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Clinical Features of Infections Caused by New Nontuberculous Mycobacteria, Part II*

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Abstract

Many of the mycobacterial species described in the past decade have been involved in human disease. In Part I of this article, the most frequent pathologies discussed included respiratory infections in elderly people, cervical lymphadenitis in children, and localized post-traumatic and post-surgical infections at various body sites. Also discussed were disseminated infections, which were frequent in AIDS patients several years ago but are now rare, with the prevalence probably higher in immunocompromised HIV-negative persons. Part II of this article describes *Mycobacterium* species associated with sepsis and other diseases. No substantial differences exist in the number of cases in which slow and rapid growers are involved in disease, with the former more commonly responsible for pulmonary infections and lymphadenitis and the latter preferentially affecting bones, joints, skin, and soft tissues.

Mycobacterium Species Associated with Sepsis

Several cases of catheter-related sepsis due to newly described mycobacteria have been reported. In three cases (20), *Mycobacterium immunogenum* was isolated from a bone marrow transplant patient, a subject with acute leukemia, and a patient with pacemaker-related sepsis.

Five isolates of *M. hackensackense* were grown from catheter and peripheral-blood specimens from a 6-year-old girl with relapsed acute lymphocytic leukemia and a history of multiple infections. A change of treatment from vancomycin to amikacin for 1 week and clarithro-

mycin for 4 weeks produced rapid and definitive improvement (77).

One case of sepsis in a 64-year-old man was due to *M. goodii* (15), whereas *M. mageritense* was the responsible agent in a 32-year-old immunosuppressed woman (30).

M. mucogenicum was grown from a blood specimen of a 47-year-old man with end stage cirrhosis and fever (78). Defervescence was achieved with a combination of imipenem and amikacin, to which the organism was susceptible in vitro, but the patient died because of hepatic and renal failure.

The only strain of *M. septicum* isolated to date was from three blood specimens and the tip of a central venous catheter from a 2-year-old child with metastatic hepatoblastoma (79).

M. wolinskyi was isolated from an infected arterial-venous shunt in a 55-year-old woman undergoing hemodialysis (16).

Mycobacterium Species in Other Diseases

Two cases of genital disease caused by newly described mycobacteria have

been reported, both in AIDS patients. A 49-year-old homosexual man with two subcutaneous penile nodules was surgically cured (80). The resected mass appeared as a spindle-cell pseudotumor and grew *M. celatum*. In the case of a 25-year-old woman with a papular vulvar lesion, histological investigation showed necrosis and granuloma, and *M. genavense* was isolated in culture. Despite improvement following multidrug therapy, mycobacterial mesenteric adenopathy developed 1 year later. The recurrence was successfully treated with 9 months of clofazimine administration (81).

Two cases of liver infection have also been reported. *M. lentiflavum* was

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isolated from a hepatic needle biopsy specimen from a severely immunocompromised 47-year-old AIDS patient (28). When the man was hospitalized for fever, weight loss, and lumbago; lung opacities, a hepatic nodular lesion, and a vertebral deformity were detected. Treatment with three antiretroviral drugs, along with rifabutin and clarithromycin, dramatically improved his pulmonary and vertebral status but did not affect his liver. A lobectomy performed several months later led to a histologic diagnosis of non-Hodgkin's lymphoma.

The second case involved an HIV-negative 51-year-old male with hepatosplenomegaly who complained of right upper quadrant pain and night sweats (82). A liver biopsy revealed noncaseating granulomatous hepatitis with necrosis. While he was hospitalized, the man's condition rapidly worsened, and he died in multi-organ failure secondary to *Acinetobacter calcoaceticus* var. *anitratus* sepsis. Postmortem cultures of hepatic tissue biopsy specimens grew *M. mucogenicum*.

Of uncertain significance is the isolation of *M. doricum* from the cerebrospinal fluid of a 50-year-old AIDS patient with concomitant infection due to *Cryptococcus neoformans* (83).

A corneal *M. immunogenum* infection has been reported in a patient with suspected keratitis (20).

M. interjectum was isolated from the stools of a 45-year-old AIDS patient with diarrhea (84). Despite the patient's improvement following treatment with clarithromycin, ofloxacin, and ethambutol, the isolate was most likely simply a colonizer.

Isolates of *M. hassiacum* (85,86) and *M. holsaticum* (36) from urine specimens almost certainly lack clinical significance.

Summary and Conclusions

The new mycobacteria described

here are only occasionally responsible for human diseases, but they include more than 50% of the species described since 1990, and the number of cases reported in the literature exceeds 200. Regardless of whether such numbers are important, without question, these case reports represent only the tip of the iceberg. Many other cases remain unpublished because the mycobacterial agent has been misidentified.

Interestingly, several features seem to distinguish the infections due to recently described mycobacteria in HIV-positive and HIV-negative patients (Table 1). With few exceptions, disseminated infections, mostly due to *M. genavense* or *M. celatum*, are reported in AIDS patients. Furthermore, the frequency of these infections has decreased dramatically following the introduction of highly active antiretroviral treatments. In contrast, in HIV-negative patients, the spectrum of mycobacterial diseases is broad, and many species are involved. Of note, slow growers are primarily involved in respiratory and lymph node infections, whereas sepsis and infections of skin, soft tissues, bone, and joints are frequently attributable to rapid growers. Also noteworthy is the high number of infections caused by rapid growers. Reconsideration of the role of such organisms, regarded for many years as minor players in disease, is due.

Little good information is available about the antimicrobial susceptibilities of nontuberculous mycobacteria (NTM), in particular, of recently described species. There is, however, a clear distinction characterizing the susceptibilities of rapidly and slowly growing mycobacteria. Generally speaking, isoniazid and pyrazinamide should not be used against the slow growers, as they are not effective; a variable degree of activity is shown by rifamycins (rifampin and rifabutin), quinolones (ciproflox-

acin, moxifloxacin, ofloxacin, and sparfloxacin), macrolides (clarithromycin), aminoglycosides (streptomycin and amikacin), and ethambutol. The resistance of *M. celatum* to rifamycins is unquestioned, and the repeatedly reported multidrug resistance of the species genetically related to *M. simiae*, such as *M. lentiflavum* and *M. triplex*, seems reliable. For rapid growers, the spectrum of effective antimycobacterial drugs is restricted to ciprofloxacin, clarithromycin, tobramycin, and amikacin, in addition to cefoxitin, doxycycline, imipenem, and sulfamethoxazole.

The most important problem is deciding whether the isolation of an NTM, and in particular one of the newly described species, is clinically relevant. Although disregarded by many, clear criteria issued by the American Thoracic Society do exist (87) and should be followed (Table 2). These recommendations refer to pulmonary disease, but their extension to mycobacterioses involving other body sites makes sense.

Finally, what about the role of the laboratory? The identification of new and rarely encountered mycobacteria is out of reach of most routine clinical laboratories. The best choice is to submit strains not identifiable with commercial DNA probes to a reference laboratory that uses genetic sequencing or, at least, high-performance liquid chromatographic analysis of cell wall mycolic acids.

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Table 1. Number of cases in which a single mycobacterial species has been isolated

Species	No. of cases by disease type ^a									
	Respiratory		Lymphadenitis	Soft tissues	Osteoarticular	Disseminated		Sepsis	Other	
	HIV ⁻	HIV ⁺				HIV ⁻	HIV ⁺		HIV ⁻	HIV ⁺
<i>M. branderi</i>	[9]+(1)				1					
<i>M. bohemicum</i>			3							
<i>M. celatum</i>	3	5	1	1			9			1 ^b
<i>M. conspicuum</i>						1	1			(1) ^c
<i>M. doricum</i>	(1)		1							
<i>M. elephantis</i>			1							
<i>M. genavense</i>			1			2	~100			1 ^b
<i>M. goodii</i>	5			1	16					
<i>M. hackensackense</i>								1		
<i>M. heckeshornense</i>	1+[1]			3						
<i>M. heidelbergense</i>	1		1							
<i>M. immunogenum</i>	1				1	2		3		1 ^d
<i>M. interjectum</i>	2+(1)	(1)	5							1 ^e
<i>M. lacus</i>					1					
<i>M. lentiflavum</i>	1+(1)		6		1	1	1			(1) ^f
<i>M. mageritense</i>	1				1			1		
<i>M. manitobense</i>				1						
<i>M. mucogenicum</i>				1				1		1 ^f
<i>M. novocastrense</i>				1						
<i>M. palustre</i>			1	1						
<i>M. parmense</i>			1							
<i>M. septicum</i>								1		
<i>M. triplex</i>	3		[2]			1	1			
<i>M. tusciae</i>			1							
<i>M. wolinskyi</i>				3	3			1		

^a Clinical information is missing for the cases in brackets. The clinical significance of the cases in parentheses is not proven.

^b Genital.

^c Cerebrospinal.

^d Ocular.

^e Intestinal.

^f Hepatic.

Table 2. Diagnostic criteria of nontuberculous mycobacterial lung disease^a

- A. If three sputum/bronchial wash results are available from the previous 12 months:
1. Three positive cultures with negative acid-fast bacillus smear results
 - or
 2. Two positive cultures and one positive acid-fast bacillus smear
- B. If only one bronchial wash is available:
1. Positive culture with a 2+, 3+, or 4+ acid-fast bacillus smear or 2+, 3+, or 4+ growth on solid media
- C. If sputum/bronchial wash evaluations are nondiagnostic or another disease cannot be excluded:
1. Transbronchial or lung biopsy yielding a nontuberculous mycobacterium
 - or
 2. Biopsy showing mycobacterial histopathologic features (granulomatous inflammation and/or acid-fast bacilli) and one or more sputa or bronchial washings are positive for a nontuberculous mycobacterium, even in low numbers.

^aFrom reference 87, with permission. Criteria refer to symptomatic patients with infiltrate, nodular or cavitary disease, or a high-resolution computed tomography scan that shows multifocal bronchiectasis and/or multiple small nodules.

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Microbial Forensics — Taking Diagnostic Microbiology to the Next Level

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Abstract

In contrast to the diagnostic needs of medical practice and the epidemiologic needs of public health, microbial forensics for legal proceedings requires much more detailed characterization of microbial isolates and special processing procedures for producing legal evidence. Microbial forensic analyses for biocrimes and acts of bioterrorism ultimately are needed in court to answer the following questions: where did the specific organism come from, and who was responsible? Detailed genomic analyses and other specific physical and chemical characterizations are critical parts of forensic analyses, as are chain of custody forms that are needed to sustain the validity of these analyses in legal proceedings.

Introduction

Clinical microbiologists in laboratories around the world routinely aid in the medical diagnoses of infectious diseases by determining the identities of etiologic agents and their antimicrobial susceptibility profiles. Clinical microbiology laboratories, specifically, are able to assist in answering the following questions: what disease does an individual have, and what drugs can be safely and effectively used to treat that disease? For clinical microbiology laboratories, this generally means identifying microorganisms to the species level and determining the range of antimicrobials to which that organism is susceptible. For example, the isolation of *Streptococcus pneumoniae* from a patient with signs of pneumococcal pneumonia. An antimicrobial susceptibility test would reveal the drugs suitable for

treatment. Such diagnostic tests have become so routine that they are often automated and accomplished within hours in clinical microbiology laboratories.

For diagnostic purposes, while it is sufficient to determine identity to the species level, e.g., *Bacillus anthracis* to aid in the diagnosis of anthrax, epidemiological investigations performed by public health laboratories generally involve identification to the level of a specific strain or type, e.g., Ames strain of *B. anthracis*, *Escherichia coli* O157:H7, or *Salmonella enteritidis* with a specific plasmid profile. Additionally, epidemiological investigations often involve environmental analyses and analyses from multiple patients to pinpoint the source of an infectious disease outbreak, the aim being to identify the source and route of transmission so that effective disease control measures can be implemented. Statistical analyses are critical for analyzing patterns of disease spread and for establishing where the etiologic agent most likely came from, how that agent most likely is being transmitted, and who else is likely to be at risk of contracting disease.

As will be discussed in greater detail below, forensic analyses are more demanding than either diagnostic identification of an etiologic agent or epidemiological investigation of the source of disease. To be used in legal proceedings, forensic analyses require documented handling procedures and detailed identification and characterization that permit the unequivocal linkage of a biologic agent with a perpetrator of a crime. Such forensic analyses of microorganisms represent a new challenge, which, in the case of a biocrime or bioterrorist attack, begins with the collection of the sample and encompasses all of the diagnostic activities of the clinical microbiology laboratory and the epidemiological investigations of the public health laboratory.

Case Study

Comparing clinical diagnosis, epidemiology, and microbial forensics

To provide a perspective on the issues that differentiate the roles of laboratory analyses in clinical diagnostics, epidemiology, and forensics, I will discuss the case of a 60-year-old male California orthodontist with which my

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laboratory was involved (1). He had no predisposing medical conditions; he was HIV negative and a non-smoker. He developed rapid onset of peracute pneumonia with progression to respiratory failure in 7 days. Treatment with anti-inflammatory drugs and antimicrobics was ineffective. Death occurred due to multi-organ system failure, with massive fluid accumulation in the lungs.

The medical diagnosis of legionellosis was based on the observed signs of pneumonia and the identification by a commercial clinical diagnostic laboratory of *Legionella dumoffii*, cultured from a respiratory sample collected upon hospital admission. The identification of *L. dumoffii* was sufficient for medical diagnostic purposes, and the culture was destroyed once the identification was completed. No formal epidemiological investigation ensued, as this was a single isolated case. The assumption was that the orthodontist had acquired the infection from an environmental water source, because this is the most common source of infection in cases of legionellosis. The specific water source, however, was unknown.

Subsequent questions of whether the source of infection was the orthodontist's dental operatory and possible civil legal action led to a pseudo-epidemiological/forensic investigation to see if the precise source of infection could be identified. Since the culture had been destroyed, there was no opportunity to directly link that culture with an environmental source as would be required for forensic evidence in a criminal legal proceeding. From an epidemiological perspective, *L. dumoffii* was assumed to be the cause of the fatal infection, and thus the question was whether *L. dumoffii* could be identified exclusively in a water source to which the orthodontist was exposed.

Using direct fluorescent antibody testing and PCR analyses, high levels of *Legionella* spp. (>10,000/ml), including high levels of *L. dumoffii* and low levels of *Legionella pneumophila*, were found in the dental unit water lines of the deceased orthodontist's operatory. Low levels (<100/ml) of *Legionella* spp. and no *L. dumoffii* were found in the drinking water of his home and other sites he frequently visited; e.g., his health club.

Direct fluorescent antibody staining showed the presence of *L. dumoffii*, *Legionella longbeachae*, and *L. pneumophila* in water lines of the dental operatory and in formalin-preserved lung tissue collected during autopsy of the orthodontist. The lung tissue provided a specimen that potentially could be used for microbial forensic analysis.

No further studies of office staff and patients exposed to the dental operatory water, e.g., for seroconversion to legionellae or for antibodies specific for *L. dumoffii*, were conducted, which could have added valuable epidemiological data. Nevertheless, the profile identity of *Legionella* spp. in the dental operatory water line and the preserved lung tissue suggested from an epidemiological, and possibly forensic, perspective that the dental unit water line was the likely source of infection. Dental water lines are known to permit growth of *Legionella* spp. in protozoans in biofilms, and dentists in Europe and the United States have been found to have higher rates of seroconversion to *Legionella* spp. than the general population. The presence of the same species of *Legionella*, including *L. dumoffii*, in the lung tissue of the deceased orthodontist and the water lines of his dental operatory provided strong presumptive evidence for the source of infection. However, the lack of the culture used for diagnosis, the lack of contemporary sampling of water lines, and the lack of more specific strain identification data made it impossible to make a definitive forensic determination. In a legal proceeding, this level of identification would be deemed insufficient to establish that the water in the dentist's own operatory was the source of his fatal infection. This case study reveals the problems of linking clinical diagnostic laboratory activities with microbial forensic analyses for attribution purposes in a criminal legal proceeding.

Laboratory Response Network (LRN)

Recognizing the limitations of the public health system to help identify rare diseases that might be unleashed in a bioterrorist attack, the CDC established the LRN. The LRN is a network that links laboratories involved in the diagnosis and epidemiological investigation of infectious diseases and provides pro-

ocols and reagents for identifying the major biothreat agents. The LRN was established to develop critical laboratory capacity in public health laboratories; to foster appropriate linkages with, and capacity in, clinical laboratories; and to integrate these capacities into overall emergency preparedness.

The clinical microbiology laboratories, which are likely to be the first to detect the agents of a biocrime or an act of bioterrorism, are considered to be the Sentinel Laboratories of the LRN; these laboratories previously had been designated as Level A Laboratories when the LRN was first established. The LRN Sentinel Laboratories, e.g., a hospital clinical microbiology laboratory, are to identify potential biothreat agents; they do not, however, make a definitive identification. For non-bioterrorism events, the same laboratory may do so to aid in medical diagnosis of a disease; e.g., a clinical microbiology laboratory in the southwestern United States would be expected to positively identify *Yersinia pestis* from a patient with plague acquired from a natural source. In a bioterrorism event, the role of the same laboratory as an LRN Sentinel Laboratory would be to refer the samples to an LRN Reference Laboratory, which would do confirmatory testing. The Sentinel Laboratories are not supposed to perform analyses of environmental samples. In essence, the Sentinel Laboratories play the role of ruling out rather than actually identifying biothreat agents.

Clinical diagnostic laboratories, however, are essential for the early detection of intentional dissemination of biological agents. They use clinical data and standard microbiological tests to decide which specimens and isolates should be forwarded to higher-level biocontainment laboratories. The Sentinel (clinical diagnostic) Laboratories should know who to call in the event a suspect agent is detected, where to refer the specimen, and how to safely ship specimens. Importantly, the Sentinel Laboratories have trained staff that are capable of safe collection, packaging, labeling, and shipping of samples that might contain dangerous pathogens to the next level laboratories in the network. As discussed below, they may also serve as the first step in microbial forensic analyses. As such, these labo-

ratories must have established standard operating procedures, reliable quality assurance and quality control procedures, and a chain-of-custody plan.

Within the LRN, a Reference Laboratory, typically a public health laboratory, confirms the identity of the biothreat agent. The Reference Laboratories of the LRN have access to specialized protocols and reagents for the definitive identification of biothreat agents that are not made available to the Sentinel Laboratories. The Reference Laboratories should have core and advanced capacities for agent isolation and presumptive testing of specific agents, and facilities for the safe containment of biothreat agents (usually at the biosafety level 3). They must be able to provide accurate identification of biothreat agents, often with specific strain information, and minimize false-positive results.

The most sophisticated analyses, requiring maximum containment, are performed at the CDC and/or U.S. Army Medical Research Institute of Infectious Diseases laboratories, which represent the tip of the pyramid for analyses performed within the LRN. These national laboratories are responsible for definitive characterization of biothreat agents.

Microbial Forensics

Microbial forensics represents the extension of epidemiology into greater detail, going beyond species identification to strain determination and characterization. To help define the requirements for microbial forensic analyses, a colloquium was convened by the American Academy of Microbiology in June 2002 (2) to consider (i) what resources would be needed to provide detailed information about the identification of a microorganism that could be used in legal proceedings, e.g., in a bioterrorism event, and (ii) what methods would be needed to identify the source of the biothreat agent that could lead to the apprehension and successful prosecution of the perpetrator(s). The colloquium participants concluded that while epidemiology and forensics have similar aims of identifying the source of infection, microbial forensic analysis has additional and more stringent requirements, including establishing a chain of custody on evidentiary

samples and providing much greater detail to determine the precise strain and substrain. Only the highest quality assurance and quality control standards for microbial forensics could lead to reliable results that would stand up in a court of law. Standard operating procedures, training of technologists, proficiency testing, secure databases, and multiple analyses are some of the steps required to meet this need.

Right from the start, individuals collecting specimens that may be used for microbial forensics must be aware of maintaining the chain of custody to preserve validity for subsequent criminal proceedings. In some biocrime events, including those that turn out to be hoaxes, the biocrime scene functions like a hospital emergency room. Within this environment, samples must be collected in an extremely organized and accurate manner, with an eye toward avoiding contamination and maintaining organism viability and the chain of custody. In other cases, the first evidence comes from a patient, and it is the clinical samples that must be preserved with appropriate chain of custody. Standards, such as those developed by the NCCLS, are critical. Procedures like the chain-of-custody system established by the Department of Health and Human Services for drug test samples (3) must be applied to clinical diagnostic specimens and microbial cultures.

To lay a proper foundation for the field of microbial forensics, the Federal Bureau of Investigation initiated the Scientific Working Group on Microbial Genetics and Forensics (SWGMPF) on 29 July 2002 (4). This working group was designed to provide an avenue for scientists from diverse disciplines within the government, academia, and the private sector to address issues collaboratively and to develop guidelines related to the operation of microbial forensics. The SWGMPF initially focused on (i) defining quality assurance guidelines for laboratories performing microbial forensic casework analyses, (ii) establishing criteria for the development and validation of methods to characterize or individualize various threat agents in ways that can be used forensically to attribute criminal acts, (iii) prioritizing efforts to those pathogens and toxins that would most likely be used in biocrimes, (iv) understanding

and enhancing microbial population genetic data so that a finding can be interpreted, and (v) establishing design criteria for information databases.

The SWGMPF has developed quality assurance guidelines to provide laboratories engaged in microbial forensic analyses with a framework to implement a quality assurance program (5). The *Quality Assurance Guidelines for Laboratories Performing Microbial Forensic Work* provides a baseline from which laboratories may structure their quality assurance practices. These quality assurance guidelines are based on the standards for human forensic DNA typing, clinical laboratory standards, and the International Standards Organization (ISO), as well as the experience of a broad range of scientists. Many of the recommendations outlined in the SWGMPF quality assurance document are used by laboratories already meeting ISO 17025 (6) and Clinical Laboratory Improvement Amendments of 1988 (7) requirements. The laboratory should have a facility that is designed to provide appropriate levels of security and safety and to minimize contamination. The laboratory should use validated methods and procedures for analyses. The laboratory should maintain a chain of custody for forensic samples from the time of their receipt in the laboratory.

For identifying an organism used in a biocrime, a number of analytical methods are needed to supplement the normal phenotypic and molecular identification methods and antimicrobial susceptibility assays used by a clinical laboratory in the diagnostic process. These additional assays are likely to include detailed molecular analyses, e.g., microarray analyses of specific genes, sequencing of DNA and/or RNA in samples, and genomic sequencing of culture isolates; determining patterns of single-nucleotide polymorphisms; and in some cases, such as anthrax, analyzing variable tandem multiple repeats. Multiple test methods must be used to avoid misidentification of agents caused by induced mutations. To this end, portions of samples should be saved for additional investigation or confirmatory testing. To aid in this effort, it will be important to establish a National Strain Repository to conserve reference material, to sequence multiple strains of biothreat agents for signature development,

and to understand biological variation. Identifying the source of a biocrime may also require gathering information about both the organism itself and the matrix in which it is found. The development of new physical analysis methods that may help identify where an organism actually grew will be important for future microbial forensic analyses.

Many of these analyses will need to be performed at specialty laboratories outside the realm of the clinical diagnostic laboratories and those of the LRN. The United States is developing the National Bioforensics Analysis Center (BFAC) as part of the National Biodefense and Countermeasures Center and the Fort Detrick interagency biodefense campus (4). The BFAC and its partner laboratory network is to serve as the national forensic reference center to support homeland security for the attribution of the use of biological weapons. Essentially, this will be a multi-tiered laboratory system analogous to, and building upon, the LRN in which the clinical diagnostic laboratories will be a critical component.

Because of the public health nature of a bioterrorism attack, much evidence initially will be sent to the public health sector, e.g., a state public health laboratory of the LRN and/or the CDC. Once the evidence is deemed to be from an act of bioterrorism, the materials would be sent by the LRN to the national bioforensic laboratory for attribution analysis. If an event was immediately recognized as an act of bioterrorism,

evidence would be sent directly by first responders, the intelligence community, or the Department of Defense to the national bioforensic laboratory. That laboratory would carry out a suite of applicable assays, as well as use the partnership network to enhance attribution characterization capabilities.

Summary

Diagnostics, epidemiology, and forensics have different aims. Diagnostics target the patient's health. Epidemiology targets public health. Forensics produces legal evidence. Microbial forensics not only differs from epidemiology in its emphasis on perpetrators, it also represents the extension of epidemiology into greater scientific detail, going beyond species identification to strain determination and characterization. To accomplish this challenging task, microbial forensics requires both basic traditional methodology and advanced molecular techniques. It begins with sample collection and the normal analyses performed as part of routine clinical microbiology laboratory procedures. It then moves into more detailed and specialized analyses. Laboratories performing microbial forensics must have documented standard operating protocols, quality control, and established chain of custody. Microbial forensics builds upon epidemiological analyses in which the clinical laboratories are sentinels of the LRN — and it goes beyond, to specialized analyses and laboratories that are still being defined and established.

Forensics for bioterrorism must encompass and link to clinical diagnostic laboratories and the LRN.

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A Special Invitation to Authors

The editors of *Clinical Microbiology Newsletter* extend an invitation to authors who may wish to contribute an article or editorial for publication in CMN. Of special interest are timely topics of interest to clinical microbiologists and infectious disease physicians. Persons with suggestions for articles or editorials should contact the Editor listed below to discuss the details of their potential submission.

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Call for Submissions

Case Reports

If your laboratory has isolated an uncommon organism, a common organism from an unusual patient, or an organism that presented a particular diagnostic challenge, why not share the information with your colleagues through the *Clinical Microbiology Newsletter*. The editors would like to receive interesting case reports from our readers for possible publication in the *Newsletter*. Submitted case reports should contain (i) a brief clinical history summarizing the symptoms and course of the illness, (ii) a description of how the organism(s) was cultured and differentiated from closely associated organisms, and (iii) the results of susceptibility tests for the isolate(s).

Letters

Letters expressing opinions or offering helpful technical hints will be considered for publication (subject to editing) provided they are signed by all authors and do not exceed two typewritten (double-spaced) pages.

Preparation and Submission of Material

All material submitted for publication in the *Newsletter* (including references) should be typed **double-spaced**, with 1-inch margins, on standard 8½ × 11-inch paper. Photographic prints should be in black and white, not color. Charts and graphs should also be sent as black-and-white hardcopy. If you submit a review

article, please include a **short abstract** of not more than 150 words. If you prepared your manuscript on a personal computer, please also send the manuscript on diskette and indicate the program used to create it. Electronic files for charts and graphs are useful only when submitted as separate files in EPS, TIFF, JPEG, or PICT format. Please include your phone and FAX numbers and e-mail address.

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Authors are responsible for the accuracy of references, which should include **complete** publication information. References should be listed in the **numerical** order by which they occur throughout the text, numbered **consecutively**, and cited in the text by these numbers. List the first author in a reference with **last name followed by initials**; list subsequent authors in the same reference with **initials followed by last names**. (Editors are always listed with initials followed by last names.) If there are more than three authors, include only the name of the first author followed by "et al." Include abstracts, materials in press, personal communications, and submitted material in the references section. Citations to personal communications must be accompanied by a letter from the person cited giving permission for the citation. Some typical examples of references are listed below.

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Oxoid Dryspot streptococcal grouping kit for grouping beta-hemolytic streptococci. *J. Clin. Microbiol.* 37:255-257.

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