European recommendations for antimicrobial resistance surveillance

G. Cornaglia[1], W. Hryniewicz[2], V. Jarlier[3], G. Kahlmeter[4], H. Mittermayer[5], L. Stratchounski[6] and F. Baquero[7], on behalf of the ESCMID Study Group for Antimicrobial Resistance Surveillance (ESGARS)

[1]Department of Pathology, University of Verona, Verona, Italy, [2]National Institute of Public Health, Warsaw, Poland, [3]Laboratoire de Bactériologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France, [4]Clinical Microbiology, Central Hospital, Växjö, Sweden, [5]Department of Hygiene, Microbiology and Tropical Medicine, Elisabethinen Hospital, Linz, Austria, [6]Department of Clinical Pharmacology, Smolensk State Medical Academy, Smolensk, Russia and [7]Servicio de Microbiologia, Hospital Universitario Ramón y Cajal, Madrid, Spain

ABSTRACT

The problem of antimicrobial resistance surveillance in Europe has been debated extensively in many excellent documents issued by national committees that often assume the value of national guidelines. However, a comprehensive document addressing the whole matter from a European perspective, as well as reviewing its present status and drafting future perspectives, has been lacking. The present recommendations have been produced by the ESCMID Study Group for Antimicrobial Resistance Surveillance (ESGARS) through a consensus process involving all members of the Study Group. The recommendations focus on the detection of bacterial resistance and its reporting to clinicians, public health officers and a wider—and ever-increasing—audience. The leading concept is that the basis for resistance monitoring is microbiological diagnostics. The prerequisites for resistance monitoring are findings of adequate quality and quantity, which have been recorded properly and evaluated correctly. Different types of surveillance studies should fulfil different requirements with regard to data collection and reporting, the expected use of data, and the prerequisites for networking such activities. To generate relevant indicators, bacterial resistance data should be reported using adequate denominators and stratification. Reporting of antimicrobial resistance data is necessary for selection of empirical therapy at the local level, for assessing the scale of the resistance problem at the local, national or international levels, for monitoring changes in resistance rates, and for detecting the emergence and spread of new resistances types. Any type of surveillance study should conclude, where appropriate, with a proposal for intervention based on the data obtained.

Keywords Antimicrobial resistance, European, guidelines, recommendations, resistance, surveillance

INTRODUCTION

The emergence and spread of antimicrobial resistance constitutes a major risk for human health. Resistance to antibiotics limits the success of these agents in the therapy and prevention of infectious diseases. Yet society should be aware of the fact that many accomplishments of modern medicine have only been possible because of the availability of a protective antibiotic umbrella. However, continuous positive selection of resistant bacterial clones, whether pathogenic, commensal or even environmental bacteria, will modify the population structure of microbial communities, leading to accelerated evolutionary trends with unpredictable consequences for human health.

Historical background: the past decade

Surveillance of bacterial resistance to antimicrobials involves important financial and intellectual resources throughout Europe, and coordination and harmonisation of these resources are

Corresponding author and reprint requests: G. Cornaglia, Department of Pathology, University of Verona, Strada Le Grazie 8, I-37134 Verona, Italy
E-mail: giuseppe.cornaglia@univr.it

beginning to rank increasingly high among the priorities of scientific societies, public health officers and legislators [1].

Even though previous efforts on the part of both individual scientists and pharmaceutical firms—mostly from Europe—must be acknowledged [2–5], most current activities in the field of antimicrobial resistance surveillance are rooted in the 1995 ASM Task Force Document [6], the primary aim of which was 'to assist the preparation of cumulative antimicrobial susceptibility reports that would prove useful to clinicians in the selection of the most appropriate agents for empirical antimicrobial therapy'.

National surveillance systems (or data collections) arose in Europe in response to two different driving forces, broadly reflecting the two main political and economic assets that dominated our continent throughout the Cold War period, namely:

- in western Europe—high-level academic interest in antibiotic resistance; creation of comprehensive networks of microbiological facilities in hospitals; availability of new technologies, e.g., new automated tools for antimicrobial susceptibility testing, entailing both locally based and large-scale data collection systems; and surveillance initiatives on the part of pharmaceutical companies;
- in eastern Europe—a heritage of centrally controlled public health organisations, frequently facilitating ongoing centralised data collection.

The need to compare the many different solutions which developed within the framework of these two broad models, and an awareness of the increasing importance of the antimicrobial resistance problem, resulted in a meeting in 1997 on 'The present status of antimicrobial resistance surveillance in Europe', organised by the World Health Organisation (WHO) in Verona, Italy [7]. Among the key findings of the workshop were the following:

- Much useful information was already being generated in Europe on antimicrobial resistance.
- The emergence and growth of antimicrobial resistance could not be addressed effectively by any one country or group working in isolation.
- Europe-wide coordination and cooperation were critical elements for any effective approach, in order to develop collaboration between existing antimicrobial resistance surveillance programmes.

A significant outcome of the Verona meeting was the establishment of the ESCMID Study Group for Antimicrobial Resistance Surveillance (ESGARS), with the following main aims:

- to provide a unifying forum for those involved actively in antimicrobial resistance surveillance;
- to promote awareness and facilitate the early detection of emerging antimicrobial resistance in Europe, and to contribute to an understanding of its epidemiology;
- to improve access to European data on surveillance;
- to provide opportunities to enhance cooperation;
- to establish links with and between networks of resistance surveillance programmes.

A further step towards harmonisation of antimicrobial resistance surveillance in Europe was the creation 1 year later of the European Antimicrobial Resistance Surveillance System (EARSS) [8]. The EARSS, funded by DG SANCO of the European Commission and coordinated by the Dutch National Institute for Public Health and the Environment (RIVM), is a European network of national surveillance systems that collects comparable and validated antimicrobial resistance data for public health purposes. Data generated routinely are collected and analysed, and on-line feedback is provided [9]. Results of antimicrobial susceptibility testing for invasive *Staphylococcus aureus* and *Streptococcus pneumoniae* isolates have been collected since 1999, and in 2001 the EARSS started collecting data for invasive *Escherichia coli* and *Enterococcus faecalis/faecium* isolates. More than 600 laboratories in 28 western and eastern European countries currently participate [9]. External quality assurance exercises carried out by the EARSS in cooperation with the UK National External Quality Assessment Scheme (UK-NEQAS) and the French Centre National de Référence des Antibiotiques (CRAB) in 2000, 2001 and 2002 showed that the laboratories involved are capable of delivering comparable and high-quality susceptibility data [10].

Large-scale exploitation of routine data

A major advance in antimicrobial resistance surveillance is the increasing availability and exploitation of routine susceptibility test data. Originally, resistance surveillance was conducted

largely in the form of ad-hoc studies, often sponsored by pharmaceutical companies. Sample sizes were often small, and most studies were beset by sampling errors or lack of a denominator. Routine susceptibility test data have a population denominator, and represent a huge and sometimes untapped source of inexpensive, accessible results, although several objections have been raised [11]:

- Standardisation of both methodology and interpretative criteria is often poor.
- Many laboratories test relatively few antimicrobial agents against most isolates, and they do not all test the same compounds.
- 'Second-line' antimicrobial agents are often tested only against isolates resistant to agents used more widely.
- Many isolates are only identified partly, meaning that data from different species may be pooled and major resistance developments in infrequent species are likely to be missed.

In spite of these concerns (see later), a major advantage is that surveillance systems can be fed simply by downloading data regularly on a wide range of organisms and specimen types. The main reason for a curb on the wider acceptance of routine data for surveillance was possibly related to the fact that the extensive availability of such data had an impact on huge interests in terms of both money and power stemming from the private control of antimicrobial resistance surveillance (which is somehow different from the control of antimicrobial resistance), as often occurs when advances in technology alter a well-established steady state [12]. It is worth noting that once the antibiotic resistance problem became a fashionable issue, routine susceptibility test data suddenly turned out to be reliable—even beyond their own limits—when a for-profit company proposed its monitoring strategy based on routine data collected from a number of hospitals. The use of routine data is now accepted widely, as opposed to the costly and labour-intensive traditional active surveillance, in that it can produce a great deal of useful, easily accessible and sufficiently accurate information.

However, it must be borne in mind that antimicrobial resistance surveillance can be based on routine data only when there is a steady flow of diagnostic samples submitted for laboratory testing. This precondition is generally met in most of western Europe, but in some parts of eastern Europe (because of either limited resources or the size of the country), there is an almost complete lack of microbiological diagnostic samples, which can preclude any meaningful surveillance based on routine samples. In such instances, surveillance strategies in the absence of widespread diagnostic sampling should be assessed.

Existing documents on antimicrobial resistance surveillance in Europe

The problem of surveillance in Europe has been debated extensively in many excellent documents issued by national committees that often assume the value of national guidelines. Among others, the documents issued by Austria [13], Belgium [14], Denmark [15], Finland [16], France [17], Ireland [18], Norway [19], Spain [20] and the UK [11] are of particular relevance. However, a comprehensive document addressing the whole matter of antimicrobial resistance surveillance from a European perspective, as well as reviewing its present status and drafting future perspectives, has been lacking. Consequently, the ESGARS committed itself to producing a wide-acceptance document through a consensus process involving all members of the Study Group.

Following this process, the present document focuses on the detection of bacterial resistance and its reporting to clinicians, public health officers and a wider—and ever-increasing—audience. The leading concept throughout the document is that 'the basis for resistance monitoring is microbiological diagnostics. The prerequisites for resistance monitoring are findings of adequate quality and quantity, which have been properly recorded and correctly evaluated.' [13].

GENERAL CONCEPTS

Surveillance: a definition

Surveillance is a systematic, ongoing data collection, analysis and reporting process that quantitatively monitors temporal trends in the occurrence and distribution of susceptibility and resistance to antimicrobial agents, and provides information useful as a guide to medical practice, including therapeutics and disease control activities.

Its main objectives are:

1. to describe and quantify trends in acquired antimicrobial resistance in important species as a rational basis for establishing empirical therapy, and for evaluating and comparing strategies to counteract the development of resistance in both hospital and community settings;

2. to inform those bodies or institutions capable of identifying effective public health interventions for resistance containment, and of developing specific public health policies for improving patient care;

3. to detect new antimicrobial resistance mechanisms, and to develop continuously updated systems for interpretative reading of antibiotic susceptibility tests;

4. to detect the threat of dissemination of especially unwanted resistance mechanisms or clones, e.g., methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), or extended-spectrum β-lactamases (ESBLs) in hospital wards, and multiresistant *Mycobacterium tuberculosis* or *Strep. pneumoniae* with high-level penicillin resistance in the community; and

5. to serve as an inspiration for standardisation and harmonisation of antimicrobial susceptibility testing among laboratories taking part in the surveillance programme.

To achieve these objectives, both regular surveys (non-directed data retrieval) and ad-hoc studies (targeted at a particular problem) are used in surveillance. Note that ad-hoc studies are often derived from observations obtained during regular surveys.

Resistance rates should be obtained:

- for well-defined microorganisms and antibiotics;
- at regular time periods;
- in well-defined spatial locations, i.e., country, town, hospital, or internal hospital area;
- in precise biological/sociological/clinical compartments, e.g., isolates from bacteraemia, from urine, from osteomyelitis, or from individuals of a certain age, or from immigrants.

Only in these circumstances can comparisons be made, and the differences analysed in such a way that specific action can be taken.

The detection of abnormal bacteriological events (e.g., low levels of acquired resistance or new patterns of resistance) is also an important objective of surveillance. Two specific aspects of

resistance can also be addressed: (1) the spread of resistance genes through the bacterial world; and (2) the consequences of bacterial resistance (e.g., treatment failure, morbidity, mortality, economic impact).

The surveillance targets

Surveillance of resistance trends can be focused theoretically on different targets.

Evolving trends in antibiotic resistance

1. *Description and quantification of biological resistance using epidemiological cut-off values*. Each species exhibits a natural relationship to each antimicrobial. It may possess intrinsically high or low sensitivity to the action of a drug, but the MICs for wild-type organisms are usually distributed over a ten-fold concentration interval, e.g., 0.008–0.064 mg/L (*Strep. pneumoniae* vs. benzylpenicillin), 0.064–0.5 mg/L (*Pseudomonas aeruginosa* vs. ciprofloxacin), or 0.25–2 mg/L (*E. coli* vs. gentamicin), or 4–32 mg/L (*E. coli* vs. nitrofurantoin).

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has utilised this to define 'epidemiological cut-off values' (WT ≤ X mg/L) which determine whether an organism is wild-type in relation to a particular antimicrobial. The epidemiological cut-off value can be used to describe and quantify biological resistance, possibly but not necessarily predicting future resistance trends regardless of clinical sensitivity or resistance. It offers the possibility of early action (measures to counter the development of further resistance) and assessing the results of such action from the modified distribution of susceptibility values, i.e., in the form of a true biological phenomenon. A further advantage of the epidemiological cut-off value is that it is not subject to differences in opinion, which is often the case with more clinically orientated breakpoints. Fig. 1 shows the natural distribution of ciprofloxacin MICs for wild-type *E. coli*, and the epidemiological cut-off value (WT ≤ 0.064 mg/L) as defined by EUCAST.

2. *Description and quantification of clinical resistance using pharmacological/clinical breakpoints*. The use of more clinically orientated breakpoints in surveillance of antimicrobial resistance meets with problems. There is as yet no consensus on how to define clinical/pharmacological
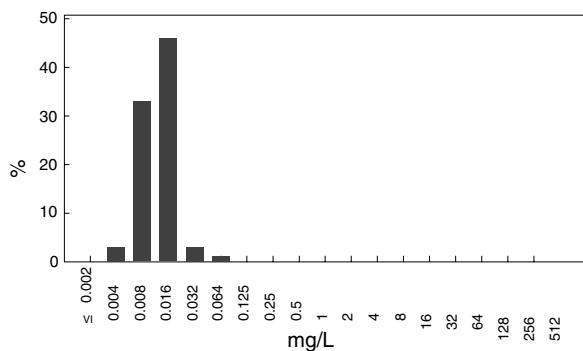
Fig. 1. Ciprofloxacin MIC distribution for wild-type *Escherichia coli*, as obtained by means of the EUCAST web-based program 'Antimicrobial wild-type distributions of microorganisms' (http://www.eucast.org). Data (4416 observations) were pooled from six sources, such as breakpoint committees, surveillance programmes, the pharmaceutical industry and scientific reports. Epidemiological cut-off: WT ≤ 0.064 mg/L.

breakpoints, which means that there are great differences in clinical breakpoints for some drug–organism combinations, and that the resulting surveillance data, based only on breakpoints and on 'S/I/R' interpretative categorisation, may be completely invalid in a broader context.

Thus, the use of clinical breakpoints for antimicrobial surveillance should be accompanied by the collection and display of MIC (or inhibition zone diameter) distributions. Fig. 2 shows that the European countries involved in EARSS [9] pro-
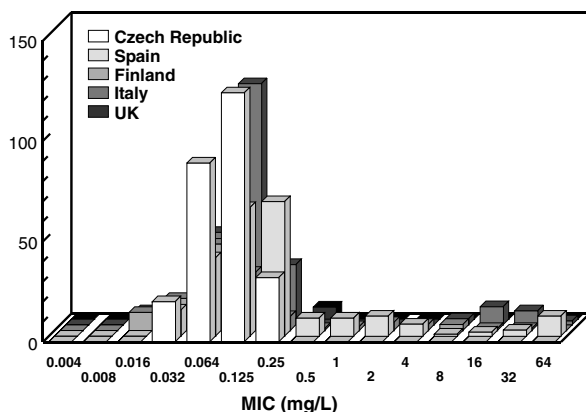

Fig. 2. *Streptococcus pneumoniae* isolates vs. erythromycin from data collected by the EARSS in 2001. The erythromycin S/I breakpoints used by countries involved in the EARSS were S < 0, 5, 1 and 2 mg/L, while the I/R breakpoints were R > 0.5, 1, 2 and 4 mg/L.

duce very comparable raw data [10], which may become extremely difficult to interpret if clinical breakpoints are applied and only the semiquantitative interpretations (S, I and R) are used to describe and quantify resistance.

Evolving trends in the incidence of particular mechanisms of resistance
For this purpose, the surveillance targets could be: (1) genes or gene combinations encoding mechanisms of resistance; (2) the specific products coded for by resistance genes (such as penicillin-binding protein (PBP) 2a, β-lactamases or CAT enzymes); (3) resistance phenotypes that are characteristic of a given mechanism of resistance. In this way, surveillance could be focused on the detection of strains producing PBP2a (detection with monoclonal PBP2a antibodies), or on strains harbouring the *mecA* gene (detection with PCR), or on singling out *S. aureus* isolates with a given susceptibility profile for further analysis with more specific methods.

Evolving trends in the incidence of particular resistant clones
In many cases, it is the spread of a particular resistant clone that influences the prevalence of antibiotic resistance. Surveillance of a particular clone whose presence is known to influence clinical antibiotic resistance can be done in different ways:
1. *Phenotype of the clone.* In some cases, when resistance emerges, there is a single clone (or a small number of clones) that is responsible for resistance. In this simple case, the resistance phenotype (resistance to the new antibiotic) serves, at least for a certain period of time, to identify the clone. In other cases, there is a particular combination of phenotypic resistance traits that may characterise the clone.
2. *Genotypic traits of the clone.* Particular genetic polymorphisms in one or more genes (not necessarily those involved in the resistance mechanism), or in non-coding regions, frequently identify a particular clone. Many techniques are available to identify particular clones, but are often used only in specialised laboratories. Nevertheless, increasing simplification of clonal detection techniques will make this type of surveillance fully accessible in the short term. Detection of a single resistant

isolate in a given patient rarely gives rise to a resistance problem in the short term, but if the strain disseminates among three or more patients, this is a presumptive sign of the epidemicity of a particular clone, and perhaps some kind of action should be taken.

Evolving trends in the incidence of antibiotic-resistant infections

Infections that fail to be cured when treated with given antibiotics (surveillance of infection response–antibiotic pairs) are of particular interest for clinicians. Very little has been done in this very important field of surveillance. Advances in research procedures for quantitative evaluation of the clinical response to antibiotics will be needed.

Surveillance studies

Different types of surveillance studies should fulfil different requirements with regard to data collection and reporting, the expected use of data, and the prerequisites for networking such activities.

Surveillance of evolving qualitative trends in low-level and high-level antibiotic resistance

Data collection requires unequivocal definition of acquired low-level and high-level resistance for each antimicrobial and in each individual species. For that purpose, consensus quantitative epidemiological cut-off values (for detecting low-level resistance) or clinical breakpoints (for high-level resistance) should be established first in order to ensure comparisons in time and space. Quantitative methods of determining MICs (or even properly measured zones of inhibition in disk tests) are therefore required. The methodology and accuracy of these methods requires standardisation among participating laboratories, as well as appropriate quality control strains with known MICs (susceptible strains and strains with known mechanisms of low-level and high-level resistance).

For reporting purposes, results could be expressed in terms of: (1) the percentage of strains belonging to the wild-type distribution, i.e., with an MIC equal to or below the epidemiological cut-off value, showing the proportion of strains devoid of any resistance level; (2) the percentage of strains exhibiting clinical resistance, i.e., above the clinical R breakpoint; (3) the percentage of strains showing high-level resistance, when a

defined breakpoint for high-level resistance exists (e.g., gentamicin high-level resistance in *Ent. faecalis*). Note that the concentrations to be tested in MICs, or the precise procedure for disk-diffusion testing, should be agreed at the start of the study. Eventually, the report should only stratify the percentage of strains in the categories 'wild-type', 'clinical resistance' and 'high-level resistance' categories. A minimal stratification of data should be included, at least according to the few basic patient features available in a typical laboratory information system (LIS), for instance: specimen type, in/outpatient, and type of ward for inpatients.

For studies focused on detecting changes in low-level antibiotic resistance, the main use will be to detect the evolution of organisms from susceptibility to low-level resistance, as a way of predicting the emergence of high-level resistance. If the studies are focused on high-level (clinical) resistance, it will be useful to define the types of infections (caused by particular organisms) in which a number of antibiotics could be of clinical interest.

For the purposes of networking organisation, many of these studies could be based on retrospective as well as prospective (continued) work ('routine surveillance' [21]); in general, participants should represent different types of laboratories (located in hospitals of different sizes, in teaching/non-teaching hospitals, or in facilities serving community patients), which should have a functional LIS.

Surveillance of evolving quantitative trends in low-level and high-level antibiotic resistance

The essential purpose of these studies is the identification of possible changes, within a bacterial species, of the proportion of strains differing in their level of sensitivity at different concentrations of a given antibiotic. Indeed, the purpose is similar to that described above, but with a more detailed examination of the number of strains inhibited by each antibiotic concentration (and not just at the cut-off values or breakpoints).

1. Data collection requires technology to quantify MICs by a pre-established test method with a common range of antibiotic concentrations, and including quality control procedures that should involve known strains with different levels of antibiotic susceptibility. Reports should be given in a population analysis

format in the form of tables and distribution graphs (number or percentage of strains inhibited at each antibiotic concentration).

2. These studies enable the trends in bacterial populations inhibited by different antibiotic concentrations to be followed. Analysis of these trends may provide interesting insights into the frequency of evolution of the members of a given species from full susceptibility to low-level resistance, and hence to clinical resistance. In the case of newly introduced drugs, these studies are of critical importance for determining the presumptive cut-off values (and sometimes the breakpoints) to categorise a given strain as being susceptible, or having intermediate or high-level resistance to the antibiotic.

3. The laboratories involved in networking in this type of activity should have facilities for conducting quantitative susceptibility studies, and for storing these results in LISs.

Surveillance of resistant clones

The aim of these surveillance studies is to detect and monitor the quantitative evolution of a number of resistant bacterial strains (clones) whose dissemination is expected to create health problems in the therapy of infectious diseases. Examples of surveillance for resistant clones are the protocols for detecting and monitoring the following:

- spread of Enterobacteriaceae and Gram-negative non-fermenting bacilli with extended-spectrum β-lactamases, acquired AmpC cephalosporinases or carbapenemases;
- vancomycin-resistant *Ent. faecium* or *S. aureus*;
- very high-level penicillin resistance in *Strep. pneumoniae*;
- methicillin- or linezolid-resistant *S. aureus*;
- isoniazid and rifampicin resistance in *M. tuberculosis*.

Several of these clones are or tend to become multiresistant to antibiotics, since selection of the clone by one particular antibiotic may lead to further enrichment and spread of the bacterial population, which facilitates the acquisition of resistance to new antibiotics.

Surveillance of resistant clones can be carried out simply by detecting strains endowed with a previously established suspicious phenotype, isolated by normal procedures. The surveillance study may be carried out to detect these resistant clones when present in low frequencies in the patient population (or also in suspected carriers). For this purpose, enrichment or selective media (containing a selective antibiotic or even a mixture of selective antibiotics) can be used to detect the clone.

- The data recovery should be done according to a very stringent protocol of strain identification; in general, these strains should be collected prospectively throughout the survey to facilitate further genetic work that will confirm and identify the clonal type. The survey should include the study of a wide range of antibiotics in susceptibility testing. These types of surveys are highly dependent on good stratification of the type of samples and data of patients (or carriers). The importance of local clonal spread means that it is important to know the precise time and compartment in which the strains were obtained (hospital/community, but also secondary compartments, such as type of ward, or intensive care unit (ICU) in hospital; or outside, e.g., day-care centres). Data should be reported as prevalence of the clone (or suspected clone) with respect to the patient frequency parameters in a given compartment, e.g., 100 admitted patients, 1000 patient-days, or 100 000 inhabitants. For comparative purposes, the number of isolates of the same species originating in the same period of time in the same compartment should be provided, but such a procedure cannot be adopted if the clonal search has been performed by applying enrichment or selective culturing. Of particular interest is the handling of the problem of repeated isolates in the same patient (see later).
- This type of surveillance is of great interest in detecting and describing outbreaks, in helping to design control measures, and in assessing the impact of control measures. Such studies will enable an understanding of the features of clonal circulation within and between compartments; the clone is a 'tagged' organism that bears witness to the bridges and gaps that exist between different environments.
- The hospital laboratories that are expected to network with others for the purposes of surveillance of clonal resistant strains should have a special interest (and experience) in the study of nosocomial infections; preferably they should have genetic facilities for clonal identification and/or be linked regularly to central

laboratories performing such tasks. In the case of community microbiological facilities, there should be adequate interest and experience in infectious diseases and epidemiology, and easy contact with larger hospital laboratories of the type mentioned above. In both cases, there should be facilities to collect (mainly prospectively) specific data on patients and their clinical and therapeutic environments.

Surveillance of antibiotic-resistant infections

The term 'antibiotic-resistant infections' can be interpreted in two ways. First, it could refer to documented infections caused by resistant bacterial organisms ('enhanced surveillance' [21]). Second, it could be used to define infections that are treated unsuccessfully ('resistant to') by antibiotics. In the latter case, there may or may not be a resistant causative organism, since antibiotic resistance is not the only feature accounting for treatment failure.

Taking the first meaning of the term, the interest in this surveillance method is to analyse trends in the frequency of documented infections caused by resistant bacteria. In other words, the high prevalence of antibiotic resistance in a given species does not automatically mean a high prevalence of a given type of antibiotic-resistant infection. With the exception of cultures taken from normally sterile sites, a substantial number of isolates in the hospital environment are obtained from infected patients, but are not necessarily the cause of the infections. Some surveillance studies have therefore focused on determining only the resistance frequencies of invasive pathogens (e.g., blood cultures in the EARSS-promoted studies). In this particular case, the number of antibiotic-resistant infections should approach closely the number of antibiotic-resistant bacteria, provided that the breakpoint is appropriate. However, patients with profound neutropenia may have antibiotic-resistant infections caused by antibiotic-susceptible bacteria.

For this type of surveillance protocol, data recovery should basically include the types of infection under surveillance, with as much detail as possible. Going into more specific detail could be critical for good stratification: e.g., resistant bacteraemia associated with intravenous catheters, resistant lower urinary tract infections (UTIs), resistant purulent effusions from chronic otitis, or resistant lower respiratory tract infections in Anthonissen II-type chronic bronchitis. Data on age and gender, the type of underlying condition (e.g., immunosuppression, diabetes), the place and time of previous hospitalisation (or stay in day-care centres), as well as any history of previous antibiotic therapy, are of crucial interest in these studies. To evaluate the medical risk associated with particular resistant clones, a comparison should be made with a group of well-matched patients suffering from the same type of illness, but from whom susceptible bacteria were isolated. Data in the analysis should include the prevalence of resistant strains in the species involved in the infection(s), as well as the number and incidence of events with resistant strains in the exposed population. An example from the WHO 2002 recommendations [21] is 'cases of ciprofloxacin-resistant *E. coli* community-acquired bacteraemia/100 000 inhabitants'.

The use of data obtained by this type of surveillance should serve to modify or refine the therapeutic indications of different antibiotics for a given type of infection, to shape treatment strategies (antibiotic policies), to alert clinicians to possible therapeutic failures, and to provide useful epidemiological indicators for public health services. The institutions that will be interested in networking this type of surveillance study should be able to engage in mostly prospective work with a standardised protocol ('enhanced surveillance' [21]). In general, such institutions should have considerable experience in dealing with infectious diseases.

REQUIRED OR DESIRABLE INFORMATION

General principles of data collection

The principles of data collection and their specific requirements differ according to the type of information desired. Retrospective studies based on LISs can be used to generate general statistics and strain population data, provided that the quality of the results is assured by the use of reference strains, and that standardised definitions are used. Given the current state of medical data computing in most institutions (hospital information system; HIS) and the linking of such systems to LISs, information on resistance in documented infections and

multidrug resistance (MDR) surveillance can be generated only by means of prospective studies. Any effort to extend and improve the HIS–LIS linkage should be encouraged.

Implementation of these recommendations can succeed only if the LIS is well-adapted to this task. LIS integration into the overall HIS may be an advantage, as clinical data can be extracted more easily, but at the same time, the tools needed for laboratory-based surveillance are often not included in these comprehensive HIS solutions. It would be an excellent achievement to promote the integration of laboratory and clinical information systems where laboratory surveillance capabilities are maintained.

Results and data elements to be included: definitions, thesaurus

Both the bacteriological results (primarily bacterial identification and susceptibility test results) and the accessory information that is usually available in LISs (patients, specimens, dates, etc.) should be based ideally on definitions and a thesaurus shared by all laboratories participating in resistance monitoring networks. These definitions and thesaurus are used during the constitution of the laboratory database, and are helpful tools for subsequent data extraction and merging processes in network databases.

Data on the laboratory
Each individual laboratory should be identified by a unique code in the network database.

Patient data
Identity. Patient identity is normally specified in an LIS, but is not included in network databases for confidentiality reasons. However, patient identification is a frequent prerequisite for identifying duplicate isolates. The patient identifier can be the family name, first name and date of birth, the coding system used in the hospital (e.g., rank of hospital admission), or any other unequivocal identifier used in the LIS.
Date of birth and sex. The date of birth can yield the patient's age, which can be extracted for the network database.
Home address. The postal (zip) code of the patient's hometown may be useful to analyse the geographical distribution of resistance, particularly in community-acquired infections.

Patient relationship vis-à-vis the healthcare system at the time of sampling
Ambulatory patients. Patients living in a private home and who are generally referred for sampling to a laboratory (only sampled occasionally at home).
Home care patients. Patients cared for at home by private or public health institutions. These patients usually have a history of recent, sometimes recurrent, hospitalisation and, consequently, may carry nosocomial bacteria. For that reason, they should not be pooled with ambulatory patients.
Patients in long-term care facilities. Patients admitted intermittently or permanently to old people's homes or similar facilities, the nature of which differs appreciably between different countries and even within a given country. These patients, as well as their infections and pathological specimens, present many peculiarities [22], so that mixing their data with those of ambulatory patients is a major mistake when it comes to tracing the epidemiology of antimicrobial resistance [23].
Patients attending day-care clinics. These patients generally have a long history of hospitalisation and must not be pooled with ambulatory patients.
Patients hospitalised in healthcare facilities for more than 24 h. This is the largest group of patients undergoing bacteriological investigation and the most diversified. In order to permit good data stratification, it is necessary to specify the type of facility (teaching hospital, general hospital, private institution, cancer treatment centre) and, particularly, the type of medical activity of the department in which the patient is hospitalised, basically:
- emergency rooms;
- gynaecology and obstetrics;
- paediatrics;
- medicine (as a whole, or specifying internal medicine, infectious diseases, cardiology, pneumology, gastroenterology, nephrology or acute geriatric units);
- haematology and oncology;
- surgery (as a whole, or specifying gastrointestinal surgery, urology, orthopaedics, cardiac and thoracic surgery, neurosurgery);
- ICUs;
- transplantation units;
- psychiatry;
- casualty departments and wards;

- rehabilitation (e.g., after surgery or neurology).

If more detailed lists are used, definitions and possible pooling should be agreed by the different laboratories included in the surveillance network.

Information on dates
For ambulatory patients, patients cared for at home, and patients attending day-care clinics, only the date of sampling is required. For patients hospitalised in healthcare facilities, the date of admission to the facility should also be provided in order to allow calculation of the interval between admission and sampling (see definitions of nosocomial vs. community-acquired infections).

Data on the specimen
Each specimen is identified in the LIS by means of a unique number, which is usually not transferred to network databases for confidentiality reasons. Environmental specimens (surfaces, water, etc.) and quality controls should be classified separately. A distinction should be made between specimens for clinical diagnosis and specimens for screening specific bacteria, since their purpose and implications in resistance surveillance are different, namely:

- Specimens for clinical diagnosis are sampled for the individual diagnosis (positive and aetiological diagnosis) of infectious diseases, i.e., to detect and identify causative bacteria. Bacterial isolates are the main source for resistance monitoring.
- Specimens for screening specific bacteria (often referred to as 'colonisation', 'screening' or 'ecological' specimens) are sampled mostly at fixed intervals of time (for monitoring purposes) or during a specific epidemiological study (cross-sectional survey). The most frequent sampling sites are the rhinopharynx, rectum or faeces, skin and vagina. These specimens are used to detect targeted species (e.g., *S. aureus*, *Salmonella enterica*, *Strep. agalactiae*) or the resistance pattern in a species (MRSA, ESBL, VRE, etc.). Consequently, whether or not screening specimens can be used for resistance monitoring depends on the aspect investigated. Thus, in the case of *S. aureus*, they can be used to survey either the resistance within this species in carriers, or the carriage prevalence of the species in a given group of patients, while in the case of MRSA, they can be used to survey

either associated resistance traits (resistance of MRSA to gentamicin, glycopeptides, etc.), or the prevalence of MRSA carriage in a given group of patients (e.g., in an ICU). They cannot, however, be used to survey the rate of MRSA among *S. aureus* isolates.

Specimens collected for screening purposes should be classified separately by the sampling site and the bacteria targeted.

It is worth considering that the resistance frequencies generated in phase II and III clinical trials are in most cases much lower than those generated when clinical isolates are surveyed from diagnostic laboratories. This is possibly because all patients are (or should be) sampled in clinical trials, while the samples sent to diagnostic laboratories in many countries come from patients who may be considered as 'abnormal', e.g., because they fail to respond to therapy, relapse after the end of treatment, or have more severe infections than normal. This is especially the case for outpatients and in healthcare systems in which those submitting specimens consider the cost of laboratory examinations to be important. For these reasons, it may be advantageous to initiate surveillance studies in which all patients with defined types of infections are sampled, i.e., to mimic for surveillance purposes the system implemented in phase II and III trials.

Minimum thesaurus for clinical specimens
The following minimum thesaurus is intentionally limited to the most frequent specimens, the interpretation of which is relatively unequivocal and allows good stratification of data. The medical significance of other types of specimens may be more controversial (e.g., wound swabs or fluid taken from drains). By classifying such specimens in the category 'others', the informative value and specificity of the statistics are improved, although the amount of detail is obviously reduced. The category 'others' can also be subdivided, as required, into broad subgroups. Specimens of fairly unequivocal interpretation include the following:

- Blood culture.
- Urine (applies strictly to urine itself, thus excluding catheter equipment). The circumstances of urine sampling, e.g., patients with indwelling catheters, should be specified, if available:
  - intravascular devices;

- peripheral catheters;
- central catheters;
- perfusion chambers.
- Serous fluid obtained by puncture:
  - cerebrospinal fluid;
  - pleural fluid;
  - joint fluid;
  - peritoneal fluid;
  - pus taken from closed, normally sterile anatomical sites by puncture or surgery (such specimens with high informative value are distinguished from those taken from drains or swabs, whose significance is uncertain).
- Protected or distal respiratory samples:
  - bronchial brush;
  - protected distal specimen;
  - bronchoalveolar lavage.
- Unprotected respiratory samples (distinct from the above, since their interpretation is more controversial).
- Bronchial aspiration;
- Sputum.
- Stools.
- Urethral and cervical/vaginal samples.
- Bile.

Indicators, denominators and data stratification

To generate relevant indicators, bacterial resistance data should be reported using adequate denominators and stratification. Denominators are adapted to the type of question addressed by the surveillance. The range of complexity levels is large, from low complexity, e.g., frequencies of resistance in a given species, to very high complexity, e.g., the proportion of a resistant organism in a given type of infection and a given epidemiological setting (Table 1). Several parameters are used for generating these indicators, some related to medical activity and some to the patient.

Medical activity parameters
Some useful parameters describe the medical activity in the exposed population or in the hospital where the monitoring takes place:
For hospital laboratories:
- number of beds, relevant for any ward or hospital—acute care (e.g., medicine, surgery, obstetrics), intensive care, rehabilitation and long-term care;

Table 1. Indicators used for resistance surveillance

Simple	Percentage of resistance in a given species, e.g., percentage of MRSA among *S. aureus* in general
Medium	Percentage of resistance in a given species isolated from a given type of sample, e.g., percentage of ciprofloxacin-resistant *E. coli* isolated from urine in acute care
Complex	Percentage of resistance in a given type of infection, e.g., percentage of ciprofloxacin-resistant *E. coli* isolated from community-acquired UTI
Very complex	Incidence of a given type of infection caused by particular resistant bacteria in a specified epidemiological setting, e.g., incidence of MRSA bacteraemia acquired in ICU/1000 hospital days

- number of direct admissions (i.e., excluding internal ward tranfers) lasting >24 h (relevant only for acute care wards and ICUs);
- number of hospitalisation days (relevant for any ward or hospital);
- number of samples (relevant for any ward or hospital).
For laboratories working for general practitioners:
- numbers of practitioners referring bacteriological samples;
- size of the population covered;
- number of samples.
Such parameters can be used for several purposes:
- as likelihood controls, e.g., expected number of strains for a given bacterial species in a given period of time;
- for stratification of results, e.g., comparative MRSA rates in a set of hospitals, according to the number of beds or admissions;
- as denominators for statistics, e.g., incidence of community-acquired pneumonia caused by penicillin-resistant pneumococci/100 admissions in acute care, or incidence of MRSA/1000 bed-days;
- to make extrapolations from the results obtained in the monitored population or set of hospitals (e.g., number of pneumococci or MRSA isolated annually in the whole country) using regional or national health statistics (e.g., total number of inhabitants and distribution by age in the case of community-acquired infections, or total number of beds, hospitalisations/year, and hospitalisation days/year in the case of hospital-acquired infections).

Parameters concerning the patient
Parameters used for community-acquired infections. Some parameters correlating with the rates of resistance in community-acquired infections,

e.g., UTIs, pneumococcal respiratory tract infections, tuberculosis, and probably other types of infections, are particularly relevant for resistance monitoring.

- *Urinary tract infections.* Several parameters influence the respective distributions of *E. coli* (the most prevalent bacterial species, which is usually sensitive to many antibiotics) and other less frequent—and generally more resistant—species (e.g., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *P. aeruginosa*), including a previous history of UTI, antibiotic therapy or hospitalisation. The same applies to the prevalence of *E. coli* strains resistant to the main antibiotics used to treat lower UTIs [17]. These parameters are of particular importance when analysing the results of monitoring based on samples from ambulatory patients, since the probability of such patients having a sample taken is much higher in the case of relapse of UTI, or in the case of risk factors such as recent indwelling catheters or surgery, than in the case of a first episode of UTI with no risk factors. As a consequence, generating rates of resistance based on indiscriminate samples from all ambulatory patients would lead to an overestimate of the rates of resistance and overuse of the most recent drugs.
- *Pneumococcal infections.* Rates of resistance to β-lactams or macrolides, as well as multidrug resistance in *Strep. pneumoniae*, are known to be associated with several parameters, namely age of the patient, recent treatment with β-lactams, macrolides or co-trimoxazole, and recurrent infections [24–29]. These parameters are helpful for analysing changes in the prevalence of *Strep. pneumoniae* resistance correctly, and for monitoring the impact of measures aimed at reducing this prevalence (e.g., a reduction in the volume of antibiotic prescriptions in children, or better control of pneumococcal transmission in day-care centres).
- *Tuberculosis.* In this case, the key parameter associated with resistance is a history of treatment with anti-tubercular drugs. This parameter makes it possible to distinguish between the rate of primary resistance (strains isolated from patients with no history of treatment, or 'new cases') and that of secondary, or acquired, resistance (strains isolated from patients with a history of treatment). Both rates should be assessed separately [30]. Since these rates differ

significantly from one country to another according to the quality of patient management, it is also recommended that resistance statistics should be presented according to patient domicile, nationality or, even better, country of origin.

Parameters used to define the community-acquired or nosocomial nature of infections in healthcare facilities. Resistance rates are higher among bacteria causing nosocomial infections than among those causing community-acquired infections for two main reasons, namely: (1) a higher proportion of naturally resistant species among bacteria of nosocomial origin (e.g., *P. aeruginosa*); and (2) a higher proportion, within a given species, of isolates with acquired resistance traits that cause nosocomial infections [31]. This is why it is so important, in the case of hospitalised patients, to distinguish between community-acquired bacteria and bacteria of nosocomial origin [21].

However, it must be borne in mind that several multiresistant organisms have appeared recently outside the hospital, particularly non-multiresistant MRSA and CTX-M β-lactamase-producing *E. coli* [32,33].

Nosocomial infections are defined as infections not present (and not incubating) at the time of admission, and which are acquired in a healthcare facility. Except for infections with a known incubation period (such as legionellosis), the incubation period is considered generally to last for 48 h. Consequently, when the interval between admission to the hospital and onset of infection is >48 h, the infection is considered to be nosocomial. Community-acquired infections are defined as infections not acquired in a healthcare institution.

In practice, a bacterial strain is presumed to be of nosocomial origin if it is isolated from a patient who has been hospitalised for at least 48 h or transferred from another healthcare facility (the latter cases should be kept separate). Conversely, a bacterial strain can be presumed to be community-acquired if isolated from an ambulatory patient or from a patient hospitalised for <48 h who was not transferred from another healthcare facility. A more precise definition, which takes account of the patient history, is helpful to ascertain the community-acquired or nosocomial nature of infection in particular cases, namely:

- Infections that are essentially of nosocomial origin (e.g., MRSA), but which can be considered wrongly as community-acquired if there was a previous unrecorded hospital stay [32].
- Infections that are essentially, or almost exclusively, acquired in the community (e.g., typhoid fever or listeriosis), but which can be considered wrongly as nosocomial if the diagnosis after admission to the hospital is delayed (e.g., blood cultures taken after the second day of the hospital stay).
- Any infection diagnosed within the first 48 h after readmission, which could be either community-acquired or linked to a previous hospital stay. Patient characteristics and the history or nature of the infection may suggest either community-acquired infection (e.g., *S. aureus* whitlow) or hospital-acquired infection (e.g., *S. aureus* surgical wound infection).

Parameters used for monitoring multidrug-resistant bacteria (MDRB) in healthcare facilities (for countries with high resistance rates). MDRB are those showing combined resistance to at least two major drugs used in therapy. Indicators are based on the following parameters:

- Numerator: number of MDRB isolated from specimens for clinical diagnosis, in patients hospitalised throughout the period, excluding duplicate isolates.
- Denominator:
  - number of strains of the same species isolated in the same conditions (will generate rates of resistance in the species);
  - number of direct admissions, i.e., excluding ward transfers, and number of days of hospitalisation throughout the period (will generate rates of incidence and incidence density).

It is also recommended:

- To calculate for each case the interval between the date of hospitalisation and the date of sampling, which gives an idea of the time taken to acquire a MDRB in the facility.
- To define the ratio of acquired to imported cases, which reflects the efficacy of the MDRB control programme within the facility.
- To stratify MDRB data according to:
  - the main types of specimen: blood culture, surgical wound and protected respiratory specimens (which are probably linked to severe infection), and other samples (which may reflect colonisation);
  - the main types of medical activity (e.g., acute care, intensive care, rehabilitation, long-term care).

THE ISSUE OF DUPLICATE ISOLATES

In any type of surveillance, data collection should include each distinct event in order to ensure sensitivity, but should include it once only in order to ensure specificity. For surveillance of bacterial resistance, each distinct event refers to a distinct bacterial isolate. Every effort should be made to exclude redundant or duplicate isolates from analysis. In human medicine, and especially in healthcare facilities, microbiologists are confronted every day with the practical problem of identifying duplicate isolates, since, for many patients, separate specimens can yield bacteria of the same species.

Justification for not counting duplicate isolates

The high proportion of duplicate isolates, and their impact on published resistance rates, has been demonstrated repeatedly, particularly in the hospital setting [17,34–37]. Indeed, when duplicate isolates are included, the rate of resistance tends to be higher, particularly for species in which drug resistance is frequent (*S. aureus*, *P. aeruginosa*, etc.), because resistant strains have a higher probability of not being cleared by antibiotic therapy, and will be isolated several times (see below; Tables 2 and 3).

For these reasons, several systems for identifying duplicate isolates have been proposed, relying upon: (1) isolation rank; (2) characteristics of the strains (mostly their antibiogram pattern); or (3) more complex principles. Whatever the system used, strains must be identified to the species level, and the duration of the reference period must be defined (generally the period covered by the surveillance).

In practice, the question of duplicate isolates arises mainly in human medicine, especially in healthcare facilities. Indeed, when resistance monitoring concerns the community or animals, repeated specimens from a given individual are unusual, and exclusion of duplicate isolates makes little difference to resistance frequencies. In contrast, repeated screening of patients colonised by resistant organisms (e.g., MRSA or VRE) adds greatly to the likelihood of duplicate

Table 2. Effect of criteria used for excluding duplicate isolates from susceptibility reports (basis = rank of isolation [36])

	Proportion of resistant isolates			
	Klebsiella spp.			
	gentamicin			
Isolates	screen incl. (*n* = 6800)	screen excl. (*n* = 5800)	*Enterococcus* spp. vancomycin (*n* = 12 000)	*E. coli* amoxycillin (*n* = 31 000)
All	31	21	13	45
First isolate, based on period of:				
5 days	26	17	12	45
30 days	19	13	10	44
365 days	15	11	10	44

Table 3. Effect of duplicate isolate exclusion on resistance rates (basis = isolate susceptibility pattern/antibiogram)

			% resistance	
Species (no. of isolates)	Duplicate isolates (%)	Antibiotic considered	Duplicate included	Duplicate excluded
E. coli (5253)	20	Nalidixic acid	13	11
P. aeruginosa (2154)	43	Ciprofloxacin	51	46
S. aureus (3684)	48	Oxacillin	36	29

Data from [17] and Groupe Hospitalier Pitié-Saltpétrière, Paris, France (unpublished data).

isolates, and affects susceptibility reports substantially (besides being in itself a strong bias towards resistance, since screening usually reports only resistant isolates [36]).

It is important to bear in mind that there is no single 'correct' way to eliminate duplicates, and that each criterion may fit different data applications and/or provide complementary views of the data [38]. Moreover, elimination of duplicates might mask trends in emerging resistance, and thus it is advisable that all 'filtered' reports be accompanied by a careful analysis of all—i.e., unfiltered—susceptibility data included in the database.

Whatever the system used for their definition, duplicate isolates must not be deleted from the LIS, since every bacteriological event is important from the patient's point of view. Duplicate isolates can be flagged in each patient chart of the LIS as 'duplicates', and then excluded only at the time of data analysis or data extraction. Indeed, in some situations (e.g., chronic infections), it might be useful to estimate how long a patient had been carrying resistant bacteria. This indicator of 'persistence of resistant bacteria' is often used in nosocomial infection surveillance, and is a further justification for keeping all bacteriological events stored in the LIS for all patients.

Besides considering which of the different criteria best fits an individual database or specific reporting needs, even greater care should be taken when comparing results where these criteria may be different or of uncertain application.

Definitions of duplicate isolates

Definition based on isolation rank (time criterion)
By this criterion, all but the first isolate of a particular species isolated from a single patient during the period covered by the surveillance are excluded from the analysis. Different filter periods obviously result in different numbers of isolates being included in the database, with more extended time periods resulting in lower resistance frequencies, as exemplified in Table 2.

Since the susceptibility frequencies obtained with the various patient- and episode-based methods do not differ very much [37], calculations that include only the first isolate of a particular species recovered from each patient during a given time interval represent an increasingly popular way of eliminating duplicates. This method is simple, reproducible and unequivocal, and can be applied by any computer, provided that there is a unique patient identification number. Therefore, automation of this process should be simple, even in the absence of sophisticated software.

Isolation rank based on the 'first isolate/patient' has been chosen by the National Committee for Clinical Laboratory Standards (NCCLS) for reporting antimicrobial susceptibility test data, 'with the primary aim of guiding clinicians in the selection of empirical therapy' [38]. Advantageous though this criterion may be, it should not be considered as the standard in all instances in which susceptibility data are reported and the problem of duplicate isolates is encountered. In particular, this procedure cannot detect selection of resistance that occurs within the observation period, thereby giving an overly optimistic view of each patient's pathological course and the percentage of susceptible strains [39].

Definition based on susceptibility pattern
With this method, all but the first isolate of a given species, obtained from the same patient during the period covered by the surveillance, and sharing the same—or a very similar—susceptibility pattern (antibiotype), are excluded from the analysis. A non-redundant ('original')

isolate is a species–antibiotype combination that has not been included previously in the database for a particular patient. A duplicate isolate is a species–antibiotype combination that does not differ—within the limits set below—from an isolate already included in the database for a particular patient. Elimination occurs regardless of the time between isolates. The effect on resistance rates of antibiogram-based duplicate isolate exclusion is shown in Table 3.

Two strains isolated from the same patient are considered different if their antibiotypes show at least one major difference (S → R, R → S). Minor differences of either the I → R or the R → I type may reflect only the variable phenotypic expression of a given resistance mechanism, or even a methodological problem (e.g., inoculum size). Conversely, minor differences of either the S → I or the I → S type may reflect real differences between strains. Examples of the main differences to be taken into account for identifying duplicate isolates are listed in Table 4.

To avoid bias, susceptibility tests should ideally include marker antibiotics that can unequivocally reveal major differences (e.g., impaired susceptibility to classical quinolones—such as nalidixic acid—that clearly points to reduced activity of the

newer fluoroquinolones). These markers must be selected specifically for each microorganism. When such markers cannot be used, the number and nature of the minor differences used to identify duplicate isolates must be specified accurately in the surveillance methodology.

Choosing the susceptibility pattern as the criterion for eliminating duplicates can reveal selection of resistance occurring within the observation period, giving a more realistic view of each patient's pathological course than when using the isolation rank. However, it is less objective and reproducible, since it requires experienced input and special care to avoid methodological errors in routine susceptibility testing by busy, understaffed departments [39].

Advantages and disadvantages of using antibiogram pattern or isolation rank for identifying duplicate isolates

The rates of resistance generated using antibiogram pattern and isolation rank seem to be similar in many cases (Table 5). However, the definition based on isolation rank leads to an underestimate of the number of infectious events, at least in the hospital setting, and for species (e.g., MRSA) causing nosocomial colonisation or infections (Table 6). In addition, isolation rank does not account for selection of resistant mutants during therapy (e.g., *S. aureus*, *P. aeruginosa* or *Ent. cloacae*), or for successive colonisation or infection with different strains of the same species. In comparison with the antibiogram pattern method, the number of events can be underestimated by 10–40% according to the species (Table 7), thus limiting the use of these data for assessing actual frequencies of resistant isolates, which is one of the important tasks of surveillance [21]. Thus, while the definition based on isolation rank represents a useful indication for appropriate empirical

Table 4. Major ('M', namely S → R and R → S) and minor ('m', namely S → I and I → S) differences in antibiotic susceptibility patterns that can be used to identify duplicate isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*

	Difference in pattern
E. coli	
Ampicillin–amoxycillin	M
Ticarcillin	M or m
Third-generation cephalosporins (ESBL production)	M or m
Gentamicin, tobramycin, netilmicin, amikacin	M or m
Tetracyclines	M or m
Co-trimoxazole	M
First-generation quinolones	M
P. aeruginosa	
Ticarcillin	M or m
Ceftazidime	M or m
Imipenem	M or m
Tobramycin, amikacin	M or m
Ciprofloxacin	M
S. aureus	
Penicillin (penicillinase production)	M
Oxacillin (specific tests)	M
Kanamycin (tobramycin, amikacin)	M
Gentamicin	M
Erythromycin	M
Tetracyclines	M
Rifampicin	M or m
Fusidic acid	M or m
Strep. pneumoniae	
Penicillin (MIC)	M or m
Erythromycin	M
Chloramphenicol	M
Tetracyclines	M

Table 5. Duplicate isolates: rates of resistance generated using isolation rank or susceptibility pattern

Species/antibiotic (no. of isolates)	% resistance		
		Duplicates excluded by	
	All	Rank	Pattern
Strep. pneumoniae/penicillin G (173)	47	46	46
S. aureus/oxacillin (1094)	37	27	28
Ent. cloacae/cefotaxime (583)	31	26	27
P. aeruginosa/imipenem (1069)	20	15	19

Groupe Hospitalier Pitié-Salpétrière, Paris, France (unpublished data).

Table 6. Identification of duplicate isolates by isolation rank or susceptibility pattern

	Criteria	Automation	Adequate for	
			% R	Event counting
Rank	One	Easy	+	−[a]
Pattern	Multiple	Possible	+	+

[a]Events that cannot be counted: (1) selection of resistant mutant in the same patient (e.g., cip^s → cpi^r MRSA; imp^s → imp^r *P. aeruginosa*; (2) successive isolation of different strains in the same patient, e.g., community-acquired MSSA followed by hospital-acquired MRSA.

Table 7. Effect of excluding duplicate isolates by isolation rank or antibiogram pattern, and the resulting underestimation of events obtained with the former method

Species (no. of isolates)	Resulting events when duplicates are excluded by		Calculated underestimation of events (rank vs. pattern)
	Rank	Pattern	
Strep. pneumoniae (173)	122	123	0%
S. aureus (1094)	526	620	18%
Ent. cloacae (583)	329	368	12%
P. aeruginosa (1069)	400	560	40%

therapy (based on the resistance pattern of the first isolate), this information should be compared with the susceptibility patterns of subsequent isolates from the same patient during a hospital stay.

Definitions based on other criteria

Several other systems have been evaluated for their ability to identify duplicate isolates. Some rely on analysis of each patient chart: (1) counting only the most susceptible isolate; (2) counting only the most resistant isolate; (3) calculating a patient's own 'rate of susceptibility'. Others rely on considering successive periods of time for each patient (e.g., periods of 7 days, 30 days, etc.) separately, and then counting only the first isolate for each of these periods [38]. Such systems are neither simple nor more accurate than others for counting distinct events, and therefore are not recommended.

SPECIES INCLUDED OR POOLED IN STATISTICS

Selecting the bacterial species to be monitored

The choice of which bacterial species to monitor depends on many criteria. It would seem reasonable to limit monitoring activity to the main species of medical interest, except when surveillance has to achieve very specific targets. These species are, first, those isolated most frequently (e.g., *E. coli*, *S. aureus*) and, second, those which are encountered less commonly, but which are responsible for important contagious diseases (e.g., *Neisseria meningitidis*, *Shigella* spp.). When surveillance focuses on specific well-documented infections, it is important to take into account all species (even the less frequent ones) involved in the infection monitored.

It should be remembered that 'surveillance' isolates are sometimes obtained from ad-hoc cultures that are taken for the purpose of determining if a patient is harbouring a particular organism, and not from cultures that are taken as part of the routine clinical evaluation of a patient's illness. These isolates introduce a frequent bias, and should not be included in the report. Bacterial species monitored normally in ambulatory patients and in healthcare facilities are listed in Tables 8 and 9, respectively.

Surveillance and speciation

Lack of careful identification to the species level may undermine the results of antibiotic resistance surveillance programmes, and constitutes one of the major obstacles to automated recovery of data from clinical laboratories for surveillance purposes. Surveillance at the genus level is frequently meaningless. Even for 'groups of species' such as viridans streptococci or coagulase-negative staphylococci, the results may be difficult to interpret. Isolates should be identified to the species level. Pathovars may be warranted in specific surveys, e.g., when there are noteworthy differences in the prevalence of resistance according to pathovars, or when changes in the distribution of the different pathovars within a given species (e.g., *Salmonella enterica*) need to be quantified.

Table 8. Bacterial species to be monitored in ambulatory patients

Escherichia coli
Proteus mirabilis
Salmonella enterica (including Typhi, Paratyphi, Typhimurium and Enteritidis)
Shigella spp.
Klebsiella pneumoniae and *K. oxytoca*
Haemophilus influenzae
Campylobacter jejuni/coli
Neisseria meningitidis and *N. gonorrhoeae*
Moraxella catarrhalis
Staphylococcus aureus
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus agalactiae
Enterococcus faecium and *Ent. faecalis*
Mycobacterium tuberculosis complex

Table 9. Bacterial species to be monitored in healthcare facilities

Escherichia coli
Proteus mirabilis
Salmonella enterica (including Typhi, Paratyphi, Typhimurium and Enteritidis)
Shigella spp.
Klebsiella pneumoniae and *K. oxytoca*
Enterobacter cloacae and *Enterobacter aerogenes*
Serratia marcescens
Citrobacter freundii
Morganella morganii
Pseudomonas aeruginosa
Acinetobacter baumannii
Stenotrophomonas maltophilia
Burkholderia cepacia
Haemophilus influenzae
Campylobacter coli
Campylobacter jejuni
Neisseria meningitidis and *N. gonorrhoeae*
Moraxella catarrhalis
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus haemolyticus
Streptococcus pneumoniae
Streptococcus pyogenes
Enterococcus faecium and *Ent. faecalis*
Bacteroides fragilis
Clostridium difficile
Mycobacterium tuberculosis complex

Accurate species identification and characterisation of the mechanism of resistance to certain antimicrobial agents may be relevant from the clinical point of view. In some instances, clinical failures may be the result of reporting an impossible (or very rare) species or phenotype, wrong species identification, or underestimation of a given resistance mechanism. Another consequence of such mistakes is the nosocomial transmission and spread of certain resistance genes to more virulent organisms (e.g., *vanA* to *S. aureus*) as a result of a lack of infection control policies. A well-known example is the case of *Enterococcus* spp., and the most frequent mistakes originating

in the clinical microbiology laboratory are listed in Table 10.

When may data for small numbers of isolates be pooled? Data from small numbers of isolates may have limited practical or scientific value. To improve the significance of the data, the denominator may be enlarged by: (1) grouping species together; (2) pooling resistance data from different clinical or environmental specimen types; (3) pooling data from several observation periods or from several institutions within a geographical area; and (4) extending the period of observation. A report must state clearly if and how data have been pooled.

No general recommendation on the method to be used for achieving an adequate sample size can be given. However, it should be taken into account that a long period of data collection may mask temporal trends associated with possible changes in the prevalence of resistance. Hence, extending the observation period over an (arbitrarily chosen) time of >1 year may not be advisable, and grouping of species or pooling of data from different healthcare facilities should be considered. When grouping species or genera together, the internal comparability with respect to resistance mechanisms and the probability of their emergence must always be considered.

Alerting systems and surveillance of rare resistance phenotypes

Reporting of unusual but potentially important resistant organisms is one of the most important goals of antimicrobial resistance surveillance. The

Table 10. Frequent mistakes made when reporting antimicrobial susceptibilities of *Enterococcus* spp.

Wrong report	Why?	Correct report	Consequences
Ent. faecalis Amp^R (at 10^5 inoculum)	Misidentification of *Ent. faecium*	*Ent. faecium* Amp^R	Probably none
Ent. faecalis Amp^S (if Bla$^+$)	Presence of β-lactamase (not described in Europe)	*Ent. faecalis* Bla$^+$	Therapeutic failure! Important consequences if patient has endocarditis
Ent. faecalis Q-D^S	Inconsistent phenotype	*Ent. faecalis* Q-D^R	Therapeutic failure!
Ent. durans Van^R	Misidentification of *E. faecium* (some *E. faecium* are asaccharolytic)	*Ent. faecium* Van^R	Under-reporting of the isolate
VanC-carrying species not tested for glycopeptide susceptibility	Presence of *vanA* or *vanB* besides *vanC*	*Ent. gallinarum* *vanA* or *vanB*	Important consequences if patient has endocarditis 1. Nosocomial transmission 2. Spread to other Gram-positive bacteria
Vancomycin-susceptible enterococcus (VSE)	Undetected presence of *vanA*, *vanB*, *vanD*, *vanE*, or *vanG*	Vancomycin-resistant enterococcus (VRE)	Therapeutic failure! Important consequences if patient has endocarditis 1. Nosocomial transmission 2. Spread to other Gram-positive bacteria

Q-D, quinupristin–dalfopristin.

availability and awareness of this list in clinical microbiology laboratories should be considered as part of the desirable quality control (and good laboratory practice) of these facilities.

Such a list of 'wanted' resistant organisms/ resistance mechanisms, to be disseminated among laboratories, should include, for instance:

- high-level penicillin resistance in *Neisseria meningitidis*;
- penicillin resistance in *Strep. pyogenes*;
- very-high-level penicillin resistance (MIC > 8 mg/L) in *Strep. pneumoniae*;
- high-level vancomycin resistance in *S. aureus*;
- presence in Enterobacteriaceae of extended-spectrum β-lactamases not inhibited by current inhibitors;
- presence in Enterobacteriaceae of carbapene-mases;
- β-lactamase inhibitor resistance in *Haemophilus influenzae*;
- high-level penicillin resistance in *Ent. faecalis*;
- gentamicin or ampicillin resistance in *Listeria monocytogenes*;
- cefotaxime or fluoroquinolone resistance in *Salmonella enterica* Typhi or Paratyphi.

Alert reporting can be established as a two-level system. The first (immediate) alert does not require full understanding of the mechanism involved, and should be considered as precautionary reporting, to be modified if the observation is not confirmed by more in-depth investigation. The alerting laboratory should be capable of keeping the resistant strain viable and giving it priority in its research activities. Furthermore, the strain should be made available to specialised research groups if it is of interest, for a better understanding of its features and/or for controlling its spread.

ANTIBIOTICS LISTED OR POOLED IN STATISTICS

Surveillance antibiotics

Antibiotics to be included in surveillance studies should be selected in such a way as to ensure the highest sensitivity in detecting the possible presence of a particular antibiotic resistance mechanism. This mechanism can be inferred for each given species from the MIC of a given antibiotic or group of antibiotics ('surveillance antibiotics'). In a second step, it should be possible to infer,

without further testing, which other antibiotics are probably inactivated by the same resistance mechanism. The rules to be applied in this process constitute the basis of the 'interpretive reading of susceptibility testing procedures' [40], and should be applied whenever a new antimicrobial agent is introduced in the test panel. So-called 'expert systems' embedded in the software of automatic susceptibility testing devices are based on this inductive approach.

In summary, surveillance studies should focus on the activities of a relatively limited number of sentinel antimicrobials, but information resulting from these activities should include a much broader list of resistances which are likely to occur with a much larger group of drugs. This broader list is the one to be proposed to and used by clinicians and epidemiologists, who do not necessarily need to be aware of all the details regarding the method of inference and its application. Unfortunately, surveillance studies often only include data regarding the antibiotics examined in routine susceptibility tests, and therefore available in LISs. A frequent problem is whether it is possible to pool the data obtained with similar but different antibiotics.

Tables 11–23 suggest appropriate surveillance antibiotics for different microorganisms, as well as the antibiotics to which each surveillance antibiotic might be considered equivalent when a particular mechanism of resistance is to be detected (inference of which may differ between different groups of microorganisms). The list of possible resistance mechanisms is not exhaustive, and resistance results often from a combination of more than one mechanism in the same microorganism.

Expression of resistance and resistance patterns

For some of the bacterium–antibiotic combinations considered in Tables 11–23, the use of semiquantitative data, i.e., reporting only the interpretative categories and not the MIC values, may fail to reveal acquired resistance (i.e., when strain susceptibility is not decreased to such an extent as to allow classification as I or R). When the underlying resistance mechanism is known and the resistance pattern is well-defined, it is possible to often detect abnormal behaviour of a strain by observing its altered susceptibility to one or more antibiotic(s) of the same family to

Table 11. β-Lactams suggested as surveillance antibiotics for Enterobacteriaceae with low-level chromosomal constitutive or non-constitutive expression of Class C β-lactamases (e.g., *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Proteus mirabilis*)

Surveillance antibiotic	Predictor for:	Main resistance mechanisms
Ampicillin	Amoxycillin	Penicillinase AmpC hyperproduction
Amoxycillin–clavulanate	Ampicillin–sulbactam	Penicillinase hyperproduction AmpC hyperproduction Oxacillinase production Inhibitor-R β-lactamase
Cefazolin	Cephalothin Cefaclor	Penicillinase hyperproduction AmpC hyperproduction
Cefotaxime	Ceftriaxone Ceftazidime Aztreonam Cefepime	ESBL AmpC hyperproduction
Ceftazidime	Ceftriaxone Cefotaxime Aztreonam Cefepime	ESBL AmpC hyperproduction
Cefoxitin	Cefotetan	AmpC hyperproduction
Imipenem	Meropenem	AmpC hyperproduction plus reduced permeability Carbapenemase

ESBL, extended-spectrum β-lactamase.

Table 12. β-Lactams suggested as surveillance antibiotics for Enterobacteriaceae producing constitutive chromosomal Class A β-lactamase (e.g., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Citrobacter koseri*)

Surveillance antibiotic	Predictor for:	Main resistance mechanisms
Piperacillin		Penicillinase ESBL K-OXY β-lactamase hyperproduction (*K. oxytoca*)
Amoxycillin–clavulanate	Ampicillin–sulbactam	Penicillinase hyperproduction Inhibitor-R β-lactamase
Cefotaxime	Ceftriaxone Ceftazidime Aztreonam Cefepime	ESBL K-OXY β-lactamase hyperproduction (*K. oxytoca*)
Ceftazidime	Ceftriaxone Cefotaxime Aztreonam Cefepime	ESBL
Cefoxitin	Cefotetan	AmpC hyperproduction Reduced permeability
Aztreonam	Ceftriaxone Cefotaxime Ceftazidime Cefepime	ESBL K-OXY β-lactamase hyperproduction (*K. oxytoca*)
Imipenem	Meropenem	AmpC hyperproduction plus reduced permeability Carbapenemase

ESBL, extended-spectrum β-lactamase.

Table 13. β-Lactams suggested as surveillance antibiotics for Enterobacteriaceae producing inducible chromosomal Class C β-lactamase (e.g., *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, *Hafnia alvei*, *Morganella morganii*, *Providencia* spp.)

Surveillance antibiotic	Predictor for:	Main resistance mechanisms
Ticarcillin	Piperacillin	Penicillinase AmpC hyperproduction ESBL
Cefotaxime	Ceftriaxone Ceftazidime Aztreonam Cefepime	AmpC hyperproduction ESBL
Ceftazidime	Ceftriaxone Cefotaxime Aztreonam Cefepime	AmpC hyperproduction derepression ESBL
Cefepime	Ceftriaxone Cefotaxime Ceftazidime Aztreonam	AmpC hyperproduction plus reduced permeability ESBL
Imipenem	Meropenem	AmpC hyperproduction plus reduced permeability Carbapenemase

ESBL, extended-spectrum β-lactamase.

Table 14. β-Lactams suggested as surveillance antibiotics for Enterobacteriaceae producing inducible chromosomal Class A β-lactamase (e.g., *Proteus vulgaris* and *Proteus penneri*)

Surveillance antibiotic	Predictor for:	Main resistance mechanisms
Piperacillin		Penicillinase Class A derepressed β-lactamase ESBL
Amoxycillin–clavulanate	Ampicillin–sulbactam	Penicillinase hyperproduction Inhibitor-R β-lactamase
Cefotaxime	Ceftriaxone Ceftazidime Aztreonam Cefepime	Class A derepressed β-lactamase ESBL
Ceftazidime	Ceftriaxone Cefotaxime Aztreonam Cefepime	Class A derepressed β-lactamase ESBL
Cefoxitin	Cefotetan	Acquired AmpC-type cephalosporinase Reduced permeability

ESBL, extended-spectrum β-lactamase.

Table 15. Non-β-lactams suggested as surveillance antibiotics for all Enterobacteriaceae

Surveillance antibiotic	Predictor for:	Main resistance mechanisms
Gentamicin		Aminoglycoside-modifying enzymes
Netilmicin[a]	Gentamicin	Reduced permeability
Tobramycin[a]	Gentamicin	
Amikacin	Tobramycin Netilmicin	
Nalidixic acid	Quinolones	Topoisomerase mutation
Ciprofloxacin	Quinolones Fluoroquinolones	Topoisomerase mutation
Co-trimoxazole		Changes in enzyme target
Nitrofurantoin		Decreased nitrofurantoin reductase

[a]Predictor for resistance to gentamicin only in amikacin-susceptible strains.

which resistance is more marked (cross-resistance).

For example, it is particularly interesting to monitor the following bacterium–antibiotic combinations indirectly, using the corresponding 'phenotypic markers' of resistance:
• *E. coli* and third-generation cephalosporins: resistance to first-generation cephalosporins but susceptibility to ticarcillin identifies cephalo-

Table 16. Antibiotics suggested as surveillance antibiotics for *Pseudomonas aeruginosa*

Surveillance antibiotic	Predictive for:	Main resistance mechanisms
Ticarcillin	Piperacillin	AmpC hyperproduction Penicillinase Efflux
Ceftazidime	Cefepime Aztreonam	AmpC hyperproduction Efflux
Imipenem	Meropenem	AmpC hyperproduction plus reduced permeability Carbapenemase
Meropenem	Imipenem	AmpC hyperproduction plus reduced permeability Efflux Carbapenemase
Ciprofloxacin	Fluoroquinolones	Topoisomerase mutations (and/or efflux)
Netilmicin[a]	Gentamicin	Aminoglycoside-modifying enzymes
Tobramycin[a]	Gentamicin	Reduced permeability
Amikacin	Tobramycin	

[a]Predictor for resistance to gentamicin only in amikacin-susceptible strains.

Table 17. Antibiotics suggested as surveillance antibiotics for *Acinetobacter baumannii*

Surveillance antibiotic	Predictive for:	Main resistance mechanisms
Imipenem	Meropenem	Reduced permeability Carbapenemase Oxacillinase PBP modification
Ciprofloxacin	Fluoroquinolones	Topoisomerase mutation
Gentamicin		Aminoglycoside-modifying enzymes
Tobramycin[a]	Gentamicin	Reduced permeability
Amikacin		Efflux

[a]Predictor for resistance to gentamicin only in amikacin-susceptible strains.
PBP, penicillin-binding protein.

Table 18. Antibiotics suggested as surveillance antibiotics for *Staphylococcus aureus*

Surveillance antibiotic	Predictor for:	Main resistance mechanisms
Penicillin	Ampicillin Amoxycillin Piperacillin	Penicillinase
Oxacillin	All β-lactams[a]	PBP2a
Vancomycin	Teicoplanin	Thick cell wall VanA
Gentamicin	Tobramycin Netilmicin Amikacin	Aminoglycoside-modifying enzymes
Netilmicin	Amikacin	
Tobramycin	Netilmicin Amikacin	
Kanamycin	Neomycin Amikacin	
Erythromycin	Clarithromycin Azithromycin Other macrolides	Ribosomal methylation Efflux
Lincomycin	Clindamycin	Ribosomal methylation Inactivating enzymes
Ciprofloxacin	Fluoroquinolones	Topoisomerase mutation
Co-trimoxazole		Changes in enzyme target
Tetracycline	–	Efflux Ribosomal protection
Chloramphenicol	–	Acetyltransferase
Mupirocin	–	Altered target
Linezolid	–	Ribosomal mutation

[a]MICs should be determined.

Table 19. Antibiotics suggested as surveillance antibiotics for *Streptococcus pneumoniae*

Surveillance antibiotic	Predictor for:	Main resistance mechanism
Penicillin	Amoxycillin[a]	Modified PBP
Cefotaxime	Ceftriaxone Cefepime	Modidifed PBP
Ciprofloxacin	Fluoroquinolones	Topoisomerase mutation
Erythromycin	Azithromycin Clarithromycin Roxithromycin	Ribosomal modification Efflux
Telithromycin		Ribosomal modification Efflux
Co-trimoxazole		Changes in enzyme target
Tetracycline		Efflux Ribosomal protection
Chloramphenicol		Acetyltransferase
Linezolid		Ribosomal mutation

[a]MICs should be determined.
PBP, penicillin-binding protein.

Table 20. Antibiotics suggested as surveillance antibiotics for *Enterococcus* spp.

Surveillance antibiotic	Predictor for:	Main resistance mechanisms
Ampicillin	Penicillin Piperacillin Carbapenem	PBP modification PBP overproduction
Gentamicin (HLR)	Amikacin[a] Tobramycin Netilmicin	Aminoglycoside-modifying enzymes
Streptomycin (HLR)		Aminoglycoside-modifying enzymes Ribosomal modification
Ciprofloxacin	Fluoroquinolones	Topoisomerase mutation
Vancomycin	Teicoplanin	VanA, VanD
Erythromycin		Ribosomal methylation Efflux
Quinupristin–dalfopristin[b]		Modifying enzymes Ribosomal methylation (high-level resistance)
Linezolid		Ribosomal mutation

[a]Infrequent isolates may exhibit synergy with amikacin despite high-level resistance to gentamicin.
[b]Only for *Ent. faecium*.
HLR, high-level resistance; PBP, penicillin-binding protein.

Table 21. Antibiotics suggested as surveillance antibiotics for *Streptococcus pyogenes*

Surveillance antibiotic	Predictor for:	Main resistance mechanisms
Penicillin		
Ciprofloxacin	Fluoroquinolones	Topoisomerase mutation
Erythromycin		Ribosomal methylation Efflux
Telithromycin		Ribosomal modification Efflux
Tetracycline		Efflux Ribosomal protection
Chloramphenicol		Chloramphenicol acetyltransferase

sporinase-hyperproducing strains, which exhibit decreased susceptibility to cefotaxime, ceftriaxone and ceftazidime (MIC 0.25–2 mg/L).

• Enterobacteriaceae and fluoroquinolones: resistance to classical quinolones (e.g., nalidixic acid) identifies strains with low-level resistance

Table 22. Antibiotics suggested as surveillance antibiotics for *Haemophilus influenzae*

Surveillance antibiotic	Predictive for:	Main resistance mechanisms
Ampicillin	Amoxycillin	Penicillinase
Amoxycillin–clavulanate		PBP modification and/ or penicillinase
Cefotaxime	Ceftriaxone	ESBL
	Cefixime	
Nalidixic acid	Fluoroquinolones	Topoisomerase mutation
Azithromycin	Clarithromycin	Efflux
Co-trimoxazole		Change in enzyme target
Tetracycline		Efflux
		Ribosomal protection
Chloramphenicol		Chloramphenicol acetyltransferase

ESBL, extended-spectrum β-lactamase; PBP, penicillin-binding protein.

Table 23. Antibiotics suggested as surveillance antibiotics for *Neisseria meningitidis*

Surveillance antibiotic	Predictive for:	Main resistance mechanisms
Penicillin		Penicillinase
		PBP modification
Cefotaxime	Ceftriaxone	PBP modification
Ciprofloxacin	Fluoroquinolones	Topoisomerase mutation

PBP, penicillin-binding protein.

to newer antibiotics of this family. This helps to monitor the evolution of rates of resistance to fluoroquinolones [41].

Possible bias in reporting

Some laboratories perform second-level susceptibility tests with selected antimicrobial agents on isolates that demonstrate resistance initially to one screening agent (or method) only. Furthermore, some second-line antimicrobial agents are only tested routinely against isolates resistant to agents used more widely. Any calculation of the susceptible percentage based on these selected subsets of isolates would bias the results towards higher levels of resistance.

Another frequent question is whether 'cascade' or selective reporting rules should be applied to reports, i.e., reporting secondary agents only if the isolate is resistant to the primary agent(s) of a specific drug class. Whatever decision is taken with regard to internal reports, which must take into account the local clinicians' ability to understand the implications of the report fully, it is important to ensure that all stored data—and not just those reported to clinicians—are analysed for epidemiological purposes. When only isolates resistant to primary agents are analysed, then results for secondary agents are biased towards higher levels of resistance.

FREQUENCY OF DATA ANALYSIS

How frequently should the cumulative susceptibility data be reported?

Surveillance studies are frequently based on comparing resistance over successive time periods.

Comparing data over short time intervals is advantageous in surveillance studies, both in theory and in practice. Studies based on time-series have shown the superiority of short time intervals (months) for some epidemiological purposes, including studies of the relationship between the use of antibiotics and antibiotic resistance [42]. Furthermore, surveillance should be designed for, or related to, an effective alert system, and therefore 'the sooner the alert, the better'. However, the actual frequency of cumulative susceptibility reports may vary according to a number of factors. These factors include the availability of sufficient, reliable data within a given period, the specific purpose of the surveillance (e.g., guiding empirical therapy, alert function, assessing the impact of therapeutic or preventive strategies, educational goals), accessibility of rapid means of distribution and circulation (peer-reviewed articles, institutional bulletins, regular internal reports, Intranet, Internet), or even funding problems.

If the main purpose of surveillance is guiding therapy in the hospital setting or in the community, or to give a general report of the resistance problem at local, national or international levels, it is sufficient usually to analyse data which have been collected over longer periods of time. However, if the surveillance system entails an alert function, it is necessary to analyse the data more frequently and to report whenever a relevant change has occurred. The emergence of unusual or rare resistance patterns, or of new mechanisms of resistance, would mandate immediate reporting. To assess the impact of interventions, the time and frequency of analysing and reporting must be related clearly to the action to be undertaken (pre-interventional, post-interventional, follow-up). Easy access to rapid electronic means for spreading information should facilitate the distribution of surveillance reports and the timely utilisation of data.

In order to provide data as a guide to empirical therapy, different cumulative susceptibility reports should be generated for each healthcare facility

served by a laboratory. For this purpose, it has been recommended to analyse and report data on at least a yearly basis [38]. When a large number of strains of a species or group of microorganisms have been tested, more frequent analyses may be carried out. It has, however, been argued that more frequent reporting may be confounded by seasonal variations.

Yearly reporting may also be appropriate if the aim of surveillance is to assess the scale of the resistance problem at the local, national or international levels. The surveillance standards recommended by the WHO [21] suggest at least yearly reports at the national and international levels ('intermediate/central level'). For monitoring changes in resistance rates, and for detecting the emergence and the spread of new resistances, yearly reporting will probably cause an unacceptable delay in the diffusion of important information. Hence, more frequent presentation of data is required. The WHO [21] recommends a daily review of unusual or important results at the local level ('peripheral level'), a weekly-to-monthly review of organism frequencies and resistance profiles for outbreaks, and a quarterly review of data for monitoring resistance trends, and a review of hospital usage policy. At the intermediate and central levels, quarterly reviews of pooled data for monitoring resistance trends by organism, antibiotic, geographical and demographic parameters, as well as a quarterly review of resistance results for possible errors in laboratory performance, are proposed.

The EARSS also collects national resistance data on invasive isolates quarterly, and gives a quarterly feedback to the national representatives, who are responsible for distributing information on important or unexpected results, as well as on unclear or doubtful data, to the individual laboratories. A report for a broader public is prepared once a year [9].

In order to quantify the effectiveness of interventions aimed at curtailing resistance, the time and frequency of analysis and reporting must be related clearly to the action taken. In general, data of the pre-interventional and the post-interventional periods, as well as those of at least one follow-up period, should be provided. Whatever the type and frequency of the report, it is essential that the information be as timely as possible and that delays in dissemination be avoided [43,44].

Do small numbers of isolates imply imprecise statistics?

If the number of observations is small, resistance rates may be biased easily—upwards or downwards—by the features of a handful of isolates. The debate about the threshold to be applied for reporting small numbers of isolates is still open. In the NCCLS Guidelines [38], a minimum of ten strains is required for separate reporting, with a proposal to use a threshold of at least 25 strains having been rejected. As a rationale for setting the minimum at 25 strains, it was argued that, for example, the impact of two resistant strains in a sample of ten is much greater than in a sample of 25 (resistance rates of 20% and 8%, respectively). However, both figures, namely ten and 25, are completely arbitrary. Such small numbers of observations are generally not suitable for comparison of resistance rates between different settings or periods of time, since random fluctuations of uncertain significance will occur readily. A more accurate example for estimating the sample sizes needed for documenting increasing or decreasing antimicrobial resistance frequencies is given in the WHO document 'Surveillance Standards for Antimicrobial Resistance' [21]. It is of crucial importance to report the exact number of observations on which resistance rates are based, i.e., the number of strains tested for each antibiotic listed in the report. Inclusion of confidence intervals helps to interpret results based on small-sized samples.

Does perception of important clinical changes imply more frequent analysis?

One of the main purposes of resistance surveillance is to detect variations in the susceptibility level of microorganisms to antimicrobials, and to make an interested public aware of clinically or epidemiologically relevant changes. Perception of clinically important changes, be it the emergence and spread of new resistances or the increasing (or decreasing) prevalence of already known resistance patterns, should imply careful evaluation of previous data and frequent analysis of newly collected information in order to verify presumed trends.

Do seasonal variations in resistance rates complicate the presentation of data?

Seasonal variations in resistance rates have been reported, particularly for pathogens causing community-acquired lower respiratory tract infections. Consistent with studies published previously, EARSS data [9] show a peak of invasive *Strep. pneumoniae* isolates around the turn of the year, a constant decline in isolates until August, and a subsequent regular increase in the autumn and winter months. The proportion of penicillin-non-susceptible *Strep. pneumoniae* (PNSSP) isolates tends to be higher in the summer months; however, this difference is not statistically significant. The variations in PNSSP proportions, and their higher prevalence in August, are as yet unexplained. Although seasonal variations in resistance rates for particular pathogens may not reflect real changes in the level of resistance, they at least complicate, and possibly bias, any presentation of data on a more frequent basis than 1 year, particularly for small-size samples.

Do larger numbers of isolates imply more frequent analysis?

Although it is true that inaccurate statistics related to small sample sizes often discourage frequent or stratified analysis, the availability of data on a larger number of strains does not imply necessarily that data should be presented more frequently. As discussed above, the frequency of reporting should be defined primarily according to the purpose of the surveillance. Detection of resistance trends is easier when more numerous data are evaluated frequently and reported (e.g., increased fluoroquinolone resistance in *E. coli*), while detection and reporting of unexpected or new resistances should not be influenced greatly by the amount of data available. In the latter case, an alert system would allow timely information, leading to prompt epidemiological intervention.

A larger amount of data could also prove useful for obtaining better stratification (e.g., data from ICUs as compared to those from other services, data from different specimen types, data from different age groups). Hence, when selecting the report periodicity, an assessment should be made as to whether additional stratification of data or more frequent reporting is appropriate.

Cross-sectional surveillance studies

For frequent pathogens, many studies are organised on the basis of collecting all isolates of this pathogen on a particular day from hospital (or community or ICU) patients. For less frequent isolates, the collection may be extended over 3 days or 1 week. These periodic cross-sectional studies permit evolutionary analysis to be conducted, and may be useful for obtaining a fixed image of the prevalence of resistance in a given organism or in a small group of organisms. A major advantage of this method is that the laboratories enrolled in the surveillance program are not burdened by the task of ad-hoc analysis. Hence, this short-time recovery method generally assures full recovery of strains and offers a non-biased sample. The strains should be collected at the laboratory by the surveillance team, and analysed in a central reference laboratory to assure comparability of results.

FORMAT FOR DATA PRESENTATION

Reporting antimicrobial resistance is generally considered necessary to allow selection of empirical therapy based on local data for: (1) assessing the scale of the resistance problem at the local, national or international levels; (2) monitoring changes in resistance rates; (3) detecting the emergence and spread of new resistances; and (4) providing a measure of the effectiveness of any interventions aimed at reducing resistance [45].

Circulation of reports

It is essential that results be reported as rapidly as possible to as wide an audience as is thought appropriate, including all those involved in antimicrobial testing, prescribing, supplying and auditing. It is also worth recalling that any information gathered from a surveillance system, although collated centrally, must be reported back to its providers, i.e., those who generated and submitted the basic data.

Earlier surveillance studies tended to rely upon yearly reports, presentations at international symposia, and publications in peer-review journals, all of which are relatively slow processes, but the growth in use of the Internet and the World Wide Web makes information accessible much more easily than previously [44]. Presentation on a

website would probably meet most needs for circulation of reports, but a printed version should always be available for wider diffusion and prompt consultation. Such a printed report should be designed mainly to meet clinicians' needs, and should be printed in a format that the clinician finds easy to access and understand. A foldout card and a laminated page to be placed at the front of each new patient file have proved useful [38].

Stratification of data, even within the same healthcare facility, may be useful in answering questions and in guiding clinicians in empirical therapy decisions [38], but can also split the data into more homogeneous subsets, which are comparable more readily between studies. This may take the form of a separate report to individual user groups rather than being part of the whole cumulative antimicrobial susceptibility report. Examples of stratification criteria include:
• clinical service or ward grouping;
• specific ward, clinic or unit;
• specimen type;
• age groups;
• special patient populations (e.g., cystic fibrosis, haematological malignancies).

Information to be included in the report

The time schedules adopted in producing the report should be indicated, as well as the date of issue and periodicity of the report. If data have been stratified and appear in separate reports, the ward, unit and specimen (or anything else) that the present report refers to should be stated clearly. All other available reports should be listed, with all relevant information about their availability, in order to allow comparison between a specific unit and other units or total hospital susceptibility data. Information on units other than those that the report refers to may be subject to restrictions in accordance with an individual hospital's privacy policies.

A note should be made when a new analytical method has been used to generate the data, or a new denominator (e.g., a different ward grouping or different ward composition) has been applied to calculate the figures, and comparison with previous reports must be made with caution.

In table headings, complete antimicrobial names should be used when possible. If space is

an issue, legend keys should be given in a footnote.

Further information reported should include:
• types of specimens considered (e.g., blood, cerebrospinal fluid, urine, respiratory, all);
• level and accuracy of microorganism identification (genus, species, subspecification, type);
• if organisms have been grouped, an exact description of species and genera, including pooling criteria;
• method of resistance testing, MIC breakpoints, interpretative criteria;
• whenever possible, information on infection, colonisation, surveillance culture;
• quality assurance.

Comparison of data from different sources

A proper comparison of data between wards, hospitals or geographical areas (at the local, national and international levels, as well as over time) is indispensable for analysing trends and emerging problems. Unfortunately, information needed for such comparisons is often scarce or not provided at all. As a result, data on non-homogeneous populations (e.g., those including mostly outpatients vs. those including mostly ICU or haematological patients) are often compared, resulting in completely erroneous conclusions.

It would be advisable to list in every report the percentage composition of the data sources or, at least, the percentages of those coming from outpatients, inpatients (excluding ICUs) and ICUs, respectively. These three main categories could be stratified further (e.g., by detailing the individual wards encompassed by the term 'inpatients', or by singling out wards with a high resistance incidence, such as haematology, or by admitting patients with specific pathologies—such as cystic fibrosis—or age ranges—such as geriatric or paediatric patients). This way of 'labelling' the data would permit easier comparison of resistance rates in different reports, including those presented in published studies, posters or oral presentations, and rapid identification of different categories (e.g., those laboratories processing many outpatient specimens, or those lacking an ICU). This would be all the more effective if expressed in graphical format (e.g., a bar or a pie chart indicating the main wards with different standard colours or grey levels). If stratification is kept to a minimum, distinguishing

between outpatients, inpatients and ICUs would in itself provide very useful information.

The report format

Reports can be compiled by manual data input or can be generated automatically. Automated and semi-automated systems for antimicrobial susceptibility testing [46] can produce standardised or customised patient test reports generated by computer software packages that are referred to as data management systems (DMS). The DMS package usually contains an epidemiology component, which can archive results, thereby providing specialised reports, organism trend reports and antibiograms. To optimise the availability and transcription accuracy of rapid patient reports, integrated data from automated and semi-automated systems can be transferred through a computer interface to the LIS. An additional level of integration can be provided by software packages that allow interfacing with a pharmacy system, so that the microbiology results can be matched against a patient's record of antimicrobial therapy.

An additional level of enhancement in automated systems is referred to as 'expert software', which examines and validates the antimicrobial susceptibility profile or phenotype of an individual isolate. These expert systems use specific rules or algorithms (pre-programed or user-defined) to flag unlikely resistance patterns and recommend changes. Expert software may also predict cross-resistance to other antimicrobial agents, and can facilitate the addition of footnotes or comments to a patient's report regarding the resistance pattern.

Qualitative definition of denominator data (e.g., what kind of population is being sampled) is essential. Wherever possible, rates should be expressed in terms of cases within a defined human population over a defined time period. Since the submission of microbiological specimens for analysis is inconsistent and varies widely, the use of laboratory specimens and isolates as denominators produces rates that are of limited epidemiological relevance unless linked to disease incidence.

Statistical handling of surveillance data is difficult. Many standard approaches to sampling human populations are not relevant because the target population in surveillance studies is highly variable and not consistent. In general, it is not possible to establish a 'sampling frame', and techniques used in ecology might be more appropriate. Sampling methods for surveillance studies have been compared with those used for more conventional surveys in a recent paper [44].

Expression of data as truly qualitative test results
For a few bacterium–antibiotic combinations, susceptibility tests are truly qualitative and are designed to detect the presence of a given resistance mechanism, such as:
- *S. aureus* resistant to all β-lactam agents, by the detection of microcolonies in the inhibition zone surrounding an oxacillin disk in specific growth conditions (salt, temperature), or PBP2a detection by immunoenzymatic methods or *mecA* gene detection;
- *H. influenzae* or *S. aureus* resistant to penicillins, by the detection of β-lactamases with enzymatic methods;
- Enterobacteriaceae resistant to third-generation cephalosporins, by the detection of ESBL production (double-disk synergy or other similar test).

Semiquantitative vs. quantitative data
A variety of different methods are used for susceptibility testing, but it is now accepted widely that all susceptibility data should be generated as quantitative endpoints regardless of the method. This mandates measurement of disk diffusion zone diameters to the nearest whole millimetre, and the expression of MIC endpoints in mg/L for dilution methods.

The commonest measure for bacterial sensitivity is the MIC, a measure that describes partly the pharmacodynamics of an antibiotic, although it ignores its bactericidal activity, which is also of great importance for the clinical value of an antimicrobial drug. Because of the ease of assessing the MIC, it is common practice to use MIC data to describe the antibacterial activity of a drug towards a bacterium, or the sensitivity of a bacterium to a drug. To make antimicrobial therapy applicable, and to avoid treatment of infections with inappropriate drugs, MIC data can be grouped into well-established interpretative categories, limited by cut-off values or breakpoints. Thus, interpretative categories must be referred to as 'semiquantitative' (and not truly qualitative) reporting of data.

To avoid misunderstandings, clear-cut definitions are needed, and any report including susceptibility tests performed by different

laboratories needs to be subjected to careful evaluation of differences that might relate to methods and breakpoints rather than to true differences in resistance [45,47].

MIC cut-off values and breakpoints should fulfil three criteria, namely epidemiological, pharmacological and clinical criteria. MIC breakpoints differ between countries, mostly because the respective national working committees attribute different importance to the two latter criteria. EUCAST makes a clear distinction between clinical breakpoints (which define clinically susceptible, clinically intermediate and clinically resistant isolates, and which can be altered by changes in circumstances such as an acquired resistance mechanism) and epidemiological cut-off values (which focus on separating the resistant strains from the wild-type strains, and which are not altered by changing circumstances such as an acquired resistance mechanism) [45,47].

For many bacterium–antibiotic combinations, acquired resistance leads to a pattern easily distinguishable from the wild-type strain, so semiquantitative data would appear to be adequate for monitoring resistance. Some examples are listed in Table 24. Interpretative categories are of particular importance when reporting on bacterial resistance within a hospital, since clinicians make daily use of S/I/R results to prescribe antibiotic therapies.

If differences in susceptibility test methods and in breakpoints make it difficult to compare semiquantitative data from different geographical regions and different published studies [45], bias and errors are of particular concern even within a given report. It is not infrequent to have, in the same laboratory, antimicrobial susceptibility tests performed by different methods (either manual or automated) according to different specimens, workload and workflow, or individual preferences. Different automated systems—or automated readers of manually performed tests—may have different breakpoints embedded in their software, or different software releases, or may have been updated manually at different times. While these considerations have an impact on the internal correctness and coherence of the report, comparing susceptibility results from different wards in the same hospital is a risky operation in that it does not take account of the huge differences existing as regards distribution of bacterial species, use of medical and surgical devices, underlying conditions of patients, and use of antimicrobials.

Table 24. Main bacterium-antibiotic combinations for which semiquantitative data can monitor resistance adequately

E. coli, P. mirabilis, Salmonella spp., *Shigella* spp.
First-generation quinolones
Tetracyclines
Aminopenicillins
Carboxypenicillins
Sulphonamides
Gentamicin
Trimethoprim
K. pneumoniae
Gentamicin
First-generation quinolones
Tetracyclines
Sulphonamides
Trimethoprim
Ent. cloacae, Ent. aerogenes, Serratia
Third-generation cephalosporins
Gentamicin
First-generation quinolones
P. aeruginosa
Ticarcillin
Ceftazidime
Imipenem
Ciprofloxacin
S. aureus
Erythromycin
Gentamicin
Tetracyclines
Sulphonamides
Fluoroquinolones
Strep. pneumoniae
Erythromycin
Chloramphenicol
Tetracyclines

Reporting semiquantitative data

Data expressed as S/I/R can be either tabulated or reported as graphs. Since breakpoints refer to given MIC values, these data are sometimes referred to as 'semiquantitative' rather than 'qualitative'. The simplest way of reporting qualitative—or semiquantitative—data is by presenting them as a table, listing the number of isolates tested, and the number and/or percentage of susceptible, intermediate and resistant strains. However, many reports only list one interpretative category, which sometimes makes it possible to compare many years of survey in different columns without compromising the overall readability of the report.

When only one interpretative category is listed, data can also be arranged in a cumulative antimicrobial susceptibility report [38], listing the microorganisms on the *y*-axis and the percentages susceptible to all tested antimicrobials on the *x*-axis; the number of tested isolates can also be placed on the *x*-axis, provided that all compounds have been tested on the same number of isolates. The statement that not testing a

subset of isolates (e.g., urine isolates) against all drugs is not likely to affect the clinical relevance of the cumulative report [38] is debatable; in such an instance, separate subset tables would be preferable.

Susceptibility rates equal to zero should be distinguished clearly from the possibility that the drug has not been tested, since in a database numerical field both cases may be represented by the figure '0'.

Data may be grouped by microorganism (and reported on several consecutive lines headed by the name of the antibiotic tested), or by antibiotic (and reported on several consecutive lines headed by the name of the microorganism tested). Microorganisms can be listed alphabetically, by organism group or by prevalence, but the alphabetical list is usually preferred. Listing by group can be helpful when comparing resistance between related microorganisms which often belong to the same genus and are also close to one another in the alphabetical list.

Antimicrobials can be listed alphabetically, by class or by rank (1st, 2nd, 3rd choice). Ranking antimicrobials may also lead to reporting lower-rank antibiotics (broader spectra, more costly, more toxic) only if a microorganism is resistant to primary agents within a given class (or even within the whole formulary). This 'cascade reporting' applies only to reports to be used in clinical settings to assist clinicians in selecting the most appropriate agents for antimicrobial therapy. In more comprehensive epidemiological reports, careful analysis of all data generated by antimicrobial susceptibility tests is essential for the timely detection of trends in emerging resistance [38].

It must always be made clear whether the intermediate isolates are reported independently or pooled with the resistant or—far less commonly—with the susceptible strains. If the intermediate isolates are pooled with the resistant isolates, this pool should be referred to as 'non-susceptible'. Such groupings are often used by analogy to clinical findings (as is the case with PNSSP), but it is also worth noting that this procedure can smooth out the differences existing between different national breakpoints. The extent of agreement on susceptible breakpoints (S/I) is often greater than that on resistant ones (I/R), and pooling intermediate and resistant isolates in a single group of 'non-susceptible' isolates may reduce the bias when international data are

compared. However, in a few instances, breakpoints categorise the wild-type organisms of a species as intermediate (or indeterminate), in which case the intermediate category must be analysed together with the susceptible category, since these microorganisms are not endowed with any acquired resistance mechanism.

The use of graphics in reporting semiquantitative data

Graphs may permit a swifter appreciation of differences between resistance levels to different antibiotics, between different microorganisms, or over different time periods, thereby giving a comprehensive overview of resistance distribution in a given species over time. Susceptibility evolution over time can be expressed by means of either linear graphs or bar graphs. Linear graphs are not entirely suitable, since they suggest continuous observations, which often is not the case. More lines, representing different antibiotics or different microorganisms, can coexist on the same linear graph. The same applies to bar graphs, where different-coloured bars represent different antibiotics or microorganisms, but usually at the expense of poor data clarity. Bars also allow S/I/R-values to be combined in a single bar with multiple colours, thus condensing complex information in a single graphic element. The amount of information is even greater with three-dimensional graphs which present, in the same chart, susceptibility values for different microorganisms and/or for different antibiotics and/or over different time periods. This type of presentation can condense several lines or pages of a table in a single graph, but overcrowded pictures can undermine the immediacy and the efficacy of the presentation. Problems can be solved by computerised presentations that introduce a few graphic elements at a time, but there is a risk of shifting from an effective dynamic presentation to a presentation in which entertainment effects are detrimental to the message itself.

Labels, figures and/or percentages may be unsuitable for a slide presentation in which the speaker can introduce the most important data and comment on them, but they should always be provided in any printed version of the graph, alongside the number of isolates considered in the study. All tabulated figures must be reported when printed graphs are not presented on the

same page as the original table, or when graphs completely replace the table.

Another popular method of reporting semi-quantitative data uses geographical maps in which the states (or other geographical areas) are coloured differently according to the different susceptibility levels. This may result in very effective reporting of large-scale studies, but normally permits the representation of only one variable at a time, usually the 'national' resistance rate. No colour key or susceptibility range is accepted internationally, but a traffic-light code is used frequently. Cold colours (green, white or light grey) are usually chosen to represent lower resistance rates, while warm colours (purple, red or black) represent higher rates. A very adequate way of presenting differences between different regions is to calculate the standard deviations of resistance rates in different areas, and present the +1 and + 2 SDs in warm colours, and the −1 and −2 SDs in cold colours, reserving white for the SD. Choosing a limited number of broad resistance ranges implies that minimal numerical variations can shift a country from one group to another, which can be disturbing, especially when maps for different time periods are compared, and smaller changes cannot be represented. On the other hand, a larger number of narrow resistance ranges may minimise the colour variations from one map to another, thus making the maps more easily comparable. However, the simultaneous presence of many colour codes on one map makes it somehow less readable, and things may be even worse if, in black-and-white prints, grey gradations or texture patterns are substituted for colours.

Reporting quantitative data
The use of quantitative data may add further details to what is expressed broadly by means of interpretative categories. Moreover, in some instances, the mere use of current breakpoints fails to reveal increased resistance, since the extent of reduced sensitivity is still not sufficient for the isolates to be classified in either the I or R category. This may occur either because the phenotypic expression of resistance is too weak, or because of the extreme natural sensitivity of the species to the antibiotic in question (see the examples listed in Table 25). In such cases it might be helpful—or even necessary—to express the results quantitatively (inhibition zone diam-

eter or MIC) in order to identify isolates with decreased susceptibility within the S category.

The correlation of quantitative data with epidemiological cut-off values would also be of value in the following cases:
- when the breakpoint divides wild-type distributions of bacteria (something which should be avoided by breakpoint committees), in which case even small methodological shifts may result in major shifts in resistance frequencies;
- wherever consensus on clinical or pharmacological breakpoints is lacking, in which case only epidemiological cut-off values allow comparison of resistance development;
- whenever resistance (to new or old drugs) and the consequent phenotypes have yet to be described, in which case efforts are concentrated primarily on recognising resistant isolates as soon as they occur;
- when studying the relationship between antibiotic use and the emergence and development of resistance.

Management of MIC results for producing quantitative reports
Currently, quantitative data on the activity of antimicrobials are reported as the range of MICs, 50% MIC (MIC_{50}), and 90% MIC (MIC_{90}). The usefulness of this way of reporting stems from its ability to condense many data into a single figure, and therefore it may be useful for abstract reporting. Nevertheless, its analytical and descriptive value remains highly controversial, particularly in Europe (see below). Two-fold dilutions are used commonly but 1.5-fold dilutions can also be found and are typical of data obtained by means of the Etest. However, Etest results can be pooled with those obtained by other susceptibility testing methods after being rounded to the nearest higher two-fold dilution value.

Endpoints are often not clear-cut, particularly in automated systems, because of the limited number of dilutions available. Thus, MIC results are reported often as either 'equal to or lower than' or 'equal to or higher than' given endpoints; since these endpoints are fairly variable, different reports are often not comparable completely, although applying restriction rules for inclusion of diverse values in the analysis could make it possible to extract valid information, even from heterogeneous quantitative reporting.

Table 25. Examples of situations where many clinical or pharmacological breakpoints may fail to disclose the development of microbiological resistance because of the large gap in MIC concentrations between the breakpoint and the MIC for a very sensitive species

Antibiotic	Examples of species	Comment
Ampicillin	*Helicobacter pylori*	Most breakpoint systems use an ampicillin breakpoint of 8 or 16 mg/L to accommodate the wild-type distribution of *E. coli*. The *Helicobacter pylori* wild-type organism does not exceed 0.125 mg/L
Cephalosporins	*E. coli* and *Shigella*	Decreased sensitivity to first-generation cephalosporins or to the amoxycillin–clavulanate combination, or to mecillinam because of penicillinase production. *E. coli* with decreased sensitivity to third-generation cephalosporins through cephalosporinase hyperproduction
Chloramphenicol	*Haemophilus influenzae*	One of the first examples (now corrected by most breakpoint committees) where a breakpoint designed for Enterobacteriaceae failed to detect CAT-producing *H. influenzae*
Fluconazole	*Candida albicans*	The NCCLS breakpoints of $S \leq 8$ mg/L and $R \geq 64$ mg/L leave a large gap between the wild-type (ending at 0.5 mg/L) and the breakpoint. Incidentally, this would never be a problem with amphotericin B, because the clinical breakpoint is perfectly in line with the epidemiological breakpoint
Fusidic acid	*Staphylococcus aureus*	Low-level fusidic acid resistance in *S. aureus* (the clinical relevance of which is still not established) goes undetected by pharmacological breakpoints
Imipenem, meropenem	Most Gram-positive bacteria; several Enterobacteriaceae	A classic example where a breakpoint designed to accommodate both Enterobacteriaceae and *Pseudomonas* often fails to disclose the production of PBP2a in MRSA
Penicillin	*Streptococcus pyogenes*	Not yet described, but it is highly likely that if resistance mechanisms do spread to or develop in *Strep. pyogenes*, only the *Strep. pneumoniae* low-level resistance breakpoint will be able to disclose the resistance
Rifampicin	*Staphylococcus aureus*	Large gap between MIC of wild type (ending at 0.064 mg/L) and breakpoints (most of which are at 1 mg/L or higher)
Fluoroquinolones	Enterobacteriaceae *Neisseria gonorrhoeae* *Neisseria meningitidis* *Haemophilus influenzae*	Large or extremely large gap between MIC of wild type and breakpoint.

The usual tables of quantitative data may list a number of fields, such as species, antibiotic, number of isolates, range, MIC_{90} and MIC_{50}. Considerations regarding the listing of species, antibiotics and number of isolates are the same as those outlined for semiquantitative data (see above).

The MIC range (expressed as mg/L or μg/mL) reports both the lowest and the highest susceptibility value obtained for a given microorganism–antimicrobial pair, but does not provide any information about the MIC distribution within these extreme figures. Moreover, even a single atypical or even misidentified isolate can affect the range strongly, which actually provides the reader with only very limited and often misleading information.

The MIC_{90} (also expressed as mg/L or μg/mL) has been used as the most common parameter for reporting and comparing antibiotic susceptibility data. By representing the MIC capable of inhibiting at least 90% of the isolates investigated, it is reasonably representative of the susceptibility of a species and provides a prudent estimate of the expected susceptibility of isolates of the same species when reports are used to assist clinicians in selection of the most appropriate agents for empirical antimicrobial therapy. However, as in the case of the MIC range, the MIC_{90} does not provide any information about the MIC

distribution above or below its own value, and its value may change significantly when even only small numbers of isolates endowed with MICs different from the remainder are included in the analysis. This, of course, is much more likely to happen when a small number of isolates is considered, since a far more conspicuous subset of aberrant isolates needs to be present in order to modify the value of the ninetieth percentile.

The MIC_{50} (also expressed as mg/L or µg/mL) can be regarded as complementary to the MIC_{90}, and has also been used as a common parameter for reporting and comparing antibiotic susceptibility data. By representing the MIC capable of inhibiting at least 50% of the isolates investigated, it is not very representative of the susceptibility of a species, and an estimate of the expected susceptibility of isolates within a given species would not be prudent if based only on MIC_{50} values. However, the calculated value is more stable than the MIC_{90}, and is less likely to undergo significant changes following inclusion of small numbers of unusual isolates. It may therefore be of some help in comparing reports from different susceptibility studies.

The combination of the MIC_{90} and MIC_{50}, rather than the MIC_{90} or MIC_{50} alone, provides a better, albeit rather static, representation of isolate susceptibility, but cannot reflect the MIC distribution accurately throughout the dilution range. This can be appreciated only in tables reporting the population distribution of MICs and, even better, by graphic presentations.

In the population distribution of MICs, data may be arranged as a cumulative antimicrobial susceptibility report, usually listing the microorganisms on the *y*-axis and the percentages corresponding to each individual MIC value in the range on the *x*-axis; the number of isolates tested is also placed on the *x*-axis. In another type of report, the percentages on the *x*-axis are increased by the additional percentages corresponding to increasing MIC values up to a final value of 100.

A population distribution of MICs, although not very popular, is the most informative way of listing antibiotic activity. This distribution is extremely important, not only for critical evaluation of susceptibility data, but also for determining epidemiological cut-off values. These do not depend on pharmacological and clinical criteria, and allow comparison of resistance rates on the basis of the microbiological characteristics of the

resistance, which do not differ between countries, while international agreement on clinical breakpoints may remain an elusive goal [45].

The use of graphics in reporting quantitative data
Visual presentation of the susceptibility distribution in the report might possibly add value to all or most of the reporting methods used currently [48]. The population distribution of antibiotic susceptibility patterns can be studied readily from a scattergram—obtained from zone diameters as related to MICs——or from a histogram showing the distribution of susceptibility values. The use of histograms as a means of conveying susceptibility test results affords a convenient and intelligent presentation of data that enables the reader to understand readily the report received, and to relate the susceptibilities of given strains to those of other members of the species and to the interpretative clinical categories.

Susceptibility distribution has various patterns. Some distributions are unimodal, with a cluster of MICs over a narrow range (e.g., glycopeptides vs. pneumococci). A unimodal distribution generally occurs when a new antibiotic is introduced. Since acquired and naturally resistant strains are encountered with increasing frequency as a result of selection through the extended use of an antibiotic, the unimodal clustering is altered, with an increasingly pronounced tendency towards a skewed or frankly bimodal distribution. Recognition of this event at an early stage is important, and such trends should be followed closely in clustering analysis.

Other susceptibility distributions are typically bimodal, with a definite cluster of resistant strains, a second cluster of susceptible strains, and only a few strains in between (e.g., penicillin vs. staphylococci). Other drugs produce bimodal distributions, with more strains falling between the sensitive and resistant clusters (e.g., penicillin vs. pneumococci) because of a wider range of expression of the resistance mechanism(s); the narrowness of the intermediate range undermines the use of a breakpoint as a means of distinguishing between resistance and susceptibility.

One virtually unexplored field is that of representing, either graphically or as a table, the resistance variations over time (a sort of 'derivative', to use mathematical terminology). The scarcity of databases spanning a sufficiently lengthy period of time that are endowed with good internal consis-

tency is probably responsible for the shortage of this kind of information, which would be useful for a more accurate analysis of resistance trends over time, and for appreciating the importance of those resistance surges which, though not attaining very high values in absolute terms, entail a sharp, rapid increase with respect to baseline values. Moreover, figures alone seldom seem to be capable of providing a clear-cut picture, while variation over time provides a picture that is not dependent upon the 'absolute' value of figures, and thus adds a further dimension to the data to ensure prompt recognition of phenomena, as well as a better appreciation of their importance.

MANAGEMENT AND OUTCOME OF ANTIMICROBIAL RESISTANCE SURVEILLANCE

Paper surveillance studies

A vast amount of information about antibiotic susceptibility and resistance is presented regularly in different scientific meetings or published in journals, books or brochures around Europe. The availability, and therefore the impact, of this information on public health is probably still only minimal. Current information retrieval systems allow the recovery of these data for surveillance purposes. These paper surveillance studies, in the form of continuous updating of published or presented data about particular bacteria–antibiotic pairs, should be carried out by experts. They should be capable of assessing the quality control selection measures, including the methods used in the research, the reputation of the journal or meeting, and the investigators and the guarantees that they offer. Such studies can take advantage of the methods afforded by previous experiences in meta-analytical research. Indeed, part of the interest of paper surveillance studies lies in their ability to establish retrospective baselines of resistance rates, which prove useful in the interpretation of recent trends. This continuous data retrieval strategy was first proposed by ESGARS in 2001, and was subsequently adopted by the EU-funded ARPAC Programme in 2003.

Surveillance and the pharmaceutical industry: from drug development to clinical trials

Surveillance studies aimed at the early detection of resistance to new drugs should start in phases II and III of the development of new or modified antibacterial agents, and should expand into phase IV. These studies should be based on the use of selective broth or plates containing different concentrations of the new drug, to be inoculated with specimens of the normal microbiota of patients, in order to evaluate the possibility of emergence of resistance. New resistant strains should be investigated properly to identify the mechanisms of resistance, the biological cost for the resistant strain, and the possibility of horizontal gene transfer. Investigations of new resistant strains should also include clinical aspects (particularly if resistance is associated with therapeutic failure) and epidemiological aspects (with special reference to their clonality).

Surveillance and the pharmaceutical industry: from surveillance studies to industrial environmental policies

During the last decade, a number of well-funded surveillance studies have been conducted at the initiative of the pharmaceutical industry, and frequently under the technical guidance of scientific experts. Some of these studies are of high quality. Unfortunately, many of them have been operative for only a few years, since an important part of their funding originated in the marketing departments. The immediate benefits for the companies are to compare the performance (frequently advantageous) of their products with those of their competitors, to publicise and expand the visibility of their antibiotics in meetings and publications, and to ensure a cooperative relationship with opinion leaders in the field of antibiotic therapy. These goals are frequently dependent on the availability of competitive products in the field. The new concepts of environmental control and ecological remediation by the industry may help to sustain these activities in future.

Information industry: surveillance companies

A number of private companies specialising in medical information, and owning powerful informatics and bioinformatics platforms, are able to construct local as well as global resistance databases on antibiotic resistance. In some cases the companies also have reference laboratory facilities (corporate or contracted) and can therefore set up a comprehensive collection of strains. While the main customers of these companies are often pharmaceutical companies, nothing should

prevent the appropriate use of the raw data, and eventually the strains themselves, for public health purposes if required by official institutions or academia to fulfil well-defined objectives. Moreover, frequent publication of relevant data by the information company itself is most welcome.

Surveillance hyper-networks

The problem of antibiotic resistance is a global risk for public health, and surveillance systems should have no frontiers. Many international, national and regional organisations, as well as scientific societies around the world, are currently developing different surveillance systems simultaneously. As stated earlier, industry (i.e., the pharmaceutical or information industry) also operates a large number of surveillance systems in parallel. Surveillance studies are flowing over from human medicine into the fields of veterinary medicine, the food industry, agriculture and environmental biology. This complex situation has led to a number of initiatives aimed at creating hyper-networks or 'networks of networks'. Surveillance hyper-networks are certainly needed to understand global trends and to increase the sensitivity of the alert function, but obviously the hub or powerhouse of most networks should coincide with the point at which measures can be taken to control undesirable deviations that might be detected.

Publishing of surveillance studies

Surveillance is a scientifically-based activity, and not a process of blind collection and dissemination of data. Any surveillance system should be aware of the intrinsic possibility of including erroneous data, and should make every effort to minimise this risk. In other words, data inputs to the surveillance system should be edited carefully before public release. When dealing with possible emerging resistances, it is sometimes difficult for surveillance systems to discard abnormal or unexpected results automatically, based on the application of so-called 'expert systems'. In case of uncertainty, the abnormal result should be released as presumptive information, and all relevant doubts should be stated clearly. At the same time, every effort should be made to ensure that the laboratory where the abnormality was detected takes all appropriate steps to confirm the suspicion, if necessary with the help of reference laboratories.

Prediction and surveillance of resistance

Surveillance needs to use landmarks or 'flags' for the early detection of antibiotic resistance. Awaiting the clinical emergence of a resistant microorganism before ascertaining its phenotype and determining the surveillance flag position (cut-off value or breakpoint) in a gradient of MICs may be a risky policy. The clinical emergence of a given bacterial variant may come too late to allow the application of counteractive control measures. This is particularly critical for new drugs in the late stages of development or recently launched on the therapeutic market. A number of procedures, many of them based on molecular genetics, and frequently using genomics and proteomics technology, may enable the likelihood of emergence of resistant variants of important bacterial pathogens to be predicted.

Carefully controlled experiments can predict the behaviour of a particular pathogen if a particular resistance gene is introduced into the organism. For instance, the introduction of ESBL genes into *H. influenzae* has suggested that NCCLS testing methods may have difficulty in detecting such strains. Stepwise selection procedures have predicted the phenotype of vancomycin-intermediate *S. aureus*, or linezolid-resistant *Ent. faecium*. The use of hyper-mutable bacterial strains has also been proposed as a predictive strategy for the early detection of resistance mechanisms. All these techniques should provide insights into the expected phenotypic features of a possible resistant strain, to be considered when interpreting particular MICs or establishing surveillance cut-off values.

Intervention-orientated surveillance

The main goal of surveillance is intervention. Brilliant academic analysis of evolutionary trends of antimicrobial resistance constitutes a by-product of surveillance, but if intervention is not implemented as a result of surveillance programmes, the final outcome will be failure. On the other hand, intervention cannot be designed, planned or controlled without appropriate surveillance programmes. Any type of surveillance study should conclude, where appropriate, with a proposal for intervention based on the data obtained. Educational programmes in the field of antibiotic therapy and epidemiological inter-

ventions should take advantage of surveillance studies. Dissemination of information in the form of periodic resistance and intervention data bulletins is advisable.

Surveillance should always suggest, or even generate automatically, some type of reaction and/or intervention. For instance, surveillance data can lead to the removal of a given drug from an accepted official list of indications. There is an important controversy regarding the resistance rate that an antibiotic has to attain in a particular setting for declassification of its use for a particular clinical indication. In other words, the discussion has to do with the critical resistance level beyond which isolates of a particular species can be regarded generally as resistant to a given drug in a given place and over a given period of time. This aspect is of major importance for empirical therapy. Several reports on the treatment of UTI with particular drugs (e.g., co-trimoxazole, quinolones) have suggested that when resistance occurs in >10–20% of isolates, the corresponding antimicrobial agents should not be used for empirical treatment [49]. The establishment of these critical percentages for particular bacterium–antibiotic combinations depends mainly on the severity of the infections and on the availability of alternative therapies. What is clear is that use of an antibiotic regardless of the aforementioned 'critical' resistance rate will lead to a further selection of resistant bacterial populations, and probably of genetic vectors associated with resistance genes. If surveillance detects resistance in a dangerous organism, with no or few alternative drugs capable of controlling it, even a very low resistance rate should be considered high risk, and appropriate action should be planned.

Defining and updating activity spectra for inclusion in summaries of product characteristics (SPCs)

This is a process that should be based on surveillance programmes. To group species in the three therapeutic classes of antibiotic activity (see below) for inclusion in SPCs, according to the European norms for antibacterial medicinal products [50], European and national agencies for health product safety need both information on strain populations and general statistics on acquired resistance for the main bacterial species of medical importance. Indeed, for each antibiotic:

- the 'susceptible' category includes bacterial species that are susceptible naturally to the antibiotic in question [51] with prevalence ranges (expressed in percentage) of acquired resistance;
- the 'moderately susceptible category' includes bacterial species that present intermediate susceptibility naturally to the antibiotic in question (e.g., enterococci for penicillin G);
- the 'resistant' category includes the naturally resistant species (e.g., Enterobacteriaceae and mycobacteria for penicillin G) and naturally susceptible species in which the prevalence of acquired resistance is high (e.g., *S. aureus and Moraxella catarrhalis* for penicillin G).

Defining therapeutic indications of antibiotics

Provided that they are presented along with the few pieces of information that come usually with clinical samples submitted to medical microbiology laboratories (e.g., sampling site, inpatient or outpatient status), general statistics on acquired resistance can contribute to defining the antibiotic indications that figure in SPCs. However, statistics on resistance in documented infections within well-defined epidemiological contexts are of primary importance in this connection.

Establishing recommendations on antibiotic therapy and good antibiotic usage

In order to help prescribers, medical scientific societies and health authorities to establish national recommendations on antibiotic therapy and appropriate antibiotic usage, the prevalence of resistance must be established for given clinical situations (documented infections) in well-defined epidemiological contexts. This is the case with common infections (e.g., UTIs, lower respiratory tract infections, acute otitis media) and particularly severe infections (e.g., community-acquired pneumonia or meningitis in patients admitted to hospital emergency units). In this connection, resistance can be provided not only for each bacterial species separately (e.g., co-trimoxazole sensitivity rates in *E. coli* strains responsible for cystitis in women with no recent history of infection, antibiotic therapy or hospitalisation), but also for the species possibly involved taken as a whole (e.g., rates of sensitivity

of bacteria responsible for community-acquired or hospital-acquired bacteraemia to third-generation cephalosporins, aminoglycosides and fluoroquinolones).

For this purpose, it is also of importance to take into account parameters that are known to be linked significantly to the prevalence of resistance, and that constitute risk factors for resistance in the type of infection considered (e.g., age, history of antibiotic therapy, previous hospitalisation) which can be evaluated easily by the prescriber.

CONTRIBUTORS TO THIS DOCUMENT

G. Bahar (Ankara, Turkey), R. Cantón (Madrid, Spain), M. Edelstein (Smolensk, Russia), R. Finch (Nottingham, UK), M. Gniadkowski (Warsaw, Poland), H. Grundmann (Bilthoven, The Netherlands), P. Huovinen (Turku, Finland), T. M. Coque (Madrid, Spain), L. Martinez Martinez (Santander, Spain), A. Mazzariol (Verona, Italy), F. Navarro (Barcelona, Spain), C. E. Nord (Huddinge, Sweden), R. Norrby (Solna, Sweden), L. Peixe (Porto, Portugal), I. Phillips (London, UK), A. Rodloff (Leipzig, Germany), P. Schrijnemakers (Bilthoven, The Netherlands), G. Skov Simonsen (Tromsø, Norway), O. Stetsiouk (Smolensk, Russia), M. Struelens (Brussels, Belgium) and J. Vila (Barcelona, Spain).

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