFU) of the much larger bacterial population present at the infected site (>10^9 CFU). The problem was recognized several years ago for methicillin-resistant staphylococci. Hence, appropriate methodological strategies were developed to recognize the phenotype. However, this is not yet the case with glycopeptide-resistant staphylococci, which have attracted attention only recently. Such organisms may still be missed by the

diagnostic laboratory. Moreover, is it not possible that glycopeptide resistance has existed for a long time, but was masked by susceptible, rapidly growing bacterial populations ?

Emerging resistance to fluoroquinolones is one of the most complex challenges facing physicians. Fluoroquinolones represent a very powerful and useful class of drugs in both human and veterinary medicine. Older molecules of this family were essentially targeted at Gram-negative pathogens. Modern fluoroquinolones have an improved anti Gram-positive spectrum, and are thus recommended for the treatment of infections due to these types of bacteria as well.



Stepwise Selection of Quinolone-Resistance in Streptococci

However, if a patient is infected by a Gram-positive bacterium that has previously acquired a resistance mutation to older quinolones, such as resistance to ciprofloxacin, this particular organism will have an increased chance of introducing a second mutation conferring high-level resistance to the newer quinolones. This hypothesis is not unrealistic since ciprofloxacin is one of the most commonly prescribed antibiotics in the world. Two treatment failures concerning levofloxacin were reported at the last ICAAC: in both cases, the failure was due to a second *gyr*A mutation appearing in a bacterium already carrying a first resistance mutation in the *par*C genes. Both of them are typical quinolone-resistance mutations in Gram-positive bacteria. Likewise, findings in Canada, where fluoroquinolones are widely prescribed for respiratory diseases, show that by combining such resistance mechanisms, pneumococci can develop resistance to the most recent molecules of this class. All together, this warrants caution against the overuse of these promising new drugs if one is to prevent the whole family of molecules from being lost at once.

The problem of extended-spectrum beta-lactamases (ESBL), which first arose in 1983, reached dramatic proportions in 1995. ESBL are present in a number of enterobacteria. One of the most recent avatars, CTX-M, seems to have caused damage in Spain and has not spared France. ESBL are also responsible for treatment failures and nosocomial epidemics of multi-resistant *K. pneumoniae*.

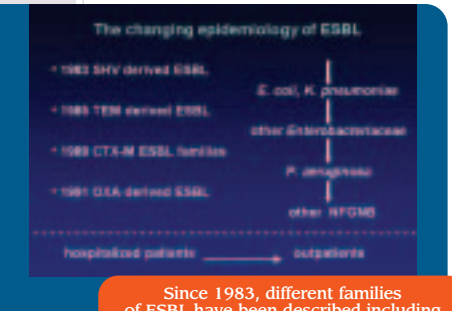
Detecting resistance is not an end in itself, but rather a means to establish the best possible treatment for a patient's bacterial infection. It also offers a still-frame picture of a never-ending story, the story of bacteria's resistance to antibiotics and of the genes responsible for resistance. Knowing the various phenotypes and genotypes is essential for both patient care and infection control. ■

Why Identify Resistance?

Epidemiology Needs

Rafael Cantón Servicio de Microbiologia, Hospital Ramón y Cajal, Madrid, Spain

Today we are fully aware that the discovery of antibiotics did not put an end to bacterial infections. Antimicrobial resistance quickly developed as a natural consequence of the ability of bacteria to adapt to antimicrobial agents. In recent years, various resistant organisms have become prominent and new resistance mechanisms have emerged, reducing the antimicrobial spectra. This is clearly the case for staphylococci, for which "dangerous" clones can rapidly emerge and spread through bacterial populations. The emergence of MRSA among non-hospitalized patients and the spread of hospital clones have been recently documented in several countries, together with the isolation of clinical MRSA displaying reduced vancomycin susceptibility. New carbapenemases and hidden carbapenemase-producing organisms have been found in Gram negative-bacteria, while a dramatic increase in ESBL has been reported during the last decade.

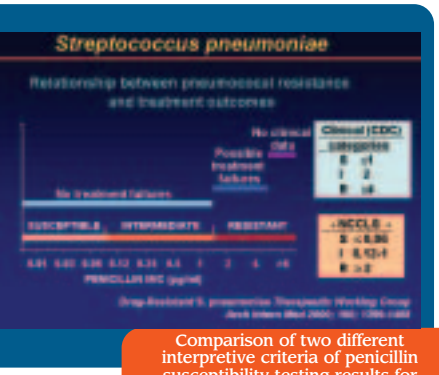


Since 1983, different families of ESBL have been described including SHV, TEM, CTX-M and OXA derivatives

The most recent developments in the never-ending story of antimicrobial resistance are troubling because they concern ubiquitous bacteria, responsible for infections in communities or hospitals, such as *Streptococcus pneumoniae*. It appears that all antibiotics are affected by this trend, including the most recently developed drugs, such as the newest fluoroquinolones. In the latter case, low level resistance mechanisms may be underestimated, particularly in quinolone-resistant bacteria, due to efflux-based mechanisms and, to a lesser extent to single gyrA mutations.

Comparison of different breakpoints proposed by different committees

What is the best response to this situation? The recognition that resistance mechanisms may evolve differently with different antibiotic policies has led to various strategies and the assessment of the ecological impact of new drugs on resistance mechanisms. It has been clearly demonstrated that antibiotic policies, including restrictive and cycling strategies, do not always lead to a decrease in resistance rates. For example, cycling policies have proven to be responsible for the accumulation of resistance mechanisms due to the incorporation of resistant genes into potential stable structures (plasmids, bacteriophages, transposons, integrons, and gen cassettes) in resistant bacteria. They are also responsible for co-selection processes, which may have played a role in the acceleration of resistance development and the increase in resistance rates.



Comparison of two different interpretive criteria of penicillin susceptibility testing results for *Streptococcus pneumoniae* isolates

The "explosion" of a few resistant mechanisms, together with the globalization of resistant mechanisms and resistant bacterial populations, requires reliable detection of resistant populations, in addition to accurate antimicrobial susceptibility testing. Epidemiological and clinical needs are now of comparable importance. However, each need brings its own constraints, and it is not certain that breakpoints established to predict favorable outcomes will bring epidemiological benefits. Among pneumococcal strains, for example, population analysis reveals PBP modifications independently of clinical breakpoints. Resistance mechanisms seem to be present in bacterial populations irrespective of clinical breakpoint criteria, and susceptible/resistant breakpoints must be re-considered by committees promoting different values.

Susceptibility testing processes in clinical laboratories will become more complex in the near future. They will be based on accurate susceptibility testing determinations, the reliable detection of resistant mechanisms and resistant populations (integrated expert system), and combined phenotypic and genotypic detection. Resistance mechanisms can be present in apparently susceptible bacterial populations, and their identification will remain the first step towards the control of the emergence – and re-emergence – of resistant bacterial populations. ■

How can we Identify Resistance?

Phenotypic Methods

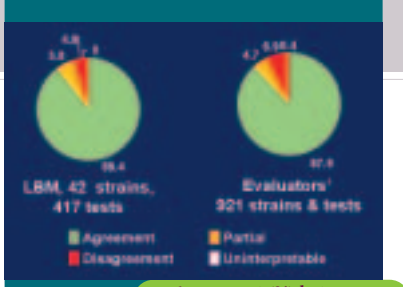
Improving Susceptibility Testing

David M. Livermore Central Public Health Laboratory, London, United Kingdom

Recording sensitivity results, the microbiologist wonders: is it actually possible to have a *Klebsiella* strain that is ampicillin and ceftazidime resistant but susceptible to piperacillin? Should I repeat the test for a third time, look at the specialized literature, call a friend for help, or send the bacteria to the reference lab? All too often time presses and the result, however odd, is recorded "as is." Yet most resistance reflects common mechanisms, with well-defined spectra. Consequently, it is usually possible to infer resistance mechanisms from resistance phenotypes, and to distinguish the unusual from the frequent.

Klebsiella, for example, may have several different resistance determinants, such as acquired TEM and extended-spectrum β-lactamases or, in the case of *K. oxytoca*, may over-produce their chromosomal "K1" β-lactamase. Each of these mechanisms has a characteristic resistance profile, as do those prevalent in other species.

"Interpretive reading" is a strategy based on analyzing the complete resistance profile for an isolate against a set of rules. The resistance mechanisms predicted from the phenotype and anomalous results are identified and, if appropriate, edited. Certain rules are simple, such as "call MRSA resistant to all beta-lactams" or "call erythromycin-resistant



Agreement (%) between VITEK® 2 AES and known mechanisms

	No tested	% Reported as: S	I	R
Aztreonam	152	10	9	81
Ceftazidime	172	10	13	77
Cefotaxime	175	37	27	36
Ceftriaxone	80	29	24	47
Cefuroxime	155	14	11	76

Reporting of resistance for 220 ESBL(+) Klebsiellae, 1994 Euro-survey

staphylococci resistant to clindamycin" and be suspicious of grossly unusual susceptibilities or resistances (tables 1 and 2), but others are much more complicated and require looking at the overall pattern of resistances and susceptibilities. The overriding principles are:

- 1 ■ to recognize and reconsider anomalous combinations of phenotype and organism;
- 2 ■ predict which further antibiotics are worth testing;
- 3 ■ eliminate susceptibilities that are tenuous in light of the inferred mechanism;
- 4 ■ conduct tentative surveillance of the prevalence of resistance mechanisms.

The problem is that -for effective interpretive reading- large numbers of profiles and mechanisms must be remembered. However, the microbiologist can now rely upon the expert systems that have been incorporated into automated susceptibility testing and zone readers. European evaluation of the Advanced Expert System (AES) on the Vitek 2 indicated 87.9% agreement to known resistance mechanisms in 921 strains and tests, and 89.4% agreement among 417 tests on 42 strains distributed by bioMérieux. Resistance mechanisms inferred with >95% agreement to reference data including *mec*A in staphylococci, *van*A and *van*B in enterococci, quinolone resistance in staphylococci and enterobacteria, *mef* and *erm*- mediated macrolide resistance in pneumococci, and acquired penicillinases and extended-spectrum β-lactmases in enterobacteria.

Expert systems are useful and efficient. However, for the systems and for microbiologists, the task at hand grows more complicated as more bacteria acquire multiple resistance mechanisms, such as combinations of impermeability and beta-lactamases or batteries of two or three different beta-lactamases or aminoglycoside-modifying enzymes. The best results are probably obtained from a careful microbiologist backed by a quality expert system.

Table 1: Unusual resistances demanding reference laboratory confirmation

Organism	*Resistances*
S. aureus	*Glycopeptides, linezolid, Synercid®*
Coag-ve staph	*Vancomycin, linezolid, Synercid®*
JK coryneforms	*Glycopeptides, linezolid, Synercid®*
S. pneumoniae	*Meropenem, glycopeptides, linezolid, Synercid®*
Group A, B, C, G beta-haemolytic streptococci	*Penicillin, glycopeptides, linezolid, Synercid®*
Enterococci	*Linezolid, both ampicillin and Synercid®*
Enterobacteria	*Meropenem, imipenem (except Proteus spp.)*
H. influenzae	*3rd-generation cephalosporins or carbapenems*
M. catarrhalis	*Ciprofloxacin, 3rd-generation cephalosporins*
N. meningitidis	*Penicillin (high level), ciprofloxacin*
N. gonorrhoeae	*3rd-generation cephalosporins*
Acinetobacter; P. aeruginosa	*Colistin*
Anaerobes	*Metronidazole, carbapenems*

Table 2: Doubtful susceptibilities: these species should be resistant to these antibiotics

Organism	*Resistances*
A. baumannii	*Ampicillin, 1 st-generation cephalosporins*
P. aeruginosa	*Ampicillin, co-amoxyclav, 1 st-2nd-generation cephalosporins, trimethoprim*
S. maltophilia	*All beta-lactams, aminoglycosides*
Klebsiella spp.	*Ampicillin, ticarcillin*
Enterobacter, C. freundii	*Ampicillin, co-amoxyclav, 1 st-generation cephalosporins, cefoxitin*
Serratia spp.	*Ampicillin, co-amoxyclav, 1 st-generation cephalosporins, cefuroxime, colistin*
P. mirabilis	*Colistin, nitrofurantoin*
P. vulgaris	*Ampicillin, cefuroxime, colistin, nitrofurantoin*

PRACTICAL ADVICE

In the past, bacteria were simply susceptible or resistant.

Microbiology testing consisted of testing a series of drugs for the use of physicians, then reporting a series of results in "S/I/R" format.
Several decades passed with a large increase in the number of drugs, the development and spread of bacterial resistance, as well as a huge accumulation of knowledge: natural resistance, acquired resistance, false susceptibility etc. Epidemiology data are available that describe the susceptibility or resistance patterns of many bacterial species.

Now, bacteria have many ways of being resistant.

Antibiotic testing is therefore increasingly becoming an identification issue: given a bacterial name we know which resistance phenotypes should be looked for. Expert systems enable automatic identification of these resistance phenotypes, using a data base and an inference engine mimicking the human mind.

Thus, testing of bacteria means answering these 5 questions:

1 ■ does this isolate need a susceptibility test?

A sorting of bacteria to be tested can be done, mainly based on medical criteria.

2 ■ which drugs should be tested to better identify resistance?

A wide knowledge of resistance mechanisms is needed to select antibiotic markers better suited to detecting resistance.

3 ■ what is the identification of the isolate?

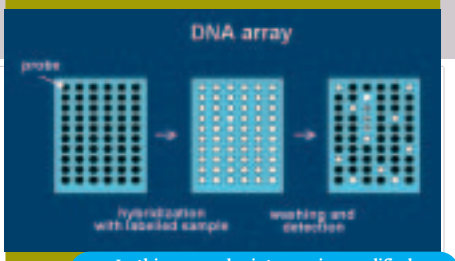
Identification of bacteria is still very important, as resistance phenotypes differ from species to species, and the matching of species identification with resistance patterns is key in the quality control process (consistency checking).

4 ■ what resistance phenotype does this bacterium have?

This is the new identification step: each species carries a series of resistance phenotypes that should be identified, while so-called "impossible" phenotypes should be ruled out.

5 ■ what is the answer for the physician?

The answer combines raw data (MIC value), interpreted data (S-I-R), plus comments about the rarity or commonness of results as well as some therapeutic advice - based on national antibiotic committees.



In this example, intragenic amplified fragments are spotted on the filter (probe). The labelled sample is composed of total bacterial DNA. A white spot on the right panel indicates homology between the probe and the sample

How can we Identify Resistance?

Genotypic Methods

Detection of Resistance Genes in the Absence of Growth

Patrice Courvalin | Unité des Agents Antibactériens, Pasteur Institute, Paris, France

Will AST as we know it, based on disk diffusion or an automated method, disappear in the coming years? Will it be replaced by new techniques of resistance characterization at the genetic level? It is likely that both the genotypic and the phenotypic approaches will coexist; microbiologists will choose the most appropriate method based on a number of parameters: the microbe responsible for the infection, the patient, the site of infection, the severity of the state of infection, whether or not the infection is nosocomial, the need for epidemiological surveillance, etc. Each approach offers advantages and disadvantages due to the type of information it provides.

Phenotypic techniques are increasingly standardized. They will probably continue to provide the basis for therapeutic decisions in straightforward cases, in which decisions are made on the basis of susceptibility data rather than on resistance mechanisms.

Genotyping techniques aim to detect the presence of genes responsible for resistance in a given bacterium, whether carried by the chromosome or by a genetic element (plasmid or transposon) able to spread among different species. These techniques are increasingly used in epidemiological studies concerning bacterial resistance. Their underlying principle is nucleic acid hybridization with a labeled sample, usually followed by detection of positive data by fluorescence. Emergence of resistance is most often due to acquisition of foreign DNA. Since acquired genes are usually closely related in the primary structure, they can be revealed by using specific DNA fragments that compose macroarrays.

By contrast, oligonucleotides (oligoarrays) can be designed for detection of a single mutation, but the existence of multiple loci leading to resistance by mutation in a housekeeping structural gene or a regulatory cistron complicates detection of this pathway of resistance. One of the most classical examples of detection of mutational resistance concerns mutations in *rpoB* leading to rifampicin resistance in *Mycobacterium tuberculosis*. All phases of the technique can be automated, but they involve high cost and low throughput technology. In addition, this method clearly does not constitute a universal solution to the problem of tuberculosis treatment, since screening indicates only that an antibiotic can no longer be used, without giving information about which antibiotics should be used.

One of the drawbacks of genotypic detection of resistance is that you only screen for what you already know. Nevertheless, this approach will probably be simple enough in the future to be used by clinical laboratories. Prior recognition of a resistance determinant will allow detection of low-level resistance and therapeutic predictions without relying on (arbitrary) clinical categories and phenotype analysis. The technique is likely to be especially useful for fastidious bacteria (anaerobes, *Capnocytophaga*, *Chlamydia*, *Legionella*, *Leptospira*, etc.) and bacteria that cannot be grown *in vitro*, such as *Rickettsia* and *Treponema*, as well as for "dangerous" micro-organisms such as *Brucella*, *Francisella*, and *Mycobacterium tuberculosis*.

ARRAY TYPES AND INDICATIONS

Characteristic	Macro-array	Micro-array	Oligo-array
Probe [a]	DNA fragments	DNA fragments	Oligonucleotides
Support	Nylon	Glass	Silicon Glass
Probe number	10,000	10,000	400,000
Labelling	Radioactive	Fluorescent	Fluorescent
Screening for:			
- acquired DNA	+	+	+/-
- mutations	-	-	+
- resequences	-	-	+
- transcripts	+	+	+/-

a. Probe: genetic information known in the reaction. In the present case, the DNA fragment or oligonucleotide is spotted on the filter. ■

THE BIOMERIEUX SOLUTION

VITEK® 2

From inoculum dilution right through to result reports, all the VITEK 2's processing steps are completely automated. Its innovative Expert System contains an extensive knowledge base of over 2,000 phenotypes to validate and interpret antibiotic susceptibility test (AST) results. This means timely and appropriate patient treatment, reducing lenghts of stay and associated costs.

The financial impact of using the VITEK system has been documented **[1]** by comparing hospital costs for patients whose samples were processed using the VITEK system vs a group whose cultured samples were processed using conventional methods. The average turnaround time was 5.2 hrs faster (39.2 hrs vs 44.4 hrs). The average variable cost was $ 1,750 less per patient ($ 4,927 vs $ 6,677), which adds up to millions of dollars in savings per year. The VITEK 2 system offers equivalent or superior turnaround times.

Several studies have attested to the accuracy of VITEK 2 performance **[2-7]**. For example, 845 strains representing 70 different taxa of *Enterobacteriaceae* or non-enteric bacilli were tested using the ID-GNB identification card **[2]**. Efficient identification was obtained in 3 hrs with an error rate of 0.8% and with only 1.2% of strains unidentified. A similar study of enterococci strains correctly identified 87% to species level, with an AST accuracy of up to 97% **[3]**.

Streptococcus pneumoniae strains showed 96% correlation with the NCCLS reference microdilution method on both challenge and routine strains **[4]**.

A study on the expert system showed an agreement of 95 to 100% with the human expert **[5]**.

The VITEK 2 system offers rapid detection of low levels of resistance, providing susceptibility testing results within 5-7 hrs, with a range of 3-18 hrs depending on the organism and the antibiotic.

REFERENCES

[1] Barenfanger, J. et al. (1999) Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. J. Clin. Microbiol. **37**, 1415-18.

[2] Funke, G. et al. (1998) Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. J. Clin. Microbiol. **36**, 1948-52.

[3] Garcia-Garrote, F. et al. (2000) Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of enterococci. J. Clin. Microbiol. **38**, 2108-11.

[4] Jorgensen, J. et al. (2000) Rapid Automated Antimicrobial Susceptibility Testing of Streptococcus pneumoniae by Use of the bioMerieux VITEK 2. J. Clin. Microbiol. **38**, 2814-2818.

[5] Sanders, C. et al (2001). Potential Impact of the VITEK 2 System and the Advanced Expert System on the Clinical Laboratory of a University-Based Hospital. J. Clin. Microbiol. **39**, 2379-2385. ■

DID YOU KNOW?

ROAR
www.healthsci.tufts.edu/apua/ROAR

***ROAR** stands for the **R**eservoirs **O**f **A**ntibiotic **R**esistance Network.*

ROAR is a unique network dedicated to generating a new impetus worldwide for research into commensal bacteria as reservoirs of resistance that can be transferred to human pathogens.

- summary and background
- definitions of key words such as antibiotic, antibiotic resistance, commensal, horizontal gene transfer, non-clinical bacteria or non-clinical isolates, reservoir of resistance
- reports, abstracts & tables: 14 papers
- annotated bibliography: 26 pages of references.
- discussion group with archive of postings
- what's new: recent awards, reports and guidelines, recent and upcoming meetings
- links: 14 sites

This site is hosted by that of APUA - Alliance for the Prudent Use of Antibiotics www.healthsci.tufts.edu/apua managed by Pr Stuart Levy.

ASM General Meeting

This year the General Meeting of the American Society for Microbiology was held in Orlando, Florida. The scientific meeting consisted of 3,970 presentations, 309 sessions, 20 colloquiums, 76 symposiums, 596 oral presentations and 3,370 posters. The exhibition brought together 380 companies. Bacterial resistance was addressed in eleven sessions. Among them:

- *the fitness cost of antibiotic resistance (session 80), chaired by B. Levin and L. Rice. Including a critical presentation of antibiotic cycling.*
- *public health issues with susceptibility testing (session 191) covered various aspects including the action plan to combat resistance with 85 action items coordinated by the CDC.*
- *perspectives in resistance of S.pneumoniae, (session 200) including "a life in chains" by Don Low, Toronto.*

Next meeting: May 20-22, 2002 in Salt Lake City, UT.

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INTERNATIONAL NEWSLETTER

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