Microwave Oven Irradiation as a Method for Bacterial Decontamination in a Clinical Microbiology Laboratory

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Exposure of 10 frequently isolated clinical pathogens to microwave irradiation resulted in total sterilization within 60 s. Time exposure experiments done with commercially prepared test strips containing *Bacillus stearothermophilus* spores indicated that a 5-min exposure was adequate to insure sterility of small, contaminated loads.

Bacterial decontamination of food products after exposure to microwave oven irradiation has been well documented, as reviewed by Culkin and Fung (2).

Although experiments done by Culkin with *Escherichia coli* and *Salmonella typhimurium* suggest that heat alone did not cause the lethality associated with microwave irradiation, definitive experiments by Goldblith and Wang (3) determined that the lethal effects of microwaves could be prevented by keeping the test organisms in an ice bath during exposure. Additional experiments conducted by Lechowich et al. (4) demonstrated that heat was the only factor effective in killing *Streptococcus faecalis* and *Saccharomyces cerevisiae* cultures by enclosing the test organisms in a Liebig condenser and varying the temperature during exposure from 25 to 55°C.

A practical application for the irradiation effect of microwave ovens on bacteria was suggested by Bush (1). Qualitative experiments done in his laboratory indicated that it was possible to use microwave ovens to decontaminate media used in clinical microbiology.

Based on the above data, a quantitative study was undertaken in this laboratory to determine the effect of timed microwave irradiation on commonly encountered bacterial pathogens and to evaluate its possible utilization in a clinical situation.

MATERIALS AND METHODS

Microwave oven. The microwave oven was furnished by Litton Industries, model 402002, 2,450 MHz.

Preliminary experiments. Selected strains of gram-positive and gram-negative bacteria were inoculated into 50 ml of tryptic soy broth (TSB; Difco Laboratories) and incubated overnight at 35°C under stationary conditions. The following organisms were included in the initial study: *Bacillus subtilis* ATCC 6633; *Enterobacter cloacae* ATCC 23355; *Klebsiella pneumoniae* ATCC 23357; *Serratia marcescens*

ATCC 8100; *E. coli* ATCC 25922; *Proteus mirabilis* (University of Utah Medical Center [UUMC] isolate); *Pseudomonas aeruginosa* ATCC 27853; alpha streptococcus, group D; *Enterococcus* (UUMC isolate); *Staphylococcus aureus* ATCC 25923; and *S. epidermidis* ATCC 12228.

After overnight incubation, total viable counts were determined in duplicate on each culture as follows. Five-milliliter portions were removed from each culture and exposed to microwave irradiation for 15, 30, 45, and 60 s. The exposed samples were then diluted in sterile, distilled water and plated on tryptic soy agar (Difco) plates to determine the total viable counts per milliliter for each time exposure. After overnight incubation at 35°C, the dilutions showing 30 to 300 colonies were counted and averaged to determine total viable organisms per milliliter.

***B. stearothermophilus* sterilization experiments.** Spore strips containing viable *B. stearothermophilus* spores (Scientific Products) were exposed to microwave irradiation for increasing time intervals as follows: 15, 30, 45, and 60 s, and 2, 3, 4, and 5 min. After exposure, strips were inoculated into TSB and incubated for 72 h at 56°C. Growth was indicated by visible turbidity in the broth culture. Unexposed positive control strips were run in parallel with each test strip.

***B. subtilis* sterilization experiment.** A spore suspension of *B. subtilis* ATCC 6633 was prepared by inoculating the organism into 5 ml of TSB, incubating overnight at 35°C, and further inoculating TSB at 24 h into a Roux bottle containing 100 ml of tryptic soy agar. The Roux bottle culture was incubated for 1 week at 35°C and examined for adequate sporulation with a Gram smear. Spores were harvested with glass beads and sterile, distilled water. The spore suspension was heated at 65°C for 30 min, washed in 50 ml of sterile, distilled water, centrifuged for 20 min at 3,000 rpm, resuspended, heated for an additional 30 min at 65°C, centrifuged, and resuspended in 25 ml of sterile, distilled water. Five-milliliter portions of this spore suspension were exposed to microwave irradiation for 15 s to 11 min. Exposed spores were plated for total viable spores per milliliter as previously indicated.

Sterilization of clinical isolates. Ten UUMC isolates, both gram-positive and gram-negative bacte-

ria, were tested to determine sensitivity of wild-type isolates to microwave irradiation compared with that of the American Type Culture Collection strains. Test organisms were grown to stationary phase in 5 ml of TSB, exposed to microwave irradiation for 5 min, and subcultured to sterile TSB. The inoculated TSBs were incubated for 48 h at 35°C and read for the presence or absence of growth.

Sterilization of contaminated plates. To test the feasibility of microwave sterilization of actual contaminated materials, autoclavable plastic bags containing discarded organisms on media in plastic petri dishes (about 100 plates per load) were exposed to microwave irradiation for 5, 10, 15, and 20 min. A spore strip from an Attest ampoule (3M Co.) was enclosed in a test tube containing 5 ml of sterile, distilled water and was placed inside the opening of each plastic bag. After autoclaving, the spore strip was placed in TSB and incubated for 48 h at 35°C. In addition, 1-ml portions of liquid remaining in each plastic bag were inoculated into TSBs and incubated for 48 h at 35°C. After incubation, the spore strip broths and media were read for the presence or absence of growth.

RESULTS

Microwave irradiation of commonly encountered clinical bacterial pathogens resulted in total sterilization within 60 s (Table 1). Typical death curves of the tested isolates are depicted in Fig. 1.

Whereas a vegetative culture of *B. subtilis* showed no viable organisms after a 30-s exposure to microwave irradiation, the time necessary to destroy 1.3×10^9 *B. subtilis* spores per ml extended beyond 11 min (Fig. 2).

Exposure of *B. stearothermophilus* strips to microwave irradiation resulted in sterilization after 5 min. Growth was observed in all control strips.

Clinical isolates (including *E. coli*, *P. mirabilis*, *P. vulgaris*, *P. aeruginosa*, *S. marcescens*, *S. aureus*, *S. epidermidis*, and enterococcus) were killed by a 5-min exposure to microwave irradiation.

Exposure of contaminated petri plates to microwave irradiation resulted in total sterilization within 5 min, with no growth evident in the control spore strips. Unexposed spore strips run in parallel showed growth after 24 h.

DISCUSSION

Data obtained in our experiments indicate that the utilization of microwave ovens for bacterial decontamination in clinical microbiology laboratories is entirely feasible. The killing times observed are consistent with those reported in Culkin and Fung (2), Goldblith and Wang (3), and others.

Although total sterilization was not attained when high concentrations of *B. subtilis* spores were exposed to microwave irradiation for 11 min, autoclaving the same spore suspension for 20 min resulted in a viable count of five organisms per milliliter compared with a viable count of six per milliliter after microwave irradiation.

For frequently isolated clinical pathogens, the data indicated that the 5-min exposure time necessary to kill *B. stearothermophilus* spores would be adequate to insure sterilization. The above data are further supported by the fact that a 5-min exposure was adequate to insure complete sterilization of contaminated petri

FIG. 1. Effect of microwave irradiation on four clinical isolates.

TABLE 1. Effect of timed microwave irradiation on 10 clinical isolates

Time(s)	Log ₁₀ viable organisms/ml									
	1 ^a	2	3	4	5	6	7	8	9	10
0	7.2	9.1	9.0	9.2	8.8	9.0	8.9	8.4	8.7	8.6
15	7.0	6.6	7.7	8.1	8.8	8.8	6.8	8.3	7.4	6.6
30	5.3	7.2	7.7	6.6	0	5.5	6.2	4.5	3.8	6.6
45	0	0	0	0	0	5.6	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0

^a Organism code is as follows: (1) *B. subtilis*; (2) *E. cloacae*; (3) *K. pneumoniae*; (4) *S. marcescens*; (5) *E. coli*; (6) *P. mirabilis*; (7) *P. aeruginosa*; (8) enterococcus; (9) *S. aureus*; and (10) *S. epidermidis*.

FIG. 2. Effect of microwave irradiation on *B. subtilis* spores.

plates. The utilization of spore strips, rather than ampoules, as sterility controls is recommended due to the probability of an explosion occurring as the broth in the ampoules expands with increasing heat. If a laboratory is already using ampoules such as Attest, however, the spore strip can be removed and used as a sterility control as previously described.

In most analogous sterilization systems, heat is the critical feature. There is, therefore, a need to define a time/volume relationship. However, in this system, the contaminated tubed media that were sterilized were in 5-ml volumes. These volumes are consistent with those used in routine laboratory testing. Therefore, we did not attempt any other time/volume studies. The lack of pressurization within the microwave oven would preclude its use in the sterilization of newly prepared media, and the sterilization of dry, wrapped materials is not recommended due

to the hazard of excessive heating of cloth and paper, which has resulted in ignition during the exposure process in this laboratory. Since microwaves do not heat glass without the presence of moisture, sterilization of glass pipettes would not be accomplished.

Utilization of microwave ovens for the decontamination of fungal, viral, and acid-fast bacterial cultures would warrant further investigation, since it would be expected that the time necessary to insure adequate sterilization would be longer.

Mention should be made of the need for good ventilation in the room where decontamination is to be carried out, due to the odors created by the microwave action on bacteria.

In conclusion, the data obtained in this laboratory suggest that microwave oven irradiation is a practical, time- and energy-saving method for decontaminating media and test tubes used in clinical microbiology, but is not advisable for preparing new media and sterilizing dry laboratory equipment. For an average load (ca. 100 plates) of contaminated material, a 5-min exposure to microwave irradiation is sufficient to insure complete sterilization of organisms commonly encountered in clinical specimens.

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