



Confronting bacterial resistance in healthcare settings: a crucial role for microbiologists

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Bacteria that are resistant to antimicrobial agents, which were previously isolated primarily in acute-care hospitals, now cause infection in a wide range of other healthcare settings. Improved detection of new resistant strains — especially by using practical and affordable screening methods and by evaluating mechanisms of resistance — is a priority for tackling this problem effectively. Standardized, effective surveillance systems for evaluating the emergence and prevalence of resistant strains are necessary to assess the success of intervention strategies.

Antimicrobial resistance in bacteria is of great concern to healthcare practitioners^{1,2}. The increase in bacteria that are resistant to frequently used anti-infective agents is now well-documented³. Some of these healthcare-associated organisms are resistant to all commonly used antimicrobial agents^{4,5}. Whereas resistant bacteria were previously common only in intensive-care units, more recently, these organisms have been recovered from non-intensive-care hospital inpatient areas and are now isolated with increasing frequency in extended-care facilities, ambulatory surgical units, home-healthcare sites and other healthcare settings^{6–8}. In addition, resistant organisms that arise in the community are now also spreading in healthcare settings⁹.

Bacterial drug resistance has many consequences, which include increased mortality and morbidity, increased expenditure for patient care and a reduction in the availability

of useful drugs for future patients^{10–13}. As the scale of the resistance problem, measured in terms of the total number of resistant organisms that are isolated, continues to increase, it is imperative that microbiologists stay abreast of the patterns and causes of antimicrobial resistance in bacteria, so that laboratory resources can be efficiently directed towards minimizing this problem.

This article highlights how changing patterns of both resistance mechanisms and their prevalence in pathogenic bacteria present problems for the medical and microbiology communities. Challenges in providing the appropriate responses to this problem are also considered.

New problems related to resistance. The emergence of resistant bacteria has taken place over several decades. However, in the past ten years, new facets of clinical resistance have emerged (TABLE 1), which have led to new challenges for microbiology service-providers.

The emergence of new patterns of resistance can alter the use of antimicrobial agents. For example, the emergence of vancomycin-intermediate strains of *Staphylococcus aureus* (VISA; minimum inhibitory concentration (MIC) 8–16 µg ml⁻¹) — which was first detected by isolation from a patient in Japan and then by isolation from patients in many other countries — was followed by reports of the isolation of two strains of vancomycin-resistant *S. aureus* (VRSA; MIC ≥32 µg ml⁻¹)

from two different areas of the United States^{14–16}. Detection of both VISA and VRSA has proved difficult in clinical laboratories using automated methods^{17,18} because changes that lead to increased MICs are not readily detected by the analyses and interpretation algorithms that are used in many automated systems^{19,20}. If resistance to vancomycin among staphylococci continues to develop and spread, the use of vancomycin, which has long been considered to be the mainstay for the treatment of methicillin-resistant strains of *S. aureus* (MRSA), could be severely compromised.

Similarly, during the past decade, strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have arisen that are resistant to all the antimicrobial agents that are usually tested^{4,21}. These organisms have become important hospital pathogens, especially in patients with hospital-onset pneumonia, so loss of the usual antimicrobial agents for treatment can dramatically interfere with patient care²². Increasing resistance in these organisms has resulted in the treatment of patients with drugs such as colistin and polymyxin B, which were essentially abandoned in the 1970s because of their unacceptable levels of toxicity compared with other antibiotics²³. Unfortunately, few laboratories now have the capability to test whether organisms are susceptible to both of these older antimicrobials, because appropriate testing materials are not widely available and the interpretation of test results is not straightforward.

Resistance to several different classes of antimicrobial agent continues to develop in organisms, such as *Acinetobacter*, that have clinically significant, but difficult to detect, resistance phenotypes²⁴. This trend fuels the demand for reliable, practical and cost-effective detection methods for identifying strains that show new resistance patterns. This need also is illustrated by the identification of bacteria that have multiple resistance mechanisms^{25,26}. Recent studies indicate that the frequency of multidrug resistance is increasing²⁷.

Table 1 | Challenges arising from emerging resistance in healthcare settings and resulting microbiological needs

| Challenges | Examples | Microbiological need |
|--|--|---|
| Emergence of resistance in clinically important organisms | VISA ¹⁴ ; VRSA ¹⁵ ; <i>Acinetobacter</i> species resistant to all routinely tested agents ⁴ | Reliable, practical and cost-effective methods for detection of new resistant strains |
| Standard testing methods are inadequate, impractical or costly | VISA ¹⁷ ; ESBL-producing Enterobacteriaceae ²⁸ | Development of new screening methods that are practical and affordable |
| Resistance to substituted drugs might emerge when their use increases | Linezolid or quinupristin-dalfopristin for MRSA and VRE ³⁴ ; colistin for <i>Acinetobacter</i> spp. ³⁵ | Reliable, practical and cost-effective methods to detect resistance to alternative and investigational drugs |
| Prevalence of resistance might increase through both transfer of genetic determinants and clonal dissemination | VRSA ¹⁵ ; <i>Enterococcus faecalis</i> ⁶⁸ ; ESBL-producing Gram-negative bacilli ⁷¹ | Genetic and immunological methods to examine modes of action and spread of organisms; typing systems to distinguish strains |
| Resistance prevalence might vary in different healthcare settings (for example, hospitals and nursing homes) | MRSA and ciprofloxacin-resistant strains of <i>Pseudomonas aeruginosa</i> in acute-care hospitals ^{41,42} | Standardized surveillance systems to monitor emergence and changes in bacterial resistance |
| Prevalence of resistance might vary by geographical location | Increases in community-onset MRSA infections span the globe ⁴⁴ | Methods to standardize and audit susceptibility-testing procedures |

ESBL, extended-spectrum β -lactamases; MRSA, methicillin-resistant *Staphylococcus aureus*; VISA, vancomycin-intermediate *S. aureus*; VRE, vancomycin-resistant enterococci; VRSA, vancomycin-resistant *S. aureus*.

Resistance to expanded-spectrum cephalosporins in strains of *Enterobacteriaceae* that contain extended-spectrum β -lactamases (ESBLs) can be difficult to detect using routine antimicrobial-susceptibility test methods²⁸. The presence of ESBLs in some organisms produces few or no phenotypic changes in MICs or in the ZONE DIAMETER of the affected organisms that can be detected by routine methods²⁹. Although the National Committee for Clinical Laboratory Standards (NCCLS, USA) has developed methods to screen for ESBLs, many other types of β -lactamases, such as AmpC enzymes, continue to pose problems of detection and interpretation³⁰. In addition, false-negative results can arise when the inoculum is not carefully controlled³¹. This makes it necessary to develop new screening methods for the detection of organisms such as VISA, VRSA and those containing ESBLs that are both practical and affordable for clinical and public-health laboratories³².

The emergence of bacterial strains that are resistant to routine therapies increases the need for susceptibility testing of alternative therapeutic agents. For example, the recent increase in the prevalence of resistance to fluoroquinolones in Gram-negative bacilli makes it necessary to rethink the usual empirical treatments for these organisms³³. Similarly, routine testing of linezolid, quinupristin/dalfopristin and daptomycin might now be requested when VISA or VRSA strains are encountered, and routine testing of colistin or polymyxin B might be added when *A. baumannii* strains are resistant to aminoglycosides, β -lactam-inhibitor combinations and carbapenems^{34–36}. Monitoring resistance to these alternative drugs has become essential for patient care, and is also important because rises in their use increase the possibility that

bacteria will become resistant to their action. Drug resistance can develop rapidly — for example, resistance to linezolid can occur through a single-step change in the ribosomal-RNA coding sequence^{37,38}. When resistance precludes the use of standard drugs, it can also increase the need for testing for susceptibility to investigational drugs (that is, those that are not yet licensed in a given country or community), such as the testing against MRSA or vancomycin-resistant enterococci (VRE) of tigecycline, which is not licensed in the United States at present³⁹.

For many bacteria that cause healthcare-associated infections, the prevalence of resistant isolates varies by location in the institution (for example, between intensive-care units (ICUs) and non-ICU areas in hospitals) (FIG. 1)^{6,40}. Patterns of resistance in *S. aureus* illustrate this point — data from Project ICARE (Intensive Care Antimicrobial Resistance Epidemiology), a cooperative surveillance initiative involving about 40 hospitals around the United States, show differences in the prevalence of MRSA isolates from ICU and non-ICU settings. It has also shown an increasing prevalence of MRSA in both ICU and non-ICU hospital areas during three periods surveyed between 1996 and 2002 (REFS 41,42) (FIG. 2). The prevalence of resistance to selected antimicrobial agents in Gram-negative aerobic bacilli also varies by healthcare unit. For example, the prevalence of resistance to ciprofloxacin in *P. aeruginosa* in the Project ICARE hospitals has rapidly increased in the ICU setting^{41,42} (FIG. 3). Similar increases have also been documented among the ICARE hospital populations for isolated organisms from non-ICU hospital areas.

In addition to these local variations, changes in resistance patterns can affect

whole cities, regions or countries, and some changes are evident across national boundaries^{34,3}. The appearance of community-onset MRSA infections has been reported from such diverse parts of the world as North America, Japan and Western Australia⁴⁴. This emphasizes the need for reliable surveillance systems that track both healthcare-associated and community-based resistance, with local, regional, national and international scope⁴⁵. However, such systems would need to be standardized and validated to make results comparable⁴⁶.

Initiatives for resistance control
Microbiological initiatives are discussed in turn, followed by a consideration of the challenges that they present.

Methods for detecting new resistant strains.

As resistant strains continue to emerge, diagnostic laboratories must be ready to respond with detection methods that are reliable, practical, and cost-effective³. For many laboratories, this entails continual validation of their testing methods. One problem that has been reported with increasing frequency is that organisms with new resistance profiles, or new mechanisms of resistance, are difficult to detect using either automated susceptibility testing or disk diffusion^{17,28,47}. However, proficiency-testing programmes that focus on antimicrobial resistance, such as the External Quality Assurance System of the World Health Organization (WHO), enable microbiologists to ensure that their testing methods are capable of detecting emerging resistance mechanisms^{48,49}. Proficiency testing is an external quality-assurance method, in which laboratories are sent simulated clinical specimens,

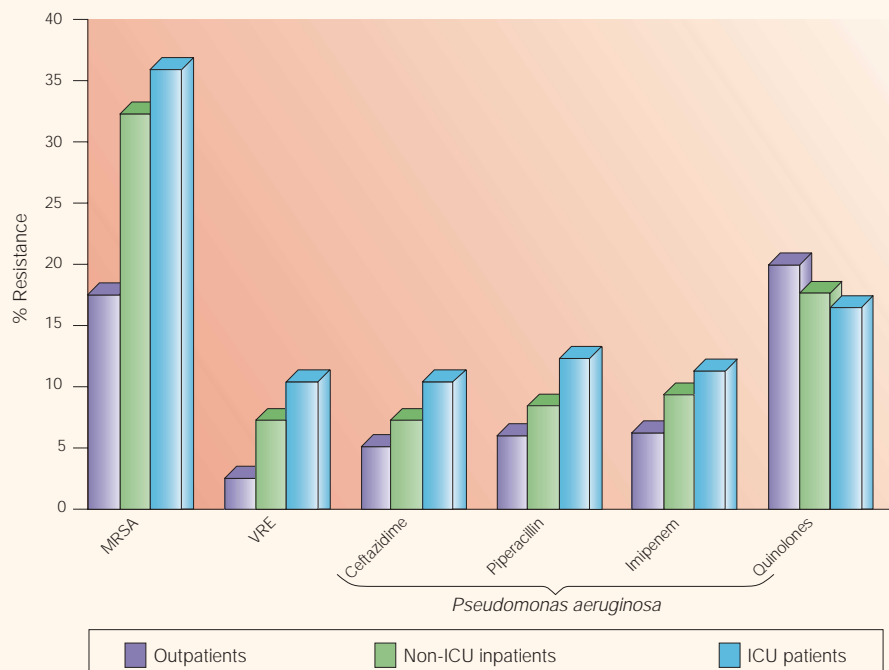


Figure 1 | Prevalence of resistance by organism/drug combination and hospital setting. Prevalence of pooled mean rates of resistance (%) in isolates of *Staphylococcus aureus* with resistance to methicillin (MRSA), strains of *Enterococcus faecalis* with resistance to vancomycin (VRE), and strains of *Pseudomonas aeruginosa* with resistance to ceftazidime, piperacillin, imipenem and quinolones (either ciprofloxacin or ofloxacin). Prevalence is shown by hospital area (outpatients, inpatients not in intensive-care units (non-ICU inpatients), and ICU patients). The data were obtained from 41 hospitals in the United States participating in Project ICARE Phase 2, from 1996–1997. The prevalence of resistance for each drug/organism combination varied significantly for each hospital area shown. $P < 0.01$ (using a chi-square test) for all comparisons. Modified with permission from REFS 6,40. © (1999) University of Chicago Press; © (1999) Mosby.

or bacterial isolates, for testing using routine laboratory methods⁴⁹. Test results from participating laboratories are then compared with those that are obtained by a reference laboratory using 'gold-standard' methods. This provides information about the accuracy of susceptibility testing, determines whether a laboratory's methods are sensitive enough to detect new resistance patterns, and allows the performance of laboratories to be compared. One aspect of susceptibility testing that is often overlooked is the importance of the correct identification of the organism to the genus and species levels prior to interpreting the test results. This is particularly important when using automated methods, such as the MicroScan Walkaway (Dade Microscan, Inc., USA) or Vitek 2 (BioMérieux Vitek, Inc., USA) systems, in which species identification may be used to select the appropriate algorithm for interpretation. Newer methods of detecting resistance are not necessarily more expensive — for example, a new method for detecting MRSA uses a ceftazidime disk test⁵⁰, which is cheap, but very accurate⁵¹.

Determining the bacterial genotype that is related to resistance determinants, rather

than the resistance phenotype of the isolate, such as the results of a disk-diffusion or MIC test, has the advantage of speed and specificity. For this purpose, commercially prepared molecular tests are becoming available — for example, several amplification-based methods have been described for the detection of methicillin-resistant *S. aureus*^{52,53}. However, not all rapid genetic tests target DNA sequences. The latex-agglutination test for the altered staphylococcal penicillin-binding protein PBP2a, which is an antibody-based test for the product of the *mecA* gene, is both sensitive and specific for MRSA⁵⁴.

Challenges. All new tests must be field-tested and validated before their widespread use can be recommended⁵⁵. In some areas, such as the United States, automated systems for susceptibility testing dominate the commercial market. Both local and national surveillance data suffer if these systems have problems in identifying certain resistant organisms^{17,20}. The standardization of test procedures and test interpretation from nation to nation and from region to region is a crucial challenge to worldwide surveillance for emerging resistance⁵⁶. It is important to remember that

molecular techniques can only detect genotypes for which polymerase chain reaction (PCR) primers or DNA probes exist. The increasing complexity of determinants of multidrug resistance means that the use of multiplex PCR assays, microarrays, matrix assays and other multifaceted assays will be needed to fully characterize new resistant organisms⁵⁷. Some progress has been made in the development of 'signature chips' for bacterial identification and susceptibility testing^{58–60}. Potential impediments to the use of signature chips for testing include intellectual-property issues, the need for training of laboratory personnel, and the requirement for the approval of all diagnostic tests by regulatory agencies. For example, would development of a new matrix assay by the addition of two or three new nucleotide sequences to detect a new resistance mechanism be slowed down significantly by the need for the developer to seek a patent for the changed panel? Would small changes of this type be hindered in the United States by the slow process of Food and Drug Administration (FDA) approval of new or modified diagnostic tests? These issues will become important as the cost of reagents and processing is substantially reduced to the point where microarray analyses become practical for use in diagnostic laboratories⁶¹.

Practical and affordable screening methods.

The difficulties of detecting vancomycin resistance in enterococci — particularly for *vanB*-containing strains, which show low-level resistance to vancomycin (MIC $>4 \mu\text{g ml}^{-1}$) — led to the recommendation that a vancomycin-agar screen-plate (Brain-Heart Infusion agar containing $6 \mu\text{g ml}^{-1}$ vancomycin) should be inoculated in conjunction with automated susceptibility-testing methods⁶². The failure of several automated susceptibility-testing methods and disk-diffusion methods to detect VISA strains reinforced the need for additional screening methods for emerging vancomycin resistance in *S. aureus*³². More recently, a VRSA strain from Pennsylvania in the United States (vancomycin MIC $32 \mu\text{g ml}^{-1}$) was not detected by automated methods, a finding that further reinforces this need²⁰. Screening tests, including the ceftazidime disk test for MRSA, the double disk test (D test) for inducible clindamycin resistance in staphylococci, and testing with clavulanic acid to detect ESBLs, are burdensome in terms of time and effort, but significantly increase the accuracy of the antimicrobial-susceptibility test reports that physicians use to select anti-infective therapies for their patients²⁸.

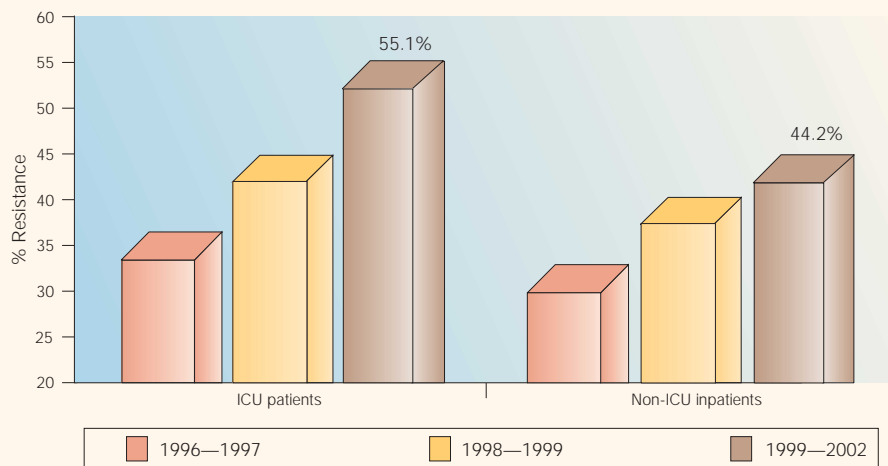


Figure 2 | Prevalence of methicillin-resistant *Staphylococcus aureus* by year and hospital area. Changes in pooled mean prevalence rates of resistance in methicillin-resistant *S. aureus* (MRSA) isolates recovered from patients in intensive-care units (ICUs) and from inpatients not in ICUs (non-ICU) at Project ICARE/Antimicrobial Use and Resistance hospitals, from 1996 to 1997, compared with 1998–1999 and 1999–2002. The x axis shows the prevalence rate of resistance of all *S. aureus* strains to methicillin; 55.1% of *S. aureus* strains from ICUs in 1999–2000 were resistant to methicillin (that is, they were MRSA strains). Modified with permission from REFS 41,42. © (1999) Centers for Disease Control and Prevention; © (1999) Mosby.

Challenges. Screening tests increase the sensitivity of detection for new resistance mechanisms; however, they can place an extra burden on the clinical laboratory when a lack of screening-test specificity generates the need for large numbers of confirmatory tests. For example, the use of NCCLS-recommended screening tests for ESBL production should not be extended to Enterobacteriaceae other than *Escherichia coli* and *Klebsiella* species, as this would result in changes in categorical interpretation for less than 2% of the organisms tested, and would require a

great deal of additional testing of isolates⁶³. Some hospitals, such as small rural hospitals in some areas of the United States, may produce unreliable results from screening tests because confirmatory tests are not available to validate screening assays for resistance⁶⁴. Workload and accuracy must both be included in the assessment of proposed new screening tests. In addition, the extra time-lag in the reporting of results that might be introduced by the sequential use of screening and confirmatory tests must be considered.

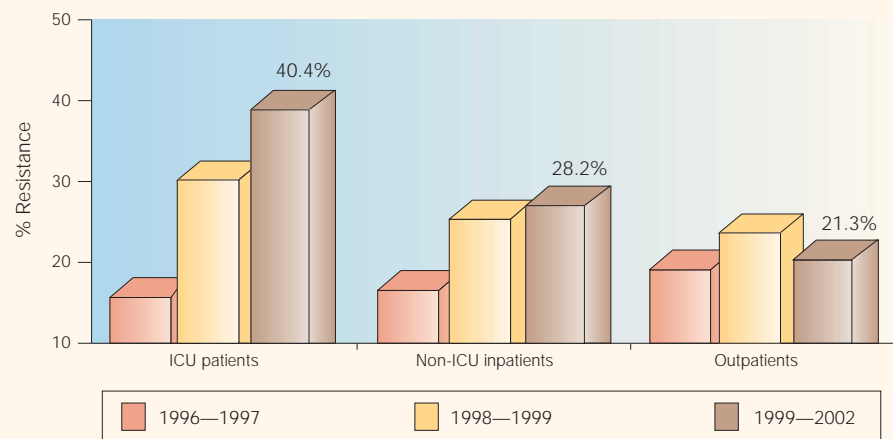


Figure 3 | Prevalence of ciprofloxacin-resistant *Pseudomonas aeruginosa* by year and hospital area. Changes in pooled mean prevalence rates (%) of ciprofloxacin resistance in *P. aeruginosa* isolates recovered from patients in intensive-care units (ICUs), from inpatients not in ICUs (non-ICU) and from outpatients at Project ICARE/Antimicrobial Use and Resistance hospitals from 1996–1997, compared with 1998–1999 and 1999–2002. Modified with permission from REFS 41,42. © (1999) Centers for Disease Control and Prevention; © (1999) Mosby.

Susceptibility to alternative and new drugs. Diagnostic-microbiology laboratories are often asked to perform tests for new compounds that might have activity against infections that are caused by multiply resistant Gram-positive and Gram-negative organisms. For Gram-positive organisms, the use of quinupristin/dalfopristin for VRE and of evernimycin for MRSA are two pertinent examples of this⁶⁵. Whereas experimental disks for agar-disk diffusion testing were provided for the quinupristin/dalfopristin combination, Etest strips (AB BioDisk, USA) were made available for testing for evernimycin. Provisional interpretive criteria were provided, but the number of non-susceptible results for the quinupristin/dalfopristin combination, due to its relatively poor activity against *Enterococcus faecalis*, complicated the interpretation of the results. For Gram-negative organisms, many laboratories now test multiply resistant *P. aeruginosa* and *A. baumannii* for susceptibility to polymyxin B or colistin using disk-diffusion assays; however, disk results often show little correlation with MIC results⁶⁶, which complicates the interpretation of test results for clinicians.

Challenges. Reliable, practical and cost-effective methods for testing the susceptibility of bacteria to new drugs are essential in an era of changing clinical needs, but such tests are often unavailable. In the United States, pharmaceutical companies should work closely with the NCCLS and the FDA to establish quality-control ranges and interpretive criteria as quickly as possible. This will provide increased assurance that susceptibility-test results that are provided for drugs in compassionate-use programmes, and those pending approval, will be accurate and clinically useful. Similar cooperation among pharmaceutical companies, diagnostic companies and regulatory agencies is also needed in other countries. Such efforts are now being made in Europe⁶⁷. Further cooperative efforts between the European Committee for Standardization (CEN), the NCCLS and the International Organization for Standardization (ISO) may also lead to progress.

Genetic and immunological methods. Public-health programmes that minimize the impact of resistance depend on an accurate assessment of the source of resistance determinants, their reservoirs and the mode of spread of the resistant organism. For example, genetic relatedness among strains of *E. faecalis* that have transposon-mediated resistance to gentamicin was associated with the dissemination of genetically related clones

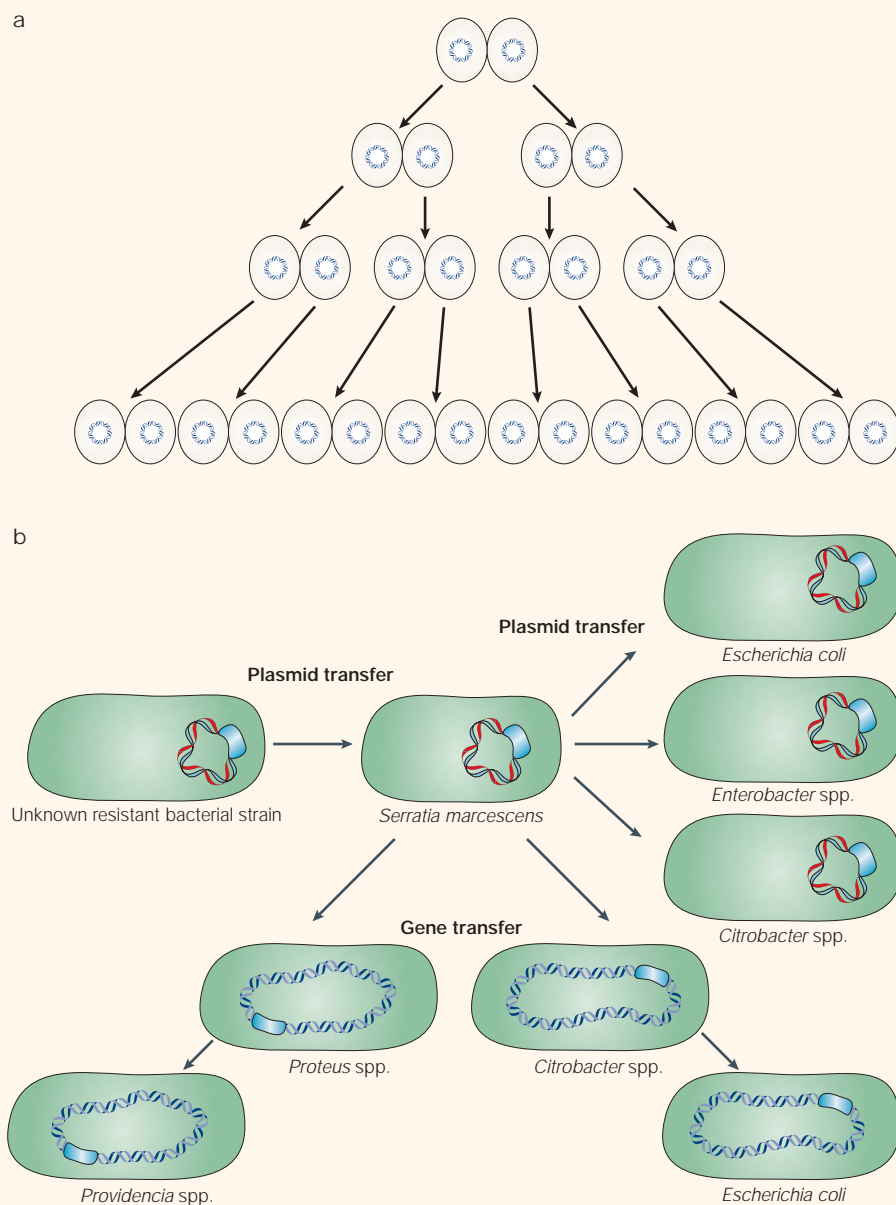


Figure 4 | Mechanisms of spread of bacterial resistance. a | Dissemination of a vancomycin-resistant enterococcus carrying the *vanA* resistance gene on a plasmid (blue circle). This represents the clonal spread of an organism in a hospital. The enterococci are shown as cell pairs, although in stained preparations single cells or clusters of cells are observed. **b** | Dissemination of an aminoglycoside-resistance gene (indicated as blue bars on plasmids) by the spread of the resistant strain, the spread of the plasmid containing the gene, and the spread of the gene on a transposable element. The plasmid that contained the resistance gene was introduced from an unknown strain into an isolate of *Serratia marcescens*, which disseminated and spread among patients in the hospital. The plasmid then transferred to other members of the Enterobacteriaceae (*Escherichia coli*, and *Enterobacter* and *Citrobacter* species) by conjugation, followed by transposition of the resistance gene to other plasmids in *Proteus* and *Citrobacter* species. The new plasmids containing the resistance gene were then transferred by conjugation to *Providencia* species, and to other strains of *E. coli*.

in southern Sweden (CLONAL DISSEMINATION)⁶⁸. By contrast, the high-level resistance to vancomycin in the VRSA strain reported from Michigan was associated with the acquisition of the *vanA* resistance determinant from a vancomycin-resistant enterococcus (LOCAL SELECTION)^{15,69}. Both of these patterns, local selection and clonal dissemination (FIG. 4),

occur in healthcare settings⁷⁰. In some cases, more than one mechanism of spread is found. For example, an outbreak of multidrug-resistant *Klebsiella pneumoniae* clinical isolates in a hospital in Mexico was associated with the spread of an epidemic strain, as well as with the dissemination of a multidrug-resistance plasmid to other clinical isolates,

which was presumably enhanced by the pattern of drug usage in the hospital⁷¹. Methods to distinguish between these types of spread are therefore crucial for devising control measures.

The design of a protocol to evaluate elements of the resistance pattern requires a method for the identification of genetic elements of resistance — in order to detect the transfer of resistance determinants from one organism to another — and a typing system that can assess similarities or differences between organisms among a collection of isolates. Rapid methods of detecting resistance genotypes using PCR have been investigated using 'in-house' methods (that is, those that are developed and used only by certain laboratories) for almost a decade, in both clinical and research settings⁷². In addition, commercial tests that use line-probe (reverse DNA-hybridization-based) assays⁵⁷ for resistance genes in *Mycobacterium tuberculosis* and *Helicobacter pylori*, cycling probe assays for *mecA* in *S. aureus*, and antibodies to the staphylococcal PBP2a protein (the presence of which also indicates oxacillin resistance in staphylococci), have been developed as rapid tests for resistance determinants.

Clinical-microbiology laboratories are often asked to provide support for epidemiological studies by processing surveillance cultures and providing strain-typing data for the isolates recovered. Laboratory data are frequently combined with epidemiological data to determine the frequency of and risk factors for the transmission of resistant organisms in healthcare settings⁷³. Examples include studies of VRE⁷⁴ and *Acinetobacter*⁷⁵. The establishment of reference panels of organisms that have been characterized by several typing systems often helps to identify the patterns of spread of resistance genes in healthcare institutions⁷⁶.

A more detailed understanding of the origins of antimicrobial-resistance genes may also facilitate successful interventions for slowing the spread of antimicrobial-resistant bacteria⁷⁷. At present, more than 120 bacterial genomes have been completely sequenced, and the sequencing of more than 100 other genomes and resistance plasmids is underway. For example, the plasmid sequence for one of the VRSA strains has been completed (see [The Institute for Genomic Research *S. aureus* Michigan VRSA genome web page](#))⁷⁸. The abundance of microbial genome sequences enables the construction of detailed phylogenies for antimicrobial-resistance genes⁷⁹. This in turn makes it possible to deduce when a gene was acquired by an ancestral strain or species⁸⁰. All organisms that are

Glossary

ANTIBIOGRAM

The results of all susceptibility tests for a given organism considered as an overall group. The term is often used to refer both to such a profile for an individual organism and to cumulative summaries of such profiles for a given period of time.

ANTIBIOTYPE

The subgroup into which a bacterial organism is classified on the basis of a comparison of its antibiogram with those of organisms of the same genus and species. The antibiotype is the result of the application of a typing system on the basis of this phenotypic characterization.

CLONAL DISSEMINATION

The spread of a single strain of a bacterial organism, or of a single resistance determinant, in a given geographical area, or the introduction of such a strain or element from a geographically distant population.

LOCAL SELECTION

The proliferation and spread, usually under the influence of local antimicrobial use, of resistant bacteria that have either accumulated mutations that reduce their susceptibility to antimicrobial agents or have acquired novel resistance genes by direct DNA transfer

PRINCIPAL-COMPONENT ANALYSIS

A means of representing multidimensional data in a reduced-dimension space to obtain an overview of the data.

ZONE DIAMETER

The measurement (in millimetres) of the diameter of the zone of inhibition of organism growth around a disk that contains a standard amount of an antimicrobial agent. Used in one method of standardized susceptibility testing (agar-disk diffusion) to define the activity of the drug against a bacterial organism.

descended from the ancestor that acquired a resistance gene are likely to contain a version of that gene. In cases in which a resistance gene has been acquired recently, phylogenetic analyses can be combined with geographical information about the location in which various resistant isolates were collected to generate hypotheses about how widely dispersed a resistance gene has become⁸¹. In addition, an experimental method for determining whether a strain contains cryptic antimicrobial-resistance genes has been described, in which a transposable element that contains a promoter is used to induce the expression of downstream genes in a microbial genome⁸².

Challenges. Relatively few laboratories take advantage of rapid techniques for detecting antimicrobial resistance. The ability to provide strain-typing data beyond profiles of antimicrobial resistance — often called ANTIBIOGRAMS — or simple biochemical profiles is usually restricted to larger clinical laboratories in academic centres or reference laboratories. The continued development of tests that are practical for clinical and public-health laboratories to implement is needed. Many typing systems that have been developed so far suffer from the need for subjective analysis. For example, the examination of pulsed-field gel electrophoresis data requires visual inspection of DNA-banding patterns, because the software that is required for gel analysis remains prohibitively expensive for many laboratories⁸³. The development of new tests that remove subjectivity from interpretation should improve the communication and comparison of typing results from different laboratories, regions and areas.

Surveillance systems for monitoring resistance. Monitoring the occurrence of — and trends in — bacterial resistance is the first step towards the development of appropriate practice guidelines for the care of patients with infectious diseases⁸⁴. In addition, these data can be used to identify outbreaks, identify organisms or organism groups for which new therapeutic agents are needed, and allow relationships between resistance and exposure variables — such as antimicrobial use — to be evaluated⁸⁵. Such data may also facilitate the development of new programmes to control and prevent resistance in different regions or nations⁸⁴. Timely information about changes in patterns of bacterial resistance helps to optimize empirical treatment. Many laboratories provide such information on susceptibility patterns to assist prescribers with empirical treatment decisions. For example, a representative study of hospitals in the United States for the period 1999–2001 found that >90% of the centres that were studied compiled summaries of antimicrobial-susceptibility patterns³. Information on resistance patterns is useful for agencies that are engaged in antimicrobial research and drug development, and for public-health planners, as well for groups that are developing guidelines⁸⁵. Of course, surveillance must also be worldwide^{44,86,87}. Recent efforts have led to the formation of cooperative networks that pool and summarize resistance information from individual hospitals into community, regional, national and international summaries^{33,41,88–91}. Examples that are available on the internet include the WHO **Antimicrobial Resistance Info Bank**, the **European Antimicrobial Resistance Surveillance System (EARSS)** and **Project ICARE** in the United

States. These programmes have provided important perspectives on national and international antimicrobial-resistance trends. Studies of this type have also been used to document epidemiological patterns that underlie resistance, such as the extent to which selected resistant bacteria cluster in individual hospitals⁹². Reports from individual microbiology laboratories form the backbone of these surveillance systems, so the quality control of data from each participating source is of paramount importance. The use of standardized definitions and interpretations by all the participating laboratories is a necessity⁴⁵. There is a specific need to develop a standardized description of multidrug-resistance patterns — which is analogous to the efforts that are being made to define families of antimicrobial-resistance genes⁹³.

Resistant organisms that arise in the community have an important role in determining resistance patterns that occur in hospitals and elsewhere in the healthcare system. Examples of this include the links between patterns of resistance inside and outside hospitals for relevant organisms, such as *E. coli*, *Acinetobacter* and *S. aureus*^{73,75,94}. This poses a new challenge, as the relevant laboratory detection and reporting systems for resistant organisms that arise in the community may not necessarily be attached to healthcare systems. So, freestanding, referral and public-health laboratories may also need to be involved in surveillance activities if a true picture of regional or national patterns is to be obtained. This important facet has been recognized in the United States by a group of Emerging Infections Program (EIP) centres, which are organized and supported by the Centers for Disease Control and Prevention (CDC). The EIP centres are a population-based network of investigators in selected geographical areas of the United States that conduct surveillance of several infection problems, including resistant bacteria. As the EIP centres include all of the laboratories in the geographical region among their information sources, information can be obtained on both community-based and healthcare-based organisms that cause resistance. Similar networks exist in other countries and regions — examples include the DAN-MAP program, which covers all of Denmark, and the EARSS, which conducts surveillance in 28 European countries^{95,96}.

Challenges. Some laboratories do not compile annual summaries of susceptibility-test results — for example, a study in the United States showed that only 50% of small rural

hospitals provide this service⁶⁴. The provision of these data in the United States may become more uniform in the future, because a requirement to provide an annual summary is being incorporated into the laboratory-accreditation requirements of the College of American Pathologists⁹⁷. Some population-based programmes for the surveillance of resistance, such as the EIP, cover only a small portion of their region or country⁹⁸. Other voluntary reporting systems enrol only a small proportion of the microbiologists and laboratories that are likely to encounter emerging resistant bacteria, such as VRSA. Problems associated with bacterial resistance that are confined to the community are also increasing, but are outside the scope of this review.

Several surveillance systems for antimicrobial resistance are directed or funded entirely by pharmaceutical manufacturers. These surveillance programmes often focus on areas or settings in which drugs that are produced by the sponsor will provide the best outcomes. To deal with the biases that are inherent in these sponsored programmes, guidelines are needed to ensure that conflicts of interest and disclosures of sponsorship are reported fairly and accurately in publications and presentations⁹⁹. It is encouraging, however, that a recent comparison of resistance frequencies from a population-based study in the United States and from commercially sponsored surveillance programmes showed comparable patterns³. Financial support for such surveillance from sources other than industry needs to be increased. Guidelines for dealing with conflicts of interest and for the reporting of payments or other business relationships must also be developed for cases in which external microbiologists are asked to review company data or surveillance information and produce a summary or review article for the medical literature^{100,101}.

Conclusion

The emergence of antimicrobial resistance has become a fact of life for those who deal with bacterial infections. Attempts to deal with the problems that result from these changes will require techniques that include the use of ANTIBIOTYPES, PRINCIPAL-COMPONENT ANALYSIS, PHYLOGENETICS, POPULATION-GENETICS analysis and the evaluation of data from large, multinational surveillance studies¹⁰². As the magnitude of resistance and its consequences continues to increase, microbiologists must constantly work to stay abreast of the patterns and causes of antimicrobial resistance in bacteria, so that laboratory resources can be efficiently directed to minimizing this problem.

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Competing interests statement

The authors declare competing financial interests; see web version for details.

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