

The Clinical Microbiology Laboratory and Infection Control: Emerging Pathogens, Antimicrobial Resistance, and New Technology

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The work required of the clinical microbiology laboratory and of the infection control program has become increasingly complex, demanding, and intertwined as the decade of the 1990s has progressed. To do their jobs effectively and efficiently, these two groups must work as a team, using the expertise from each discipline to improve patient care. As in the past, the microbiology laboratory must be able to detect and identify microorganisms so that the clinicians can diagnose and treat established infections and the infection control team can monitor, prevent, and control infections in the hospital environment.

However, the number and types of pathogens that the laboratory must detect have increased dramatically. New technology, developed to detect, identify, and characterize microorganisms, has improved significantly the laboratory's ability to keep up with the rapidly changing nosocomial pathogens. In particular, molecular biological techniques have enhanced the speed and sensitivity of detection methods and have allowed the laboratory to identify organisms that do not grow or grow slowly in culture. Molecular biological techniques also enable the microbiologist to identify antibiotic-resistance genes and to "finger-print" hospital organisms, thereby facilitating studies of nosocomial transmission.

In addition to performing their traditional roles, albeit with new tools, laboratory personnel must perform some tasks that are specifically designed to facilitate infection control activities. For example, the laboratory should also participate actively in surveillance efforts, and laboratory staff members should help plan and execute microbiological and molecular epidemiological investigations of nosocomial infections. The laboratory staff also must provide the infection control team with high-quality data in a timely fashion and teach the infection control personnel how to use laboratory resources appropriately during epidemiological investigations.

The clinical microbiologist (doctoral level microbiologist, pathologist, microbiology supervisor, or designated laboratory personnel), hospital epidemiologist (or infectious disease clinician), and infection control professional must work as a team to prevent and control nosocomial infections effectively [1].

Given the rapid changes in nosocomial pathogens, in medical care, and in health care delivery, staff members from the laboratory and from infection control must collaborate continuously and must communicate openly. The relationship between the microbiology laboratory and the infection control program is critical to the success of both groups. In this review we discuss the microbiology laboratory's role in this essential collaboration.

The Clinical Microbiologist and the Infection Control Committee

The clinical microbiologist, or the microbiology supervisor in an institution without a doctoral level microbiologist, is an integral part of the infection control team and thus must be an active member of the infection control committee. Because the infection control committee frequently bases its decisions on the results of microbiological tests, the clinical microbiologist must teach the committee how to interpret culture results and which microbiological approaches could be used to solve specific infection control problems. Furthermore, the microbiologist must explain the resources necessary to accomplish the committee's goals. Conversely, while serving on the committee, the microbiologist will learn about the problems confronting hospital epidemiology and infection control personnel and thus will be better able to organize the laboratory's response to such problems.

The microbiologist must educate the committee about several important issues. Because most infection control personnel have not worked in laboratories, the microbiologist will need to ensure that these individuals understand basic microbiology principles and techniques. The microbiologist must also explain the advantages and limitations, the scope and adequacy (i.e., sensitivity and specificity), and the costs of microbiological methods used to detect, identify, and assess the antimicrobial susceptibility of the most common nosocomial pathogens.

In addition, the microbiologist should inform the committee about changes in methods, reagents, or instrumentation that may substantially affect the laboratory's ability to detect and characterize nosocomial pathogens. Because most laboratories have limited financial and staff resources, the microbiologist must help the infection control staff and the committee understand the costs and appropriate indications for the microbiological tests most commonly used to support epidemiological investigations so that these limited resources are used effectively.

Members of the infection control team must communicate with each other to accomplish their goals. Communication can

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be enhanced if the infection control staff members regularly make rounds in the laboratory to ask questions, review microbiological and molecular epidemiological results, and discuss current problems and issues. Likewise, the microbiology staff should attend conferences at which infection control personnel discuss epidemiological principles and contemporary topics.

Accurate Identification of Nosocomial Pathogens

In many instances, the results of routine culture and identification procedures are the first indications that patients have acquired nosocomial infections. For most epidemiological investigations, the routine procedures performed in the microbiology laboratory are satisfactory. However, in selected instances, certain laboratory services and expertise, which may extend beyond routine practice and knowledge, may be necessary. Regardless of which tests are performed, the laboratory must perform the tests quickly, accurately, and reproducibly to ensure that the infection control team can identify and assess nosocomial infections properly.

Rapid Diagnostic Tests

The increased repertoire of immunologic and molecular methods has enhanced the ability of the microbiology laboratory to detect a variety of bacteria, fungi, and protozoa [2–5]. Investigators have used a variety of molecular biological techniques, including nucleic acid probe hybridization, target amplification, and signal-generating formats, to detect pathogenic microorganisms directly in clinical specimens (table 1) [6–13]. The most widely used method amplifies the target DNA by PCR. The newer amplification technologies such as ligase chain reaction and transcription-mediated amplification will form the basis of additional diagnostic systems that are or are soon likely to be available for use in the clinical laboratory (table 1).

The clinical microbiologist, in consultation with appropriate clinicians and members of the infection control team, should determine whether the laboratory should perform rapid microbiological tests. The group should base the decision on data in the literature, data generated by their laboratory (if possible), and data on their patient population. In addition, the group should try to determine whether the test would improve patient care substantially. In this era of competition and cost-consciousness, such critical evaluations have become even more important than they were previously [14–17].

A critical review of the literature indicates that most existing molecular diagnostic tests are of limited or no use for infection control purposes. However, rapid and sensitive amplification-based methods that detect *Mycobacterium tuberculosis* in sputum specimens may be very important tools for infection control. Likewise, molecular methods for detection and characterization of HIV and hepatitis C virus (HCV) might help infection control and employee-health staff quickly assess the

seriousness of needlestick injuries or other exposures and determine whether prophylaxis is warranted. Recently, detection of HCV RNA by PCR, coupled with genotyping of the virus, was useful in defining the epidemiology of an outbreak of HCV infection among recipients of human intravenous immunoglobulin [18].

Automated Identification and Susceptibility Testing

The clinical laboratory's ability to identify nosocomial pathogens accurately is challenged continuously by the expanding spectrum of organisms that colonize and infect seriously ill patients. Pathogens such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, enterococci, *Candida albicans*, and the Enterobacteriaceae, which are easy to detect and identify, cause most nosocomial infections. However, an array of unusual or fastidious pathogens also cause nosocomial infections. Current examples include bacteria such as *Acinetobacter* species, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia*; fungi such as *Aspergillus* species, non-*albicans* species of *Candida*, *Fusarium* species, and *Pneumocystis carinii*; viruses such as respiratory syncytial virus, rotavirus, and cytomegalovirus; and parasites such as *Cryptosporidium*, *Cyclospora*, and *Microsporidium* species. Consequently, the diagnostic microbiology laboratory frequently must update the methods used to identify and characterize pathogens. In addition, the laboratory staff must read and attend conferences to enhance their knowledge.

Most microbiology laboratories can isolate organisms from clinical material, identify isolates to species level, and perform antimicrobial susceptibility testing. The classic manual methods of identification and susceptibility testing have largely been replaced by automated or semiautomated commercial systems that use frozen or dried microdilution panels [14]. Currently, semiautomated or fully automated microbiology systems are available in a variety of formats, including instruments that perform only identification or testing of susceptibility to antimicrobial agents and instruments that can identify the organism and test its susceptibility to antimicrobial agents simultaneously. Automated systems can test common aerobic and facultative gram-negative and gram-positive bacterial pathogens and also anaerobes and fastidious organisms such as *Neisseria*, *Haemophilus*, and *Moraxella* species and yeasts [19–21].

The commercial identification and antimicrobial susceptibility testing systems offer several advantages over the older, manual methods. First, the commercial systems have standardized the performance and interpretation of microbiological tests among various laboratories. Second, the instruments have simplified many tedious, labor-intensive steps. Third, systems that use photometric or fluorometric technology assess results more rapidly and objectively than do manual methods. Fourth, commercial microbiology systems can perform tests more reproducibly than their conventional counterparts [19–21], and fifth, compared with the conventional methods, the accuracy of the

Table 1. Commercially available nucleic acid probes and amplification systems for direct pathogen detection.

Technology	Organism	Specimen	Format	Detection/reporter system
Nucleic acid probe	Cytomegalovirus	Buffy coat	Solution phase	DNA/RNA hybrid capture and chemiluminescence*
	Human papillomavirus	Cervical	Solution phase	DNA/RNA hybrid capture and chemiluminescence*
	Hepatitis B virus	Serum	Solid-phase microtiter tray	Branched chain DNA [†]
			Solution phase	DNA/RNA hybrid capture and chemiluminescence*
	Hepatitis C virus	Serum	Solid-phase microtiter tray	Branched chain DNA [†]
	<i>Chlamydia trachomatis</i>	Urogenital	Solution phase	Acridinium ester [‡]
	<i>Neisseria gonorrhoeae</i>	Urogenital	Solution phase	Acridinium ester [‡]
Nucleic acid amplification	<i>Streptococcus pyogenes</i>	Throat	Solution phase	Acridinium ester [‡]
	Hepatitis C virus	Serum	PCR	Microtiter capture [§]
	HIV	Serum	PCR	Microtiter capture [§]
	<i>Mycobacterium tuberculosis</i>	Sputum	Strand displacement amplification	Ethidium bromide–stained gel [#]
			Transcription-mediated amplification	Acridinium ester probe [‡]
			PCR	Microtiter capture [§]
	<i>Chlamydia trachomatis</i>	Urogenital	PCR	Microtiter capture [§]
			LCR	Antibody capture
	<i>Neisseria gonorrhoeae</i>	Urogenital	PCR	Microtiter capture [§]
			LCR	Antibody capture

NOTE. The table contains examples of available systems and is not intended to be all inclusive. LCR = ligase chain reaction.

* Digene, Silver Spring, MD.

[†] Chiron, Emeryville, CA.

[‡] Gen-Probe, San Diego.

[§] Roche, Branchburg, NJ.

[#] Becton Dickinson, Cockeysville, MD.

^{||} Abbott, Chicago.

automated and semiautomated identification and susceptibility testing systems is 90%–95% [19–24].

The newer methods allow laboratories to perform a wider variety of tests than they could with manual methods, thereby decreasing the number of specimens that must be sent to reference laboratories. On-site microbiological testing is clearly an advantage when the infection control program must investigate nosocomial infections because the results can be obtained quickly and the isolates are available in the laboratory if additional tests are necessary.

Despite their advantages, the commercial microbiology systems may have significant limitations that are especially relevant with regard to infection control [19]. The limitations are most prominent among the systems that use short (3- to 5-hour) incubation periods to decrease the time needed for testing antimicrobial susceptibility [25–31]. These systems can misclassify organisms that display heteroresistance to β -lactam antibiotics, have inducible resistance mechanisms, or have high mutation rates in the genes controlling susceptibility because their resistance phenotype might become apparent only after an incubation period longer than 3–5 hours.

Thus, many commercial systems underestimate the frequency with which pneumococci are resistant to penicillin [27], enterococci are resistant to glycopeptides [29, 32, 33], staphylo-

cocci are resistant to oxacillin [31, 34], and Enterobacteriaceae are resistant to β -lactam drugs [19]. Conversely, some highly automated systems falsely identify certain gram-negative bacilli as being resistant to agents such as imipenem and aztreonam [25]. The combination of these problems (false susceptibility and false resistance) could cause the infection control team to direct their efforts away from true problems and toward spurious problems.

Because automated systems have serious limitations and because new pathogens and resistance mechanisms evolve, many laboratories supplement the automated systems with additional manual susceptibility tests such as disk diffusion, agar dilution, broth dilution, or the new stable gradient technology (Etest, AB BIODISK, Solna, Sweden), all of which have performed satisfactorily for many of the problematic organism-drug combinations [27, 33, 35, 36]. For example, at the University of Iowa we employ the Vitek System (bioMérieux, Hazelwood, MO) for susceptibility testing of Enterobacteriaceae, a microdilution broth panel for testing gram-positives and nonenteric gram-negative bacilli (e.g., *Acinetobacter*, *Pseudomonas*, and *Stenotrophomonas* species), and Etest for testing fastidious organisms such as *Streptococcus pneumoniae*.

The rapid automated identification systems appear to make fewer errors than do antimicrobial susceptibility testing sys-

tems. However, Singer et al. recently reported a pseudoepidemic of vancomycin-resistant enterococci (VRE) that resulted when a fully automated microbiology system misidentified a species of *Enterococcus* [37]. Furthermore, the automated identification systems and other commercial and conventional systems that employ longer incubation times have similar problems identifying certain organisms [21].

Finally, automated microbiology systems are often useful for routine testing. However, they inherently lack flexibility and, thus, these systems might hinder infection control efforts. Consequently, microbiology laboratories must be able to implement less automated and more labor-intensive testing if the clinical and epidemiological evidence indicates that these methods are warranted. Hence, the microbiologist constantly must consider whether techniques in addition to those used for routine testing will enhance the infection control efforts.

Emerging Pathogens and Antimicrobial Resistance

In recent years, gram-positive cocci and *Candida* species have replaced gram-negative bacilli as the most common causes of nosocomial infections [38–42]. For example, the frequency of infections caused by *E. coli* has declined and the frequency of infections caused by staphylococci (coagulase-negative staphylococci and *S. aureus*), enterococci, and *C. albicans* has increased [39, 43]. Serious infections caused by streptococci (*S. pneumoniae* and viridans group streptococci) and *Mycobacterium* species have also become quite common [44–48].

One of the more alarming recent trends has been the increasing frequency with which resistant pathogens are causing nosocomial infections. Even among species that are usually susceptible, the strains causing nosocomial infections are often resistant to agents in more than one antimicrobial class [43]. Thus, the infection control team must track not only antibiotic-resistant gram-negative pathogens but also quinolone and methicillin resistance in staphylococci, multiple antibiotic resistance in enterococci, penicillin resistance in pneumococci and other streptococci, vancomycin resistance in gram-positive cocci, and polyene and azole resistance in *Candida* and other fungal pathogens (table 2) [33, 40, 41, 49–52].

The microbiology laboratory's role in monitoring resistance is extremely important to the success of the infection control effort. Laboratory personnel must notify infection control staff immediately when resistant organisms are identified and when new or unusual phenotypic resistance patterns are found so that appropriate isolation precautions can be instituted.

Methicillin-resistant strains of *S. aureus* and coagulase-negative staphylococci have long been recognized as important nosocomial pathogens; however, their numbers are increasing. The problem of methicillin-resistant staphylococci has been compounded by the development of resistance to fluoroquinolones [39, 51]. The fluoroquinolone class of antimicrobials generally affords excellent staphylococcal coverage. However, given the appropriate selective pressure, staphylococci, particularly those

that are resistant to methicillin, can become resistant to fluoroquinolones very quickly. Moreover, methicillin-resistant staphylococci are often resistant to macrolides, aminoglycosides, tetracyclines, trimethoprim-sulfamethoxazole, and chloramphenicol.

Naturally occurring vancomycin-resistant strains of *S. aureus* have not been described. However, vancomycin-resistant isolates of *Staphylococcus haemolyticus*, enterococci, and other less common gram-positive cocci have been obtained from clinical specimens (table 2) [33, 39, 40, 50, 53]. VRE, particularly *Enterococcus faecium*, are epidemic in many centers [33, 50, 54], and nosocomial bloodstream infections caused by these organisms have an extremely high attributable mortality [55].

The mechanism of resistance to vancomycin in *S. haemolyticus* and other nonenterococcal gram-positive cocci is chromosomally encoded and is unrelated to the plasmid-mediated resistance in *E. faecium* and *Enterococcus faecalis* [56]. Because the vancomycin-resistance gene in those enterococcal species is on a transmissible element, many microbiologists and epidemiologists are concerned that VRE might transmit this gene to staphylococci [43]. In addition, strains of enterococci that are resistant to β -lactam antibiotics, aminoglycosides, and vancomycin have become widespread [33, 50]. Thus, multiresistant enterococci can be a reservoir for resistance genes that can be transferred to other organisms [56].

Multiresistant enterococci are easily transmitted in hospitals because they frequently contaminate the rooms where the patients are housed. Furthermore, no currently available antimicrobial agent or combination of agents has bactericidal activity against the multiresistant enterococci. Consequently, these organisms present a major challenge to clinicians and to infection control personnel. To help control the spread of multiresistant enterococci, clinical microbiology laboratories might need to participate in active surveillance for these organisms. Such surveillance could be limited to obtaining full susceptibility profiles on all enterococci obtained from normally sterile body sites but could also involve conducting either point prevalence or routine stool culture surveys on high-risk units to identify resistant enterococci.

Regardless of their approach to controlling resistant enterococci, microbiologists and the infection control staff must remember that many automated systems detect resistant enterococci poorly. Although most automated systems are capable of detecting high-level (*van A*-mediated) resistance to vancomycin, many have difficulty detecting the *van B*-containing strains [32]. Thus, the laboratory must supplement the automated systems with appropriate manual methods to ensure that these important nosocomial pathogens are identified and that patients can be isolated [29, 30, 32]. At the University of Iowa, enterococci are tested with use of an in-house-prepared microdilution broth panel, supplemented by Etest as needed, for precise confirmation of vancomycin resistance.

S. pneumoniae and the viridans group streptococci, which in the past were always exquisitely susceptible to penicillin,

Table 2. Emerging antimicrobial resistance problems in nosocomial pathogens.

Organism group	Type of antimicrobial resistance	Mechanism
Staphylococci (coagulase-negative and <i>S. aureus</i>)	Methicillin	Altered penicillin-binding proteins
Enterococci	Quinolones	Altered DNA gyrase
	β -Lactam drugs	Altered penicillin-binding proteins, β -lactamase
	Aminoglycosides	Aminoglycoside-modifying enzymes
	Glycopeptides	Altered pentapeptide D-ala-D-ala terminus
Gram-positive	Vancomycin	Altered pentapeptide D-ala-D-ala terminus
<i>Staphylococcus haemolyticus</i>		
<i>Lactobacillus</i> species		
<i>Pediococcus</i> species		
<i>Leuconostoc</i> species		
Gram-negative bacilli	Extended-spectrum β -lactam drugs, β -lactamase inhibitor combinations	Extended-spectrum β -lactamases; Amp C β -lactamases; reduced permeability
	Quinolones	Altered DNA gyrase
Fungi	Polyenes and azoles	Reduced permeability; altered target; overexpression of target; efflux pump
<i>Aspergillus</i> species		
<i>Candida krusei</i>		
<i>Candida lusitanae</i>		
<i>Candida glabrata</i>		
<i>Trichosporon beigelii</i>		

have now acquired resistance to that antibiotic [45, 52, 57, 58]. Penicillin-resistant pneumococci have been transmitted on occasion within hospitals [45, 59]. In addition, some strains of *S. pneumoniae* and of the viridans group streptococci have become resistant to cephalosporins, macrolides, and trimethoprim-sulfamethoxazole. The laboratory should perform appropriate susceptibility tests so that these multiresistant strains can be identified promptly. Laboratory staff should notify the clinicians and the infection control staff immediately upon detection of multiresistant strains of *S. pneumoniae* so that the patient receives effective therapy and spread of the organisms can be prevented.

Despite the significant increase in the frequency of nosocomial infections caused by gram-positive organisms, gram-negative bacilli as a group remain frequent and important causes of nosocomial infections [39]. In addition to the enteric bacilli and *Pseudomonas aeruginosa*, other gram-negative organisms such as *Acinetobacter* species, *S. maltophilia*, and *B. cepacia* have become important nosocomial pathogens worldwide [60, 61].

Many gram-negative nosocomial pathogens have developed resistance to β -lactam antibiotics through small changes in the structure of enzymes (usually TEM-1, TEM-3, and SHV-1 β -lactamase enzymes) that they already possess. These novel extended-spectrum β -lactamases (ESBLs) are increasing in frequency among *Klebsiella* species and other Enterobacteriaceae such as *Escherichia coli* and *Citrobacter* species, and have been identified most frequently in teaching hospitals and intensive care units [62]. The ESBLs are located on transmissible plasmids and may be present with other resistance factors, resulting in multiresistant pathogens [40, 63]. Organisms that produce ESBLs can disseminate easily in units that use broad-spectrum cephalosporins extensively. In some cases, a single

bacterial strain may be transmitted from patient to patient; in other situations a resistance plasmid may be transmitted among various bacterial strains [64, 65].

An inducible chromosomal type I cephalosporinase that confers resistance to almost all β -lactam drugs, including third-generation cephalosporins and β -lactamase-inhibitor combinations, can be produced by strains of *Enterobacter*, *Citrobacter*, and *Serratia* species, indole-positive *Proteus* species, and *P. aeruginosa* that carry the *amp C* gene. The Amp C β -lactamase is not normally produced at high concentrations, but production of the enzyme can be induced when the organisms are exposed to the newer cephalosporins, resulting in strains that are resistant to numerous antimicrobial agents [66]. *Enterobacter* species can become resistant to newer cephalosporins while the patient is receiving therapy. These organisms may become resistant when a mutation permanently "switches on" production of the enzyme, eliminating the need for induction. These organisms are called stably derepressed mutants [64].

Routine testing may indicate that these organisms are susceptible to newer cephalosporins, but ESBL- and Amp C-producing organisms are not truly susceptible to these agents (e.g., cefotaxime, ceftriaxone, ceftazidime, and cefoperazone). In addition, many in vitro susceptibility assays used in the clinical microbiology laboratory do not detect resistance unless the organism has been induced or the enzyme production is derepressed. In particular, rapid (3- to 5-hour incubation) automated or semiautomated methods and microdilution assays in which a relatively low inoculum of organism (10^5 cfu/mL) is tested are prone to make these very serious errors.

The laboratory must use a macrodilution broth method (1- to 5-mL total volume), an agar disk diffusion method, the Etest,

or a microdilution assay with sufficient inoculum (5×10^5 cfu/mL) and an incubation time of 18–24 hours to correctly identify organisms that carry these resistance determinants. If such strains are identified, the laboratory must undertake surveillance to determine the extent to which these organisms have spread within the hospital [39, 67]. Such laboratory-based surveillance complements the efforts of the infection control program to prevent person-to-person spread and the efforts of the pharmacy committee to limit antibiotic use and thus limit the selection of such strains.

Finally, fungal pathogens, such as *Candida* species, *Trichosporon beigelii*, and *Fusarium* and *Aspergillus* species, have become important nosocomial pathogens. Consequently, established antifungals such as amphotericin B are used more frequently and newer classes of antifungal agents such as the triazoles, lipid formulations of amphotericin B, and echinocandin derivatives have been introduced [42, 49, 68]. Standardized antifungal susceptibility testing methods [69] have demonstrated that fungi such as *Candida lusitanae*, *T. beigelii*, and *Aspergillus* species may be relatively resistant to amphotericin B [68, 70].

Furthermore, extensive use of triazole antifungals (e.g., fluconazole) for prophylaxis and therapy has been associated in some institutions with the emergence of azole-resistant strains of *C. albicans* and with an increasing number of infections caused by *Candida krusei* and *Candida glabrata*, which are inherently more resistant to these antifungal agents [42, 49, 71]. Two recent outbreaks caused respectively by a strain of *C. albicans* resistant to fluconazole [72] and a strain of *C. lusitanae* resistant to amphotericin B and 5-fluorocytosine [73] should warn microbiologists and infection control personnel that multiresistant strains of *Candida* may develop and spread within the hospital environment.

Microbiology laboratories in hospitals caring for patients at high risk of fungal infections may need to conduct surveillance to detect such organisms and prevent outbreaks. To this end, clinical microbiology laboratories may need to expand their ability to identify yeasts to the species level [49].

Reporting Laboratory Data

The laboratory should report all results as quickly as possible. In most situations, routine reporting on the hospital's computer or on paper will be adequate for clinical and epidemiological purposes. However, the results of some cultures or tests have a higher priority than others because they will affect the patient's care substantially or because they require an immediate response by the infection control staff. Examples of results that must be reported immediately include positive cultures of blood and normally sterile body fluids, smears and cultures positive for acid-fast bacilli, cultures yielding enteric pathogens such as *Salmonella* or *Shigella* species, and cultures that yield multiply resistant organisms.

Infection control staff members need the microbiological data generated routinely by the laboratory to do their job. Thus, they usually review microbiology reports daily. However, infection control personnel who limit their interaction with the laboratory to phone conversations and computer screens or printouts will miss much of what the laboratory can offer. Infection control personnel who visit frequently in the laboratory will not only gather important data efficiently but also establish relationships that will be critical during crisis times such as outbreaks. Laboratory rounds enable the infection control team to clarify whether patients are colonized or infected, ensure that specimens from epidemiological investigations are evaluated properly, and focus the laboratory's efforts such that infection control issues are addressed optimally.

The microbiology laboratory must store the data it generates so that aggregate data can be analyzed to establish the frequency with which specific organisms cause infection and to allow infection control personnel to assess trends or patterns in nosocomial infections. Modern laboratory information systems can store and analyze the basic data needed by infection control programs. However, certain information that traditionally was saved on laboratory work cards (e.g., the results of specific biochemical reactions and the morphological features of the colony) may not be retained and thus will be unavailable for epidemiological investigations [1]. The microbiologist and the infection control staff should review which information is stored so that the needs of the infection control program are met and the resources of the microbiology laboratory are used wisely.

Periodic summaries of selected microbiology results may be quite useful to both clinicians and infection control staff. A table or pie graph that illustrates the frequency with which particular nosocomial pathogens are isolated from specific sites and the antimicrobial susceptibility profiles of these organisms will help clinicians choose appropriate empirical therapy and will allow the microbiologist and the infection control staff to follow trends in nosocomial pathogens and antibiotic resistance within the hospital [74].

When developing this report the microbiologist should exclude the results of repeated cultures performed for the same patient and yielding the same organism so that the data are not biased. In addition, the hospital's efforts to save money might be enhanced if the cost of each antimicrobial agent is listed in the report.

Role of the Microbiology Laboratory in Outbreak Investigation

The microbiology laboratory often serves as an "early warning" system [1], by identifying clusters of organisms with unique phenotypic characteristics and communicating the observations promptly to infection control personnel. When confronted with a cluster or outbreak of nosocomial infections, the infection control team must act quickly to characterize and

define the extent of the outbreak, to identify possible causes, and to design and implement effective control measures (table 3) [1]. Members of the infection control team must communicate clearly with each other during outbreaks to ensure that the investigation proceeds quickly and efficiently and to ensure that all affected persons are identified and the specimens are processed appropriately. Given the stress inherent in an outbreak investigation and the speed with which important decisions must be made, the infection control team, including laboratory personnel, might need to meet daily to discuss new findings and make decisions.

Outbreak investigations can be facilitated if the infection control team prepares in advance. One step in this process is to identify the most common types of outbreaks that have occurred in the hospital (e.g., *S. aureus* wound infections in the surgical intensive care unit or VRE bacteremia on the liver transplant service). Laboratory and infection control personnel can then determine what resources (e.g., personnel, time, money, materials, space, or special tests) would be required to investigate a "typical" outbreak. The infection control team and the administration should determine in advance how to pay the extra costs associated with outbreak investigation so that squabbles over priorities or financing do not impair important investigations. The excess costs should not be charged to the affected patients, nor should these costs be paid from the laboratory's operating budget.

It is important to emphasize that the laboratory should not undertake any epidemiological investigation without prior consultation with the hospital epidemiologist or infection control professional. The practice of "ad hoc" epidemiological workup by physicians and nurses who are not part of the infection control team, while well-meaning, is inefficient, wasteful, and possibly more confusing than useful in controlling nosocomial infections.

Staff members in both the infection control program and the laboratory have important unique responsibilities during outbreak investigations. One of the laboratory's critical responsibilities is to save all potentially relevant organisms in case further analysis is needed. Regardless of their ability to perform special tests to characterize the organisms, all microbiology laboratories should save isolates during outbreaks. If the laboratory cannot do necessary tests, the isolates can be sent to a reference laboratory. Similarly, the laboratory should save all organisms that might be even remotely related to the outbreak, because organisms can be discarded if they are not needed but cannot be retrieved once they have been thrown away.

Supplementary Cultures

During the course of an epidemiological investigation, the microbiology laboratory might be asked to perform specialized or supplementary cultures of specimens from patients, hospital

Table 3. Role of the laboratory in the epidemiological investigation of an outbreak of nosocomial infections.

Investigative step	Laboratory participation
Identify the problem	Laboratory surveillance
Form case definition	Communication (early warning)
Look for additional cases	Microbiological confirmation
Calculate rates	Identification
	Susceptibility testing
	Archive data on occurrences
	Store isolates
Characterize the outbreak	Characterize outbreak-related isolates
Who	Type isolates
Where	Phenotypic methods
When	Molecular methods
What	Assess the number and distribution (clustering) of strains
Consider possible causes	Conduct supplementary studies
Define the mode of transmission	Obtain cultures of specimens from personnel, patients, and environment
Identify potential reservoirs	Select isolates from these cultures on basis of phenotypic characteristics
Identify potential vectors	Type selected phenotypically identical isolates to determine whether they match the outbreak strain
Control or terminate the outbreak	Adjust laboratory procedures to support control activities
Define and implement control measures	Continue laboratory surveillance
Evaluate the efficacy of control measures	Store isolates
Continue surveillance for new cases	Maintain communication

NOTE. Adapted in part from [1].

personnel, or the environment (table 3). Before processing hundreds of specimens, the infection control and laboratory staff must review the epidemiological data, their previous experience, and the literature to determine which tests are necessary for the specific investigation.

Special culture media might improve the laboratory's ability to identify the reservoir. For example, selective media (i.e., which inhibits the growth of species other than that of interest) or differential media (i.e., which reveals distinctive morphological features that differentiate the species of interest from other species), or both, might allow staff members to process specimens expeditiously and to reduce their work. In addition, enrichment cultures might be necessary to optimize the laboratory's ability to detect specific nosocomial pathogens (e.g., *Candida* [75] or methicillin-resistant staphylococci [76]) present in low numbers.

The etiologic agent's primary reservoir and mode of transmission will determine whether cultures of specimens from hospital personnel, medical devices, and the hospital environment (e.g., surfaces, air, or water) are necessary (table 3). Cultures of infusion-related products and devices (e.g., blood products, parenteral fluids, and intravascular devices), environmental surfaces, disinfectants, respiratory therapy equipment, air, water, and ice rarely provide useful information when done in the absence of epidemiological data implicating a source [1, 77].

Because the hands of hospital personnel can transfer nosocomial pathogens from patient to patient, cultures using the broth-bag method [75, 78] may help confirm the hypothesis that the etiologic agent was spread in this manner [79–81]. These specialized cultures should be performed rarely and only when epidemiological evidence suggests that a particular person or object might have transmitted the epidemic strain. In rare circumstances, cultures of specimens from personnel or the environment could either save time and effort for the team or identify a reservoir that would be difficult to identify through classical epidemiological methods.

Before embarking on a huge culture survey, the infection control and laboratory staffs must agree that this process is essential. Furthermore, infection control and laboratory staff members must understand that cultures of environmental samples are labor-intensive and nonstandardized and might provide data that are difficult to interpret [77].

In general, surveillance culture specimens should not be obtained from hospital personnel and the environment during nonepidemic periods. Investigators have documented that routine cultures of specimens from patients or hospital personnel (surveillance cultures), antiseptics and disinfectants, blood units, respiratory therapy equipment, commercially prepared patient-care items, peritoneal dialysate, air, and environmental surfaces are very expensive and do not provide significant clinical and epidemiological data [77, 82]. In rare instances such cultures might be of benefit if they are performed because patients have become ill, because epidemiological data indicate

that the cultures are warranted, or because the results are needed for a research project or for an important educational program [67, 80].

Epidemiological Typing

The laboratory characterization of nosocomial pathogens to provide evidence regarding their biological and genetic relatedness is frequently useful to epidemiologists as an aid in the investigation of nosocomial infections. In many situations, species identification and antimicrobial susceptibility testing can determine whether the isolates are epidemiologically related. If a cluster of nosocomial infections is caused by an organism such as *E. coli*, *S. epidermidis*, or *P. aeruginosa* that is a frequent or universal member of the normal flora or environment, additional tests might be required to determine whether the isolates are related. Subspecies delineation or strain or subtype identification is done to determine whether various isolates yield the same or different results in one or more tests. If isolates from different patients yield the same result or "fingerprint," the isolates probably originated from a single clone and were transmitted from patient to patient from a common source or by a common mechanism [83–86]. Similarly, if the same strain or subtype of an organism is repeatedly isolated from a single patient, the organism most likely is infecting or colonizing the patient and is unlikely to be a contaminant.

Epidemiological typing methods include both phenotypic (traditional and protein-based) and genotypic (DNA-based) methods (table 4). Phenotypic methods such as antimicrobial susceptibility profiles, biochemical profiles, bacteriophage susceptibility patterns, multilocus enzyme electrophoresis profiles,

Table 4. Phenotypic and genotypic methods for epidemiological typing of nosocomial pathogens.

Phenotypic typing methods
Traditional
Antibiotic susceptibility testing
Biotyping
Serotyping
Bacteriocin typing
Phage typing
Protein-based
Multilocus enzyme electrophoresis (MLEE)
Polyacrylamide gel electrophoresis (PAGE) of cellular proteins
Immunoblot fingerprinting
Genotypic typing methods
Plasmid fingerprinting
Restriction endonuclease analysis of chromosomal DNA with conventional electrophoresis
Restriction fragment–length polymorphism (RFLP) analysis of chromosomal DNA, supplemented with nucleic acid probes
Pulsed-field gel electrophoresis (PFGE)
Random amplification of polymorphic DNA (RAPD) and other PCR-based methods

and immunoblot fingerprinting have allowed investigators to describe the epidemiology of some nosocomial infections. However, the phenotypic methods discriminate poorly among strains, frequently require labor-intensive, long procedures, and often produce variable results [83–85, 87–89]. Consequently, newer genotypic or DNA-based typing methods, which have eliminated most of these limitations, have become the preferred techniques for epidemiological typing [83–86].

Investigators have used a variety of DNA-based methods to genotype nosocomial pathogens (tables 4 and 5). All of these methods use electric fields to separate DNA—either restriction endonuclease digestion fragments, amplified DNA fragments, or whole chromosomes or plasmids—into unique patterns or “fingerprints” that are visualized by staining the DNA with ethidium bromide or by nucleic acid probe hybridization. Epidemiologically related isolates share the same DNA profile or “fingerprint” pattern, whereas epidemiologically unrelated isolates have distinctly different patterns.

In addition to identifying “gross fingerprints,” molecular methods can be adapted to identify specific genes. For example, researchers have used probe hybridization or DNA amplification techniques to detect several different genes that encode antibiotic resistance, thus providing an antibiotic-resistance genotype (table 6) [90–92]. Microbiologists can use molecular detection of antibiotic resistance to calibrate conventional susceptibility tests [93]. These methods might also allow clinicians to choose antibiotic therapy that would be least likely to select resistant organisms, given the isolates’ genetic background.

Furthermore, these methods enhance the ability of the microbiologist and the infection control personnel to track the spread of specific resistance genes within and among health care facili-

ties and communities [64, 65]. Antibiotic-resistance genotyping alone is not highly discriminatory [94]. However, the combination of antibiotic-resistance genotyping with other genotyping methods is a very powerful means of characterizing the epidemiology of antimicrobial resistance among nosocomial pathogens [64, 65, 92].

In contrast with many of the phenotypic methods, genotypic typing methods can be used with only minor modifications in equipment, reagents, or procedures to assess a wide variety of bacterial, fungal, viral, and protozoan species [95]. In general, the DNA-based methods are relatively simple to perform and give highly reproducible results that are stable over time [83, 84]. These methods are capable of identifying a large number of polymorphisms, and most methods have been tested to determine whether they can distinguish epidemiologically related from unrelated isolates [83–85, 94].

DNA-based typing methods have enabled investigators to study the relationship between colonizing and infecting isolates in individual patients [96, 97], distinguish contaminating from infecting strains [98], document cross-infection among hospitalized patients [99–102], and evaluate reinfection vs. relapse in patients being treated for an infection [103–106]. Many DNA-based typing methods can be used to study nosocomial infections, but certain methods may be more useful or are more easily applied to some organisms than others (table 5) [84, 85]. Several excellent and comprehensive reviews provide more detailed information on each technique and discuss the practical applications, strengths, and weaknesses of each test [83–85].

All laboratory tests have limitations, and the genotypic typing methods are no exception to this rule. Microbiologists and infection control staff members who want to use these tech-

Table 5. DNA-based methods for epidemiological typing of microorganisms.

Method	Substrate	Principal characteristics	Examples
Plasmid fingerprinting	Plasmid DNA	Technically simple and inexpensive; potentially unstable owing to loss of plasmids; may be augmented by restriction endonuclease digestions	<i>S. aureus</i> , coagulase-negative staphylococci, <i>Klebsiella</i> species, <i>Serratia</i> species, <i>Enterobacter</i> species
Restriction endonuclease analysis of chromosomal DNA with conventional electrophoresis	Chromosomal DNA	Reproducible and broadly applicable; complex banding patterns; very difficult to interpret	<i>Clostridium difficile</i> , <i>Enterococcus faecium</i> , <i>S. aureus</i>
Restriction fragment–length polymorphism (RFLP) analysis with nucleic acid probes	Chromosomal DNA	Includes insertion sequence analysis and ribotyping; broadly applicable; multistep process; automated system available; limited discriminatory power (ribotyping)	<i>M. tuberculosis</i> (IS6110), <i>S. aureus</i> , <i>C. albicans</i> , Enterobacteriaceae
Pulsed-field gel electrophoresis	Chromosomal DNA	Broadly applicable; excellent reproducibility and discriminatory power; employs rare cutting restriction enzymes to generate large DNA fragments (10–800 kb); fewer bands; slow turnaround time; expensive equipment	Staphylococci, enterococci, Enterobacteriaceae, <i>Pseudomonas</i> species, <i>Candida</i> species
PCR	Chromosomal DNA	Rapid and relatively easy to perform; universally applicable when random primers are used (RAPD); only moderate reproducibility and discriminatory power	<i>S. aureus</i> , <i>C. difficile</i> , Enterobacteriaceae, <i>Candida</i> species

Table 6. Molecular methods used to determine antibiotic-resistance genotypes.

Organism	Target gene	Antimicrobial resistance	Molecular technique*
<i>Staphylococcus</i> species	<i>mec A</i>	Methicillin/oxacillin	Probe, PCR
<i>Streptococcus pneumoniae</i>	<i>pbp</i> 1A, 2B, 2X	β -lactam drugs	PCR
<i>Enterococcus</i> species	<i>van A, B, C, D</i>	Glycopeptides	Probe, PCR
Enterobacteriaceae	<i>tem, shv</i> , other β -lactamase genes	β -lactam drugs	Probe, PCR
<i>Mycobacterium tuberculosis</i>	<i>rpo B</i>	Rifamycins	PCR
	<i>kat G</i>	Isoniazid	PCR

NOTE. This list is not all-inclusive but includes genotyping methods used in epidemiological studies.

* PCR is frequently used to amplify a gene relevant to antimicrobial resistance, which must be characterized by other methods such as DNA sequence analysis or single-strand conformational polymorphism analysis to determine if a gene mutation that confers resistance is present.

niques as tools for epidemiological investigations must understand these limitations so that they do not misinterpret the test results. The DNA patterns generated by these techniques are often highly complex and quite difficult to analyze. Thus, investigators must become well-versed in the basic principles of molecular biology and epidemiology and must learn the art of reading the patterns.

Computer-assisted systems can help investigators compare complex banding patterns [107]; however, these systems still require the user to do considerable editing. Problems with DNA extraction and digestion or differences in the conditions of amplification (PCR-based methods) or electrophoresis can cause variations in the final profile, further complicating the analysis of DNA banding patterns. Consequently, two or more isolates can be compared only if they were typed under identical conditions. The laboratory ideally should use one test run to assess all isolates that must be compared.

Finally, the methodology, nomenclature, and reference strains have not been standardized, a limitation which can impede the interpretation and comparison of results obtained by different methods or different laboratories [108]. Flexible and sophisticated computer-based analysis systems such as Dendron (Solltech, Iowa City, IA) and fully automated molecular typing systems such as the RiboPrinter (Qualicon, Wilmington, DE) are major steps toward standardization and quantitative analysis of molecular typing results [105, 107, 109]. In addition, groups of investigators have begun developing standards and guidelines for the use of DNA-based typing methods, but more work must be done in this area [108–110].

Molecular epidemiological typing methods allow microbiologists and the infection control staff to identify specific strains within a given species, which in turn allows the team to study the epidemiology of nosocomial pathogens and then develop effective measures to prevent their spread within hospitals. When the infection control team considers using molecular typing in an epidemiological investigation, the members must understand that there is not one best method. Although investigators have used all of the typing methods listed in table 5 to study nosocomial infections successfully, particular methods work better for certain organisms than they do for others [84].

Pulsed-field gel electrophoresis and certain PCR-based typing methods perform well for a wide array of nosocomial pathogens and are the molecular typing methods most frequently used to investigate nosocomial infections. However, as aptly stated by Maslow and Mulligan, the best method for a given epidemiological situation is the one that works [84]. Despite theoretical or actual limitations, many typing methods work quite well when used in the context of a careful epidemiological investigation [84, 86]. In contrast, if investigators use the most powerful and sophisticated typing methods indiscriminately in the absence of sound epidemiological data, these techniques may provide conflicting and confusing information.

Summary and Conclusions

The clinical microbiology laboratory is an essential component of an effective infection control program. Laboratory personnel have a broad range of technologies, from traditional methods of detecting and identifying organisms to modern molecular typing methods, that they can use to support and enhance the efforts of the infection control staff. If the infection control team applies these technologies appropriately, it can prevent problems and solve nosocomial mysteries efficiently. In this era of cost-containment, staff members in the laboratory and in the infection control program must work hard to communicate their unique and shared goals, needs, and problems. If the laboratory and infection control personnel cooperate and collaborate rather than compete, both programs will be successful and the patients and the hospital will benefit because the risk of nosocomial infections and the frequency of resistant organisms will be reduced.

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