Evaluation of Disinfectants on Military NATO and DECON Litters

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OBJECTIVE: This study evaluated the effectiveness of five disinfectants: A33, 10% bleach, 1% bleach, SPOROX, and 3% H_2O_2, on military NATO and DECON litters.

DESIGN: Suspensions of *Acinetobacter baumannii, Staphylococcus aureus*, and spore-enhanced *Bacillus subtilis*, with five percent albumin, were inoculated onto litters and dried overnight. The litters were saturated with disinfectant solutions and sampled after 10 minutes. The Log_{10} reduction in the number of bacteria recovered was calculated.

SETTING: 59th Medical Wing, 59th Clinical Research Division, Lackland AFB TX.

MAIN OUTCOME MEASURES: A reduction of ≥3 Log_{10} in the number of bacteria recovered from the test squares compared to the control squares was considered effective disinfection.

RESULTS: On the NATO litter 10% bleach and SPOROX were effective against vegetative cells. On the DECON litter A33, 10% bleach, and SPOROX were effective against vegetative cells. After the 10 minute exposure none of the disinfectants evaluated were effective against spore-enhanced *B. subtilis*.

CONCLUSION: When contaminated with vegetative cells military NATO and DECON litters can be effectively disinfected with a 10 minute exposure to some disinfectants. Further research is needed to find an effective disinfectant for spore contamination.

ABBREVIATIONS: AFB = Air Force Base; ATCC = American Type Culture Collection; BAMC = Brooke Army Medical Center; BSA = bovine serum albumin; CFU = colony forming units; D/E = Dey/Engley; DECON = decontamination; H_2O_2 = hydrogen peroxide; NAM = nutrient agar with manganese sulfate (50 µg/mL); NATO = North Atlantic Treaty Organization; QAC = quaternary ammonium compound; SBA = Trypticase soy agar with five percent sheep's blood.

INDEX TERMS: *A. baumannii*; disinfection; infection control; surface disinfection.

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Reports in the literature cite an increase in *Acinetobacter baumannii* infections among patients at military medical facilities treating US service members who have been injured in Iraq and Afghanistan. Two medical centers, Landstuhl Regional Medical Center and Walter Reed Army Medical Center, identified 102 patients with blood cultures positive for *A. baumannii* during the period January 1, 2002-August 31, 2004. The two medical centers had a combined total of three cases of *A. baumannii* positive blood culture during the previous two years.[1] Brooke Army Medical Center (BAMC) reported twenty-three soldiers wounded in Iraq and subsequently admitted to BAMC who had wounds that were culture positive for *A. baumannii* during the period of March 2003 to May 2004. Eighteen of the twenty-three patients had osteomyelitis which had not been identified at BAMC during the 14 months preceding March 2003.[2]

Studies involving environmental and colonization cultures indicate that the source of the *A. baumannii* infections may be nosocomial in origin. A study conducted at BAMC that included 293 soldiers with no history of deployment and who were not healthcare workers found no *Acinetobacter* nares colonization in any of the participants, indicating that *A. baumannii* nares colonization in a normal healthy population is very low.[3] A study assessing the bacteriology of war wounds

at the time of injury sampled 61 separate acute traumatic injury wounds from 49 casualties upon arrival at the 31st Combat Support Hospital in Baghdad. The study revealed a predominance of gram positive organisms of low virulence and pathogenicity. No multi-drug resistant gram negative organisms were recovered.[4] A study conducted in Iraq and Kuwait by the Walter Reed Army Institute of Research found skin colonization in only 1 of 160 patients who were screened and in only 1 of 49 soil samples, but *A. baumannii-calcoaceticus* complex isolates were recovered from treatment areas in all seven of seven field hospitals sampled.[5]

Bacteria, including *Acinetobacter* spp., have been shown to survive for long periods of time, greater than four months, on dry inanimate surfaces.[6-10] Some of these surfaces, such as beds, tables, hygroscopic bandages, a stretcher, and infusion pumps, have been implicated as reservoirs for transmission of disease in hospital settings.[11-16] Surface disinfection has been cited as a contributing factor in controlling and eliminating the transmission of disease from inanimate objects.[11-13] Many factors can influence surface survival of bacteria and the effectiveness of surface disinfection: type of disinfectant used, type of organisms present, concentration of organisms present, porosity of the material, type of material, and presence of bioload.[6,8-10,17-25] Additionally, it has been reported that the bacterial binding capacity of a fabric varies with organism and type of fabric. For example, *Staphylococcus aureus* and *Pseudomonas aeruginosa* bind more efficiently to polyester, acrylic, and wool than to cotton.[9,17,18] Standard North Atlantic Treaty Organization (NATO) litters are made of tightly woven cotton duck canvas and decontamination (DECON) litters are made of loosely woven plastic and nylon. There are few reports in the literature of bacterial disinfection studies performed on porous surfaces and none that address the presence of bioload.[19] This study was undertaken to evaluate the effectiveness of various disinfectants on military NATO and DECON litters in the presence of simulated bioload.

METHODS

Staphylococcus aureus American Type Culture Collection (ATCC) 29213 and *Bacillus subtilis* ATCC 6633 were obtained in lyophilized form from MicroBioLogics, St. Cloud MN. *A. baumannii* was isolated in the Wilford Hall Medical Center clinical microbiology laboratory from a patient who had served in Iraq. The isolate was stored at -70°C in trypticase soy broth with 20% glycerol until used. These organisms were chosen for the study due to their relevance to current events and as characteristic organisms to represent gram positive cocci, gram negative rods, and spore forming gram positive rods. All cultures for this study were incubated at 37±2°C in ambient air and each isolate was subcultured twice before testing. *S. aureus* and *A. baumannii* were grown on trypticase soy agar with five percent sheep's blood (SBA). To enhance spore production *B. subtilis* was grown on nutrient agar with manganese sulfate (50 µg/mL) (NAM) and incubated for five days before use to achieve >90% spores. Spore production was confirmed by spore stain. Bacterial suspensions were prepared by harvesting cells from 18 hour to 24 hour growth of *A. baumannii* and *S. aureus* on SBA or five day growth of *B. subtilis* on NAM and transferring the cells to 0.9% sterile saline. To simulate bioload bovine serum albumin (BSA) was added to the bacterial suspensions to achieve a five percent BSA concentration. The suspensions were adjusted spectrophotometrically to an absorbance at 660 nm of 0.15 for *S. aureus* and *A. baumannii* and 0.5 for *B. subtilis.* Absorbance was determined on a Beckman DU Series 600 spectrophotometer. The resulting suspensions contained approximately 1 x 10^8 colony forming units per milliliter (CFU/mL). The concentrations of the inoculation suspensions were verified by preparing 1:10 serial dilutions of the suspensions in sterile saline and plating the dilutions on SBA. The resulting colony counts were used to calculate the suspension concentrations.

To create a simulated bacterial reservoir 100 µL aliquots of the suspensions, a total inoculation of approximately 1 x 10^7 CFU, were transferred onto 1.5 inch test squares (n=3 positive control and n=3 test) on clean litters and allowed to dry overnight. The test squares were saturated with the disinfectant solutions: A33 (a quaternary ammonium compound (QAC) disinfectant, Airkem Professional Products, Division of Ecolab, Inc), prepared according to manufacturer's instructions; 10% solution of 6.0% household bleach in sterile deionized water (10% bleach); 1% solution of 6% household bleach in sterile deionized water (1% bleach); SPOROX (7.5% hydrogen peroxide (H_2O_2) plus 0.85% phosphoric acid, Sultan Chemists), supplied ready to use; or 3% H_2O_2, prepared by diluting 30% H_2O_2 (Sigma-Aldrich) with sterile deionized water. The disinfectants were prepared fresh each day of use. The control squares were saturated with sterile deionized water. After a 10 minute contact time the tip of a sterile cotton tipped swab, moistened with sterile water, was placed in one of the corners of the square. The swab was moved over the surface of the litter, spiraling inward until the entire surface area of the square had been sampled. The sampling time was approximately 15 seconds per square. The swabs were placed in one mL of saline (for A33, SPOROX, and H_2O_2) or Dey/Engley (D/E) neutralizing broth (for bleach) and vortex mixed vigorously for three

to five seconds The excess moisture was expressed from the swab by rolling it against the side of the tube and then the swab was discarded. The samples were serially diluted, 1:10, in sterile saline and plated on SBA for quantitation. The mean CFU/mL recovered was calculated for each test and control group. The Log_{10} reduction in the number of bacteria recovered from the disinfectant test squares compared to the water control squares was calculated. The organism/disinfectant combinations were set up as separate experiments and each set of test squares was compared to the control squares from the same experiment. A minimum of 1×10^4 CFU/mL had to be recovered from the control squares for the results to be accepted. A reduction of ≥3 Log_{10} (99.9%) in the number of bacteria recovered was considered effective disinfection.

RESULTS

As shown in Table 1, the mean number of CFU/mL recovered from the control squares for all test runs on the NATO litter was slightly lower than the number recovered from the DECON litter. The day to day variability in the CFU/mL recovered from the control squares was greater on the NATO litter than on the DECON litter (Table 2). The mean CFU/mL recovered from each set of test and control squares was used to calculate the Log_{10} reduction for each disinfectant/organism/litter combination (Table 3).

In this study none of the disinfectants tested were effective against the spore-enhanced *B. subtilis* on either litter, but A33, 10% bleach, and SPOROX were effective against *A. baumannii* and *S. aureus* on the DECON litter and 10% bleach and SPOROX were effective against *A. baumannii* and *S. aureus* on the NATO litter. Under these test conditions 1% bleach and 3% H_2O_2 were not effective against *A. baumannii* or *S. aureus* on either litter.

DISCUSSION

Finding and eliminating reservoirs and routes of transmission for nosocomial infections remains a high priority for healthcare workers. The increase in the number of multi-resistant organisms and the volume of international travel add to the urgency of this problem. Disinfection of porous surfaces is an area that is largely unexplored. This study evaluated the effect of five disinfectants on selected bacteria inoculated on military NATO and DECON litters. The 10% bleach and 3% H_2O_2 were included as disinfectants commonly used by healthcare workers. The 1% bleach was evaluated to determine if a lower concentration of bleach could be substituted as a safer and less costly alternative for the 10% bleach. Under the test conditions in our study the 1% bleach was not acceptable as an alternative to 10% bleach. A33 is currently in use as a disinfectant in some military deployment settings. To our knowledge there are no published reports in the literature addressing the effectiveness of A33 or other QACs on porous material. SPOROX is marketed for use as a high level disinfecting solution for heat-sensitive dental instruments. We chose to include SPOROX in the study for several reasons. The high concentration of H_2O_2 in SPOROX (7.5%) results in oxidation of biological debris, an important consideration for use in trauma settings, which may result in more effective disinfection. SPOROX is commercially available and comes ready to use. If shown to be effective SPOROX could provide an easy, effective means of disinfection in deployment settings. In our study SPOROX was more effective than the 3% H_2O_2. To achieve effective disinfection the manufacturer of A33 recommends a contact time of 10 minutes. We chose to use the same contact time for each disinfectant challenge. It is probable that increasing contact time would result in effective disinfection for some of the disinfectant/organism/litter combinations that were ineffective at the 10 minute contact time. The amount of time that could be allotted for disinfection of litters would vary greatly in real world situations, from little or no time in a mass casualty situation to hours in low demand situations. It was not within the scope of this study to evaluate multiple contact times.

The NATO litter is made of a tightly woven cotton duck material and the DECON litter is a loosely woven plastic and nylon mesh. Both types of litters are currently in use by our military forces deployed throughout the world though the NATO litters are gradually being phased out in favor of the newer DECON litter. In this study both litter types were inoculated with equal numbers of bacteria but we recovered a greater number of CFUs from test squares on the DECON litter than those on the NATO litter for each of the three bacteria used. Possible explanations for this are: 1) the bacteria bind better to the NATO litter (cotton) than to the DECON litter (plastic and nylon), making recovery harder; or 2) the material construction, tightly woven (NATO) versus loosely woven (DECON), contributed to recovery differences

Table 1. Mean colony forming units per milliliter recovered from control squares

Litter	*A. baumannii*	*S. aureus*	*B. subtilis*
NATO	4.91E+05	3.52E+05	1.63E+05
DECON	1.32E+06	2.91E+06	3.16E+06

by allowing increased access to bacteria on the more loosely woven material of the DECON litter. Previous reports in the literature[9,17,18] indicate that *S. aureus* binds more strongly to polyester than cotton, supporting the theory that construction of the material being disinfected plays an important role in determining recovery. There was greater between run variability in the CFU/mL recovered from the control squares on the NATO litter than on the DECON litter. This could also be explained by the differences in the construction of the litters. It is interesting to note that on the NATO litter the tests that were determined to be ineffective resulted in Log_{10} reductions ranging from 0.32 to 2.92 but on the DECON litter the reductions for the ineffective tests were all ≤0.73 Log_{10}, indicating an "all or nothing" type of result. It is possible that the more loosely woven fabric allows a more even distribution of the disinfectant or a more reproducible access for recovery. The same question (material composition or construction?) can be asked for the disinfection results. If the data is classified as either "effective" or "non effective" disinfection, the only difference between the two litters is the A33 with the vegetative cells. A33 was effective against vegetative cells, i.e., *A. baumannii* and *S. aureus,* on the DECON litter but not on the NATO litter. The activity of quaternary ammonium compounds may be reduced by materials such as cotton[26] indicating that material composition may have played a role in the effectiveness of disinfection by A33.

There are many variables that affect disinfection. Scrubbing before or during disinfection and rinsing after disinfection were not evaluated as part of this study, but these and other mechanical procedures that may be part of a routine disinfection procedure could influence the total reduction in the number of CFU/mL recovered from a porous surface. It is also important to mention that the actual state of litters in use, especially in a trauma setting, will vary dramatically. The overall cleanliness of the litter, the presence or absence of blood, the amount of time that could be dedicated to disinfection procedures before the litter is needed again, and other variables would all influence the effectiveness of any disinfection procedure. It would not be possible to reconstruct every disinfection situation. We limited this study to disinfection of litters contaminated with bacterial suspensions containing a simulated bioload of five percent bovine serum albumin with application of the disinfectants for 10 minutes. With the continuing problem of hospital acquired infections, the rising incidence of community acquired infections, and the growing number of multi-resistant organisms, infection control is gaining in importance. Additional research into surface disinfection of porous materials is needed to fully answer the questions that arise concerning protection of the public from infectious disease transmission.

Table 2. Mean colony forming units per milliliter recovered from test and control squares

NATO litter

	A. baumannii		*S. aureus*		*B. subtilis*	
Disinfectant	Control	Test	Control	Test	Control	Test
A33	1.2E+05	5.3E+03	7.5E+04	3.6E+04	5.2E+04	5.5E+04
10% bleach	5.23E+04	0	4.7E+04	0	2.73E+05	4.15E+04
1% bleach	5.23E+04	2.5E+02	4.7E+04	5.67E+01	2.73E+05	2.76E+05
SPOROX	1.3E+06	0	9.33E+05	3.00E+02	2.33E+05	8.07E+04
3% H_2O_2	1.3E+06	5.23E+03	9.33E+05	2.17E+04	2.33E+05	3.2E+05

DECON litter

	A. baumannii		*S. aureus*		*B. subtilis*	
Disinfectant	Control	Test	Control	Test	Control	Test
A33	1.1E+06	0	1.6E+06	1.6E+02	1.4E+06	1.4E+06
10% bleach	1.6E+06	0	5.9E+06	5.0E+00	7.8E+06	5.6E+06
1% bleach	1.6E+06	7.76E+05	5.9E+06	1.1E+06	7.8E+06	4.7E+06
SPOROX	1.27E+06	0	1.23E+06	3.33E+00	2.67E+05	4.73E+05
3% H_2O_2	1.27E+06	7.63E+05	1.23E+06	4.5E+05	2.67E+05	5.47E+05

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REFERENCES

1. Centers for Disease Control and Prevention. Acinetobacter baumannii infections among patients at military medical facilities treating injured US service members, 2002-2004. MMWR Morb Mortal Wkly Rep 2004;53:1063-6.
2. Davis KA, Moran KA, McAllister CK, Gray PJ. Multi-drug resistant Acinetobacter extremity infections in soldiers. Emerg Infec Dis 2005;11(8):1218-24.
3. Griffith ME, Ellis MW, Murray CK. Acinetobacter nares colonization of healthy US soldiers. Inf Cont and Hosp Epidem 2006;27(7):787-8.
4. Murray CK, Roop SA, Hospenthal DR, and others. Bacteriology of war wounds at the time of injury. Military Med 2006;171(9):826-9.
5. Scott P, Deye G, Srinivasan A, and others. An outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus complex infection in the US military health care system associated with military operations in Iraq. Clin Inf Dis 2007;44(12):1577-84.
6. Wendt C, Dietze B, Dietz E, Ruden H. Survival of Acinetobacter baumannii on dry surfaces. J Clin Micro 1997;35(6):1394-7.
7. Jawad A, Seifert H, Snelling AM, and others. Survival of Acinetobacter baumannii on dry surfaces: comparison of outbreak and sporadic isolates. J Clin Micro 1998;36(7):1938-41.
8. Jawad A, Heritage J, Snelling AM, and others. Influence of relative humidity and suspending menstrua on survival of Acinetobacter spp. on dry surfaces. J Clin Micro 1996;34(12):2881-7.
9. Neely AN, Maley MP. Survival of Enterococci and Staphylococci on hospital fabrics and plastic. J Clin Micro 2000;38(2):724-6.
10. Neely AN. A survey of gram-negative bacteria survival on hospital fabrics and plastics. J Burn Care Rehabil 2000;21:523-7.
11. Aygun G, Demirkiran O, Utku T, and others. Environmental contamination during a Carbapenem-resistant Acinetobacter baumannii outbreak in an intensive care unit. J Hosp Inf 2002;52:259-62.
12. Wang SH, Sheng WH, Chang YY, and others. Healthcare-associated outbreak due to pan-drug resistant Acinetobacter baumannii in a surgical intensive care unit. J Hosp Infect 2003;53(2):97-102.
13. Denton M, Wilcox MH, Parnell P, and others. Role of environmental cleaning in controlling an outbreak of Acinetobacter baumannii on a neurosurgical intensive care unit. J Hosp Infect 2004;56(2):106-10.
14. Catalano M, Quelle LS, Jeric PE, and others. Survival of Acinetobacter baumannii on bed rails during an outbreak and during sporadic cases. J Hosp Inf 1999;42: 27-35.
15. Melamed R, Greenberg D, Porat N, and others. Successful control of an Acinetobacter baumannii outbreak in a neonatal intensive care unit. J Hosp Infect 2003;53(1):31-8.
16. Al-Barrak A, McLeod J, Embil J, and others. Putting out the fire: extinguishing an outbreak of methicillin-resistant Staphylococcus aureus (MRSA) on a burn unit. Am J Infect Control 1998;26(2):189.
17. Takashima M, Shirai F, Safeshima M, and others. Distinctive bacteria-binding property of cloth materials. Am J Infect Control 2004;32(1):27-30.
18. Hseih YL, Merry J. The adherence of Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli on cotton, polyester and their blends. J Appl Bacteriol 1986;60(6):535-44.
19. Neely AN, Maley MP. The 1999 LINDBERGH AWARD: 3% hydrogen peroxide for the gram-positive disinfection of fabrics. The J Burn Care Rehabil 1999;20(6):471-7.
20. Sattar S.A. Survival of microorganisms on animate and inanimate surfaces and their disinfection. In: International Symposium on Disinfection, Sterilization, and Antisepsis: Principles and Practices in Healthcare Facilities, WA Rutala editor. Washington DC: Associated Practitioners in Infection Control (APIC);2001: 195-205.
21. Exner M, Vacata V, Hornei B, and others. Household cleaning and surface disinfection: new insights and strategies. J Hosp Infect 2004;Apr;56 Suppl 2:s70-5.
22. Spicher G, Peters J. Effect on the microbicidal efficacy of formaldehyde, glutardialdehyde, peracetic acid, chloramine T (N-chloro-4-toluenesulfonamide), m-cresol, ethanol and benzyldimethyldodecacylammonium bromide by blood (model experiments for chemical disinfection of instruments). Zentralbl Hyg Umweltmed 1998;200(5-6):465-77.
23. Dharan S, Mourouga P, Copin P, and others. Routine disinfection of patients' environmental surfaces. Myth or reality? J Hosp Inf 1999;42:113-17.
24. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Micro Rev 1999;12(1):147-79.
25. Russell AD. Bacterial resistance to disinfectants: present knowledge and future problems. J Hosp Inf 1998;43(Supplement):S57-S68.
26. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. Manual of Clinical Microbiology, 9th ed. Washington DC: ASM Press;2007:76-7.

Table 3. Log_{10} reduction after 10 minute exposure to disinfectant

NATO litter

Disinfectant	*A. baumannii*	*S. aureus*	*B. subtilis*
A33	1.35	0.32	0.00
10% Bleach	4.72	4.67	0.81
1% Bleach	2.32	2.92	0.00
SPOROX	6.11	3.49	0.46
3% H_2O_2	2.40	1.63	0.00

DECON litter

Disinfectant	*A. baumannii*	*S. aureus*	*B. subtilis*
A33	6.04	4.00	0.00
10% Bleach	6.20	6.07	0.14
1% Bleach	0.31	0.73	0.22
SPOROX	6.10	5.57	0.00
3% H_2O_2	0.22	0.44	0.00