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Resistance of *Mycobacterium chelonei*-Like Organisms to Formaldehyde

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Mycobacterium chelonei-like organisms have been isolated from patients in two outbreaks of peritonitis involving chronic peritoneal dialysis machines routinely disinfected with 2 to 3% formaldehyde. Susceptibility studies revealed that water-adapted *M. chelonei*-like organism strains could survive 2 h of exposure to 10% formaldehyde.

Two outbreaks of peritonitis caused by a *Mycobacterium chelonei*-like organism (MCLO) recently occurred in patients using automated chronic peritoneal dialysis (CPD) machines that were contaminated with MCLO (2a). The CPD machines were routinely disinfected according to the recommendations of the manufacturers by filling all tubes and reservoirs with 2 to 3% formaldehyde and leaving it there for at least 2 h. However, Carson et al. (5) have shown that MCLO is relatively resistant to 2% formaldehyde: viable organisms persist after 24 h of exposure. This study was undertaken: (i) to determine the concentration of formaldehyde and time of exposure required to kill MCLO, (ii) to determine whether MCLO strains freshly isolated from dialysis patients and those adapted to water differ in susceptibility to formaldehyde from each other or from a standard reference *M. chelonei* strain, and (iii) to compare the efficiency of colony counting done by membrane filtration versus direct plating. Sampling times and formaldehyde concentrations were chosen to correspond with practical regimens for disinfecting CPD machines. Sterile tap water was used as the diluent in the susceptibility tests because tap water may be a natural reservoir for MCLO (2a), and it is used in CPD machines.

Six strains of mycobacteria were used for susceptibility studies: (i) one reference strain of *M. chelonei* subsp. *abscessus* (strain A); (ii) two MCLO strains isolated from peritoneal fluid of dialysis patients (strains B and C); and (iii) three MCLO strains which had been water adapted, including two from the peritoneal fluid of patients (strains D and E) and one from a CPD machine (strain F). The Mycobacteriology Branch, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Ga., identified all isolates and provided the reference strain. Medium-grown strains A, B, and C were each subcultured from Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) into Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) and grown at 25°C for 72 h in air on a rotary shaker at 60 rpm. Cells were washed twice by centrifugation and suspended in autoclaved tap water (121°C for 20 min) to an optical density of 0.2 at 420 nm (spectronic 20 spectrophotometer; Bausch & Lomb, Inc., Rochester, N.Y.). These served as stock suspensions. Strains D, E, and F had been adapted to water for another study by storage in sterile tap water at room temperature for 2 years. Each of the water-adapted strains was diluted to 1:100 in sterile tap water, which was then incubated at 25°C for 5 days in air on a rotary shaker at 60 rpm.

Colony counts for all strains were done by direct plating of 10-fold serial dilutions to determine the number of colony-forming units per milliliter. A sample of each serial dilution of the reference strain was filtered through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.) to compare colony counts obtained by direct plating and filtration.

Formaldehyde solution (certified ACS; St. Louis, Mo.) was added to sterile, uninoculated tap water to obtain concentrations of 0, 2, 5, and 10% (vol/vol) in which the medium-grown stock suspensions were inoculated for a final dilution of 1:100 in a total volume of 250 ml. For each water-adapted strain, 37% formaldehyde in sterile tap water was added to a fixed volume of MCLO-inoculated tap water to obtain the same formaldehyde concentrations in a total volume of 370 ml.

Exposure to formaldehyde was maintained at 25°C with no agitation. Susceptibility to formaldehyde was determined by the membrane filter technique (2). Each sample was filtered (0.45 μm; Millipore Corp.) after 0, 2, 6, 18, and 24 h of HCHO exposure. At zero time, 10 ml of each bacterial strain, diluted to equal the inoculum

TABLE 1. Growth of *M. chelonei* and MCLO on membrane filters after exposure to formaldehyde

Strain[a]	Formaldehyde concn[b] (%)	Colonies of MCLO per ml				
		0[c]	2	6	18	24
A	2	TNTC[d]	0	0	0	0
B		TNTC	1	1	3	0
C		TNTC	1	1	1	1
D		TNTC	0	0	0	0
E		TNTC	TNTC	17	0	1
F		TNTC	12	5	10	2
A, B, C, D	5	TNTC	0	0	0	0
E		TNTC	14	3	0	0
F		TNTC	6	1	0	0
A, B, C, D	10	TNTC	0	0	0	0
E		TNTC	1	0	0	0
F		TNTC	0	0	0	0

[a] A, Reference *M. chelonei*, 10^6/ml; B and C, patient isolates of MCLO, 10^6 and 10^5/ml, respectively; D, E, and F, water-adapted MCLO, 10^3/ml.

[b] At 0% formaldehyde, at all hours, the colonies on the filters were too numerous to count.

[c] Hours of exposure.

[d] TNTC, Too numerous to count.

exposed to HCHO, was passed through the filter. Filters were then flooded with 10 ml of HCHO at the appropriate concentration, followed by 20 ml of Trypticase soy broth (BBL) to wash the exposed cells. At 2-, 6-, 18-, and 24-h time increments, 10-ml aliquots of the bacterium-HCHO mixture were poured through the filters, followed by a 20-ml Trypticase soy broth rinse. The filters were placed on Trypticase soy agar plates and incubated at 25°C in air. All testing was done in triplicate, and the arithmetic means are reported in Table 1. After 2 weeks, the colonies on each filter were counted. Filters showing no growth were transferred to 7H9 broth, incubated for 1 week at 25°C, and then plated to Trypticase soy agar and examined for growth after 2 weeks of incubation.

Standard plate counts, performed at zero time and with 0% HCHO, established that the colony counts of the total volume (250 ml) for the medium-grown strains were 10^5 to 10^6/ml, and the colony counts of the total volume (370 ml) for the water-adapted strains were 10^3/ml. Organisms exposed to 0% HCHO for 2, 6, 18, and 24 h showed confluent growth on the filters, indicating that MCLO survived the processing procedure.

The *M. chelonei* reference strain did not survive 2 h of exposure in 2% HCHO. In contrast, three of five MCLO strains survived 24 h of exposure in 2% HCHO. Two of the water-adapted MCLO strains, E and F, survived 6 h of exposure in 5% HCHO; strain E also survived 2 h of exposure in 10% HCHO (Table 1). Filters showing no growth continued to show no growth upon subculture.

Colony counts of the reference strain done by membrane filtration and direct plating differed by 0.5 log, 7.4×10^5 and 1.2×10^6/ml, respectively, indicating that membrane filtration was an effective method for determining colony counts. In addition, membrane filtration was a useful method for removing formaldehyde by rinsing the filters without further diluting the inoculum.

These studies confirm the observation of Carson et al. that MCLO can survive 2% formaldehyde for 2 h. We also found that water-adapted MCLO could survive 10% formaldehyde for 2 h. Although the exposure size of our medium-grown strains (10^6/ml) was 3 logs higher than that of the water-adapted strains (10^3/ml), use of the 10^6/ml concentration was not unrealistic because MCLO has been shown to multiply in commercial distilled water to that concentration (5). In fact, if the concentration of water-adapted MCLO had been 10^6/ml, it is suggested that the MCLO would have survived even longer exposure times and higher formaldehyde concentrations.

Factors which may cause contamination of CPD machines and which may affect disinfection of these machines have been studied previously (1, 2, 3, 4, 6–11). We suggest that if a machine has malfunctioned or a break in sterile technique has taken place, specimens from both patient and machine should be cultured for possible contamination, including MCLO. We recommend that machines known to be contaminated with MCLO be disinfected with at least 5% formaldehyde for 18 h or 10% formaldehyde for 6 h.

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