

New Technologies for Infection Diagnosis and Control **6**

Three major options exist for the control of bacterial diseases: 1) disrupt or halt transfer of bacteria from person to person and from the environment to people, 2) treat cases of disease with antibiotics, and 3) prevent disease through vaccination. This chapter describes diagnostic methods that guide the selection and use of antibiotics, the use of vaccines, methods for delivery of high concentrations of antibiotics to areas of localized infections, devices and materials designed to reduce the transfer of bacteria in the hospital, and some treatment methods used before the antibiotic age.

The cartoon, which is adapted from one that originally appeared in *Science*, is a humorous look at the serious problem posed by bacteria resistant to all available antibiotics. Some bacteria are expected to develop resistance to any antibiotic introduced into medical practice. Therefore, continued improvement in infection diagnosis and control is necessary to optimize the use of antibiotics and slow the spread of resistant bacteria.

DIAGNOSTIC METHODS

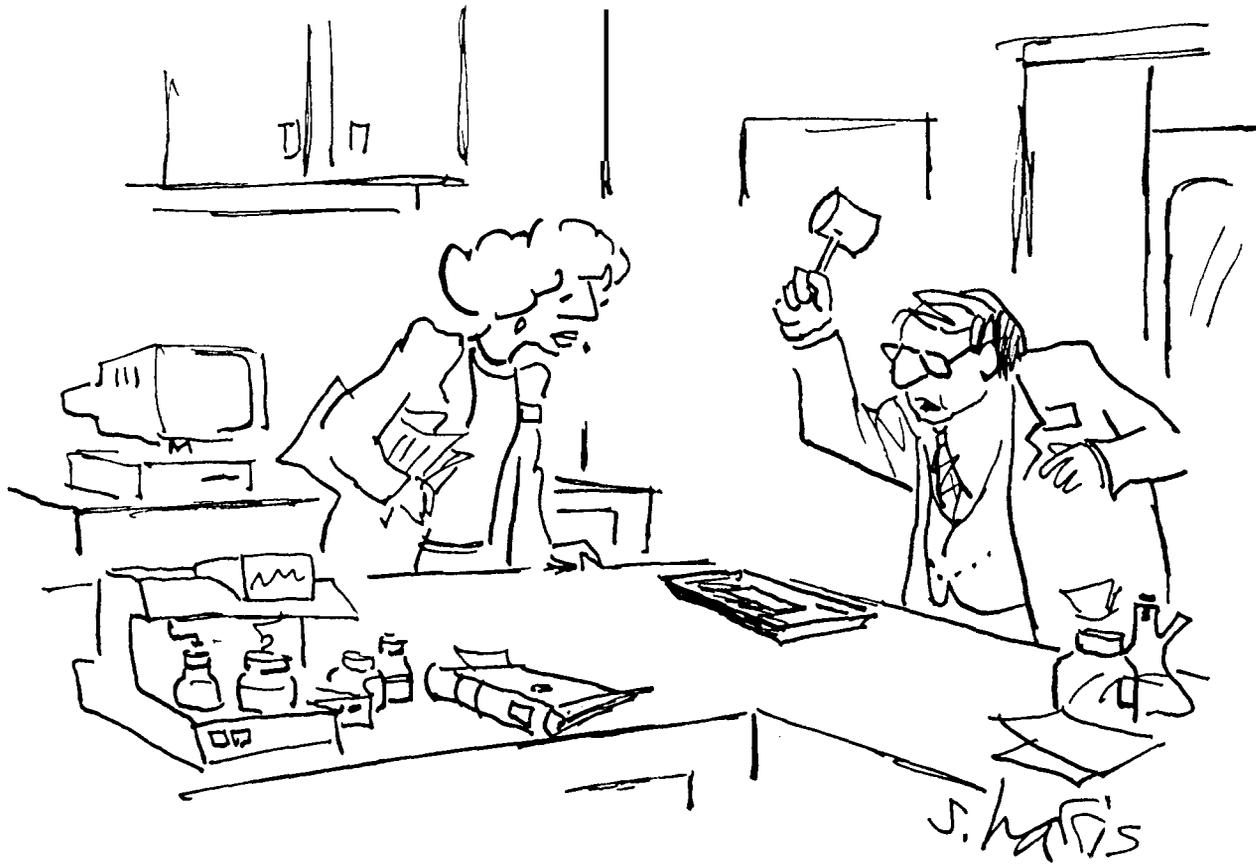
In the future, science may develop a small device, such as the “tricorder” used in the TV series *Star Trek*, that physicians can pass over the

body of a sick person to identify the cause of a disease. Such methods are far in the future, and current techniques used to identify bacteria and susceptibility patterns are “traditional methods” that have been developed over the last century. Newer methods that involve techniques from molecular biology and modern instrumentation—not immediately at the level of *Star Trek*—promise to make identification and characterization faster and more certain.

■ Traditional Methods for the Identification of Bacteria

Some experts estimate that there may be a million different bacteria and that scientists have identified only one percent (10,000 species) of that total. Of those 10,000, only a fraction have been associated with human diseases.

When seeing a patient, a physician will ask questions, make observations, and perform tests to determine which bacteria are likely to be associated with an illness and to choose an antibiotic treatment. The physician may swab the throat in the case of a sore throat or obtain a sample of urine in the case of a urinary tract infection. The collected material on the swab or the urine can be stained with diagnostic dyes, such as the Gram stain (see chapter 2), and examined under a



"NOW FOR THOSE DRUG-RESISTANT BACTERIA..."

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microscope. Distinctive shapes and staining properties facilitate reliable preliminary rapid identification of the bacteria causing infection.

Collected samples may contain such low numbers of bacteria as to make finding them under the microscope difficult. The staining properties and shapes of the bacteria may not be unique and therefore not identifiable. The sample may contain a mixture of bacteria, as is common in faecal samples. To identify the bacteria in those cases, the physician sends a biological sample of some kind—a volume of blood **or** pus **or** other exudate, a scraping or swab from the throat or other orifice, a sample of urine or feces—to a microbiology laboratory.

In the laboratory, the sample is transferred to culture media specifically designed to encourage

the growth of certain pathogenic bacteria and to prevent the growth of others such as commensal bacteria that may be present in samples from both healthy and sick individuals. The bacteria that are able to grow form visible colonies on agar-based media in a Petri dish or grow in broth so that the broth becomes turbid, as apple cider does when yeast grow in it. In both the collection and handling of the sample, health care personnel must be careful to avoid contamination with the bacteria that grow literally everywhere, on the patient's and physician's skin, on the surfaces of furniture and unsterilized devices in the examining room, and on apparatus in the diagnostic laboratory.

Microbiologists can sometimes look at and smell the colonies or liquid cultures and, based

on their knowledge and experience, identify the bacteria in the sample. They may be able to dismiss some bacteria from further consideration by recognizing them as contaminants. Iterative tests with more selective media and biochemical tests may be used for more specific identification.

Culturing and identification take time. The shigella that might be present in a fecal sample, or the *Escherichia coli* that frequently cause urinary tract infections, grow quickly, forming colonies in 24 hours or so, and a laboratory would probably identify them in one or two days. *Mycobacterium tuberculosis* grows far more slowly, and six weeks may pass before traditional methods can be used to identify it.

Identifying the bacteria is often critical for choosing the most appropriate antibiotic therapy because some antibiotics work better against certain bacteria. But identification does not provide information about whether the bacteria are resistant to the antibiotic or susceptible to it. "Susceptibility tests" are used to determine that.

Traditional Susceptibility Tests

Information about antibiotic-resistance/susceptibility is developed by testing the bacteria isolated from the infection against six to 12 different antibiotics, or more if necessary. The results from these tests may support the use of the antibiotic that was empirically selected by the physician, indicate that other antibiotics would work as well, or show that the disease-causing bacteria are resistant to the antibiotic empirically chosen.

Jorgensen (1995) describes four methods that are currently used to determine the antibiotic susceptibility or resistance of bacteria: 1) disk diffusion tests, 2) broth dilution tests, 3) agar dilution tests, and 4) agar gradient methods.

Disk diffusion tests

Disk diffusion tests measure the size of a clear area of no bacterial growth around a sterile paper disk containing antibiotic. The size of this area, called the "zone of inhibition," can be measured and reported directly, or the measurement can be compared to criteria established by the National Committee for Clinical Laboratory Standards

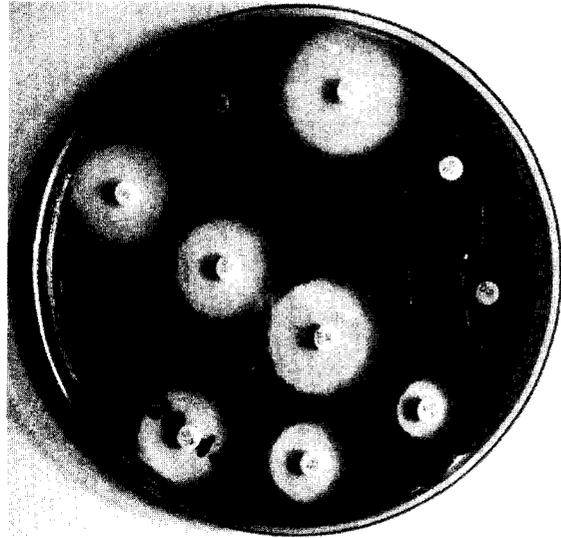


Photo of a disk diffusion (Kirby-Bauer) susceptibility test plate. Courtesy of James H. Jorgensen, University of Texas Health Science Center, San Antonio, TX, 7995.

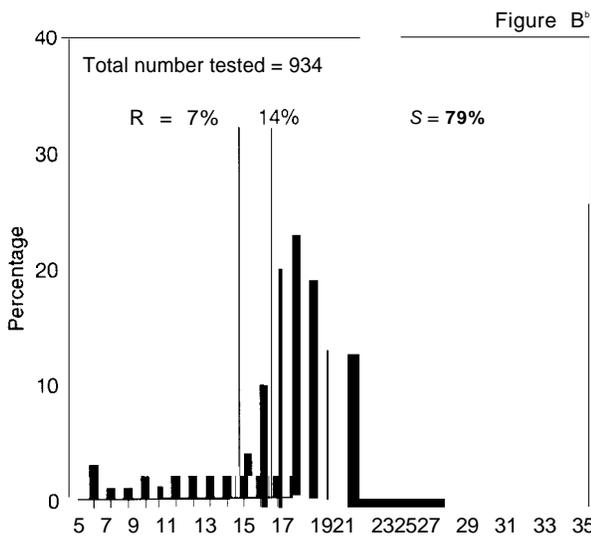
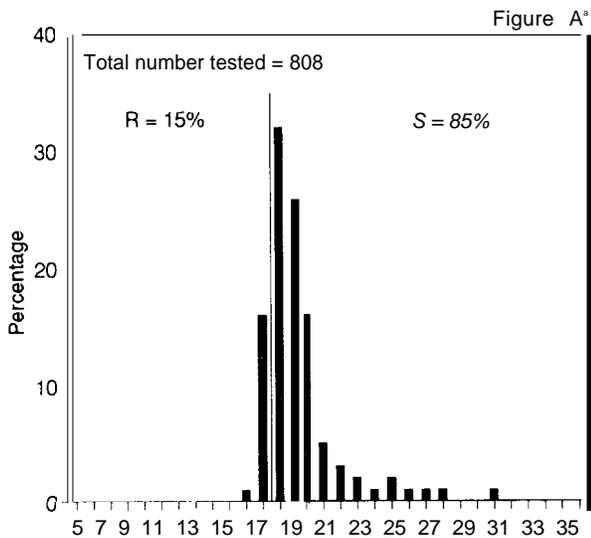
(NCCLS) to classify the bacteria as susceptible, intermediate or resistant (S, I, or R). These tests are well standardized for certain bacteria and may be highly reproducible. However, disk tests are influenced by many laboratory variables that can limit accuracy unless tightly controlled.

O'Brien (1994), who initiated and runs WHO-NET, the World Health Organization-sponsored surveillance system for antibiotic-resistant bacteria, emphasizes the importance of requiring laboratories to report raw data about the size of the zones of inhibition (figures 6-1 and 6-2) to surveillance organizations. While laboratories in Europe and North America are consistent in their measurement and reporting of the diameters of zones of inhibition around antibiotic disks, they interpret the meaning of the measurements differently (figure 6-1). Therefore, data reported as zones of inhibition rather than as interpretations are necessary to make any valid international comparisons about the prevalence of antibiotic-resistant bacteria.

Broth dilution tests

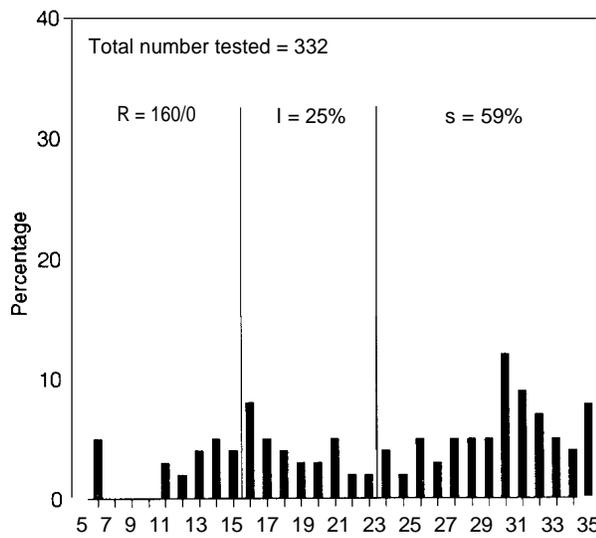
Dilution tests measure the concentration of antibiotic that is necessary to prevent the growth of bacteria. In these tests, known amounts of bacteria are deposited into small test tubes containing

FIGURE 6-1: Histograms of the Range of Zone Diameters for Enterococci Tested Against Vancomycin, During 1992



^aFigure A represents a center in Europe (Center 1).
^bFigure B represents a center in North America (Center 5).
 NOTE: European and North American centers measured similar zones of inhibition, illustrating the reproducibility of the methods. However, the use of different break points in the two centers would result in the centers reporting different percentages of resistant organisms. Even if the laboratory data were identical, the centers would report different percentages of resistant organisms. This example demonstrates the importance of reporting raw data for making comparisons between laboratories.
 SOURCE: WHONET, 1994.

FIGURE 6-2: *Klebsiella pneumoniae*: S, I, R Distribution Cefotaxime Susceptibility Data



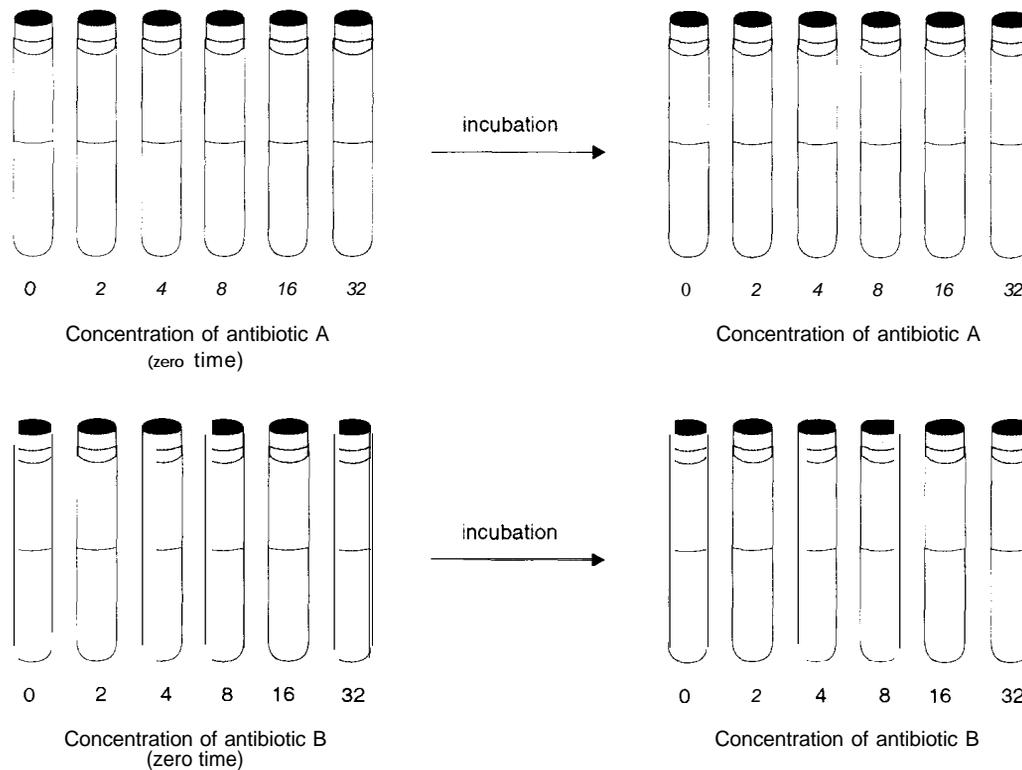
NOTE: This histogram illustrates the ambiguity of setting breakpoints for classifying bacteria as susceptible, intermediate, or resistant. In particular, the breakpoint for the division between resistant and intermediate appears to fall at a peak in measured zones of inhibition.
 SOURCE: WHONET, 1994

1 to 2 milliliters (a teaspoonful is about 5 milliliters) of sterile nutrient growth medium (“broth”) containing different concentrations of antibiotic (figure 6-3). The lowest concentration of antibiotic that prevents growth of the bacteria defines the “Minimum Inhibitory Concentration” (MIC).

While the MIC provides information about the concentration that will inhibit the growth of a bacterium, it does not say whether that concentration can be reached in the treated patient or what dose of antibiotics is needed to reach the critical concentration. Interpretive guidelines provided by NCCLS publications help clinical microbiologists and physicians interpret MICs as clinical categories of S (“susceptible”), I (“intermediate”), and R (“resistant”).

A disadvantage of this method is the large number of test tubes and racks and large volumes of media that are required. To test a single bacterial culture against six antibiotics would require 42,48, or 54 tubes, depending on the lowest concentration used. The miniaturization of this

FIGURE 6-3: Broth Dilution Test



NOTE: In both A and B, a small measured volume of bacterial culture is added to each test tube at time zero. Following incubation (the time of Incubation depends on the growth characteristics of the bacteria), the test tubes in which bacteria were able to grow are turbid (the first three tubes in A and all tubes in B). The absence of growth in the last three tubes that contain antibiotic A indicates that the bacteria are killed or inhibited by concentrations of A equal to 8 units or more. Growth in all tubes that contain antibiotic B indicates that the bacteria are resistant to all tested concentrations of antibiotic B.

SOURCE: Office of Technology Assessment, 1995.

method with microdilution trays solved that problem. The broth *micro* dilution test is currently the most popular antibiotic sensitivity test in the U.S. (table 6-I; the test using test tubes is called the “broth *macro* dilution test”). The small size of the wells and the small volumes, about 0.1 milliliter (about a drop from an eye-dropper), require that some viewing device be used to determine which of the wells in the test plate are clear and which are turbid. There are a number of commercial devices that make that determination, and some plot out the MICs from the tests.

To hold down costs and reduce the space needed for incubation of test cultures, many laboratories do not use the entire series of dilutions

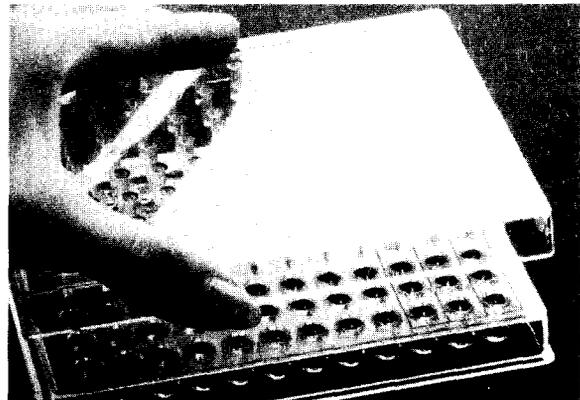


Photo of a broth microdilution susceptibility test tray with disposable inoculator.

Courtesy of James H. Jorgensen, University of Texas Health Science Center, San Antonio, TX, 7995.

**TABLE 6-1: Most Commonly Used
Antibiotic Susceptibility Testing Methods**

Testing method	Percent of laboratories reporting routine use
Broth macrodilution	1.8
Broth microdilution	
Commercially prepared	46.2
User prepared	0.4
Agar dilution	0.2
Disk diffusion	31.8
Rapid automated	19.7

SOURCE: Jorgensen, 1995. From data collected in a 1991 Proficiency Survey of 3414 laboratories conducted by the College of American Pathologists.

as diagrammed on figure 6-3. Instead, based on the NCCLS interpretive criteria, only two to three dilutions of each antibiotic are used. One of the dilutions is set to match the “break-point” that defines the division between the resistant and intermediate response; another dilution matches the concentration that defines the break-point between the intermediate and susceptible responses (see figure 6-2 for examples of break-points using disk diffusion tests). When only two or three dilutions are used for each antibiotic, the tests provide only an estimate rather than a quantitative measurement of the MIC. The true break-point might be somewhat different from the guidelines, and this fact can cause errors in classifying the bacteria as resistant or susceptible.

Agar dilution tests

Agar dilution tests are similar to the broth dilution tests in that they measure the MIC. In these tests, a small volume of a bacterial suspension, usually 1–2 microliters, is transferred to each of a series of agar plates containing known concentrations of antibiotics. Multi-prong devices are used to transfer approximately 100 colonies at one time.

Antibiotic gradient susceptibility test methods

Two commercial methods, the Etest¹ (AB BIO-DISK, Solna, Sweden) and the Spiral Gradient Endpoint System (Spiral Biotech Inc., Bethesda, Maryland), use antibiotic concentration gradients on agar plates. Both tests establish MICs that compare closely with those determined in the disk diffusion or broth dilution tests, and both are useful for testing anaerobic and other hard-to-grow bacteria.

The Etest has been cleared by the FDA for clinical use in the U.S. The Spiral Gradient End-

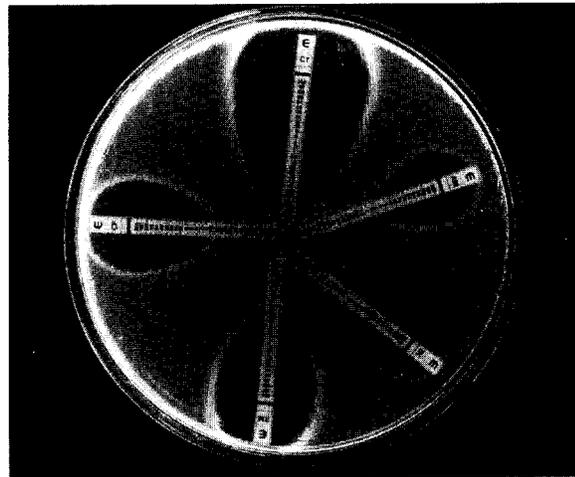


Photo of five Etest strips on the surface of an agar plate. Intersection of the growth ellipses with the strips indicate the MICs. Courtesy of James H. Jorgensen, University of Texas Health Science Center, San Antonio, TX, 1995.

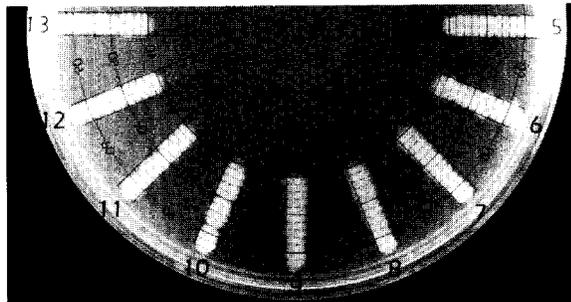


Photo of the Spiral Assay System Susceptibility Test. Courtesy of Spiral Biotech, Inc., Bethesda, MD, 1995.

¹ OTA mention of a company or product does not constitute an endorsement. Furthermore, companies and products are mentioned as examples; competing companies and products exist.

point System has not yet been cleared by the FDA for clinical use.

These tests may have a special advantage for resistance surveillance because they have a continuous concentration gradient and are able to show subtle changes in susceptibility, and the wide concentration gradients of these tests cover the MIC ranges of susceptibility of a wide variety of pathogens and allow both low-level and high-level resistance to be detected. The Etest is reportedly easy to use in most laboratory settings and requires no complicated procedures.

Modifications of Traditional Methods To Shorten Times Necessary To Obtain Results

The four methods discussed require at least overnight incubation to obtain results. That time can be shortened to four to 10 hours for certain antibiotics and organisms by using optical devices (sometimes coupled with fluorescent indicators) more sensitive than the human eye to detect growth in microdilution tubes. Two commercially available automated systems can produce results in four to 10 hours.

The AutoSCAN Walk/Away (Dade Microscan, USA, Miami, Florida) uses standard microdilution trays that are inoculated in the standard way and placed in an automated incubator that uses a fluorometer to detect the presence or absence of growth at different antibiotic concentrations. The Vitek System (bioMerieux Vitek, Hazelwood, Missouri) was developed by NASA to diagnose urinary tract infections in astronauts in space in the 1970s. It uses credit-card size reagent cards, each of which has 30 tiny wells for the testing of different antibiotic concentrations, and the assays can be completed in three to 10 hours. While both systems provide rapid results, each requires backup cultures and other tests in case of power or mechanical failures.

In some cases, the automated machines can fail to detect resistance. To deal with this problem, manufacturers of both of these instruments have developed computer software that reviews the results to identify those that may be false. Some of these systems can also identify unex-

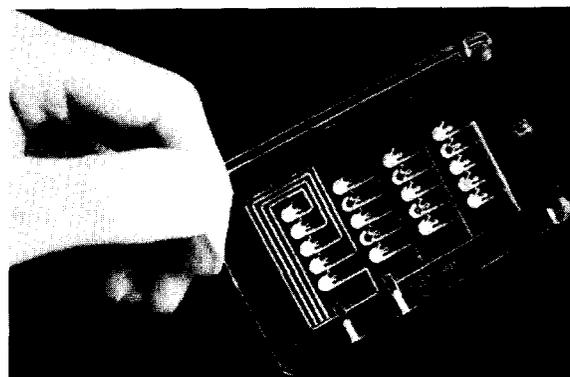


Photo of bioMerieux Vitek susceptibility testing reagent card. It was originally developed by NASA to diagnose urinary tract infections in astronauts in space and had to be very small. Courtesy of James H. Jorgensen, University of Texas Health Science Center, San Antonio, TX, 1995.

pected resistance patterns and offer suggestions for antibiotic treatment (Jorgensen, 1993). Computer analysis of the test results can also be linked to the hospital pharmacy's computer to alert the pharmacy personnel when the wrong antibiotic therapy is being used. As discussed in chapter 4, computer networks such as this can improve patient care and reduce costs.

Summary of the Test Methods

Table 6-1 shows the reported frequency of use of the various test methods in a survey of American laboratories, and table 6-2 provides information about the relative costs of the most commonly used methods. None of the methods differs very much in labor costs. Based on the costs of equipment and supplies, the disk diffusion method is the least costly. O'Brien (1994) argues that it can also be the most informative under most conditions because the sizes of the zones of inhibition (see photograph) provide raw data that have not been subject to interpretation, and zone of inhibition information is more quantitative than broth dilution tests that are sometimes based on only one or two dilutions.

Will Faster Tests Make a Difference?

A test result that shows that bacteria are resistant to the empirically chosen antibiotic will certainly cause the physician to substitute another antibi-

Method or instrument	Test format	Means of growth detection/ endpoint determination/time	Equipment required	Approximate cost of equipment	Approximate cost/test*
Broth microdilution (prepared in-laboratory)	96-well plastic microtiter trays	Visual determination of MICs by turbidity; 18–24 hours	Tray dispenser, manual reader	\$35,000	\$6–7
Broth microdilution (commercial frozen or dried trays)	96-well plastic microtiter trays	Visual determination or automated reader interpretation of MICs; 18–24 hours	Choice of manual reader or auto-reader	\$500 \$50,000	\$7–9
Disk diffusion	6–12 disks on 100–150 mm petri plates	Visual measurement of zones of inhibition; suscept. categories; 16–18 hours	None (only standard lab incubator)	None	\$1.50–4
Etest	1–5 strips on 100–150 mm petri plates	Visual determination of MICs; 16–18 hours	None (only standard lab incubator)	None	\$3–11
Dade MicroScan WalkAway	96-well plastic microtiter trays	Fluorogenic substrate auto-incubator/ reader MICs; 3.5–15 hours	Auto-incubator/reader	\$85,000 \$125,000	\$10–12
bioMerieux Vitek	30–45-well plastic cards	Kinetic turbidimetric MICs or categories; 4–15 hours	Auto-inoculator/ incuba- tor/reader	\$40,000 \$152,000	\$4–6

* Includes cost of consumable supplies only; labor requirements do not differ substantially between methods.

SOURCE: J.H. Jorgensen. March 1995. Current and future phenotypic methods for detection of resistant bacteria. Report prepared for the U.S. Congress, Office of Technology Assessment.

otic if the patient is not improving. Sometimes, however, the patient improves despite the presence of apparently resistant bacteria. This can occur because the patient's immune system is successfully controlling the bacteria, or because the antibiotic reached a higher concentration in the patient's body than in the laboratory test, so that the bacteria were killed or inhibited.

Up to 40 percent of antibiotic therapy was inappropriate as judged by a comparison of physicians' prescriptions to an analysis of the laboratory results for bacterial identification and antibiotic susceptibility tests (see, for example, Jorgensen and Matsen, 1987). While older reports in the literature (see Edwards, Levin, Balogtas, et al., 1973) indicated that physicians pay little attention to microbiology test results, more recent publications (Doern, Scott, and Rashad, 1982; Weinstein, Murphy, Reller, et al., 1983; Jorgensen and Matsen, 1987) indicate that some physicians do modify their prescriptions upon receiving additional laboratory information. In particular, rapid susceptibility tests, which can be completed in four to 10 hours, resulted in more appropriate therapy, and Doern, Vautour, Gaudet, et al. (1994) found that rapid tests resulted in fewer additional laboratory tests, fewer invasive procedures, shortened time in intensive care, and reduced mortality.

Physicians typically receive the results of antibiotic susceptibility tests on the morning of the second or third day after specimens are submitted to the laboratory. The faster methods produce the results more quickly, but unless the physicians and nursing staff are prepared to use the information at the earlier time, it will not be considered until the next morning. In this case, technological improvements can only be useful if accompanied by changes in habits.

Jorgensen (1995) discusses another obstacle to the use of rapid methods. Laboratory managers often confirm the results of the faster methods with backup tests using the older, slower methods. However, this requires performing the tests more than once and therefore increases costs. Trade-offs must be made among the objectives

of speeding up the process, ensuring accuracy by performing backup tests, and saving money.

■ New Technologies for Identifying Bacteria

Antigen Tests

Antigen tests use antibodies to recognize specific molecules on or in bacterial or other cells. For instance, the home pregnancy test detects antigens that are produced only during pregnancy.

There are many versions of antigen tests to detect the presence of strep A bacteria in sore throats, but the usefulness of these tests is limited by low sensitivity. Traditional cultures are still recommended when tests are negative. Antigen tests are also available to detect *Clostridium difficile* in patients with diarrhea and to determine the bacterial cause of meningitis.

Methicillin-resistant *Staphylococcus aureus* (MRSA) presents identification problems because it and all other *S. aureus* grow slowly. Further, its identification is usually accomplished by a specific test for protein antigens on its surface, and these tests failed to identify between 1 and 25 percent of *S. aureus*. Kuusela, Hilden, Savolainen, et al. (1994) discovered another protein on the surface of *S. aureus* and developed a test for it that detects both methicillin-susceptible *S. aureus* and MRSA.

Tests which Directly Measure the Presence of a Bacterial or Antibiotic Resistance Gene

Tests which directly measure the presence of a bacterial gene (discussion of tests for antibiotic resistance genes follows) are fundamentally different from the traditional tests, which measure a property of an organism such as its ability to grow in the presence of a certain concentration of antibiotic. The new gene tests bring with them a new set of considerations: A bacterium might contain a gene for resistance, but not "express" it under the conditions of the traditional diagnostic tests, or a resistance gene may have undergone a mutation that does not affect its function but that makes its presence undetectable, or the genes of dead bacteria may be detected with DNA tests.

For example, samples from a patient who is being successfully treated with anti-TB drugs often test positive in DNA tests, but negative in culture-based tests that rely on growing the organism. These are problems that must be considered in designing new genetic tests and using them in clinical practice.

One huge advantage of tests that measure the presence of a bacterial gene is that they are quick; many tests take only a few hours or less. Another advantage is that they generally have much higher sensitivity than the antigen and other enzymatic tests described above, although in some cases the sensitivity is not as high as that of traditional culture methods. The speed combined with the sensitivity is very useful. However, some tests require culturing, i.e., the growing of bacteria from the clinical samples, before the genetic test can be performed. The culturing requirement adds time to the process. To date there exists no test so rapid that it will confirm bacterial identification and susceptibility before a patient leaves a physician's office. The development of faster and more susceptible genetically based tests for bacteria started in the early 1980s, but most are still not available for routine use. Nevertheless, some of these tests, such as those that are able to diagnose tuberculosis in a few hours instead of a few weeks, represent a significant technological advancement that has improved clinical practice.

DNA probe assays

Single-stranded fragments of DNA or RNA that are complementary to a target DNA or RNA sequence will form a double-stranded molecule, known as a "stable hybrid," under certain reaction conditions. Diagnostic fragments, or probes, which will bind to target DNAs or RNAs, are labeled with enzymes or dyes so that the binding of the probe to the target can be detected.

At the present time, several commercial DNA probe tests in clinical use are manufactured by Gen-Probe, Inc. (San Diego, California), Gene-Trak (Framingham, Massachusetts), Ortho Diagnostic Systems (Raritan, New Jersey), and others. Some of these tests are designed to con-

firm the identification of cultivated colonies, such as tests for *M. tuberculosis*, *M. avium* (an important pathogen in patients with AIDS), and *Neisseria gonorrhoeae* (the agent of gonorrhea). Other tests can be used for the direct detection of bacteria in clinical samples, such as *Neisseria*, *Chlamydia trachomatis* (an agent of urethritis, cervicitis, and pelvic inflammatory disease) and *S. pyogenes* (a cause of suppurative tonsillitis or "strep throat"). These are organisms that for the most part are difficult or slow to cultivate and identify in the laboratory. The tests require approximately two to four hours to complete and cost the patient approximately \$20–40 per test.

One important disadvantage of probe-based methods to date has been their low sensitivity compared to culture-based methods. Probe assays for *M. pneumoniae* (an agent of atypical or "walking" pneumonia) and *Legionella pneumophila* (the agent of Legionnaire's Disease) are no longer much used, primarily because of this problem. New technologies in development, which provide the ability to amplify probe or probe-linked signals after binding to the target, may help increase the sensitivity of these tests.

One promising probe-based test that does have adequate sensitivity is a rapid direct DNA probe test from Gen-Probe that can identify Group A Streptococcus directly from throat swabs. In comparative studies, test results agreed closely with those from older and slower tests (see, for example, Rippin, et al., 1994; Heiter and Bourbeau, 1993), unlike the quick antigen strep tests described above. However, Heiter and Bourbeau conclude that because this test requires several instruments not routinely found in doctors' offices and because it still requires two hours, the test will not be useful for point-of-care testing in doctors' offices or emergency room clinics.

Target amplification methods

One of the most promising approaches for increasing the sensitivity of probe-based DNA tests is to amplify the target DNA sequence through such methods as polymerase chain reaction (PCR), which can rapidly generate millions

of copies of bacterial or resistance gene DNA or RNA sequences. PCR requires identifying and synthesizing short sequences complementary to the target gene that act as “primers” for the synthesis of the DNA. It is relatively easy to synthesize millions of these short sequences, but it would be difficult to synthesize larger pieces of DNA. Starting with as little as one strand of DNA from the sample, PCR uses enzymes to elongate the “primers” into full copies of the DNA. PCR can generate millions of copies of a particular DNA in hours.

Species-specific PCR detection assays have been developed for at least 50 different bacterial pathogens, and specific sequences are available from a much larger number of species, for which PCR primers can be designed. However, only a few standardized kits for performing these tests on specific bacterial species are commercially available in the U.S. Among those kits that either have been cleared, or are nearing clearance, by the Food and Drug Administration are PCR assays for *C. trachomatis*, *N. gonorrhoeae*, and *M. tuberculosis*. Even without a commercially available standardized kit, the tests can still be set up individually by service labs. However, there are several disadvantages to performing these tests without using a standard kit. First, most of these assays do not perform as well in detecting bacteria in clinical samples as they do in purified cultures; suboptimal sample preparation procedures and reaction conditions are probably to blame. Second, unless physical, chemical, or enzymatic precautions are in place, PCR and other target amplification methods are easily jeopardized by contaminating nucleic acid, either from prior amplification reactions or from positive clinical samples. Third, there is dramatic interlaboratory variability in the test results for the same group of clinical samples. Many of these problems may be solved by the availability of standardized commercial kits.

After the nucleic acid is isolated and amplified by a technique such as PCR, the nucleic acid can be sequenced to identify the organism. Automated sequencers marketed by Applied Biosystems can determine 48 independent DNA

sequences of 400–500 nucleotides in length in approximately 8 hours, and speed and sequence length capabilities are continually being improved. Automated sequencing systems require an initial investment of approximately \$55,000 (Molecular Dynamics) to \$125,000 (Applied Biosystems, including sequence analysis software). It is estimated that identification of a single bacterial isolate with an automated procedure will cost approximately \$75.

Another way to identify the organism is to bind the nucleic acid to probes that recognize specific sequences. Currently, sequences prepared from specific reference strains of bacteria are used. New strategies are expected to use random sequences of nucleic acid bound in orderly arrays on micro-scale photolithographic silicon chips, and the nucleic acid can be identified by determining which probes bind to it. Because of the microscopic scale of these tests, the bound nucleic acid must be detected with a laser confocal microscope. This approach has already been shown to be useful for the detection of single base pair mutations in the human immunodeficiency virus. This technology offers significant potential for rapid sequence determination of specific gene targets and for the detection of specific identifying signature sequences or antibiotic-resistance-associated sequences. First-generation chip-based sequencing systems may be available for research by 1996.

Using rapid DNA tests to diagnose tuberculosis

Diagnosing tuberculosis is difficult because it has many different clinical manifestations. Moreover, many physicians were not trained to recognize tuberculosis because its prevalence was decreasing until about 10 years ago. The recent resurgence of this disease is a huge problem, both in the United States and around the world, and rapid diagnosis is critical so that patients can be treated before they pass this highly infectious disease to others. Quick determination of the susceptibility of the infecting organism is also becoming increasingly important because many

drugs are inactive against some of the multi-resistant strains of tuberculosis.

The tuberculin skin test is often used as the first diagnostic indication that a person has been infected with tuberculosis. A positive tuberculin skin test does not mean that the person has active disease, only that the person has been exposed to tuberculosis. Haas and Des Prez (1995) review studies of the interpretation of positive tuberculin skin tests in nursing homes which show that 3.8 percent of men who were tuberculin-positive on admission to nursing homes subsequently developed active disease, and that 11.6 percent of men who became positive while in the nursing home later developed the disease. The percentage developing active disease could be reduced to 0.2–0.3 percent with the prophylactic use of the antibiotic INH. However, the level of hepatic toxicity from INH was 3–4 percent, and there were other side effects. Deciding when to prescribe antibiotics for a patient with a positive skin test but no other symptoms is very complicated because the toxicity of the drug must be weighed against the probability that the patient will develop tuberculosis. The same considerations apply to new diagnostic tests based on the detection of the DNA of *M. tuberculosis*.

Isolating the mycobacteria causing tuberculosis requires from three to eight weeks, and susceptibility testing by agar dilution methods requires another three to six weeks. Highly variable results have been observed between two different clinical laboratories using culture methods (Hewlett, Horn, and Alfalla, 1995). The identification and susceptibility testing of drug-resistant TB can be significantly hastened by using the BACTEC radiometric method, but the time required is still 20 days or more. Recent data on the Etest for susceptibility testing of mycobacteria suggest that MIC values can be obtained in five to 10 days, a significant improvement over current methods (Wanger and Mills, 1995).

With PCR and probe-based DNA tests, physicians will have the ability to identify mycobacteria in the sputum of patients within a few hours to a few days. All tests that are currently cleared by the FDA require some culturing of the clinical

sample, but newer tests in development will allow identification of mycobacteria directly from clinical samples. These tests are used in many other countries, including much of Europe. Some laboratories are promoting clinical use of PCR tests in the U.S. Macher and Goosby (1995) document a difficulty in interpretation of PCR tests in the absence of other clinical signs of tuberculosis. On the basis of two (out of three) positive PCR tests, the patient received antituberculosis chemotherapy and was placed in isolation. Later, six cultures turned out to be negative for tuberculosis, and the patient was taken off drugs for active tuberculosis and placed on INH alone for preventive therapy. This case study indicates that the DNA probe tests might be too sensitive: they might detect non-viable mycobacteria from a previous exposure. This result is comparable to a positive tuberculin skin test, which, as discussed above, indicates past exposure to mycobacteria but does not necessarily signify active tuberculosis.

■ New Technologies for Detecting Antibiotic Resistance

The increasing prevalence of antibiotic-resistant bacteria is leading manufacturers to develop tests specifically to identify resistant strains. In general, these tests are designed to produce results in a few hours. Discrepancies may arise between the results of old and new methods. The older methods directly measure whether an organism expresses resistance and can grow in the presence of an antibiotic. Some of the newer methods indicate whether an organism has a gene encoding for resistance. However, the organism may not “express” this resistance even if it has the gene. In some cases, it is unknown whether the presence of the gene or the expression of the gene under laboratory conditions is the more important predictor of clinical outcome.

Enzymatic Tests

Enzymatic tests can directly measure the presence of an enzyme that confers antibiotic resistance, such as β -lactamases that inactivate

penicillins and other β -lactam antibiotics and the enzyme that inactivates chloramphenicol. The detection of the β -lactamases requires only a few minutes (Stratton and Cooksey, 1990), but it is limited to only a few bacterial species. Moreover, it does not detect penicillin resistance caused by other mechanisms, such as the production of modified penicillin binding proteins. The test for the chloramphenicol inactivating enzyme requires one to two hours and can be used to detect the most common form of chloramphenicol resistance, but it has decreasing utility because of the declining use of this antibiotic.

Tests Based on Indicator Dyes or Light-Producing Enzymes

Some tests add indicator dyes to a bacterial culture and then detect the presence of living organisms by a color change in the indicator dye. An example is the Crystal MRSA Rapid ID test from Beckton Dickinson. This test, which can detect MRSA in four hours in cultured bacteria, uses an indicator dye that can be observed under an ultraviolet light source in the absence of oxygen. In this test, three samples of bacteria are incubated with the indicator dye. In addition, one of the samples is incubated with oxacillin (a semi-synthetic penicillin similar to methicillin) and one of the samples is incubated with vancomycin. If the bacteria survive, they will use the oxygen in the samples and the dye changes color. If the sample contains MRSA, the organism will survive in the presence of oxacillin but not in the presence of vancomycin. If the organism is susceptible to oxacillin, it will not survive either antibiotic. The test, which costs about five dollars, does not require expensive instrumentation. Kohner, Kolbert, Geha, et al. (1994) found that this system is an effective rapid screening method for MRSA but has poor performance for coagulase-negative Staphylococci, which often present a greater diagnostic dilemma.

A more complicated test for multiresistant tuberculosis is currently in very early development (Jacobs, Barletta, Udani, et al., 1993). In this test, the gene for the light-producing enzyme from fireflies was cloned into a virus that infects *M. tuberculosis*. The virus is added to a sample of sputum from the patient. The virus will infect any mycobacteria that are present. If the virus infects living mycobacteria, the viral DNA is activated, and the firefly enzyme will cause the culture to give off light. When antibiotics are added to the test, only resistant mycobacteria will support viral growth; susceptible ones will not, and susceptible cultures will not light up. Thus susceptibility can be determined. Research is currently underway to determine if this test can measure as few as 100 live *M. tuberculosis* bacteria, and would therefore work directly on patient samples in a few hours (Jacobs, NIH Grant R01AI27235). However, this sensitivity may be difficult or impossible to achieve because of background signals in the sample.²

DNA-Based Methods for Testing Antibiotic Resistance

Current susceptibility patterns suggest that rifampin resistance in *M. tuberculosis* can be used as a predictive marker of multidrug resistance. In general, surveillance indicates that resistance to rifampin correlates well with resistance to three or more antituberculosis drugs. Furthermore, virtually all of the highly resistant mycobacterial strains (resistant to greater than five drugs) are rifampin-resistant. However, this may change in the future if *M. tuberculosis* undergoes further genetic mutation.

PCR tests are in development to detect rifampin resistance in *M. tuberculosis* caused by the *rpoB* gene. The use of the signature sequences in the *rpoB* gene assumes that there are not significant numbers of rifampin-resistant *M. tuberculosis* strains in the community with other, uncharacterized *rpoB* mutations in the gene.

² All samples “glow in the dark”—some background signals are detected. This test will only achieve high sensitivity if the signal from the firefly enzyme is significantly larger than the background signal.

MRSAs are currently identified by using the traditional tests discussed in the first part of this chapter. The performance of these tests may be variable. Factors such as the inoculum size, incubation time and temperature, pH of the medium, salt concentration of the medium, and prior exposure to β -lactam antibiotics all influence the expression of resistance. To complicate matters further, only some bacteria in a culture may express methicillin resistance, even if all have the gene. Taking into account these factors, the National Committee for Clinical Laboratory Standards (NCCLS) has recommended guidelines to optimize the detection of resistance. However, occasional organisms have been isolated that are difficult to characterize by these methods. The results produced by various methods of disk diffusion and those from agar dilution methods are often not consistent. In addition, it is difficult to separate organisms that are highly resistant due to overproduction of β -lactamase from organisms that have the *mecA* gene encoding for an altered penicillin binding protein. Organisms resistant due to the *mecA* gene often require vancomycin therapy, while organisms resistant due to overproduction of β -lactamase might actually respond better to treatment with β -lactam antibiotic/ β -lactamase inhibitor combinations than vancomycin.

PCR and DNA-probe techniques have now been developed to identify the *mecA* gene. In general, the studies to date show a high degree of correlation between traditional and DNA-based tests and allow accurate classification of highly resistant and borderline resistant strains.

Guidelines for interpretation of the *mecA* detection result will need to be formally addressed as more laboratories begin to use this and other genetic methods. Proposals have been made to regard *mecA*-positive organisms (both coagulase-negative staphylococci and *S. aureus*) as intrinsically resistant to all antibiotics except vancomycin and to report immediately all *mecA*-positive results, which can be available well before results from traditional methods. There are situations where the *mecA*-positive organism does not express resistance clinically and may

respond to β -lactam therapy. It is important to document these cases carefully to avoid unnecessary use or overuse of vancomycin. Nevertheless, all *mecA*-positive organisms may be highly resistant if the organism expresses the *mecA* gene. This may lead to treatment failures if β -lactam antibiotics are chosen.

Surveillance and DNA-based diagnostics

Surveillance of genetic mutations in bacteria will be essential in the use of new DNA diagnostics, which measure the presence of specific genetic sequences. Mutations might alter these sequences, or new genes conferring resistance may spread. For example, widespread surveillance efforts are necessary to insure that signature sequences represent the majority of mutations in the *rpoB* gene that confer rifampin resistance in *M. tuberculosis*.

■ Regulation of Diagnostic Tests

The Clinical Laboratory Improvement Amendments (CLIA '88) were passed by Congress to regulate the quality of diagnostic testing. Regulations under CLIA, which became effective in September of 1992, require all clinical laboratories that perform certain diagnostic tests to register with the federal government and perform quality control tests and document quality assurance. However, certain tests are "waived" under CLIA; this means that the test can be performed in any physician's office, whether or not the office is registered under CLIA. Other tests, generally the more complex ones, can be performed only in offices that comply with the CLIA regulations for laboratories.

The CLIA regulations may be a disincentive to performing tests. Complying with them increases the cost of testing and may delay results. For example, physicians may choose not to register their offices under CLIA and will therefore be compelled to send out numerous tests that they formerly performed. This may result in the performance of fewer diagnostic tests, which could contribute to the overuse of antibiotics. A physician might decide that it is

easier and more cost-effective to prescribe antibiotics for all sore throats rather than perform throat cultures. However, this negative potential consequence of the CLIA regulations must be weighed against whatever positive effects they have had on the quality and consistency of testing that is done in the clinical laboratories that meet CLIA standards.

■ Getting New Tests to Market

The worst outcome for a sensitivity test is to indicate that bacteria are susceptible to an antibiotic when the antibiotic has no effect against that strain. Such an error, which can result in a patient's death, is called a "very major error" in testing. The second worst outcome is to report that bacteria are resistant to an antibiotic that is in fact effective against them. That error, which could result in treatment with a more toxic, more expensive antibiotic than necessary, is termed a "major error" (Jorgensen 1995).

It is impossible to design and perform tests that are completely error free. The manufacturers, the FDA, health care providers, and the public have to decide what levels of errors are acceptable. Often, new tests are compared with a "gold standard"—an older test that has been proved to be reliable. However, the "gold standard" is also not completely error free. Therefore it is sometimes difficult to interpret differences between a new test and a "gold standard." For example, culturing *M. tuberculosis* is considered the "gold standard" for the diagnosis of tuberculosis. However, Abe, et al. (1993) found that some patient samples were positive for *M. tuberculosis* by DNA-based techniques but negative when cultured; these patients had clinical signs of tuberculosis, including characteristic radiographs, clinical manifestations of the disease and/or clinical response to antituberculosis chemotherapy.

Two FDA centers are involved in approving test methods for antibiotic susceptibility. The FDA Center for Drug Evaluation and Research certifies that disks are available for each antibiotic on the market in the United States, and it

assures the potency of the disks and that criteria for interpretation of the disk assays are available when an antibiotic goes on the market. The FDA Center for Devices and Radiological Health has responsibility for determining the safety and effectiveness of other devices and materials, including computer software, for susceptibility testing.

A new diagnostic device can be reviewed by FDA under two different procedures. A device or method that employs principles similar to those used by products already on the market and that requires an incubation period of 16 hours or more is reviewed under the "510(k) clearance" process. The performance of the new device or method is compared to the performance of the product already marketed to determine whether the two are "substantially equivalent." If they are, the new device or method is cleared for marketing without undergoing the more extensive procedures, known as "pre-market approval." The 510(k) process is also used when a manufacturer wants to add a new antibiotic to the battery of antibiotics already included in a test kit.

New diagnostic tests that are not "substantially equivalent" to any product on the market must submit an application for "pre-market approval" (PMA) to the FDA. Because the approval process under the PMA review is substantially more difficult, manufacturers have a disincentive to develop novel products.

Any device that requires less than 16 hours' incubation is required to undergo the pre-market approval process, which takes longer and is substantially more difficult to complete than the 510(k) clearance process. Jorgensen (1995) claims that there is no clear justification for the 16-hour incubation period serving as the cutoff between a 510(k) review and a PMA review because there is no indication that more rapid devices are inherently less accurate than others. The difference in the time required to obtain a 510(k) clearance, as opposed to a pre-market approval, is a matter of contention. According to Jorgensen (1995), the requirements for a 510(k) clearance have grown since 1990, and they are now approaching those required for a PMA. On

the other hand, FDA (1995) asserts that there have been marked improvements in the processing of 510(k) applications.

VACCINES

Perhaps the ultimate weapon against antibiotic-resistant bacteria is the development of vaccines and pre-emptive immunization. In concept, vaccines are simple. When a person receives an inoculation of a preparation of killed or attenuated (“weakened”) disease-causing bacteria or virus, a component of such an agent, or a related organism that does not cause disease, the inoculated person’s immune system will respond and produce antibodies to antigens on the injected materials. The immune system has a “memory.” As a result, if the person is subsequently infected by the organism for which the vaccine was prepared, he or she will produce antibodies that can inactivate the agent and remove it from the body. “Natural immunity” is produced in a similar way; once a person has had a disease, the immune system recognizes the organism that caused it and eliminates it from the body.

In practice, preparation of the specific material for the inoculation—the antigen—can be difficult. Preparing it so that the production of antibody is stimulated without objectionable toxicity, either at the time of inoculation or later, may not be simple.

The success of *Haemophilus influenzae* type B (Hib) vaccines, which were introduced in 1988, demonstrates that antibacterial vaccines can be quite successful. Countering that great success is the more than 75 years’ experience with an antituberculosis vaccine.

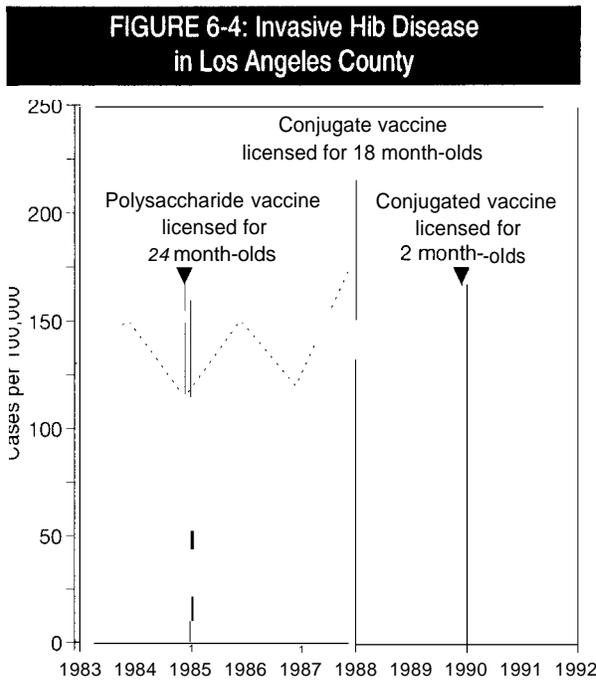
■ Hib Vaccines, a Success Story

Before the introduction of vaccines against it, Hib (*Haemophilus influenzae* type B) was the leading cause of invasive bacterial disease in children under five years of age, and it caused about 20,000 cases of meningitis and another 3,000 to 5,000 cases of invasive Hib disease annually. The mortality rate was 3 to 5 percent; moreover, up to 20 percent of the survivors of

meningitis suffered hearing loss or mental retardation, and resistance to ampicillin was increasing (Adams, Deaver, Cochi, et al. 1993).

In 1993, five years after the introduction of Hib vaccines, a number of researchers published reports about the incidence of Hib diseases in children up to five years old. Those vaccinated with Hib vaccine generally had disease rates 80 to 90 percent below the rates seen in unvaccinated children (Wenger 1994). The rates of Hib meningitis began to fall in 1989, after the introduction of the vaccine, and they continued to fall through 1991 (the last year for which data were available). In contrast, the rates of meningitis from *Neisseria meningitidis* and *Streptococcus pneumoniae* remained unchanged, ruling out a general decline in meningitis as the explanation for the Hib results. An unexpected result of the Hib vaccination program was a reduction in the number of children who carry Hib in their upper airways. That, in turn, reduced the number of children who could infect others, and the rates of Hib disease have fallen in both vaccinated and unvaccinated children.

A polysaccharide (a polymer of sugar molecules that is unique to the Hib bacteria) vaccine licensed in 1985 had no effect on the occurrence of invasive Hib disease in Los Angeles County (see figure 6-4) and, in fact, it was of little value in disease prevention. Three years later, a conjugate vaccine, prepared by chemically joining the polysaccharide to a protein that was known to stimulate antibody production, was licensed. This vaccine was very successful. Even when use of this vaccine was restricted to children older than 18 months (from 1988 through 1990), there was a drop in the Hib invasive disease rate in younger children. Vaccination of the older children had reduced infections of the younger ones, due to reduced transmission of the bacteria. Licensing of a vaccine for 2-month-old children in 1990 led to great reductions in the disease in Los Angeles County by 1992.



SOURCE: Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases. 1994. Annual Report, p. 1

■ BCG Vaccine, 75 Years' Experience

Albert Calmette and Camille Guerin at the Pasteur Institute in Paris first produced BCG as a vaccine for the prevention of TB. BCG is made from preparations of a live, attenuated strain of *M. bovis*, which is closely related to *M. tuberculosis*, and over 70 percent of children worldwide now receive the vaccine. It is compulsory in 64 countries and recommended in 118 others (OTA, 1993). People who have received this vaccine typically show a positive response to tuberculin skin tests. This is considered a great disadvantage in the U. S., where tuberculin skin tests are used to screen for exposure to tuberculosis.

Colditz, Brewer, Berkey, et al. (1994) reported the most thorough review of the efficacy of BCG vaccine. Their meta-analysis of the worldwide literature led to the conclusion that BCG reduced the risk of TB by about 50 percent, but the success rate varies from batch to batch of the vaccine, which is prepared in different laboratories under different conditions around the world. The 50 percent effectiveness conclusion was chal-

lenged by a number of scientists (Benin, 1994; Wheeler, Rodrigues, and Diwan, 1994; Comstock, 1994), but the authors replied that “. . . meta-analysis shows that the preponderance of evidence reveals that BCG vaccine is effective in preventing TB” (Colditz, Brewer, Berkey, et al., 1994a).

The United States has never required the vaccine because of questions about its efficacy and its usefulness in a population with a low incidence of TB. In 1979 the Centers for Disease Control and Prevention (CDC) recommended the vaccine for health care workers in contact with TB patients, but CDC's 1988 policy statement reversed that recommendation because of the lack of evidence for increased TB among health care workers (OTA, 1993). CDC recommend BCG for members of high risk groups who have limited access to health care. However, the CDC believes that the rate of tuberculosis is so low in the general population of the U.S. that the advantages conferred by vaccination are outweighed by the disadvantage of being unable to screen the population by using the tuberculin skin test.

■ Vaccine Research

Successful vaccines are available for use against viral diseases such as measles, mumps, and rubella, and against bacterial diseases such as diphtheria, tetanus, and pertussis (whooping cough). Currently, researchers are pursuing new vaccines against bacterial pathogens, such as the *Streptococcus* species, *Staph. aureus* and *Helicobacter pylori*, which are common problems, in part because of high rates of antibiotic resistance.

Active Systemic Immunization

Active immunization is the process of administering specific microbial antigens that stimulate the host's immune system to produce protective antibodies. Active immunization can be systemic—the traditional method—or mucosal (discussed below). Systemic immunity is accomplished by injection, the result being long-lived production of circulating immunoglobulin G

(IgG) antibodies. For bacterial vaccines, polysaccharides from the outside capsule of the bacteria are generally employed, but as was seen with the Hib vaccines (Wenger, 1994), capsular polysaccharides alone do not always stimulate sufficient antibody production. To raise sufficient levels of antibodies, the polysaccharides may have to be conjugated with “carrier proteins,” potent immunogens that provoke an immune system response to the entire complex (i.e., to polysaccharide antigen and protein carrier). The combination of polysaccharide and protein is called a “conjugate vaccine.” Finding the proper carrier is one of the more difficult aspects of vaccine development, but four different proteins—all of bacterial origin—work well in Hib vaccines.

***Streptococcus pneumoniae* vaccines**

Vaccine development is further complicated because different strains of the same bacteria have different polysaccharide antigens. For instance, *Streptococcus pneumoniae* has 84 distinct capsular polysaccharides. A vaccine that contains 23 different polysaccharides provides protection against 90 percent of invasive pneumococcal strains (Siber, 1994). That vaccine is 75 percent effective when administered to immunocompetent adults, and its wider use might prevent half of the 80,000 annual pneumococcal pneumonia deaths among older people (Medical World News, 1993). It is not, however, reliable in children under two years of age. For the vaccination of children, several companies are developing conjugated vaccines against the polysaccharides of the seven strains of pneumococcus that most commonly infect children, and these are currently undergoing human trials. In addition, researchers are investigating the possibility of using a polysaccharide that is common to all pneumococcus conjugated to one or more of several proteins that are common to all pneumococcus as vaccines, but there is no definitive evidence for their usefulness (Siber 1994).

Vaccines against otitis media

S. pneumonia is one of several bacteria that cause otitis media. That illness is so notorious that phy-

sicians who surveyed parents about their willingness to have their children immunized against the disease titled their report “The Surprisingly High Acceptability of Low-Efficacy Vaccines for Otitis Media...” (Wischnack, Jacobson, Poland, et al., 1995). Although no such vaccine is now in use, the interviewers asked about five hypothetical vaccines that had different efficiencies in disease prevention, and side effects that ranged from the temporary discomfort of a “shot” in all children to a few days of flu-like symptoms in up to half of vaccinated children. About half of the 601 interviewed parents would accept any vaccine if it would prevent three or more infections in the next six months. Parents were less accepting of vaccines with lower efficiencies and worse side effects. The authors of the study conclude that parents, even of children who have not had otitis media, are willing to accept some discomfort in their children to obtain protection against the disease and that parents’ willingness is greater than the medical establishment’s or FDA’s.

A biotech firm, MicroCarb (Bethesda, Maryland), has licensed one vaccine against *Haemophilus influenza*, another cause of otitis media, to Pasteur Merieux Serums et Vaccines S.A. If this vaccine proves successful, it will be of value against both antibiotic susceptible and resistant *H. influenza*, which are increasingly common.

***Staphylococcus aureus* vaccines**

Vaccines against *Staph. aureus*, which is often antibiotic resistant, would be helpful to patients at high risk for infection with this organism, including renal dialysis patients, or patients receiving prosthetic devices like hips or vascular grafts, which act as sites for infection (Univax, 1994). Researchers are pursuing vaccines made of capsular polysaccharide types 5 and 8, which would encompass 90 percent of *Staphylococcus* systemic infections. Recent research has shown that high levels of biologically active antibodies against *Staph. aureus* types 5 and 8 can be stimulated in human subjects when the antigens are combined with a protein from *P. aeruginosa* as

the carrier (Fatton, et al., 1990; Fatton, et al., 1993).

Active Mucosal Immunization

The second approach to active immunization is to stimulate the immune defenses of the mucosal linings of the gastrointestinal, respiratory and urogenital tracts, the nasal passages, and the inner ear. These mucosal linings produce immunoglobulin A (IgA). IgA diminishes microbial virulence by preventing microbial adherence to host cells. It also coats the surface of the antigen, making an antigen/IgA complex that stimulates white blood cells to recognize, engulf, and destroy any pathogen expressing that antigen. Mucosal lymphocytes also trigger production of circulating IgG antibodies. Current targets for mucosal immunity include *Helicobacter pylori*, *Clostridium difficile*, *Shigella flexneri*, Campylobacter strains, and certain strains of *Escherichia coli*.

Mucosal vaccines are immunogenic only if they reach specific immune response tissues beyond the stomach, which requires their surviving passage through stomach acid and enzymes. Some researchers are testing synthetic polymers to protect their vaccines. Another strategy is to use liposomes, lipid-containing vesicles made from the same natural materials that compose mammalian cell membranes.

Helicobacter pylori vaccines

The discovery by Marchetti, Arico, Burrone, et al. (1995) that bacteria isolated from humans with ulcers could infect mice and cause disease processes that mimic those seen in humans has spurred progress toward a vaccine against *H. pylori*, the causative organism. Those researchers found that mice were protected from infection after administration of disrupted *H. pylori* bacteria. This finding, characterized as “of extreme practical importance” (Tompkins and Falkow 1995) may lead to a vaccine to protect the 50 percent of the world’s population that are currently infected by *H. pylori*. While *H. pylori* is most often associated with gastritis and ulcers in

the United States, elsewhere in the world it is also a common cause of stomach cancers.

Campylobacter vaccines

Campylobacter strains have recently emerged as one of the common causes of diarrhea and may cause 2.5 million cases annually in the United States. Treatment is increasingly complicated by antibiotic resistance. In 1994, the U.S. Navy signed a Cooperative Research and Development Agreement (CRADA) with MicroCarb Inc. for clinical trials of a vaccine against Campylobacter.

Passive immunization

Passive immunization involves administering antibodies directed against specific pathogens to individuals who have not developed such antibodies on their own but who are at risk for infection. This may be due to lack of prior exposure to the pathogen, or due to immunosuppression, which renders the individual’s immune system unable to produce antibodies. The antibodies are purified from the blood of healthy donors whose antibody levels are raised by active immunization. The most common example of passive immunization is the administration of Hepatitis B virus-specific gamma-globulin to travelers. Researchers are currently focusing efforts to develop antibodies against *Staph. aureus* and *P. aeruginosa*, both of which are often antibiotic resistant, as well as against other bacteria.

Passive immunization does not always work. Low birthweight babies are at high risk of nosocomial infections because of long hospitalizations and immature immune systems. Injections of pooled human antibodies (“immune globulin”) into very low birthweight babies did not reduce the incidence of nosocomial infections compared to the incidence in very low birthweight babies who did not receive the immune globulin (Fonaroff, Korones, Wright, et al., 1994). This failure does not invalidate the idea of passive immunization, even in low birthweight babies, but it underlines the importance of trials of the efficacy of interventions before they are introduced widely into practice.

Vaccine Summary

Vaccines are not high-profit items. UNICEF estimates that the entire global vaccine market is about \$3 billion, which can be compared to the \$3.5 billion market for a single ulcer drug. While vaccine development against bacteria that have high frequencies of antibiotic-resistant strains, such as *Staph. aureus* or *S. pneumonia*, would reduce infections by those bacteria, few vaccines will be developed for bacteria solely because of the problems raised by antibiotic resistance. Instead, the general problems raised by the bacteria may lead to development of a vaccine that will protect against both antibiotic susceptible and resistant strains.

STIMULATING THE IMMUNE SYSTEM

Granulocyte colony-stimulating factor (G-CSF) is a growth factor that stimulates the proliferation of neutrophil cells, important components of the immune system. Crawford, Ozer, Stoller, et al. (1991) have shown that the administration of G-CSF to cancer patients on chemotherapy led to a 51 percent reduction in culture-confirmed infections, a 47 percent reduction in the mean number of days of antibiotic use, and a 45 percent reduction in the mean number of days of hospitalization. G-CSF in the form of filgrastim (Amgen, Thousand Oaks, California) has been approved by the FDA and is clinically available.

TARGETED DELIVERY OF ANTIBIOTICS

Some sites of infection or potential infection are localized, such as wounds or the area around a joint replacement. Delivery of antibiotics directly to those sites may stop the growth of susceptible bacteria, and if the concentration can be raised high enough, it may even stop the growth of many resistant bacteria. Direct delivery of antibiotics in this way has the additional advantage of producing only very low levels of circulating antibiotics, thus reducing pressure for the selection of resistant bacteria elsewhere in the body.

■ Microencapsulation

Entry into the body, whether surgical or traumatic, opens pathways for infection. Surgical patients who develop wound infections spent, on average, 14.3 days longer in the hospital than uninfected matched controls (Maderazo, Judson, and Pasternak, 1988), at an increased cost of \$36,000 to \$45,000 per patient (Cohen, 1994; Daly, Eliopoulos, Reiszner, et al., 1988). Twenty-four percent of United States servicemen who sustained open fracture wounds in Panama during Operation “Just Cause” developed wound infections (Jacob, Erpelding, and Murphy, 1992), and 48 percent of wounded United States soldiers in the Persian Gulf conflict who sustained open fractures developed postoperative infections (Travis and Cosio, 1993). Gustilo, Mendoza, and Williams (1984) report similar infection rates in civilians with severe open fractures of the tibia. Many of these infections occur in patients who receive very large doses of systemic antibiotics.

Researchers at the Walter Reed Army Institute of Research (WRAIR) have developed a novel biodegradable local antibiotic delivery system that promises to decrease infections in wounds. They encapsulate an antibiotic in a copolymer of poly (DL-lactide-coglycolide) to produce microspheres 50 to 250 micrometers (μm) in diameter. Dusted into wounds after surgery, these microspheres provide an initial burst of the antibiotic within the first few hours and prolonged drug release over a period of up to 21 days. After 2 to 3 months, the microspheres completely degrade. As of March 1995, the WRAIR researchers had constructed microspheres containing ampicillin, cefazolin, cefamandole, and tobramycin.

Cefazolin-containing microspheres were used to treat wounds in rats that had been intentionally infected with cefazolin-resistant MRSA, and they were as effective as free cefazolin powder in eliminating MRSA. Systemic administration of cefazolin, on the other hand, had no effect on the MRSA infections. In a similar experiment involving ampicillin-resistant MRSA, microspheres containing ampicillin were more effec-

tive than free ampicillin powder, and systemic ampicillin had no effect.

The United States Army, which developed this technique (Setterstrom, Tice, and Myers, 1994; Jacob, Setterstrom, Bach, et al., 1991; Jacob, Cierny, Fallon, et al., 1993), has a patent pending on it. Further development will require private funding to take the research from the pre-clinical stage to trials in humans.

■ Antibiotic-Impregnated Cement

Bone infections and infections of joint prostheses are hard to treat with systemic antibiotics, partially because limited blood flow to the skeletal tissues does not allow high concentrations of the drug to reach the area of infection. An antibiotic-impregnated polymer, poly (methyl-methacrylate) (PMMA), has been used to cement bone fractures and prostheses in place, and has shown clinical success, but its usefulness is limited by the toxicity of the material and shrinkage which leaves marginal mechanical support for the remaining bone. Yu, et al. (1992) described hydroxyapatite (HAP) cement, which has the same chemical composition as bone mineral. This material can be molded to fill the space left by the absence of bone, and Yu, et al. demonstrated that antibiotics impregnated in this material are slowly released. They concluded that this material is very promising for preventing infections in bone fractures and in joining prostheses, and they propose future *in vivo* experiments.

■ Biological Substances to Facilitate the Entry of Antibiotics into Bacteria

One mechanism of resistance involves bacterial cell walls in excluding antibiotics from the bacterial cell. Research is underway on biological substances that allow antibiotics to penetrate into such bacteria. For example, because iron is insoluble but necessary for bacterial metabolism, bacteria synthesize and excrete compounds that can bind iron ions, called “siderophores.” These compounds scavenge iron outside the cell, and the cell then transports the iron-siderophore compound back inside the cell. Inside the cell, the

iron-siderophore complex is metabolized by the bacteria, releasing iron for bacterial use. Siderophores may be modified to carry antibiotics into the bacteria. These may be especially useful in the treatment of Gram-negative bacterial infections. Although the outer cell membrane channels (“porins”) of Gram-negative bacteria are too small to accommodate many antibiotics, siderophores enter the cell via a non-porin route, and researchers reason that antibiotics attached to siderophores might be “dragged” inside.

Over 200 siderophore molecular structures are known. Often, only portions of the siderophores are required to penetrate the cell. One goal of current research is to optimize synthetic siderophores in order to make their transport into bacterial cells more efficient. Synthetic siderophores, when conjugated with beta-lactam antibiotics or erythromycin, can carry the antibiotic across bacterial cell membranes with high efficiency. These antibiotics kill bacteria when delivered inside the cell in this manner (Miller, 1989; McKee, Sharma, and Miller, 1991). Siderophores are also being explored for their potential to transport vancomycins. Although siderophore/antibiotic conjugates have thus far been used only as antibacterials, researchers are currently attempting to apply the same methodology to antifungal/siderophore conjugates.

REDUCING INFECTIONS BY MODIFYING DEVICES

Several hundred thousand cases of hospital acquired infection per year are related to the use of medical devices such as catheters, endotracheal tubes and mechanical ventilators (IOM, 1992). These devices provide extra opportunities for bacteria to enter the body. Experience with dialysis, the filtering of the blood of patients with kidney disease, indicates that changing the design and materials of medical devices can minimize infections.

■ Infections in Dialysis Patients

In 1991, there were approximately 120,000 patients on maintenance dialysis (Favero, Alter,

and Bland, 1992) with 45,000 new patients added per year. Infections are the cause of death in 15 to 30 percent of dialysis patients.

The technique called hemodialysis is used to treat approximately 85 percent of dialysis patients. Simulating the function normally performed by the kidney, it filters the patient's blood through a membrane which separates out unwanted components and adds needed components. Cupraphane membranes, the most commonly used filtration membrane in hemodialysis, are made from cotton fibers dissolved in an ammonia solution of cupric oxide. Recently, membranes made of synthetic polymers such as polysulfone (PS), polymethylmethacrylate (PMMA) and polyacrylonitrile (PAN) have been developed. A recent review of the properties of hemodialysis membrane (Hakim, 1993) describes how the interaction of blood with cotton fiber membranes such as Cupraphane produces a decrease in the immune functions in the blood, leaving the patient more susceptible to infection. The membranes made of synthetic polymers do not seem to decrease the immune functions in the blood. Retrospective studies showed that replacing a Cupraphane membrane with a polysulfone membrane eliminated 50 percent of the infections.

Another 15 percent of patients are on peritoneal dialysis. In this technique, fluid is pumped into the patient's abdomen, allowing exchange of blood components through the peritoneal lining of the abdomen. A recent review (Diaz-Buxo, 1993) shows that the incidence of peritonitis (peritoneal infection) was twice as high when older CAPD (continuous ambulatory peritoneal dialysis) machines were used than when new dialysis machines of different design, such as CCPD (continuous cyclic peritoneal dialysis) machines and Y-set connections for CAPD, were used. This may be because the order of flow is reversed in CCPD and Y-set CAPD compared to other forms of CAPD, so that the connections (and contaminating bacteria) are washed out before fluid is pumped into the body. Diaz-Buxo comments that CAPD machines are more common than CCPD machines, partially because of

the lower cost of the machine itself. When the total costs of the two systems were calculated, including the cost of the machine and the cost of hospitalization for peritoneal infections, the total costs were the same (King, et al., 1992).

Analyzing the costs of dialysis for kidney patients is especially interesting because dialysis patients have been covered by Medicare since 1973 regardless of their age. Medicare pays a set amount per patient for dialysis and pays separately for any hospitalization necessitated by complications. Under this system, physicians have a financial incentive to use the least expensive equipment. However, it would be beneficial to the patients, and probably cheaper for Medicare, to use the more expensive equipment and prevent infections that may require hospitalization. Outpatient costs, primarily dialysis, accounted for 33 percent of total costs compared with 44 percent of total costs attributable to hospitalizations (Smits, 1995). (The remainder of the costs were for physician services, skilled nursing care, and home health care.) This demonstrates that investing in new technologies that prevent infections and hospitalizations can be cost-effective. These investments would also reduce antibiotic resistance by preventing infections and thus reducing the use of antibiotics.

■ Infections from Sutures and Catheters

Improvements in the materials used for other medical devices such as sutures and catheters could also greatly reduce the rate of infection. In particular, sutures made of synthetic materials such as dacron and nylon have lower infection rates compared to natural sutures such as cotton, silk and catgut, and monofilament sutures have lower infection rates compared to polyfilament sutures.

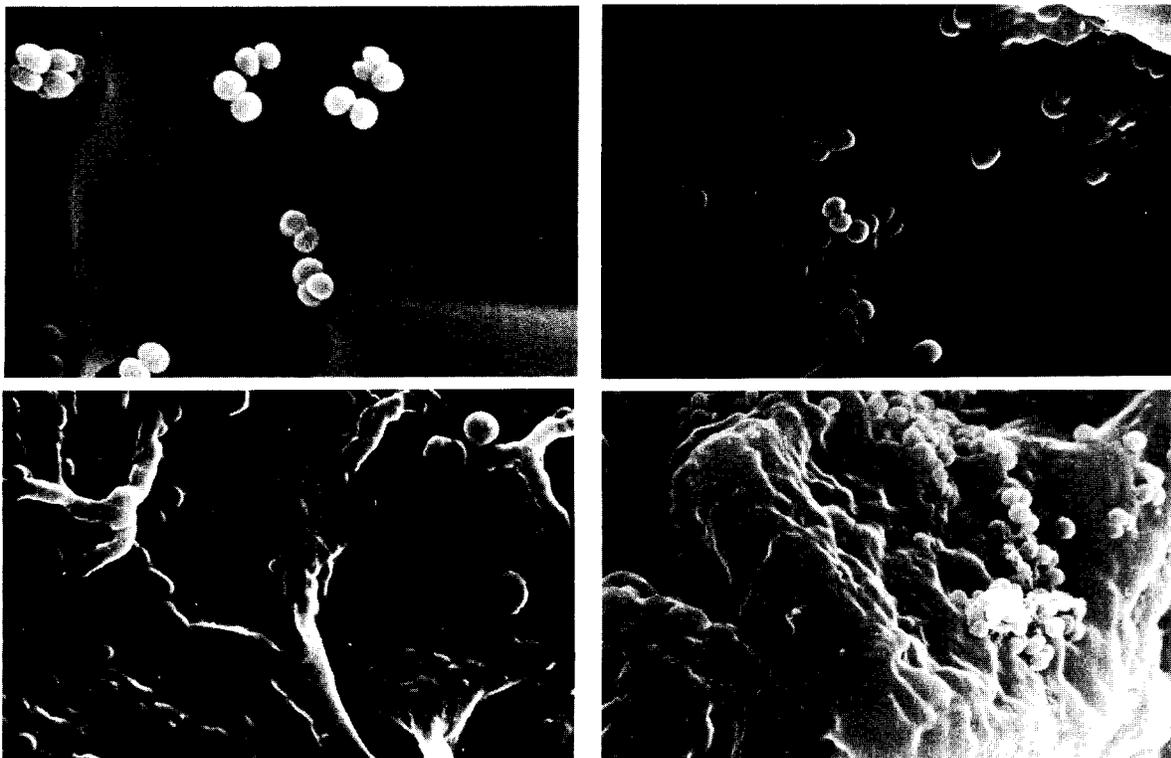
Studies of the colonization of medical devices by coagulase-negative staphylococci (Christensen, Baldassarri, and Simpson, 1994) provides some insight into why some suture materials are associated with infections more than others. The process of colonization of non-biological surfaces by coagulase-negative staphylococci is

shown in the photograph. The first step in colonization is binding and/or trapping a “unique site” on the surface, such as a microscopic crack or depression in the surface of the material. Synthetic materials such as nylon and plastics are generally much smoother than natural materials such as cotton and silk and therefore have fewer unique sites. Similarly, monofilament are smoother than polyfilaments. Therefore, it is not surprising that the natural materials and polyfilaments are more often associated with infections than the synthetic materials and monofilaments. Knowledge about the colonization and infection process for non-biological materials will help guide new designs of medical devices that may minimize infections and reduce the need for antibiotics.

Maki (1994) reviewed innovative designs that help prevent infections in intravascular catheters used for infusion therapy. Some catheters have a new design that creates mechanical barriers against infection at the entrance of the catheter to

the skin. Other designs create a closed system; for example, they replace the stopcocks used to obtain blood specimens from arterial lines with a diaphragm. Such closed systems reduce the rate of infection.

Another strategy for preventing infections is to coat the materials used in medical devices with antibiotics or other antibacterial agents. Like the microencapsulated antibiotics and antibiotic-impregnated cement, these coated catheters may have the advantage of delivering high concentrations of antibiotics to the site of potential infection with much lower systemic antibiotic concentrations. In one system, the catheters are coated or impregnated with silver ions, which are bactericidal but non-toxic to humans. (Manufacturers include Arrow International and C.R. Bard Urological Division; Maki, et al., 1991; Stamm, 1991). In another system, catheters are coated with materials bearing positively charged chemicals, to which negatively charged antibiot-



Series of four microphotograph demonstrating the colonization of a non-biological surface.
 Courtesy of Meryl E. Olson, University of Calgary, Calgary, Canada

ics are bound (Cook Bio-Guard AB coated catheters, Cook Critical Care). A trial with these catheters coated with cefazolin showed a seven-fold decrease in the infection rate (Kamal, Pfaller, Rempe, et al., 1991). Further, a reduction in the infection rate was seen even if the catheters were changed only once every seven days (compared to once every four days for standard catheters; Kamal, Divishek, Adams, et al., 1994). The longer life of the coated catheter compensates for its higher cost (about \$4.50 more per catheter).

OLD THERAPIES

In the pre-antibiotic era, scientists and physicians tried different methods to treat bacterial infections. Two of those methods, “phage therapy” and “serum therapy,” are now mentioned as possible treatments in a post-antibiotic era.

■ Phage Therapy

While most people may not recognize the term “phage therapy,” many people read about it in *Arrowsmith*. The hero of that novel tried to treat bacterial infections by the use of viruses that would specifically attack the bacteria, and in real life, many physicians tried the same method in the early part of this century. Because viruses that infect bacteria are called “bacteriophages” (literally, eaters of bacteria) or “phages” for short, the treatment is called “phage therapy.” Phage therapy has remained outside the mainstream of medicine because of doubts about its efficacy and the success of antibiotics.

Phages recognize specific binding sites on the bacteria. Therefore, phages that infect *E. coli* generally do not infect other bacteria, and, in fact, sometimes will only recognize a single strain of bacteria. This specificity offers the promise of being able to prepare phages to attack particular bacteria.

Levin and Bull (1995) and Levin, DeRouin, Moore, et al. (1995) review the literature about phage therapy. They focus on some recent experiments with systems that involve mice infected with *E. coli* and argue that phage therapy is

worth renewed investigation. While they do not think that it will replace antibiotics, they believe that it may have some future use in treating antibiotic-resistant bacteria. They also argue that the time to develop alternatives to antibiotic therapy is now, when antibiotics remain effective against most diseases.

■ Serum Therapy

Textbooks of medicine and of microbiology published before 1940 are filled with instructions for serum therapy. In some respects similar to passive immunization, serum therapy involves taking blood serum from horses or rabbits that have survived an intentional bacterial infection and injecting it into a patient suffering from an infection by the same organism.

Serum is still used in the treatment of some diseases that involve bacterial toxins; in particular, tetanus and botulism are treated with horse serum. Serum for the treatment of botulism is kept at several major airports around the country, ready for shipment to hospitals that diagnose the rare disease. (According to the CDC [1979], there were about 10 outbreaks of botulism, involving about 2.5 people per outbreak, each year in the period 1899 through 1977.) For other infections, serum therapy was replaced as antibiotics became available. A patient’s possible anaphylactic response to chemical substances in the animal serum is the chief danger.

Serum therapy may have application in treating *Escherichia coli* O157:H7, which became famous as the cause of more than 500 cases of disease and perhaps four deaths in people who ate under-cooked fast-food hamburgers in the Pacific Northwest in early 1993. The usual treatment for the disease does not include antibiotics (Salyers and Whitt, 1994). Antibiotics have not been shown to shorten the course of the disease or to reduce the occurrence of kidney complication. Further, antibiotic treatment may cause the bacteria to increase the production of the bacterial toxin that causes the disease. The cause of disease in *E. coli* O157:H7 infections is a toxin that resembles the Shigella toxin that causes dys-

entry. That toxin has been isolated and purified. Antibodies generated against the toxin have potential in treating *E. coli* O157:H7-caused diseases, but the market for such a drug is small, and no trials are in progress.

SUMMARY

This chapter reviews some new technologies that will help health care providers use antibiotics more effectively. Diagnostic technologies help the clinician identify the specific bacteria causing the infection and its susceptibility to antibiotics. This information is critical for choosing the most appropriate antibiotic. New technologies, such as DNA identification of antibiotic resistance genes, have the potential to provide this information more quickly than is possible with traditional diagnostic tests, which require growing the bacteria in cultures. These new diagnostic technologies have already proven useful in diagnosing tuberculosis. Many companies are rapidly developing additional tests for TB and other bacteria. There are unresolved issues with respect to the accuracy, sensitivity, and reproducibility of these tests. These issues may not be resolved until the tests have received FDA review and are widely used in clinical settings. This chapter discusses some of these issues.

Preventing infections is another way to slow the increase of antibiotic-resistant bacteria because prevention will reduce the total use of antibiotics. Methods of preventing infection include vaccines and changes in the design and composition of medical devices to prevent the growth of bacteria. The recent introduction of a vaccine against *Haemophilus influenzae B* resulted in a dramatic reduction in the incidence of childhood diseases caused by this bacteria. A number of other vaccines are under development, including those for *Staphylococcus aureus*, as well as better vaccines for *Streptococcus pneumoniae*. Indwelling devices and sutures are often ports of entry for bacteria into the body, and improved devices and materials have been shown to reduce infection rates. Further research and application could produce further reductions.

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