Al-Anbar University

College of Sciences

Biology department

Subject name: Microbial Identification

Educational level: Master

Lecture title: Bacterial Identification Methods

Subject teacher

Assistant prof. Dr. Jamal A. Rahman

Chapter 6

Bacterial Identification Methods

Introduction

Previous chapters have covered microscopes (see Chapter 1), microscopic observation (see Chapter 2), staining (see Chapter 3), cultivation (see Chapter 4), and biochemical characterization (see Chapter 5). In this chapter these techniques are applied to one of the most fundamental aspects of microbiology: the identification of an unknown bacterial culture. The identification of unknown cultures is especially important in clinical settings, because knowing the identity of a pathogen and its antibiotic susceptibility (see Chapter 7) is essential for determining effective treatment. Three methods of identification are discussed in this chapter: (1) conventional methods; (2) multitest systems; and (3) automated methods.

IDENTIFICATION USING CONVENTIONAL METHODS

Bacterial identification, regardless of the method used, first requires the cultivation, isolation, and staining of a pure culture. After colony characteristics, cell morphology, and Gram reaction have been determined, conventional identification methods require microbiologists to perform biochemical tests specified in an **identification scheme**, which is a key of bacterial characteristics (Figures 6.1 and 6.2). The biochemical tests specified in the key are done in a specific sequence to make a species identification.

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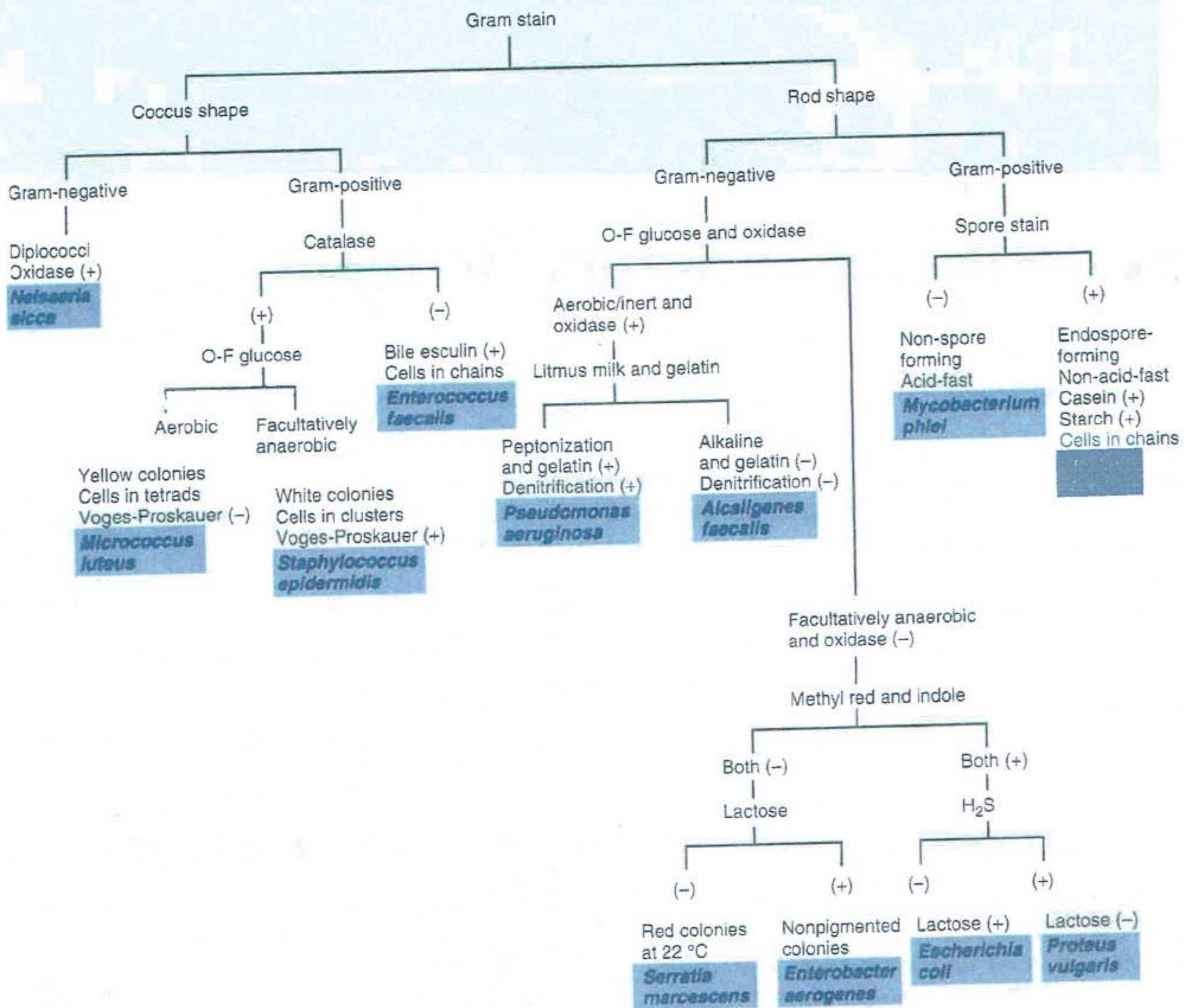

FIGURE 6.1 Identification scheme for 12 microbiology laboratory unknowns. You can use this scheme when identifying bacteria commonly used in microbiology teaching laboratories.

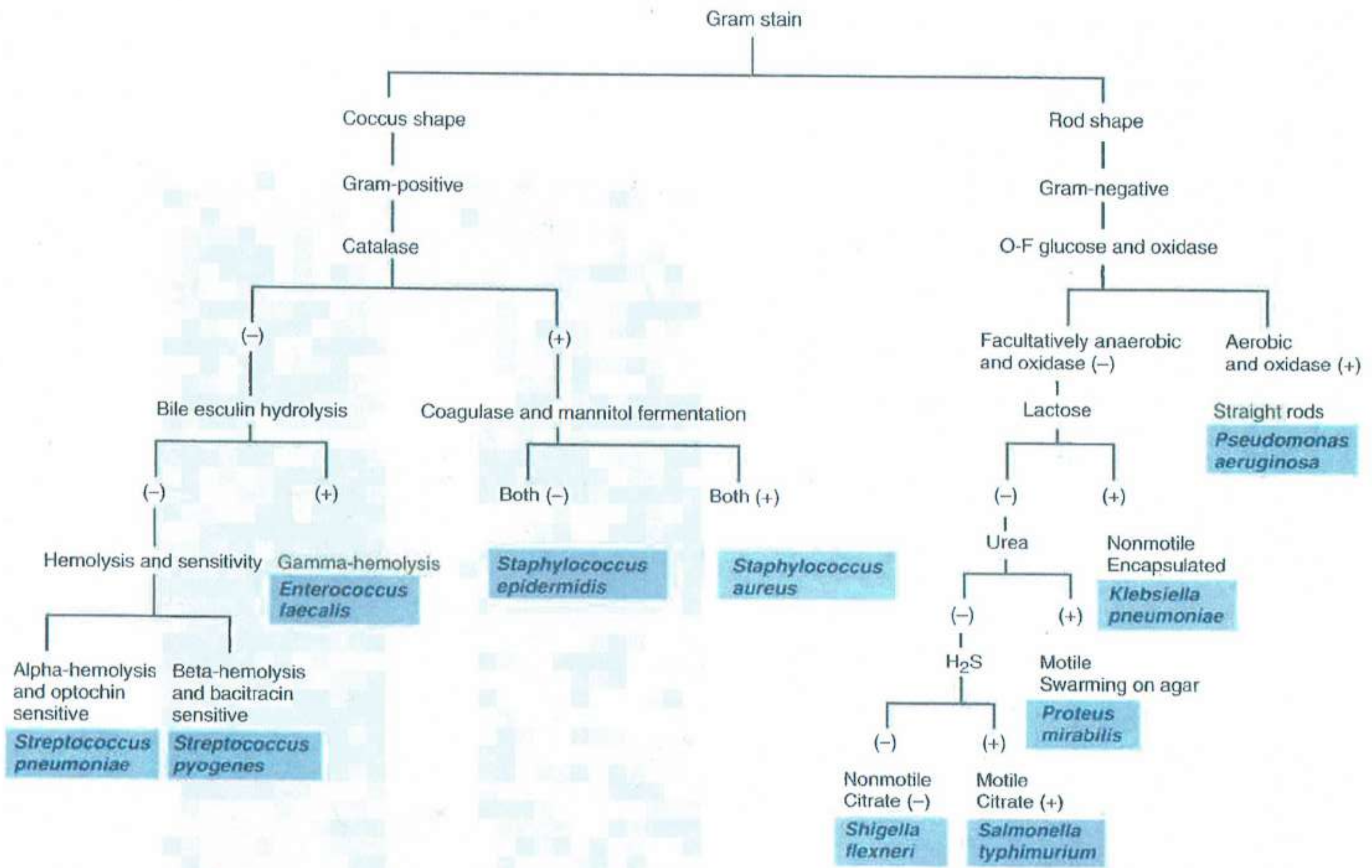

FIGURE 6.2 Identification scheme for 10 clinical laboratory unknowns. You can use this scheme when identifying bacteria commonly encountered in clinical laboratories.

IDENTIFICATION USING MULTITEST SYSTEMS

In recent years, a variety of commercially available multitest systems for bacterial identification have been developed. These systems contain up to 22 biochemical tests in a single unit and allow for rapid bacterial identification without time-consuming and costly media preparation. Here we describe two widely used multitest systems.

API® identification systems each consist of a plastic strip that contains 20 individual microtubes. Each microtube contains a dehydrated test medium that is rehydrated with a suspension of the test organism. After inoculation, strips are incubated in plastic trays at 35°C for 18–24 hours. Microtubes are then examined for color changes, and tests are recorded as either positive or negative. Test results are used to determine an identification number that corresponds to a bacterial species. A number of API® systems are available: for *Enterobacteriaceae*, API® 20E (Figure 6.3); for *Staphylococcus*, API® STAPH; and for streptococci, API® 20 STREP (Figure 6.4).

The **Enterotube® II** is used for the identification of *Enterobacteriaceae*. This system consists of a 12-compartment tube that accommodates 15 biochemical tests (Figure 6.5). The compartments are inoculated by touching an enclosed inoculating wire to a culture and then pulling the wire through each compartment. After inoculation, the tube is incubated at 35°C for 24 hours. Compartments are then examined and tests recorded as either positive or negative based on color changes. Enterotube® II biochemical tests, with colors indicating positive and negative results, as well as results for the test organism in Figure 6.5, are listed in Table 6.1. Positive and negative test results are used to determine an identification number, which is then matched with the corresponding bacterial species.

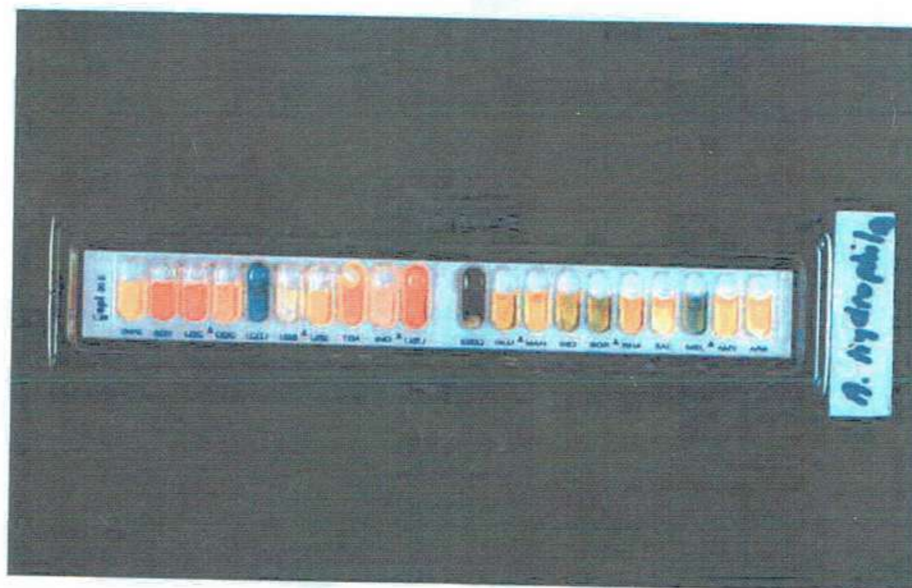

FIGURE 6.3 A multitest system: API® 20E for the identification of *Enterobacteriaceae*. Based on the results shown here, this clinical isolate was identified as *Aeromonas hydrophila*.

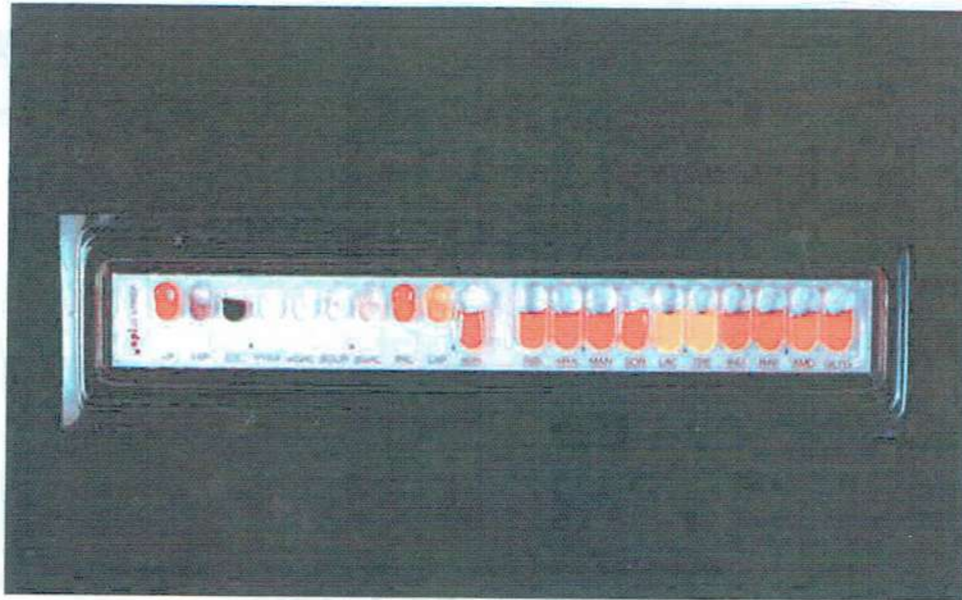

FIGURE 6.4 A multitest system: API® 20 STREP for the identification of *Streptococcus*. Based on the test results shown here, this clinical isolate was identified as *Streptococcus milleri*.

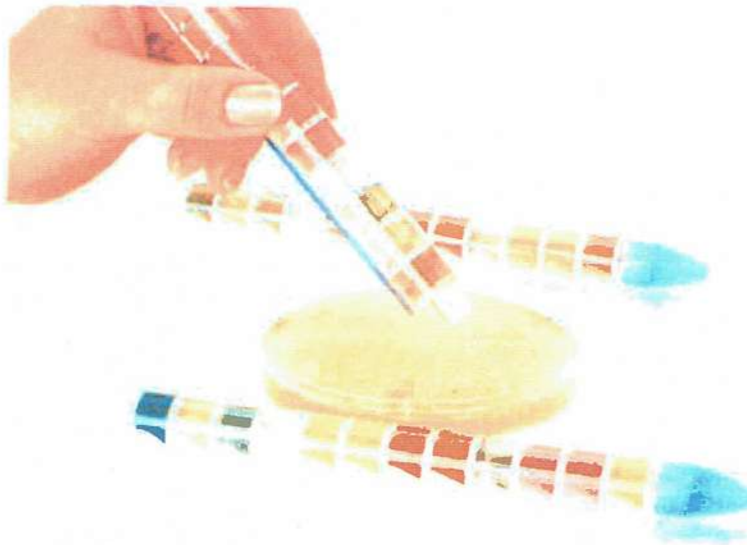

FIGURE 6.5 A multitest system: Enterotube® II. The rear tube is an uninoculated control. The center tube is being inoculated by touching the end of the inoculating wire to the culture and then drawing the wire through the compartments. The front tube has been inoculated with a test organism. Notice the color changes in the compartments of the front tube compared to the uninoculated tube. The results for this test organism are listed in Table 6.1.

TABLE 6.1

Enterotube® II biochemical tests/reactions and their use in the identification of a test organism

Test	Explanation	Indicator for		Results for test organism in Figure 6.5*
		Positive test	Negative test	
GLU	Fermentation of glucose	Yellow	Red	Positive
GAS	Gas production from fermentation of glucose	Wax separation	No wax separation	Positive
LYS	Decarboxylation of lysine	Purple	Yellow	Positive
ORN	Decarboxylation of ornithine	Purple	Yellow	Positive
H_2S	Hydrogen sulfide production	Blackening	No blackening	Positive
IND	Indole production from tryptophan	Red	Beige	Negative
ADON	Fermentation of adonitol	Yellow	Red	Negative
LAC	Fermentation of lactose	Yellow	Red	Negative
ARAB	Fermentation of arabinose	Yellow	Red	Positive
SORB	Fermentation of sorbitol	Yellow	Red	Positive
V-P	Acetoin production (Voges-Proskauer)	Red	Beige	Negative
DUL	Fermentation of dulcitol	Yellow	Green	Negative
PA	Phenylalanine deamination	Blackening	No blackening	Negative
UREA	Urea utilization	Pink	Yellow	Negative
CIT	Citrate utilization	Blue	Green	Positive

*These results yield an identification number corresponding to *Salmonella typhimurium*.
Source: Becton Dickinson Microbiology Systems, Cockeysville, MD 21030.

IDENTIFICATION USING AUTOMATED METHODS

Automated methods are the quickest and most accurate of the bacterial identification methods. They are also the most expensive. The expense of these systems is justified in clinical settings in which clinicians must identify large numbers of isolates daily. Here we describe two widely used automated systems.

The **Vitek® automated system** consists of a thin, plastic card containing 30 biochemical tests plus an instrument that reads the card. Several types of cards are available, including those for Gram-negative identification (GNI) and Gram-positive identification (GPI). Cards are inoculated with a culture suspension that is introduced through an inlet port. During incubation, cards are scanned by the instrument hourly until scans match one species in a database of known bacteria. In many instances, this system can provide a species identification in 4–6 hours. An inoculated Vitek® GNI card is depicted in Figure 6.6, while the Vitek® instrument/printer is depicted in Figure 6.7. A separate Vitek® card is used for antibiotic susceptibility testing, which is discussed in Chapter 7.

One portion of the **MicroScan® automated system**, designed for Gram-negative bacteria, consists of a panel containing numerous individual wells (Figure 6.8). The panel contains 26 wells for biochemical tests and 60 wells for antibiotic susceptibility tests. The wells are inoculated with a bacterial suspension. During incubation, the biochemical test wells are periodically scanned by a MicroScan® instrument (Figure 6.9). MicroScan® biochemical tests, with colors indicating positive and negative results, as well as results for the test organism in Figure 6.8, are depicted in Table 6.2. When test results match those of an organism in its database, the system prints out the species identification.

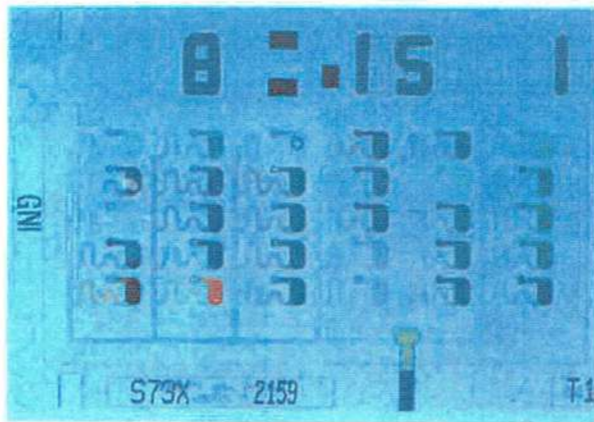

FIGURE 6.6 A component of an automated system: a Vitek® Gram-negative identification (GNI) card. This card has been inoculated with a test organism.

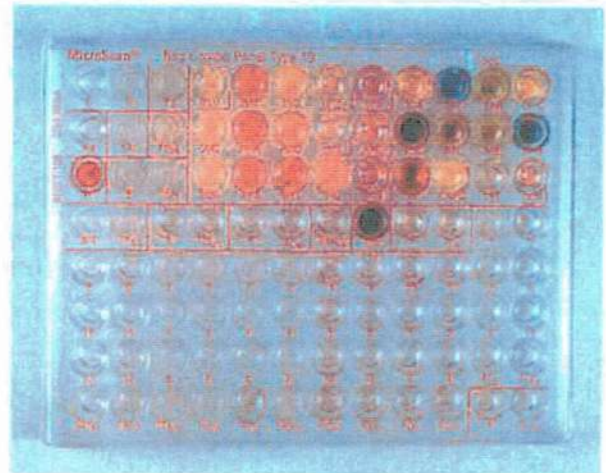

FIGURE 6.8 A component of an automated system: a MicroScan® panel. This panel contains 26 wells for biochemical tests (colored wells at the top) and 60 wells for antibiotic susceptibility tests (colorless wells at the bottom). The panel shown here has been inoculated with a test organism. The results for this test organism are listed in Table 6.2.

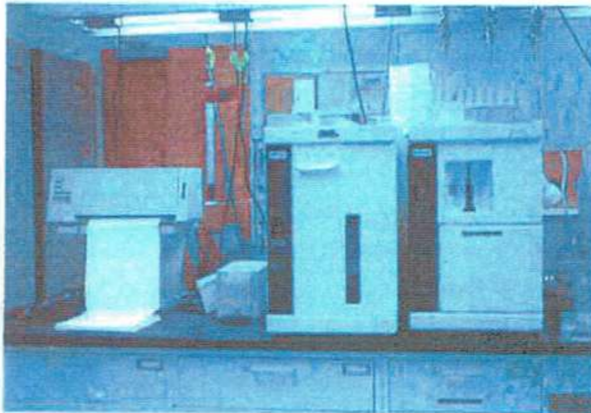

FIGURE 6.7 A component of an automated system: the Vitek® instrument/printer.

FIGURE 6.9 A component of an automated system: the MicroScan® instrument.

TABLE 6.2

MicroScan® biochemical tests/reactions and their use in the identification of a test organism

Test	Explanation	Indicator for: Positive test	Indicator for: Negative test	Results for test organism in Figure 6.8*
GLU	Fermentation of glucose	Yellow to yellow/orange	Orange to red	Positive
SUC	Fermentation of sucrose			Positive
SOR	Fermentation of sorbitol			Positive
RAF	Fermentation of raffinose			Negative
RHA	Fermentation of rhamnose			Negative
ARA	Fermentation of arabinose			Negative
INO	Fermentation of inositol			Positive
ADO	Fermentation of adonitol			Negative
MEL	Fermentation of melibiose			Negative
URE	Urea hydrolysis	Pink	Yellow to orange	Negative
H_2S	Hydrogen sulfide production	Blackening	No blackening	Negative
IND	Indole production from tryptophan	Pink to red	Yellow to orange	Negative
LYS	Decarboxylation of lysine	Purple	Yellow or colorless to gray	Positive
ARG	Decarboxylation of arginine			Negative
ORN	Decarboxylation of ornithine			Positive
TDA	Tryptophan deamination	Brown	Yellow to orange	Negative
ESC	Esculin hydrolysis	Brown to black	Beige to colorless	Positive
VP	Acetoin production (Voges-Proskauer)	Red	Colorless	Positive
CIT	Citrate utilization	Blue	Green to yellow	Positive
MAL	Malonate utilization	Blue	Green to yellow	Negative
ONPG	Beta-galactosidase production	Yellow	Colorless	Positive
TAR	Tartrate utilization	Blue	Green to yellow	Negative
ACE	Acetamide utilization	Blue	Green to yellow	Negative
CET	Cetrimide tolerance	Growth	No growth	Negative
OF/G	Oxidation-fermentation of glucose	Yellow	Green to blue	Positive
NIT	Nitrate reduction	Red	Colorless to pink	Positive

*These results yield a database match corresponding to *Serratia marcescens*. This test organism was isolated from a patient's blood.
Source: Dade Behring, Inc., 1584 Enterprise Blvd., West Sacramento, CA 95691.

Chapter 7

Evaluating Antibacterial Chemical Agents

Introduction

Microbiologists use a variety of chemical agents to control bacterial growth, including antiseptics, disinfectants, and antibiotics. Both **antiseptics** and **disinfectants** are nonselective in their action, which means they kill or inhibit different types of cells, not just bacterial cells. Antiseptics are used on the skin, while disinfectants are used on nonliving surfaces, such as floors and countertops. **Antibiotics** are selective in their action, which means they kill or inhibit bacterial cells only. For this reason, antibiotics can be introduced into the body to treat bacterial infections without harming human cells.

This chapter covers methods used to evaluate the effectiveness of various antibacterial chemical agents. The tests described help determine which chemical agent will be effective in a particular situation.

Lab Procedure

EVALUATING HANDWASHING EFFECTIVENESS

Purpose and Procedure Summary

Commercial handwashing products containing antibacterial chemical agents are widely used in the home and workplace. Two types of products are available: hand soaps containing the phenolic antiseptic called **triclosan** and hand gels containing **ethyl alcohol**. Hand soaps require the addition of water, while hand gels can be used without water.

To test the effectiveness of a particular handwashing product, an area of the hand is swabbed before and after handwashing. Bacteria on the swabs are transferred to agar plates. After incubation, colonies on the plates are counted and the numbers used to calculate a percent decrease in bacteria. The procedure for a simple handwashing experiment is outlined in Figure 7.1.

Tips for Success

- *Duplicate exactly* the procedure for swabbing the before and after agar plates so that comparisons are valid.
- *Use duplicate areas* on one or both hands to calculate an average percent decrease.

Expected Results

Bacteria cultivated from the hand before and after washing with an antibacterial hand soap are shown in Figure 7.2. In this instance, a comparison of bacterial numbers before and after handwashing indicates a 57% decrease. Given that triclosan is known to be effective primarily against Gram-positive bacteria, it is likely that a large number of surviving

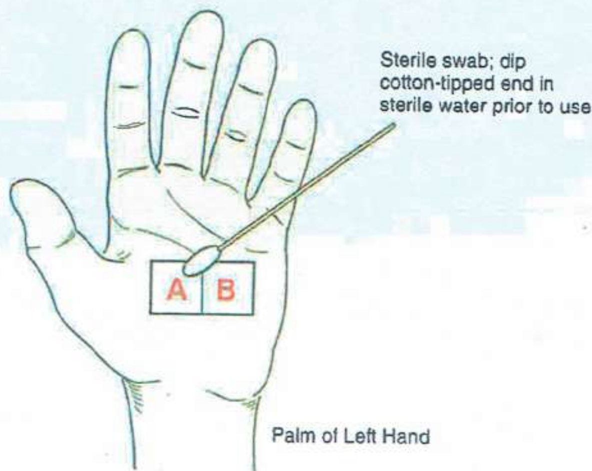

FIGURE 7.1 Handwashing experiment. After sampling area A using a sterile swab, wash hands as follows: Use hand soap for 30 seconds followed by a 30-second rinse, or use hand gel for 30 seconds. After washing, hands should be allowed to air-dry before sampling area B.

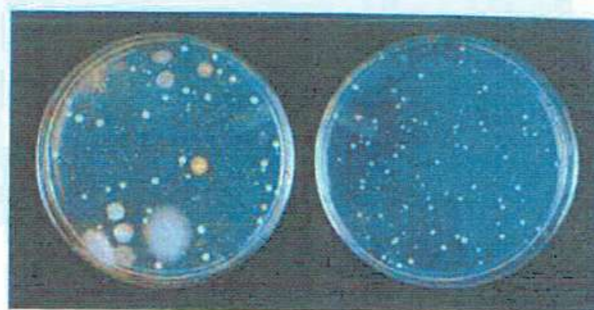

FIGURE 7.2 Bacteria cultured from a hand before (left) and after (right) washing with an antibacterial hand soap containing triclosan. The number of bacteria before (433) and after (188) indicates that handwashing removed or killed 57% of the bacteria.

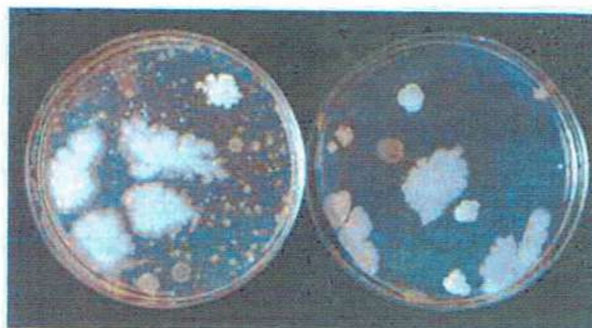

FIGURE 7.3 Bacteria cultured from a hand before (left) and after (right) washing with an antibacterial hand gel containing ethyl alcohol. The number of bacteria before (414) and after (15) indicates that handwashing removed or killed 96% of the bacteria.

bacteria are Gram negative. A Gram stain could verify this assumption. If the survivors are indeed Gram negative, the reliability of this product in preventing the spread of diseases caused by Gram-negative bacteria is called into question.

Bacteria cultivated from the hand before and after washing with an antibacterial hand gel are depicted in Figure 7.3. In this case, a comparison of bacterial numbers before and after washing indicates a 96% decrease. The greater effectiveness of the hand gel is likely due to the presence of ethyl alcohol, a chemical agent known to be effective against a wide range of bacteria. Based on their appearance, it is likely that a majority of the surviving bacteria are Gram positive. A Gram stain could verify this assumption. If the survivors are Gram positive, the reliability of this product in preventing the spread of diseases caused by Gram-positive bacteria is called into question.

Lab Procedure

EVALUATING ANTISEPTICS AND DISINFECTANTS: THE FILTER PAPER METHOD

Purpose and Procedure Summary

Antiseptics and disinfectants include a variety of chemical agents used in everyday life. For example, **isopropyl alcohol, hydrogen peroxide, tincture of iodine,** and **mercurochrome** are common antiseptics used to treat cuts and scrapes. Bleach and mildew removers, typically containing the disinfectant **sodium hypochlorite** (NaClO), are used to clean nonliving surfaces such as floors and countertops in kitchens and bathrooms. Other cleaning agents con-

tain the disinfectant **ortho-phenylphenol** or **pine oil**, a disinfectant extract from pine trees.

The effectiveness of these chemical agents can be tested using the **filter paper method.** Filter paper disks are dipped into a chemical agent and placed on an agar plate that has already been inoculated with a bacterial culture. The plate is incubated and then examined for bacterial growth.

Tips for Success

- *Cover the entire agar surface with bacteria* by swabbing the agar surface three or four times with a broth culture of the test organism. If this is done incorrectly (Figure 7.4), it is difficult to distinguish zones of inhibition.
- *Blot excess chemical off the disk* prior to its placement on the agar plate, so that excess chemical does not flow away from the disk and cause excessive zones of inhibition.
- *Handle disks with sterile forceps* to prevent contamination of the culture.
- *Place disks as far apart as possible* on the plate to minimize the overlap of zones of inhibition.

Expected Results

During incubation, the chemical in the disk diffuses out into the agar to create a concentration gradient ranging from high near the disk to low away from the disk. If the chemical is effective against bacteria, there will be an area around the disk where there is no growth. This area is called the **zone of inhibition.**

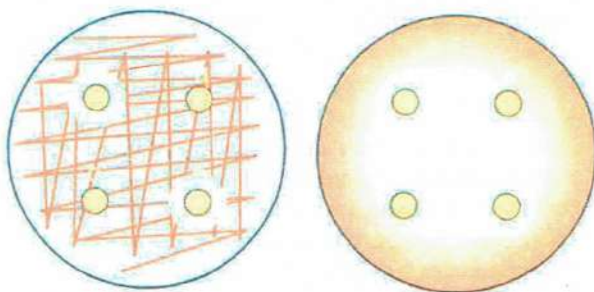

FIGURE 7.4 Growth on an agar plate swabbed incorrectly (left) compared to that on an agar plate swabbed correctly (right).

The zones of inhibition apparent in Figure 7.5 indicate the effectiveness of the common antiseptics mercurochrome (M), hydrogen peroxide (H_2O_2), and tincture of iodine (I) against *Bacillus cereus.* Although alcohol is effective against bacteria when rubbed on the skin, it is shown not to be effective in this application, probably because it evaporates from the disk before coming into contact with bacterial cells.

The effectiveness of three common disinfecting agents against *Bacillus cereus* is shown in Figure 7.6. Two of the products contained sodium hypochlorite (HC) as the active ingredient, while one contained ortho-phenylphenol (PP), and one contained pine oil (PO). A zone of inhibition around each disk indicates that all three agents were effective against *Bacillus cereus.*

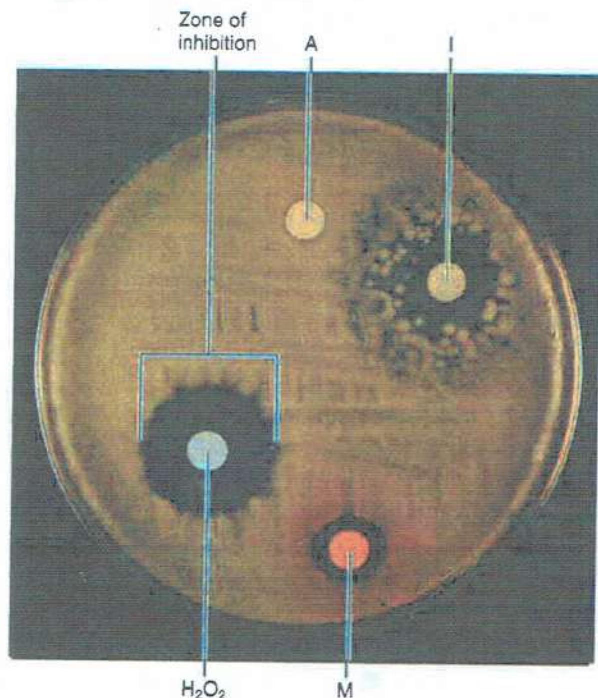

FIGURE 7.5 The effectiveness of four common antiseptics on *Bacillus cereus.* The disks were soaked in mercurochrome (M), hydrogen peroxide (H_2O_2), isopropyl alcohol (A), and tincture of iodine (I). All these antiseptics, except alcohol, inhibited growth around disks, indicating their effectiveness. Alcohol probably evaporated from the disk before it could make contact with bacterial cells.

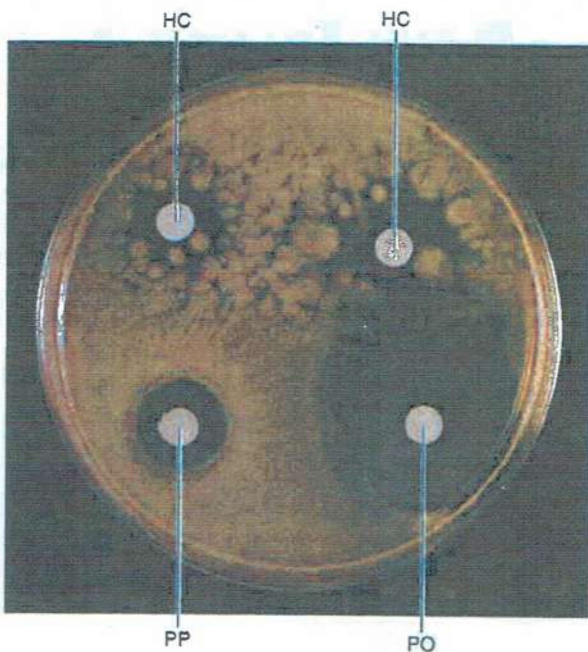

FIGURE 7.6 The effectiveness of three common disinfectants on *Bacillus cereus.* The disks were soaked in cleaning agents containing the disinfectants sodium hypochlorite (HC), ortho-phenylphenol (PP), and pine oil (PO). All three cleaning agents inhibited growth around disks, indicating their effectiveness.

ANTIBIOTIC EFFECTIVENESS

Antibiotics are an important class of chemical agents because they are used in the body to treat disease. These chemical agents are selective in the body, which means that they target specific components of the bacterial cell, such as the cell wall, the cell membrane, and ribosomes. For example, the antibiotics **ampicillin** and **penicillin** target the bacterial cell wall by disrupting the formation of the peptidoglycan layer. As a result, these antibiotics are effective against Gram-positive bacteria, which contain a thick peptidoglycan layer. These antibiotics are not as effective against Gram-negative bacteria, because they do not readily pass through the outer lipopolysaccharide layer of the cell wall. Because they affect only certain bacteria, ampicillin and penicillin are referred to as **narrow-spectrum antibiotics.** These antibiotics do not affect human cells because human cells do not have cell walls.

Other antibiotics, such as **chloramphenicol,** affect both Gram-positive and Gram-negative bacteria, because they bind to bacterial ribosomes and prevent protein synthesis. Antibiotics that affect a variety of bacteria are considered **wide-spectrum antibiotics.** These antibiotics have little effect on human cells, because human cells have ribosomes that are different enough from bacterial ribosomes so that binding does not occur.

The spectrum of activity of eight selected antibiotics is presented in Table 7.1.

TABLE 7.1

Spectrum of activity of selected antibiotics

Antibiotic	Activity against Gram (−) rods	Gram (+) rods	Gram (+) cocci
Ampicillin	Some		Yes
Chloramphenicol	Yes	Yes	Yes
Erythromycin		Yes	Yes
Gentamicin	Yes	Yes	Yes
Penicillin G			Yes
Polymyxin B	Yes		
Streptomycin	Yes	Yes	Yes
Tetracycline	Yes	Yes	Yes

Lab Procedure

EVALUATING ANTIBIOTICS: THE KIRBY-BAUER METHOD

Purpose and Procedure Summary

The Kirby-Bauer method is used to determine the effectiveness of various antibiotics by comparing results to an **interpretive standard**. This comparison allows the determination of whether a test organism is resistant or susceptible to a particular drug. Physicians use this information to make decisions about which drugs to use in treating bacterial infections.

An agar plate is inoculated with a broth culture using a swab. After inoculation, disks containing antibiotics are placed on the plate. Plates are incubated and then inspected for zones of inhibition around disks. The zone of inhibition around each disk is measured to the nearest millimeter and compared to an interpretive standard. The interpretive standard for eight selected antibiotics is presented in Table 7.2.

TABLE 7.2

Zone diameter interpretive standards for selected antibiotics used in the Kirby-Bauer method

Antibiotic	Disk symbol	Disk content	Zone diameter standard (mm) Resistant	Intermediate*	Susceptible
Ampicillin (for staphylococci)	AM 10	10 µg	≤28	–	≥29
Chloramphenicol	C 30	30 µg	≤12	13–17	≥18
Erythromycin	E 15	15 µg	≤13	14–22	≥23
Gentamicin	GM 10	10 µg	≤12	13–14	≥15
Penicillin G (for staphylococci)	P 10	10 units	≤28	–	≥29
Polymyxin B	PB 300	300 units	≤8	9–11	≥12
Streptomycin	S 10	10 µg	≤11	12–14	≥15
Tetracycline	TE 30	30 µg	≤14	15–18	≥19

*Intermediate values are interpreted as "indefinite" results.
Source: Based on data from the National Committee for Clinical Laboratory Standards (NCCLS).

Tips for Success

- *Cover the entire agar surface with bacteria,* as described previously for the filter paper method.
- *Handle disks with sterile forceps* to prevent contamination of the culture.
- *Place disks as far apart as possible* on the plate to minimize the overlap of zones of inhibition. Even so, overlap does occur occasionally, as seen in Figure 7.10. If this occurs, measure the zone of inhibition around one of the disks first; then calculate the zone of inhibition around adjacent disks by difference.

Expected Results

Results of the effectiveness of eight antibiotics against four bacteria using the Kirby-Bauer method are presented as follows: for *Bacillus cereus,* in Figure 7.7; for *Escherichia coli,* in Figure 7.8; for *Staphylococcus aureus,* in Figure 7.9; and for *Staphylococcus epidermidis,* in Figure 7.10. The zones of inhibition and their evaluation based on interpretive standards are presented in Table 7.3. Results confirm the spectrum of activity presented in Table 7.1 for these antibiotics: Ampicillin and penicillin are effective against the Gram-positive coccus *Staphylococcus epidermidis;* erythromycin is effective against Gram-positive bacteria only; polymyxin B is effective only against the Gram-negative *Escherichia coli;* and chloramphenicol, gentamicin, streptomycin, and tetracycline are effective against both Gram-positive and Gram-negative bacteria. Notice, however, that ampicillin and penicillin, typically effective against Gram-positive cocci, are ineffective against the Gram-positive coccus *Staphylococcus aureus* in this test. The reason for this resistance is discussed at the end of this chapter.

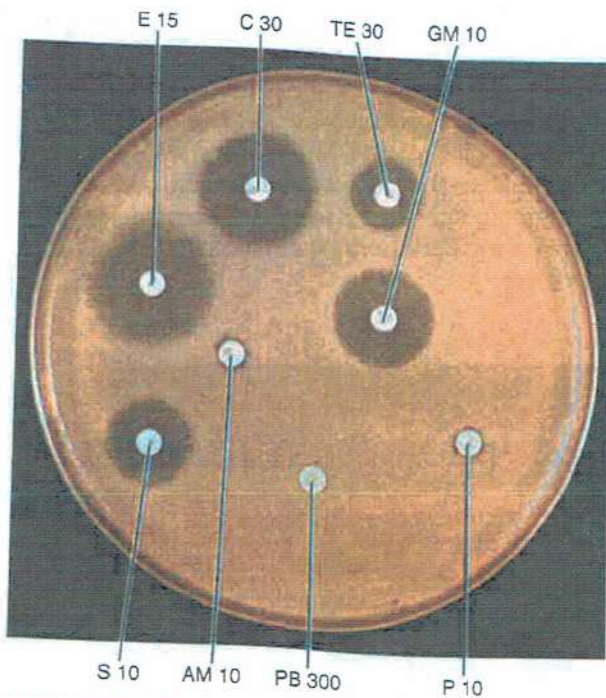

FIGURE 7.7 The effectiveness of eight antibiotics against the Gram-positive rod *Bacillus cereus.* The zones of inhibition and evaluations based on the interpretive standard for each antibiotic are presented in Table 7.3.

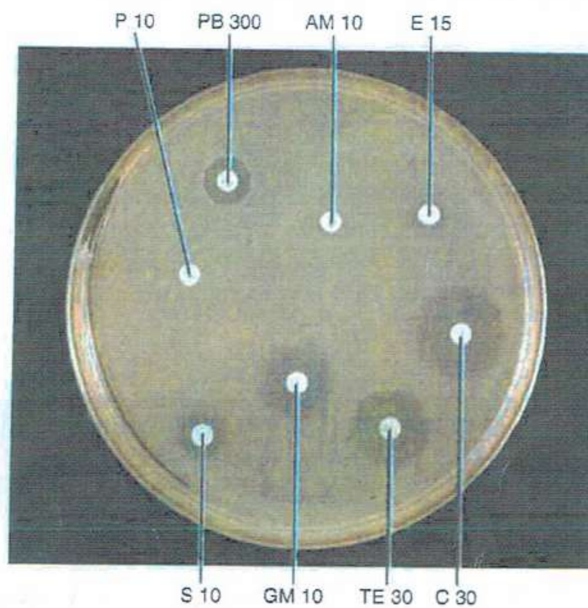

FIGURE 7.8 The effectiveness of eight antibiotics against the Gram-negative rod *Escherichia coli.* The zones of inhibition and evaluations based on the interpretive standard for each antibiotic are presented in Table 7.3.

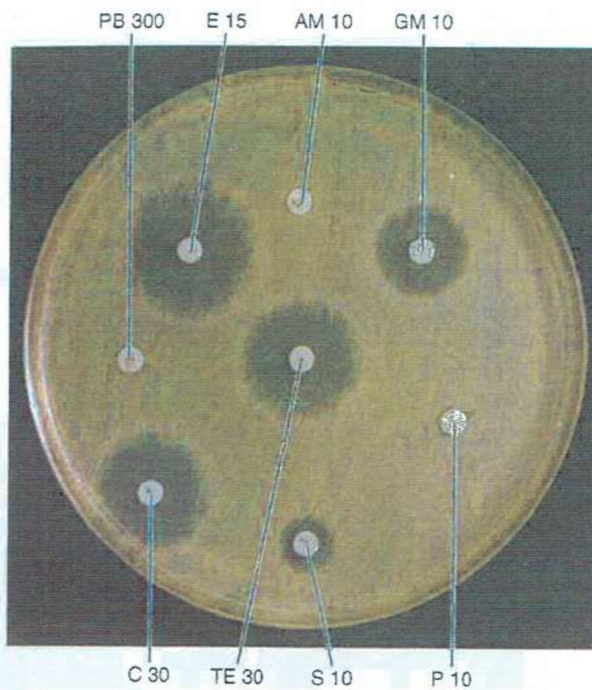

FIGURE 7.9 The effectiveness of eight antibiotics against the Gram-positive coccus *Staphylococcus aureus*. The zones of inhibition and evaluations based on the interpretive standard for each antibiotic are presented in Table 7.3.

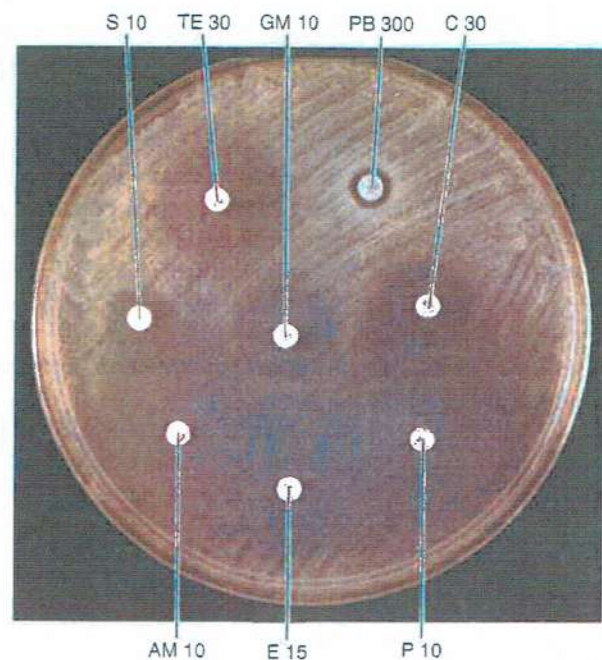

FIGURE 7.10 The effectiveness of eight antibiotics against the Gram-positive coccus *Staphylococcus epidermidis*. The zones of inhibition and evaluations based on the interpretive standard for each antibiotic are presented in Table 7.3. Notice that zones of inhibition overlap at the bottom. This situation can be handled as follows: First, measure the zone of inhibition around one of the disks; then calculate the zone of inhibition around adjacent disks by difference.

TABLE 7.3

Antibiotic effectiveness against selected bacteria

	Zone of inhibition (mm)				Evaluation (based on standard)			
Antibiotic	*Bacillus cereus* (BC)	*Escherichia coli* (EC)	*Staphylococcus aureus* (SA)	*Staphylococcus epidermidis* (SE)	BC	EC	SA	SE
Ampicillin (AM 10)	7	0	0	30	R	R	R*	S
Chloramphenicol (C 30)	29	26	28	20	S	S	S	S
Erythromycin (E 15)	30	10	33	25	S	R	S	S
Gentamicin (GM 10)	25	23	25	20	S	S	S	S
Penicillin G (P 10)	8	0	0	30	R	R	R*	S
Polymyxin B (PB 300)	0	14	0	10	R	S	R	I
Streptomycin (S 10)	21	12	14	15	S	I	I	S
Tetracycline (TE 30)	19	22	28	25	S	S	S	S

R = resistant; S = susceptible; I = intermediate

*Resistance results from production of a penicillinase effective against ampicillin and penicillin.

EVALUATING ANTIBIOTICS: ETEST®

Purpose and Procedure Summary

The Etest® uses a plastic-coated strip containing a gradient of antibiotic concentrations. This gradient allows the determination of the lowest level of antibiotic that inhibits bacterial growth. This value is called the **minimum inhibitory concentration** (MIC). This value is compared to a standard to determine if the test organism is resistant or susceptible to the drug.

An Etest® strip is placed on an agar plate that has been inoculated with a test organism. After incubation, growth of the organism around the strip is examined to determine the MIC.

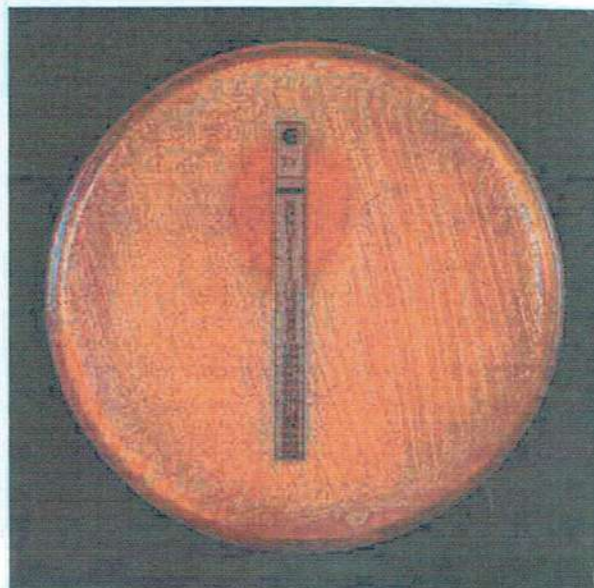

FIGURE 7.11 The Etest® using an antibiotic strip containing ceftriaxone against the Gram-negative coccus *Neisseria gonorrhoeae*. The antibiotic concentration increases along the strip from 0.002 μg/ml at the bottom to 32 μg/ml at the top. The minimum inhibitory concentration (MIC) for this antibiotic against this culture is 1.5 μg/ml.

Tips for Success

- *Cover the entire agar surface with bacteria,* as described previously for the filter paper method.
- *Handle the strip with sterile forceps* to prevent contamination of the culture.
- When using multiple strips on large plates, *place strips as far apart as possible* to minimize the overlap of zones of inhibition.

Expected Results

The antibiotic **ceftriaxone** is often used to treat patients with gonorrhea, a bacterial disease caused by *Neisseria gonorrhoeae*. However, the MIC of the drug must be determined for each isolate from a patient. Notice that the isolate depicted in Figure 7.11 grows adjacent to the ceftriaxone strip at the lowest concentrations of the drug but is inhibited at concentrations of 1.5 μg/ml and above. Therefore, the value of 1.5 μg/ml represents the MIC. Because the interpretive standard for susceptibility of Gram-negative bacteria is < 8 μg/ml, these results indicate that ceftriaxone can be used in treating this patient.

Evaluating Antibiotics: Automated Methods

Clinical laboratories must quickly determine drug susceptibility for a number of isolates daily. For this reason, laboratory technicians often use automated methods to test susceptibility quickly and accurately. Automated methods typically consist of a plastic card or panel with multiple wells, each of which contains a growth medium and an antibiotic. The wells are inoculated with a culture from a patient, and a machine detects growth in the wells to determine antibiotic susceptibility. Here we describe two widely used automated systems.

The plastic **Vitek® susceptibility card** shown in Figure 7.12 has been inoculated with a culture from a patient. Notice that growth (as indicated by turbidity) has occurred in most of the wells, demonstrating the ineffectiveness of these antibiotics. However, notice that growth is inhibited (as indicated by clarity) in wells 4 and 30, demonstrating the effectiveness of these antibiotics. A printout of this information is used to select the appropriate antibiotic for treating the patient.

The plastic **MicroScan® panel** in Figure 6.8 has been inoculated with a culture from a patient. Notice that growth has occurred in some of the antibiotic susceptibility wells at the bottom, but has been inhibited in others. A printout of this information is used to select the appropriate antibiotic for treating the patient.

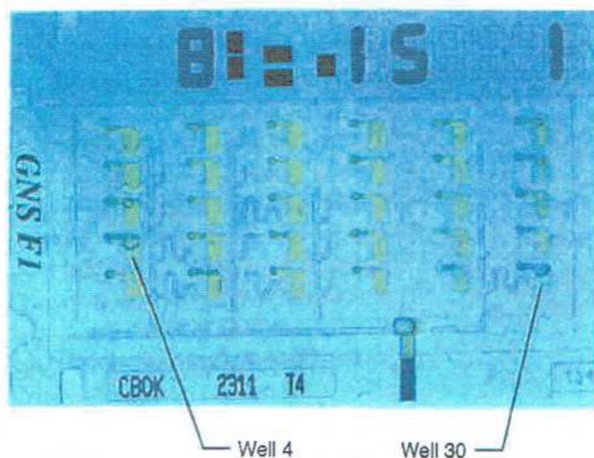

FIGURE 7.12 Results in a Vitek® antibiotic susceptibility card inoculated with a culture from a patient. After incubation, growth has occurred in every well except wells 4 and 30, which contain 32 μg/ml of amikacin and 8 μg/ml of tobramycin, respectively. Because these two antibiotics inhibited growth, they would be candidates for use in treating the patient.

ANTIBIOTIC RESISTANCE

Antibiotic resistance has become a serious problem in disease treatment. For example, when penicillin was first used in the late 1940s and early 1950s, less than 3% of all isolates of *Staphylococcus aureus* were resistant to penicillin. Today, after almost 50 years of widespread use, more than 90% of all isolates are resistant. As a result, penicillin can no longer be relied upon to treat infections caused by this organism.

The use of antibiotics in disease treatment selects for those bacteria that are resistant to the antibiotic. Susceptible bacteria die, leaving only antibiotic-resistant survivors, which multiply to increase the prevalence of resistant forms. The more widespread and long-term the use of a particular antibiotic, the higher the prevalence of resistant forms.

Bacteria become resistant to antibiotics by several mechanisms, such as the production of an enzyme that destroys the antibiotic or a change in the structure of the target site of the antibiotic. Specific examples of these two mechanisms are described next.

Resistance to penicillin is typically due to the production by bacteria of an enzyme called **penicillinase.** This enzyme breaks a bond in the beta-lactam ring of penicillin, resulting in a new molecule called penicilloic acid, which is not effective against bacteria (Figure 7.13).

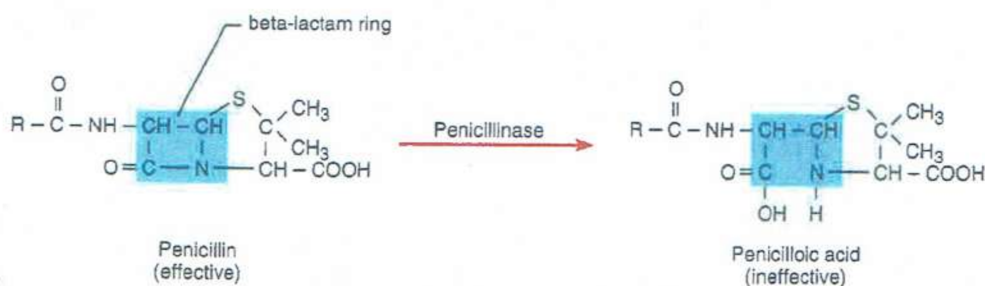

FIGURE 7.13 How penicillinase produces resistance to penicillin. Penicillinase breaks a bond in the beta-lactam ring of penicillin, resulting in an ineffective molecule called penicilloic acid.

The presence of penicillinase in a culture can be detected using a molecule called **nitrocefin**. Nitrocefin contains a beta-lactam ring similar to that found in penicillin. However, when the beta-lactam ring of nitrocefin is broken, it changes to a red color. This reaction on a nitrocefin DrySlide™ is shown in Figure 7.14. The culture on the left, *Staphylococcus aureus*, produces a penicillinase that has turned the nitrocefin red. The presence of the enzyme in this organism explains why it was resistant to ampicillin and penicillin in the Kirby-Bauer test (see Table 7.3). The culture on the right, *Staphylococcus epidermidis*, does not produce a penicillinase, so no color change occurs.

A change in the target site is another mechanism that renders bacteria resistant to antibiotics. For example, the structure of an organism's ribosomes might change, such that an antibiotic can no longer bind to them and prevent protein synthesis. Therefore, the bacterial cells can continue to grow. A change in ribosome structure may explain the presence of a few *Staphylococcus aureus* colonies near the erythromycin disk in Figure 7.15. Erythromycin inhibits protein synthesis by binding to ribosomes. The appearance of a few colonies close to the disk may indicate resistance, but these colonies should be isolated and tested to verify this possibility.

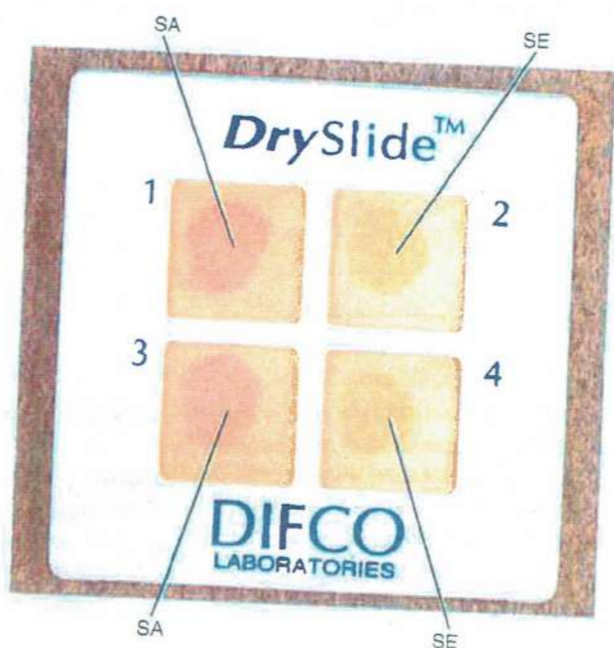

FIGURE 7.14 A nitrocefin DrySlide™ inoculated with a penicillinase-producing *Staphylococcus aureus* (SA) and a penicillinase-negative *Staphylococcus epidermidis* (SE). The enzyme penicillinase breaks open the beta-lactam ring of nitrocefin to produce the red color on the left, a positive test. There is no color change on the right, a negative test.

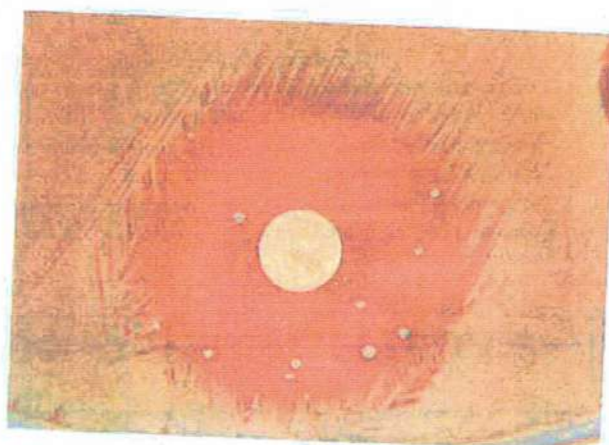

FIGURE 7.15 A few colonies of *Staphylococcus aureus* within the zone of inhibition around an erythromycin disk. The growth of these bacteria near the disk may indicate erythromycin resistance, but additional testing is required for verification.