

1 **Survival of nosocomial bacteria and spores on surfaces and inactivation by**  
2 **hydrogen peroxide vapour (HPV)**

3

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8 Work was performed in the laboratories of both institutions.

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18

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22

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24 nosocomial bacteria dried in air and killing by hydrogen peroxide vapour (HPV). 44<sup>th</sup>  
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28 **ABSTRACT**

29

30 With inocula of 6-7 log<sub>10</sub> cfu, most vegetative bacteria and spores tested survived on  
31 surfaces for more than five weeks but all were inactivated within 90 minutes exposure to  
32 hydrogen peroxide vapour in a 100m<sup>3</sup> test room, even in the presence of 0.3% bovine  
33 serum albumin to simulate biological soiling.

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35 Certain nosocomial pathogens such as methicillin-resistant *Staphylococcus aureus*  
36 (MRSA) (4), vancomycin-resistant enterococci (VRE) (3), *Clostridium difficile* (1) and  
37 *Acinetobacter* sp. (5) can contaminate hospital surfaces, survive for extended periods  
38 (13), may not be eradicated by conventional cleaning (1,4) and surface contamination can  
39 contribute to transmission (2,3,8,15). Hydrogen peroxide vapour (HPV) is a sporicidal  
40 and mycobactericidal (7,12) vapour-phase method for decontamination of surfaces and  
41 medical equipment (1,4). HPV has been shown to inactivate several nosocomial pathogens  
42 in situ (1,4) but no in vitro efficacy data are available for common nosocomial pathogens.  
43 We investigated the surface survival of common nosocomial pathogens and the in vitro  
44 effectiveness of HPV.

45  
46 Five strains of MRSA and three strains of VRE, *Acinetobacter* sp., *Klebsiella*  
47 *pneumoniae* and *C. difficile* were tested. *C. difficile* spore suspensions were prepared by  
48 harvesting colonies from seven anaerobe blood agar plates (ABA, Oxoid, Basingstoke,  
49 Hampshire, UK) grown anaerobically then held aerobically at room temperature for seven  
50 days into 5 mL sterile distilled water (SDW). Staining with Malachite Green indicated the  
51 presence of >90% spores. 5mL of absolute ethanol was added and the spore suspension  
52 was stored at room temperature. To prepare test discs, overnight broth cultures for  
53 vegetative bacteria or *C. difficile* spore suspensions were washed in SDW and 10 $\mu$ L  
54 volumes were air-dried overnight onto stainless steel discs (Apex Laboratories Inc.,  
55 Sanford, NC, USA) to achieve recoverable inocula of 6-7 log<sub>10</sub> cfu per disc. To  
56 investigate the impact of biological soiling, suspensions were air-dried for four hours in  
57 0.3% bovine serum albumin. To investigate the impact of reduced inoculum, suspensions  
58 were air-dried in SDW for four hours to achieve recoverable inocula of 5 log<sub>10</sub> cfu per

59 disc. Inoculated discs were sonicated at 60Hz for 20 minutes (Decon FS200b Ultrasonic  
60 Bath, Decon Laboratories, Hove, East Sussex, UK) in 1mL SDW and enumerated by  
61 serial 10-fold dilutions using 10µL volumes; the remaining 990µL of the original volume  
62 was pour-plated to detect a low concentration.

63  
64 To test desiccation resistance, inoculated discs were stored at ambient room temperature  
65 and humidity in a laboratory and viable counts were performed on three discs per strain  
66 weekly over six weeks. Viable counts were additionally performed for *E. faecium* NCTC  
67 12204 and the *C. difficile* clinical isolate after 12 weeks drying.

68  
69 To test HPV resistance, inoculated discs were placed inside a purpose built 100m<sup>3</sup> room  
70 sized to simulate a large hospital room or small bay. HPV decontamination using a Clarus  
71 R® suite (BIOQUELL (UK) Ltd., Andover, Hampshire, UK) was conducted as described  
72 previously (7). Discs were removed via an air-lock for viable counting after 0, 10, 20, 30,  
73 40, 50, 60, 75 and 90 minutes. One disc for each organism was removed at each time  
74 point and cycles were repeated three times for each strain.

75  
76 Relative resistance to drying at ambient temperature (21-27°C) and humidity (40-63%)  
77 was *C. difficile* > VRE > MRSA = *Acinetobacter* sp. > *K. pneumoniae* (figure 1). *E.*  
78 *faecium* NCTC 12204 and the *C. difficile* clinical isolate had a <3-log reduction in  
79 concentration after 12 weeks drying. There were species and strain differences in  
80 survival, but there was no consistent difference between reference and clinical strains of  
81 the same species. Other studies have reported extended survival times for nosocomial

82 bacteria (13,16). Variation in reported survival times is partly due to species and strain  
83 variation but also to differences in experimental conditions including inoculum size,  
84 humidity, the suspending medium and the substrate (11,18)

85  
86 The starting temperature and relative humidity ranged from 18-24°C and 30-50%,  
87 respectively. HPV concentration and relative humidity peaked at levels consistent with  
88 the onset of 'micro-condensation' on surfaces, which is critical for rapid inactivation  
89 (7,17). Relative resistance to HPV was *Acinetobacter* > MRSA = *K. pneumoniae* > *C.*  
90 *difficile* > VRE; all organisms were inactivated by 90 minutes exposure to HPV (figure  
91 2). Differences in the starting temperature, relative humidity and inoculum resulted in  
92 large standard deviations between cycles (17,19). VRE, which lack catalase, were  
93 inactivated most rapidly, with no organisms recoverable after 10 minutes exposure to  
94 HPV representing a >6-log reduction (data not shown). *C. difficile* spores, which are  
95 metabolically inert, were more susceptible to HPV than the catalase-positive bacteria.  
96 Hydrogen peroxide is an oxidising agent and catalase-peroxidase systems are known to  
97 play a key role in bacterial defence against oxidative stress (6,9,10). Therefore, the  
98 presence of catalase would appear to account for the relative resistance of these  
99 organisms to HPV.

100

101 Inocula above 7 log<sub>10</sub> cfu per disc were difficult to inactivate, especially for the catalase-  
102 positive bacteria (data not shown). In contrast, low inocula of MRSA NCTC 19939  
103 (5.1±0.2 log<sub>10</sub> cfu per disc), *A. baumannii* NCTC 12156 (5.1±0.5 log<sub>10</sub> cfu per disc) and  
104 *K. pneumoniae* NCTC 9633 (5.0±0.2 log<sub>10</sub> cfu per disc) were inactivated within 10

105 minutes exposure to HPV. 6-7 log<sub>10</sub> cfu per disc (0.8cm<sup>2</sup>) is considerably higher than the  
106 concentration of bacteria likely to be encountered in the hospital environment (1,14).

107

108 All strains tested were inactivated within 90 minutes in the presence of 0.3% BSA: *K.*  
109 *pneumoniae* NCTC 9633 (7.4±0.7 log<sub>10</sub> cfu per disc) by 90 minutes, *S. aureus* NCTC  
110 11939 (6.9±0.1 log<sub>10</sub> cfu per disc) by 50 minutes, *A. baumannii* NCTC 12156 (6.7±0.4  
111 log<sub>10</sub> cfu per disc) by 40 minutes and *C. difficile* 106 (6.4±0.3 log<sub>10</sub> cfu per disc) by 30  
112 minutes. The resistance of organisms dried in BSA and the low inoculum experiments  
113 cannot be directly compared with those dried in SDW because of differences in the  
114 drying time (overnight vs. four hours). As with any other disinfection method, HPV is  
115 applied after cleaning so levels of soiling encountered in the field should be low; indeed,  
116 HPV has been shown to be effective in rooms that have not been cleaned (4). Further  
117 research is required to examine the impact of increased soiling levels on HPV resistance.

118

119 In summary, we found that dried inocula of a range of nosocomial pathogens survived on  
120 surfaces for several weeks but were rapidly inactivated by HPV in a 100m<sup>3</sup> room. HPV  
121 has a potential role in decontaminating surfaces and equipment contaminated with such  
122 organisms.

123

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133 **FIGURE LEGENDS**

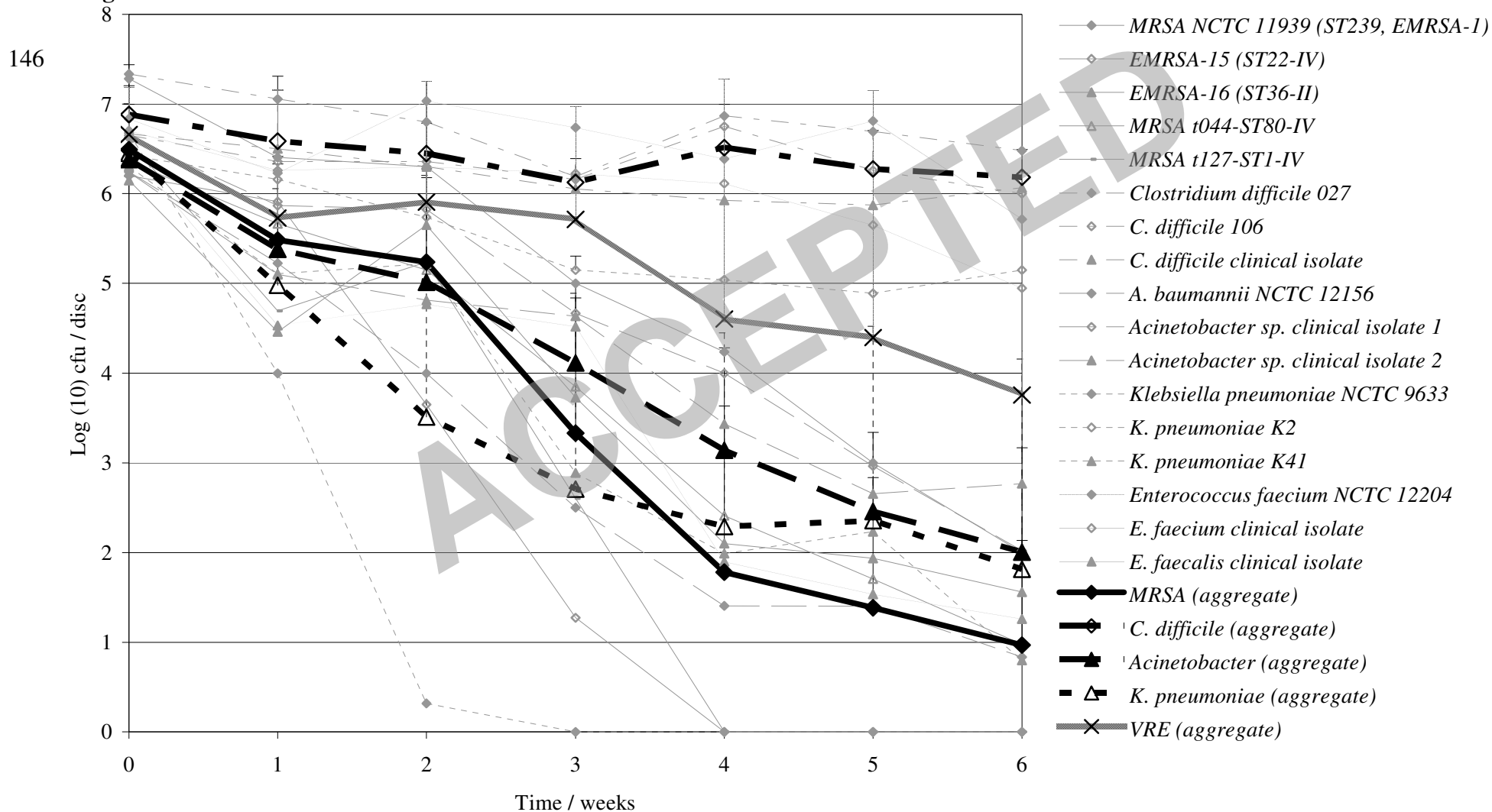
134

135 **Figure 1.** Resistance of nosocomial bacteria and spores to drying over a six week  
136 sampling period when exposed to room air at ambient relative temperature and humidity  
137 in a laboratory. Black lines: aggregate of all data for each species; error bars represent  
138 plus one standard deviation. Grey lines: each data point represents a mean of three  
139 replicate runs for each strain.

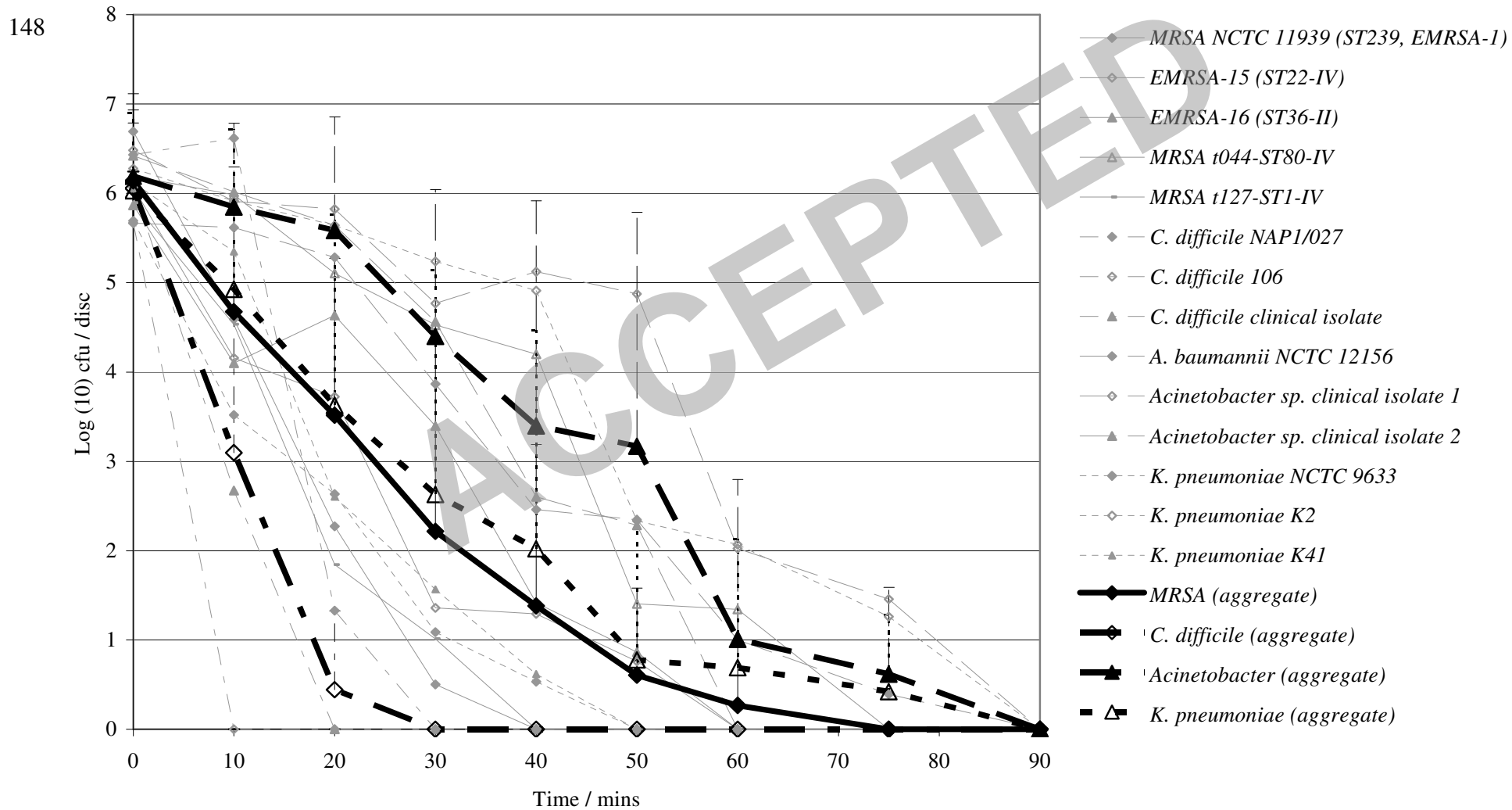
140

141 **Figure 2.** Resistance of nosocomial bacteria and spores to hydrogen peroxide vapour in a  
142 100m<sup>3</sup> test room over a 90 minute exposure period. Black lines: aggregate of all data for  
143 each species; error bars represent plus one standard deviation. Grey lines: each data point  
144 represents a mean of three replicate runs for each strain.

145 **Figure 1**



147 **Figure 2**



149 **REFERENCE**

150

- 151 1. **Boyce, J. M., N. L. Havill, J. A. Otter, L. C. McDonald, N. M. Adams, T.**  
152 **Cooper, A. Thompson, L. Wiggs, G. Killgore, A. Tauman, and J. Noble-Wang.**  
153 2008. Impact of hydrogen peroxide vapor room decontamination on *Clostridium*  
154 *difficile* environmental contamination and transmission in a healthcare setting.  
155 *Infect. Control Hosp Epidemiol.* **29**:723-729.
- 156 2. **Dancer, S. J.** 2008. Importance of the environment in meticillin-resistant  
157 *Staphylococcus aureus* acquisition: the case for hospital cleaning. *Lancet Infect.*  
158 *Dis.* **8**:101-113.
- 159 3. **Drees, M., D. Snyderman, C. Schmid, L. Barefoot, K. Hansjosten, P. Vue, M.**  
160 **Cronin, S. Nasraway, and Y. Golan.** 2008. Prior Environmental Contamination  
161 Increases the Risk of Acquisition of Vancomycin-Resistant Enterococci. *Clin.*  
162 *Infect. Dis.* **46**:678-685.
- 163 4. **French, G. L., J. A. Otter, K. P. Shannon, N. M. Adams, D. Watling, and M. J.**  
164 **Parks.** 2004. Tackling contamination of the hospital environment by methicillin-  
165 resistant *Staphylococcus aureus* (MRSA): a comparison between conventional  
166 terminal cleaning and hydrogen peroxide vapour decontamination. *J. Hosp. Infect*  
167 **57**:31-37.
- 168 5. **Getchell-White, S. I., L. G. Donowitz, and D. H. Groschel.** 1989. The inanimate  
169 environment of an intensive care unit as a potential source of nosocomial bacteria:

- 170 evidence for long survival of *Acinetobacter calcoaceticus*. Infect. Control Hosp.  
171 Epidemiol. **10**:402-407.
- 172 6. **Goldberg, I. and A. Hochman**. 1989. Three different types of catalases in  
173 *Klebsiella pneumoniae*. Arch. Biochem. Biophys. **268**:124-128.
- 174 7. **Hall, L., J. A. Otter, J. Chewins, and N. L. Wengenack**. 2007. Use of Hydrogen  
175 Peroxide Vapor for Deactivation of *Mycobacterium tuberculosis* in a Biological  
176 Safety Cabinet and a Room. J. Clin. Microbiol. **45**:810-815.
- 177 8. **Huang, S. S., R. Datta, and R. Platt**. 2006. Risk of acquiring antibiotic-resistant  
178 bacteria from prior room occupants. Arch. Intern. Med. **166**:1945-1951.
- 179 9. **Imlay, J. A., S. M. Chin, and S. Linn**. 1988. Toxic DNA damage by hydrogen  
180 peroxide through the Fenton reaction in vivo and in vitro. Science **240**:640-642.
- 181 10. **Imlay, J. A. and S. Linn**. 1988. DNA damage and oxygen radical toxicity. Science  
182 **240**:1302-1309.
- 183 11. **Jawad, A., J. Heritage, A. M. Snelling, D. M. Gascoyne-Binzi, and P. M.**  
184 **Hawkey**. 1996. Influence of relative humidity and suspending menstrua on survival  
185 of *Acinetobacter* spp. on dry surfaces. J. Clin. Microbiol. **34**:2881-2887.
- 186 12. **Johnston, M. D., S. Lawson, and J. A. Otter**. 2005. Evaluation of hydrogen  
187 peroxide vapour as a method for the decontamination of surfaces contaminated with  
188 *Clostridium botulinum* spores. J. Microbiol. Methods **60**:403-411.

- 189 13. **Kramer, A., I. Schwebke, and G. Kampf.** 2006. How long do nosocomial  
190 pathogens persist on inanimate surfaces? A systematic review. *BMC. Infect. Dis.*  
191 **6:130.**
- 192 14. **Oie, S. and A. Kamiya.** 1996. Survival of methicillin-resistant *Staphylococcus*  
193 *aureus* (MRSA) on naturally contaminated dry mops. *J. Hosp. Infect.* **34:145-149.**
- 194 15. **Samore, M. H., L. Venkataraman, P. C. DeGirolami, R. D. Arbeit, and A. W.**  
195 **Karchmer.** 1996. Clinical and molecular epidemiology of sporadic and clustered  
196 cases of nosocomial *Clostridium difficile* diarrhea. *Am. J. Med.* **100:32-40.**
- 197 16. **Smith, S. M., R. H. Eng, and F. T. Padberg, Jr.** 1996. Survival of nosocomial  
198 pathogenic bacteria at ambient temperature. *J. Med.* **27:293-302.**
- 199 17. **Unger-Bimczok, B., V. Kottke, C. Hertel, and J. Rauschnabel.** 2008. The  
200 Influence of Humidity, Hydrogen Peroxide Concentration, and Condensation on the  
201 Inactivation of *Geobacillus stearothermophilus* Spores with Hydrogen Peroxide  
202 Vapor. *J. Pharm. Innov.* **3:123-133.**
- 203 18. **Wagenvoort, J. H., W. Sluijsmans, and R. J. Penders.** 2000. Better  
204 environmental survival of outbreak vs. sporadic MRSA isolates. *J. Hosp. Infect.*  
205 **45:231-234.**
- 206 19. **Watling, D., C. Ryle, M. Parks, and M. Christopher.** 2002. Theoretical analysis  
207 of the condensation of hydrogen peroxide gas and water vapour as used in surface  
208 decontamination. *PDA. J. Pharm. Sci. Technol.* **56:291-299.**
- 209