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A review of the science regarding dental unit waterlines

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preponderance of scientific evidence has documented that the water used as a coolant and irrigant during dental procedures can be heavily contaminated with microorganisms. These microbial species are primarily naturally occurring slime-producing bacteria and fungi that form microbial biofilms on the walls of the small-bore tubing that delivers water to the dental unit and

is needed to determine the constitution pathogenicity of microbial biofilm in dental unit waterlines and the actual contribution of exposure to biofilm on human disease.

attached instrumentation. Small parti-More research cles of this biofilm may break off as the water passes through the tubing, thereby contaminating the water that is expressed into the patient's mouth and aerosolized into the dental operatory environment. If untreated, the microbial populations often exceed 10⁴ to 10⁵ colony-forming unit per milliliter, or CFU/mL, of water, which greatly exceeds the recommended drinking water standard in the United States of less than 500 CFU/mL of noncoliform bacteria.1 Furthermore, human pathogens that include Legionella pneumophila, the causative agent of legionnaires' disease; Pseudomonas aeruginosa; and nontubercular mycobacterium species frequently have been isolated from dental unit waterlines, or DUWLs.

> Does the presence of these pathogens and other vet-to-be-isolated potentially

pathogenic microorganisms contained in DUWLs constitute a health risk to dental patients and the members of the dental team providing oral health care?

To try to answer that and other questions sur-

Background. The National Institute of

Dental and Craniofacial Research, or NIDCR; the American Dental Association, or ADA; and the Organization for Safety & Asepsis Procedures, or OSAP, sponsored a workshop on the topic of dental



unit waterlines, or DUWLs, on Sept. 29, 2000, at the National Institutes of Health in Bethesda, Md. These organizations invited a group of experts from the ADA, NIDCR, OSAP, the U.S. Food and Drug Administration, the Centers for Disease Control and Prevention, the U.S. Department of Defense, academia and private industry to participate.

Types of Studies Reviewed. The sponsors asked the participants to critically review the scientific literature on the subject in an attempt to determine the evidence basis for management of DUWL contamination and potential health risks, if any, in dental procedures. The ultimate goal of the workshop was to determine if a research agenda in the area of DUWLs should be pursued and what questions such an agenda should involve.

Results. The workshop yielded four questions that need to be addressed in future research: What is the safest and most effective agent(s)/device(s) for achieving microbial levels of no more than 200 colonyforming units per milliliter, or CFU/mL, in the effluent dental water? How should these products be evaluated and by whom? What are the adverse health effects, if any, of chronic exposure to dental bioaerosol or to the agents introduced into the dental unit to treat the waterlines for both dental staff members and patients? How could these health issues be evaluated?

Clinical Implications. Developing evidence-based parameters for the management of biofilm contamination that are efficacious and cost-effective will allow clinicians to meet in proposed ADA standard of no more than 200 CFU/mL of effluent water.

rounding biofilms and dental equipment, a workshop on the topic was sponsored Sept. 29, 2000, by the National Institute of Dental and Craniofacial Research, or NIDCR; the American Dental Association, or ADA; and the Organization for Safety & Asepsis Procedures, or OSAP. The event was held at the National Institutes of Health Conference Center in Bethesda, Md. The organizers invited a group of experts that included representatives from the ADA; NIDCR; OSAP; the U.S. Food and Drug Administration, or FDA; the Centers for Disease Control and Prevention, or CDC; the U.S. Department of Defense; academia; and private industry. They asked these experts to review the scientific literature on the subject critically in an attempt to determine the evidential basis for management of DUWL con-

tamination and the potential health risks, if any, of using contaminated water as a coolant in dental procedures. The ultimate goal of the workshop was to determine if a research agenda in the area of DUWLs should be pursued by the NIDCR, the ADA and private industry and what questions such an agenda should involve.

The actual impact of biofilm may be significantly underrecognized and underreported in the literature.

occurring antibodies and cell-mediated immunity. Furthermore, current culturing techniques heavily favor and select for organisms in the planktonic state and may not represent the true microbial flora. Thus, the actual impact of biofilm may be significantly underrecognized and underreported in the literature.

In one investigation, Costerton and colleagues² presented evidence that exposure to fragments of biofilms constitutes a serious insult to the pulmonary system. Biofilm fragments are inhaled or aspirated, and the bacteria persist because the phagocytes cannot process them. The lungs are colonized by biofilm fragments, often without symptoms, but stress can cause the bacteria to proliferate, and an acute infection can ensue.^{2,3} Although no disease transmission related to

> DUWLs and related biofilm has been documented, there is potential for infection with pathogens such as P. aeruginosa and other organisms, and the long-term impact of this colonization should be investigated.

John Bartlett, M.D. Dr. John Bartlett, who is a professor of the medicine and the chief, Johns Hopkins University School of Medicine, Division of Infectious Diseases,

Department of Medicine, Baltimore, addressed the relationship between pulmonary disease and DUWLs. He stated that water supplies commonly used in dental procedures often are contaminated by multiple bacteria, including waterborne nonfermenting bacteria, the most well-known being P. aeruginosa. These organisms rarely, if ever, are implicated in infections associated with dental procedures, probably because virulence is low and the inoculum also is very small owing to very low concentrations. The major recognized risk for dental infections, according to Dr. Bartlett, is posed by the components of the host flora, primarily anaerobic bacteria and streptococci that reach concentrations of 10¹²/gram in the gingival crevice. This is the geometric limit with which bacteria can occupy space.

The organism that has attracted recent attention is *L. pneumophila*, which is a waterborne pulmonary pathogen that often is isolated in water sources in hospitals and other health care settings. These organisms survive and grow in water at temperature ranges of 25 to 42 C (77–108 F), especially if water flow is stagnant. The organisms die rapidly at 55 C (131 F). Out-

BIOFILM AND HUMAN DISEASE

What are the effects of exposure to microbial contamination via biofilm on human health? Two speakers at the workshop addressed this issue.

William Costerton, Ph.D. Dr. William Costerton, who is the director of the Center for Biofilm Engineering at Montana State University-Bozeman, addressed the overall issue of biofilm in human disease. In the medical setting, he said, biofilm rapidly forms on many devices such as endotracheal tubing, intraveneous catheters and other indwelling apparatuses. He further noted that many chronic diseases might be related to biofilm colonization. Various organisms have developed the ability to form into a biofilm. Once microbial populations form into a biofilm, they are protected by a glycocalyx, a coating that forms on the bacteria. The minimum inhibitory concentration, or MIC, of antibiotics in relation to the organisms in that biofilm is as much as 1,000 times higher than the MIC in relation to the same organism in a free-floating, planktonic state.2 In the biofilm state, microbial populations are protected from both naturally

breaks of legionnaires' disease have been noted in hospitals and hotels; when an identifiable source is found, it generally is potable hot water systems (building plumbing systems that distribute water for human contact) and water in cooling towers. Surveys of hospital water supplies since the initial outbreak of legionnaires' disease in Philadelphia in 1976 indicate that 23 to 73 percent are colonized with legionella.4 Recommendations for dealing with this vary. The CDC recommends identification of sources of legionella in the presence of documented outbreaks of the disease.4 Four states mandate routine water cultures for legionella with treatment of positives using one of five methods: superheating, hyperchlorination,

copper-silver ionization, monochloraminae or ultraviolet treatments.4 The most vulnerable patients are those with compromised cell-mediated immunity, especially those who have undergone organ transplantation and those who have received cancer chemotherapy. Thus, when surveillance for legionella is conducted, areas of hospitals in which these patients are treated are given the highest priority.

With respect to dental procedures, Dr. Bartlett was aware neither of any case of legionnaires' disease ever being associated with a dental procedure nor of any specific recommendation for preventing legionella infection that would apply to dental practice.

DENTAL UNIT WATERLINES: CURRENT CONCEPTS, TREATMENT AND **TECHNOLOGY**

Jean Barbeau, Ph.D. Dr. Jean Barbeau, who is an associate professor and the director of graduate studies, Department of Stomatology, Faculty of Dentistry, University of Montréal, addressed current concepts regarding the issue of DUWLs. He stated that it now is well-known that bacteria heavily populate water inside DUWLs. The lumens of the small-bore hoses are colonized by a tenacious freshwater biofilm that acts as a reservoir for certain opportunistic pathogens, such as P. aeruginosa (more than one clone of *P. aeruginosa* may be found in a given dental unit water sample), L. pneumophila and nontubercular mycobacteria, among others.⁵ As a consequence, bacterial counts in water samples can reach up to several million

per milliliter.

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biofilm chronically.

One of the explanations for these high concentrations may be the large (6:1) area-to-volume ratio of small waterlines, which gives biofilm plenty of surfaces on which they can spread and a relatively small volume of liquid to fill with shedding daughter cells. The cultivable microflora represents less than 4 percent of the actual bacterial load. Thus, the majority of bacteria are either dead or viable but noncultivable. Duchaine and colleagues⁷ showed that waterborne bacteria are aerosolized during dental procedures and that dental personnel may be exposed to those microorganisms and fragments of biofilm chronically.

It is known that different free-living amebae

like Naegleria, Hartmannella and Acanthamoeba species may carry legionella species or mycobacterium species as an endosymbiote. The reported high concentrations of legionella and mycobacteria in dental unit water samples may be, in part, the result of the presence of amebae. On the other hand, biofilm formation would favor the proliferation of amebae, thus creating an ecological loop. Water taken from a dental unit before any flushing shows a significantly greater amebae population than that in tap

water.8 The number of amebae in a dental unit may be up to 300 times higher than that found in tap water.8 Pathogenic Acanthamoeba species may be present in 40 percent of the samples.8 Flushing the DUWLs for two minutes reduces the concentration of amoebae by 66 percent.6

Commenting on the Canadian response to the DUWL issue, Dr. Barbeau said that the Canadian Dental Association has set guidelines for DUWLs⁹ similar to those adopted by the ADA. Following are the Canadian recommendations:

- Avoid heating dental unit water.
- At the beginning of each clinic day, purge all lines by removing handpieces, air-water syringe tips and ultrasonic tips, and by flushing thoroughly with water.
- Run high-speed handpieces for 20 to 30 seconds after each patient to purge all air and water.
- Use sterile water or sterile saline when flushing open vascular sites and when cutting bone during invasive procedures.
- Follow the unit manufacturer's instructions for daily or weekly maintenance if using bottles of

water or other special delivery system.

Shannon E. Mills, D.D.S. Dr. Shannon Mills, who is a colonel, U.S. Air Force; the dental program manager, Office of the U.S. Surgeon General, Bolling Air Force Base, Washington; and a member of the board of directors at OSAP in Annapolis, Md., provided an update on current modalities and technology for DUWL treatment. He stated that biofilm colonization of DUWLs is primarily an engineering problem that must be overcome to improve the quality of water used in dental treatment. Currently available means to accomplish this include independent reservoirs, chemical treatment (both continuous and intermittent), sterile water delivery systems, filtration and combinations of these methods. Following is a brief review of the advantages and disadvantages of each of these approaches.

Independent reservoirs. Independent reservoirs isolate the unit from municipal water and permit the use of water of known microbiological quality. The user can introduce cleaners and germicides to control or eliminate biofilm formation within the water delivery system. Independent reservoirs are among the most economical devices for treating water, with initial costs typically ranging from \$100 to

\$250. Recurring costs for water and chemicals usually are minimal. Chemical treatment using independent reservoirs is the water treatment method with the strongest support in the scientific literature. A number of major manufacturers offer independent reservoirs as standard equipment or as optional accessories.

Chemical treatment. Some manufacturers of units with independent reservoirs have recommended specific treatment regimens for use with their equipment. The most widely evaluated agent for use with reservoir systems is 5.25 percent sodium hypochlorite (500 parts per million) diluted 1:10. In three studies, weekly 10-minute treatment with diluted household bleach was effective in improving the quality of effluent water and reducing the amount of biofilm in the systems. 10-12 The investigators found, however, that multiple treatments were required to effect results. Treatment protocols also appear to be technique-sensitive; operator compliance was identified in one study as an important contributor to clinical treatment failure.11

Commercial sources offer various proprietary chemicals that purport to improve the quality of dental treatment water by controlling or eliminating biofilm. The active ingredients in these products include chlorhexidine gluconate, alkaline peroxide, citric acid and iodine compounds. While there is little peer-reviewed information on most of these products, they employ agents and regimens that have been demonstrated to be effective in other treatment settings. Some of these agents may pose less risk to staff and dental equipment than does sodium hypochlorite.

Sterile water delivery systems. Irrespective of the agent used to treat the system, the quality of water delivered can be no better than the source water used in the reservoir bottle. Irrigating with sterile water or other solutions of known microbi-

ological quality should decrease the likelihood of clinical failure.

Sterile water delivery systems are designed to provide irrigation during surgical or implantation procedures. These systems employ single-use disposable or autoclavable tubing to bypass the dental unit and provide sterile irrigating solutions directly to dental handpieces. Their disadvantages include higher purchase costs and their need for packaged sterile

Though widely advocated, pretreatment flushing—unless carried out for five to 15 minutes—has only poor support in the literature.

solutions.

Filtration. Other methods that can improve water quality include the use of microfiltration and ultraviolet germicidal irradiation of incoming water. Chemical treatment protocols also have been developed that involve continuous introduction of low levels of agents intended to inhibit biofilm formation. In some cases, automated systems are less compliance-sensitive. Lower concentrations of germicides also may reduce risk of damage to equipment.

Filtration of incoming water reduces the need for chemical treatment and the associated risk of exposure for patient and health care worker alike. It also permits units to remain connected to municipal water supplies. The disadvantages of filters include the need for frequent changes and a lack of any effect on biofilms distal to the filter.

Other water quality improvement methods. The evidence basis for various means intended to improve dental water quality is variable, and there are conflicting reports in the literature. Though widely advocated, pretreatment

flushing—unless carried out for five to 15 minutes—has only poor support in the literature. $^{13-15}$ The effects typically are transient, and in several studies, the results failed to meet the ADA goal of no more than 200 CFU/mL. $^{13-15}$

There is better literature support for chemical treatment regimens, particularly the use of diluted sodium hypochlorite, ^{10,11,16} though compliance and material compatibility problems have been observed with it. Although there are fewer studies evaluating other chemical agents in the peer-reviewed literature, several agents used either intermittently or continuously have shown promise. There are few data on the efficacy of filters in dental settings, but filters have been shown to be effective for trapping bacteria in other medical and industrial settings. ¹⁷

Standards for quality improvement products. Efforts are under way to develop national standards for products intended to improve the quality of dental treatment water. In 1994, the **ADA Standards Committee on Dental Products** began work on a specification for antimicrobial agents and other chemicals for the prevention, inactivation and removal of biofilm in dental water systems. This specification should be finalized by 2003. Proposed American National Standards Institute/ADA Specification No. 107 for Antimicrobial Agents and Other Chemicals for Prevention, Inactivation and Removal of Biofilm in Dental Unit Water System addresses efficacy, biosafety and compatibility of the various chemical agents with dental equipment and materials. 18 While the ADA Standards Committee on Dental Products reviewed various methodologies for improving water quality, it did not discuss the degree of efficacy of the existing agents and devices. A current listing of the commercially available water treatment products can be found on OSAP's Web site.¹⁹

Validation and monitoring are related processes used to evaluate the effectiveness of devices or protocols aimed at improving the quality of dental treatment water. Validation is a complex and rigorous process of testing and evaluation performed by the manufacturer to demonstrate the safety and efficacy of devices and procedures. Monitoring is a less rigorous test performed by the end-user in the clinical setting to assess the in-use performance of the manufacturer-validated device or protocol. The purpose of monitoring is to assess clinical performance of manufacturer-recommended procedures.

Noncompliance and technique errors are the most likely reasons for clinical failure that can be identified using a monitoring protocol. Monitoring does not substitute for proper device or process validation by the manufacturer. There is no valid rationale for testing untreated systems or for identification of specific organisms unless directed to do so as part of an investigation of suspected waterborne illness. Monitoring methods should be consistent with Method 9215 as presented in the current edition of Standard Methods for the Examination of Water and Wastewater.²⁰ Inexpensive commercial test kits for this purpose are available.

CLINICAL

THE AMERICAN DENTAL ASSOCIATION'S POSITION ON DENTAL UNIT WATERLINES

Brian Shearer, Ph.D. Dr. Brian Shearer, who at the time of the workshop was the director of scientific information and policy, ADA Council on Scientific Affairs, and now is manager, Scientific Communications, Bayer Corp., Westhaven, Conn., provided further commentary and reiterated the ADA's position on DUWL contamination.

Dr. Shearer related that as early as 1978, the ADA had recommendations for the flushing of DUWLs with chemical germicides. However, as a result of concerns relating to the compatibility of chemical germicides and the materials of which dental units are composed, later infection control recommendations directed dentists to follow manufacturers' instructions for the proper maintenance of waterlines. 22

The issue of biofilm contamination of DUWLs remained in the background for many years after 1978. Around 1990, however, an increasing number of scientific reports was published in the literature clearly documenting that the microbiological quality of dental unit water was poor—and that the water even may harbor opportunistic bacterial pathogens. 11,14,23-27

The ADA began holding a series of workshops that brought together representatives from the dental profession, dental manufacturers and various governmental agencies, as well as scientific researchers. Although these workshops served as an arena for the exchange of information and ideas, they did not address the vital question: what should the quality of dental unit water be? This led the ADA to convene an expert panel in August 1995. The panel was charged with defining the optimal microbiological quality of dental unit water and identifying possible

might apply to the dental unit.28

methodologies and technologies that would allow dentists to control or prevent biofilm formation and improve dental unit water quality. The ADA also asked the panel to develop a research agenda that would promote the development of such methodology and technology. As a direct result of this expert panel meeting, the ADA issued its Statement on Dental Unit Waterlines in December 1995. The ADA's justification for the statement was as follows²⁸:

- There is irrefutable scientific evidence that the water delivered to most dental patients is of poor microbiological quality and often would fail to meet U.S. drinking water standards.
- There is also scientific evidence that dental personnel are being exposed to potentially pathogenic microorganisms as a result of aerosolization of dental unit water, and, furthermore, disease transmission in association with biofilm formation has been well-documented in other settings.
- Increasing numbers of people with diminished resistance to overt and opportunistic pathogens are seeking dental treatment. This population not only includes people with human immunodeficiency virus infection, but also elderly people, people who smoke, people with alcohol dependency, organ transplant and blood transfusion recipients, people with cancer, people with diabetes and people with other chronic organic disorders. Some of these people may be particularly susceptible to infection as a result of exposure to dental unit water.
- Finally, microbial populations are becoming increasingly resistant to antibiotics, and therefore any reasonable measure to avoid exposure to potential pathogens would seem a sensible course of action.

Dr. Shearer addressed the rationale for the goal that the expert panel set for the quality of dental unit water. The panel proposed that by the year 2000, water delivered to dental patients during nonsurgical dental procedures via the unfiltered output of the dental unit should consistently contain no more than 200 CFU/mL of aerobic heterotrophic bacteria at any time. This goal of no more than 200 CFU/mL is similar to that applied to hemodialysis units. Studies have shown that if the water used to prepare dialysis fluid contains more than 200 CFU/mL, the bacterial count rapidly can amplify the bacterial colonization of the hemodialysis unit and the subsequent bacterial

The panel discussed methods that might help dentists achieve this goal. The panel was aware of available technology and also of methodologies under research that might assist dentists in improving water quality. These options included dental units with independent water reservoirs, a chemical treatment regimen, a daily draining and air purging regimen, and point-of-use filters. However, only preliminary data were available as to these options' safety and effectiveness, and it was considered premature to make a national recommendation at that time. The panel therefore went on to develop a research agenda directed at substantiating the preliminary data that already existed and encouraging the development of new technology and methodology for the control or prevention of biofilm formation.²⁸

After convening the first expert panel and setting the goal of no more than 200 CFU/mL for the year 2000, the ADA called a second panel meeting in October 1998. A full report from this second meeting was published in the November 1999 issue of The Journal of the American Dental Association. The aim of this second panel meeting was, in part, to assess research and progress toward meeting the target of no more than 200 CFU/mL. At the time of the meeting, the panel noted some success in meeting the ADA's goal, in that the FDA had cleared approximately 26 products intended to improve the quality of dental unit water. The majority of these products fell into four main categories:

- independent water systems;
- chemical treatment protocols;
- point-of-use filters;
- sterile water delivery systems.

While recognizing this success, an important charge of this second expert panel also was to identify future directions for research. The panel identified several areas for future basic and applied research, all of which were described in the November 1999 article.³¹ Basic research questions that were proposed by the panel included the following:

— How does biofilm structure and composition change from one dental unit, dental office or geographical location to the next?

- What specific polymers or biofilm structures hold the dental unit biofilm together, and how can these structures be attacked?
- Should new approaches to biofilm control attack specific microbes or the total biofilm structure?

Applied research questions included the following:

- How are the biofilm and the dental unit affected by different water quality conditions?
- What are the effects of the approach, especially if chemicals and their residues are involved, on dental materials used in patient care?
- Can dental unit designs be developed for general practice that eliminate biofilm and its associated water quality concerns?

The ADA encouraged further research to refine current means for improving water quality, as well as further research into more esoteric means for eliminating and/or inhibiting biofilm formation.

Further supporting the position of the ADA, the CDC disseminated a statement regarding biofilm and dental unit water quality in October 1999³² (box).

SUMMARY AND RECOMMENDATIONS

Although no disease transmission arising from DUWL microbial contamination has been conclusively documented, there is irrefutable scientific evidence that the water delivered to most dental patients is of poor microbiological quality and often would fail to meet U.S. drinking water standards. 23-28,31,33-36 Furthermore, evidence suggests that dental personnel and the increasing number of immunocompromised dental patients are being exposed to potentially pathogenic and resistant microorganisms as a result of aerosolization of dental unit water.³⁶⁻³⁸ Of perhaps greater significance is the fact that disease transmission in association with biofilm formation has been welldocumented in other health care settings.²⁸ The potential for transmission of disease from contaminated DUWLs exists, in at least some populations. To minimize the potential health impact of DUWL contamination, the prudent dental practi-

BOX

INFECTION CONTROL: STATEMENT FROM THE CENTERS FOR DISEASE CONTROL AND PREVENTION REGARDING BIOFILM AND DENTAL UNIT WATER QUALITY.

Biofilms are microscopic communities consisting primarily of naturally occurring water bacteria and fungi that form thin layers on virtually all surfaces (including dental water delivery systems) that remain in contact with water. The presence of biofilms in dental unit waterlines has been well-established. As a result of biofilm formation, relatively high numbers of common water bacteria can be found in some dental water systems. To date, however, scientific evidence indicates there is little risk of significant adverse health effects due to contact with water from a dental unit. Nonetheless, exposing patients or dental personnel to water of uncertain microbiological quality is not consistent with universally accepted infection control principles. In 1993, the Centers for Disease Control and Prevention issued guidelines relative to water quality in a dental setting. The CDC Infection Control Guidelines for Dentistry state that sterile irrigating solutions should be used when surgical procedures involving the cutting of bone are performed. The guidelines also recommend that all dental instruments that use water (including high-speed handpieces) be run to discharge water for 20 to 30 seconds after each patient and for several minutes before the start of each clinic day. These recommendations are designed to help reduce the number of microorganisms present in treatment water, but do not appear to reduce biofilm formation in the

CDC's 1993 Infection Control Guidelines for Dentistry are being updated and will contain more specific guidance for dental unit waterlines. CDC is encouraged by the response of dental equipment manufacturers in developing products to address the issue of dental unit water quality. To date, the U.S. Food and Drug Administration has cleared over 25 products designed to improve the quality of water used in dental treatment. Dentists should consult with the manufacturer of their dental unit to determine the best method for maintaining good water quality.

Source: United States Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Division of Oral Health.

> tioner should institute measures that will bring the microbial content of effluent dental water to no more than 200 CFU/mL. More research is needed, however, to determine the constitution and pathogenicity of microbial biofilm and the actual contribution of exposure to biofilm on human disease. There are several questions that need to be addressed in future research:

- What is the safest and most effective agent(s)/device(s) for achieving microbial levels of no more than 200 CFU/mL in the effluent dental water?
- How should these products be evaluated and
- What are the adverse health effects, if any, of chronic exposure to dental bioaerosol or to the agents introduced into the dental unit to treat the waterlines for both dental staff members and patients?
- ─ How could these health issues be evaluated? ■

CLINICAL PRACTICE

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- Dr. Costerton is the director, Center for Biofilm Engineering, Montana State University–Bozeman.
- Dr. Barbeau is an associate professor and the director of graduate studies, Department of Stomatology, Faculty of Dentistry, University of Montréal.
- $\mbox{Dr.}$ Shearer is the manager, Scientific Communications, Bayer Corp. Westhaven, Conn.
- Dr. Bartlett is a professor of medicine and chief, Johns Hopkins University School of Medicine, Division of Infectious Diseases, Department of Medicine, Baltimore.
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