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Disinfection of surfaces contaminated with Carbapenemase producing *Acinetobacter baumannii* using ozone under complex room conditions

Abstract

Introduction *Acinetobacter baumannii* is an emerging multiresistant Gram negative rod, which has caused multiple hospital outbreaks. *A. baumannii* can display a high ability to survive on inanimate surfaces. Therefore, cleaning and disinfection is an important part in the prevention of *A. baumannii* transmission. No-touch room decontamination is performed with increasing frequency to reach more standardization in hospital cleaning. In this study, we investigated the efficacy of an automated room decontamination using ozone (Sterisafe^{TM} pro) against *A. baumannii* under complex room conditions.

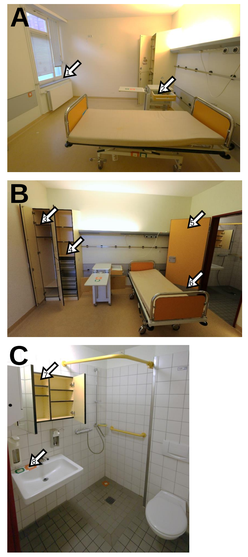
Material & Methods A Carbapenemase-producing *A. baumannii* outbreak strain was analyzed with respect to its ability to survive on dry surfaces. The Sterisafe^{TM} pro instrument was used in a patient room with an attached bathroom. The *A. baumannii* strain was dried on three different carriers (ceramic tiles, stainless steel, solid core furniture board) and placed at eight different positions in the rooms. A standard disinfection cycle (80 ppm ozone; 90 % RH; 60 min) was conducted in three independent experiments.

Results The *A. baumannii* strain displayed a long term survival on surfaces under dry conditions sufficient for further disinfection experiments. Interestingly, the mean reduction rates of *A. baumannii* dried on three surfaces displayed significant differences. Reduction rates greater than 5 log were reached on all stainless steel and ceramic carriers even under the complex room conditions using the standard disinfection cycle of the Sterisafe^{TM} pro instrument. In contrast, on furniture board individual carriers displayed reduction rates of even less than 4 log. The mean reduction rate was still 5 log for *A. baumannii* on furniture board.

Conclusions *A. baumannii* dried on different surfaces display a differential susceptibility against automated ozone disinfection. However, the Sterisafe^{TM} pro instrument displayed a sufficient reduction of *A. baumannii* for all tested surfaces even under complex room conditions. The individual behavior of *A. baumannii* on different materials indicate the necessity for the validation of automated room decontamination systems under varying conditions.

Materials und Methods

Figure 1 Three different inanimate surfaces were used for survival and disinfection experiments. A fresh overnight culture of an outbreak strain of *Acinetobacter baumannii* was resuspended in a 0.85 % sodium cloride solution containing 0,3 %/L fetal bovine albumin to reach an McFarland of 3.0 (about 5 x 10^8 cfu/mL). Of this suspension, 20 µl were plated on the different surfaces using a Drigalski spatula. For the determination of the survival rate quantitative culture was performed after recovery of the surviving bacteria using moistened Copan Liquid Amies Elution Swab (eSWAB^{TM}). Samples were taken immediately after complete desiccation as well as 4 and 24 hours storage under normal room conditions. For the whole-room decontamination experiments the contaminated surfaces were placed at different positions of a patient room (**Figure 2**). For each experiment and surface one target was placed in a room beside the decontaminated patient room as control. The survival of *Acinetobacter baumannii* after ozone disinfection as well as on the control surfaces was determined by quantitative culture. The reduction rate was calculated as the relative $\log_{10}$-difference between the control and treated surfaces.



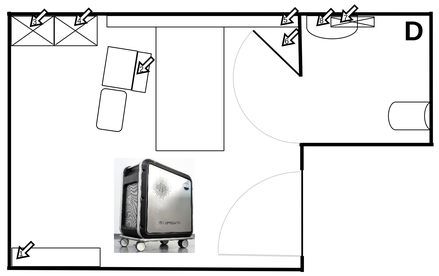


Figure 2 Positioning of contaminated inanimate surfaces in a patient room with an associated bathroom (**A** to **C**: pictures of the rooms; **D**: ground plot) used for testing under complex room conditions. The contaminated surfaces with a high contamination (ca. 10^5 cfu/25 cm^2 *Acinetobacter baumannii*) were placed open in the room (arrows 1, 5, 6 and 8) or inside of furnishings (arrows 2, 3, 4, and 7). The Sterisafe^{TM}-PRO instrument was placed in front of the patient bed and the door to the bathroom was completely opened. For the whole-room decontamination process the door of the patient room was sealed with gastight adhesive tape.

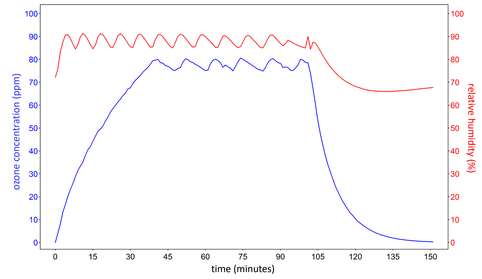


Figure 3 For the automated whole-room disinfection process with the Sterisafe^{TM}-PRO instrument (Infuser Deutschland GmbH, Mannheim, Germany) a standard disinfection cycle with the parameters of 80 ppm ozone and 90 % relative humidity with a hold time of 60 minutes was performed. The process was started by remote control using a mobile device (tablet-computer) in front of the pre-sealed door. The integrated sensors of the instrument (ozone and humidity) monitored the process continuously and the completed cycle with fully degraded ozone in the room was confirmed on the mobile device. A representative cycle of a whole-room decontamination process is displayed. Three independent experiments with all surfaces were performed (in total 24 contaminated targets for each surface).

Results

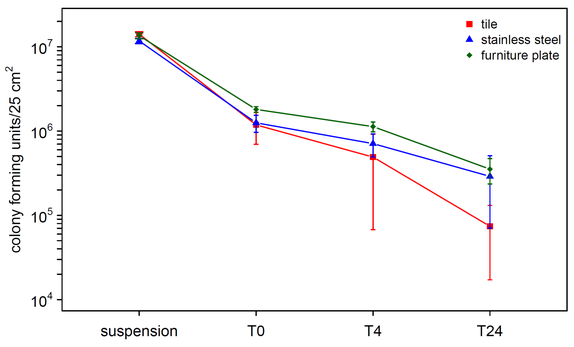


Figure 4 Survival of an *Acinetobacter baumannii* outbreak strain on different inanimate surfaces. The inserted number of bacteria (suspension) as well as the surviving number immediately (T0) as well as 4 (T4) and 24 (T24) hours after complete desiccation were observed. The mean results of three independent experiments are displayed. On furniture plates the strain displayed the highest stability with less than 1 $\log_{10}$ reduction during the desiccation and only a 0.72 $\log_{10}$ reduction over 24 h after complete desiccation. The lowest relative stability was observed on ceramic tiles. However, on all surfaces sufficient survival was observed for further experiments.

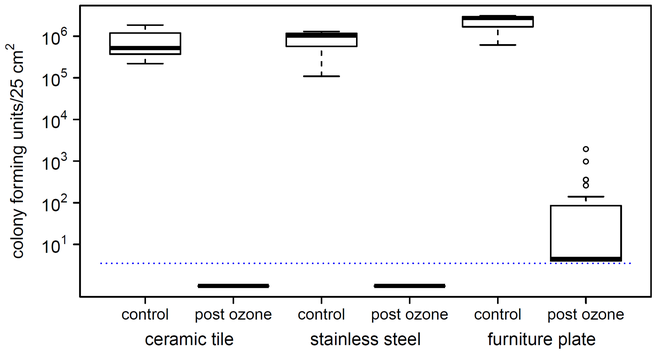


Figure 5 Activity of whole-room disinfection against an *Acinetobacter baumannii* outbreak strain on different inanimate surfaces. The number of bacteria remaining on a control surfaces (control) and after disinfection with a standard disinfection cycle (post ozone) were observed. A boxplot of all results from three independent experiments for the different surfaces is displayed. The limit of detection of the quantitative culture is marked by a blue dotted line. On ceramic tiles as well as on stainless steel all Acinetobacter were eliminated by the standard cycle. On furniture plates single bacterial cells were able to survive. However, on furniture plates the initial cell numbers were the highest in all experiments.

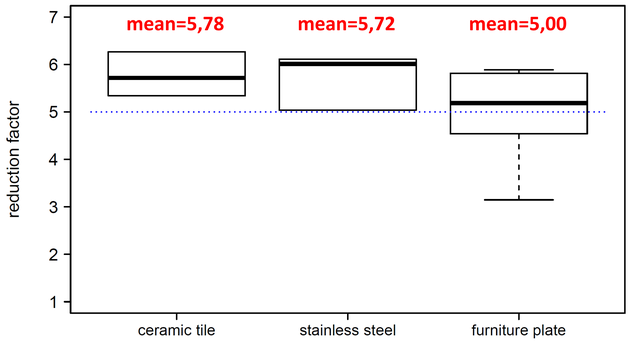


Figure 6 Reduction rates after whole-room disinfection on different inanimate surfaces. A boxplot of all results for the different surfaces is displayed. The reduction rate requested to claim a method as disinfection method against bacteria (5 $\log_{10}$ reduction) is marked by a blue dotted line. On ceramic tiles as well as on stainless steel for all tested surfaces a reduction rate of > 5 $\log_{10}$ was gained. On furniture plates individual targets displayed a reduction of < 5 $\log_{10}$. However, the mean reduction of all tested surfaces was still sufficient to claim the method as a whole-room disinfection method also against *Acinetobacter baumannii*.

Conclusions

- ***Acinetobacter baumannii* dried on different surfaces displays a differential survival on the inanimate surface and susceptibility against automated ozone disinfection.**
- **The Sterisafe^{TM} pro instrument displayed a sufficient reduction of *A. baumannii* for all tested surfaces even under complex room conditions.**
- **The individual behavior of *A. baumannii* on different materials indicates the necessity for the validation of automated whole-room decontamination under varying conditions.**