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Contamination of Ambient Air with *Acinetobacter baumannii* on Consecutive Inpatient Days

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***Acinetobacter*-positive patients had their ambient air tested for up to 10 consecutive days. The air was *Acinetobacter* positive for an average of 21% of the days; the rate of contamination was higher among patients colonized in the rectum than in the airways (relative risk [RR], 2.35; $P = 0.006$). Of the 6 air/clinical isolate pairs available, 4 pairs were closely related according to rep-PCR results.**

Acinetobacter baumannii is one of the most clinically significant multidrug-resistant organisms in intensive care units (ICUs) worldwide (1, 2), with the hospital environment serving as a major reservoir (3–5). Recently, we described a situation in which carbapenem-resistant *A. baumannii* (CRAB) was endemic and persisted within the same hospital for almost 2 decades (6). We were able to identify environmental contamination with CRAB in patient rooms occupied by CRAB-positive patients (4). As part of an ongoing surveillance program, we conducted a point prevalence study of the ambient air in our trauma ICU and found that 23% of patient air zones contained *A. baumannii* (7). However, this was a cross-sectional study of air contamination within the unit, irrespective of the room's occupant. The aims of the present study were to characterize the ambient air contamination of CRAB-positive patients using repeated measurements (for up to 10 days) and to evaluate the effect of the anatomic source of CRAB on the persistence of ambient air contamination.

This project was performed in adult ICUs at a 1,500-bed hospital in Miami, FL, where surveillance cultures for CRAB started in 2009 as an infection prevention initiative (4). Surveillance cultures were obtained upon entry to any adult ICU and weekly thereafter from both the rectum and, if intubated, the respiratory tract. Subjects evaluated were consecutive ICU patients in whom CRAB grew from any source, including clinical or surveillance cultures. Electronic medical records were not accessed; data collected were solely based on microbiology feeds received by the infection control department. This activity was approved by the institutional review board at the University of Miami.

All consecutive cases identified from 7 March to 30 July 2013 had the ambient air cultured daily for up to 10 days. Ambient air was cultured, relying on sedimentation, by placing open agar plates (BBL; Becton Dickinson, Franklin Lakes, NJ) at a height of approximately 90 cm from the headboard and within 90 cm of the roof tile. These plates were exchanged every 24 h for 10 days or until the patient was transferred outside the ICU.

Upon collection, the plates were thoroughly swabbed, regardless of apparent growth, using premoistened sterile swabs (7). These swabs were inoculated on Trypticase soy broth, left at 37°C overnight, and then resuspended and streaked on MacConkey agar plates (Becton Dickinson, Franklin Lakes, NJ). After overnight incubation at 37°C, plates were observed for growth, and colonies were selected and isolated to purity based on morphology and color. Species identification and susceptibility testing were performed using Vitek II (bioMérieux, Hazelwood, MO). When *A. baumannii* was identified, we proceeded to test carbapenem susceptibility by the disk diffusion method using meropenem disks, and results were interpreted based on CLSI standards.

Air and patient isolates were typed by repetitive extragenic palindromic PCR (rep-PCR) using the DiversiLab *Acinetobacter* kit (bioMérieux), as previously described (8). Genomic DNA was extracted using the UltraClean microbial DNA isolation kit (MO BIO Laboratories, Carlsbad, CA), which was used as the template for PCR. PCR was conducted with AmpliTaq DNA polymerase (Invitrogen, Carlsbad, CA), as recommended. The amplicons were then subjected to electrophoresis in a chip in an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Closely related isolates were defined as those sharing ≥95% similarity of the banding patterns based on an analysis with the Pearson correlation coefficient.

Poisson regression was used to evaluate the presence of air contamination (binary outcome) based on the presumed source of colonization. Analyses were performed as repeated measures clustered within individual subjects using SAS 9.2 (SAS Institute, Cary, NC).

During the patient stays in the ICU, we performed daily cultures

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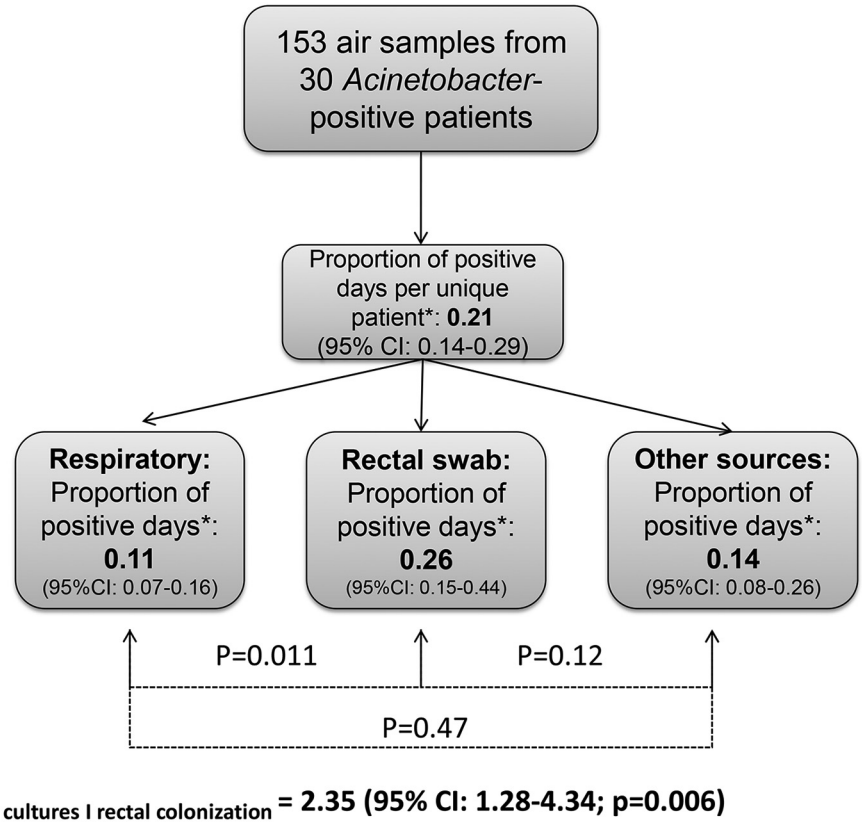

FIG 1 Proportion of days in which *Acinetobacter baumannii* was detected in the ambient air of individual patients. * Statistical differences of the proportion of days *A. baumannii* was detected in the ambient air of individual patients based on their anatomic sources of colonization. *P* values represent the difference in means between the groups. CI, confidence interval; RR, relative risk of having *A. baumannii* recovered from the ambient air given a patient with rectal colonization compared to a patient with respiratory colonization.

of the ambient air of the rooms of 30 CRAB-positive patients. Seventeen (57%) of these 30 patients grew CRAB from the respiratory tract secretions; 5 (17%), from rectal swabs; and the remaining 8 (27%), from other sources (two blood samples, two urine samples, two spinal fluid samples, one wound sample, and one catheter tip). Of note, none of the patients who were followed had an overlap on the anatomic source of *A. baumannii*. Air samples were collected for a total of 153 days, with an average of 5.1 days per patient (range, 1 to 10 days). Throughout the longitudinal observations, patients who were rectally colonized had an average of 26% of their days with CRAB in ambient air (95% confidence interval [CI], 15% to 44%), compared to 11% (95% CI, 7% to 16%) and 14% (95% CI, 8% to 26%) among respiratory and other sources, respectively (Fig. 1). Differences among the proportion of positive days between patients rectally colonized and with respiratory sources were statistically significant (Fig. 1). Furthermore, the relative risk of obtaining a positive ambient air culture among patients colonized in the rectum compared to patients colonized only in the respiratory tract was 2.35 (95% CI, 1.28 to 4.34; $P = 0.006$).

Of the 29 air isolates, 10 isolates corresponding to 8 different patients were available for rep-PCR. The matching patient isolate was not available for one air isolate, and another isolate was later confirmed as *Acinetobacter radioresistens*, not *A. baumannii*. The remaining 8 air isolates from 6 patients were typed along with the corresponding patient isolates using rep-PCR. In 4 of the 6 cases, the patient and air isolates shared ≥95% similarity, defining them as closely related. In one of them, 3 air isolates serially collected within 4 days were all closely related to the patient isolate, suggesting a higher level of air contamination (Fig. 2).

In this longitudinal observational study, we found that the air was contaminated with CRAB in 21% of sampled days for each individual patient. Additionally, the proportion of days with air contamination was higher among patients colonized in the rectum than among patients colonized in the respiratory tract. This finding might be due to the closed circuit used by mechanically ventilated patients versus the possibility of the rectum being a more efficient aerosolizer than the respiratory tract. Interestingly, our preliminary results from a different project show higher degrees of contamination of horizontal surfaces, in addition to air, among patients colonized with CRAB in the rectum versus colonization elsewhere (L. S. Munoz-Price, unpublished data). Even if these results are indeed due to the closed circuit, we believe it is important to describe this potential aerosolization observed among patients who were solely rectally colonized.

The limitations of this project include being a small, single-center study conducted in a hospital where CRAB has been endemic for 2 decades (6). The air-sampling method we used is qualitative, with a level of sensitivity that might not be optimal, especially for low bacterial loads. Given that the electronic medical records were not accessed, we did not collect other potentially significant covariates (e.g., fecal incontinence, diarrhea) that might have played a role in our primary association. Unfortu-

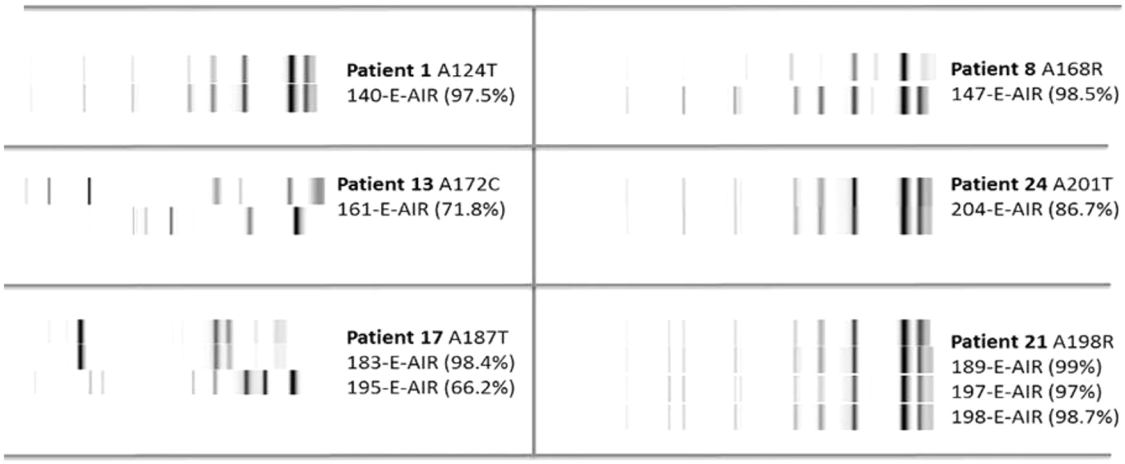

FIG 2 Results of rep-PCR on the isolates belonging to *A. baumannii*-positive patients and their corresponding matching air (E-AIR) isolates.

nately, not all of the isolates were available for PCR testing. This was due to ongoing remodeling in the microbiology laboratory, which caused some of the samples to be mistakenly thrown away, and others failed to grow after thawing.

A recent publication by Rock and colleagues at the University of Maryland showed that only 1 out of 12 air samples belonging to rooms occupied by *A. baumannii*-positive patients were positive for this organism (9). This is indeed an interesting finding, especially compared to our initial publication, which showed up to 40% of ambient air contamination with *A. baumannii* (7). We believe that these differences might be caused in part by different utilization of the heating, ventilation, and air conditioning (HVAC) systems, especially in locations with high outside temperatures and humidity (10). More research should be done evaluating the impact of HVAC systems on the association between the room occupant, the anatomic source of colonization, and the presence of air contamination with pathogens such as *A. baumannii*, especially in hospitals located in warmer climates.

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REFERENCES

1. **Munoz-Price LS, Weinstein RA.** 2008. *Acinetobacter* infection. N Engl J Med **358:**1271–1281. http://dx.doi.org/10.1056/NEJMra070741.
2. **Centers for Disease Control and Prevention.** 2013. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.
3. **Hess AS, Shardell M, Johnson JK, Thom KA, Roghmann MC, Netzer G, Amr S, Morgan DJ, Harris AD.** 2013. A randomized, controlled trial of enhanced cleaning to reduce contamination of healthcare worker gowns and gloves with multidrug-resistant bacteria. Infect Control Hosp Epidemiol **34:**487–493. http://dx.doi.org/10.1086/670205.
4. **Munoz-Price LS, Namias N, Cleary T, Fajardo-Aquino Y, Depascale D, Arheart KL, Rivera JI, Doi Y.** 2013. *Acinetobacter baumannii*: association between environmental contamination of patient rooms and occupant status. Infect Control Hosp Epidemiol **34:**517–520. http://dx.doi.org/10.1086/670209.
5. **La FC, Franke J, Hacek DM, Thomson RB, Jr, Robicsek A, Peterson LR.** 2010. Management of a multidrug-resistant *Acinetobacter baumannii* outbreak in an intensive care unit using novel environmental disinfection: a 38-month report. Am J Infect Control **38:**259–263. http://dx.doi.org/10.1016/j.ajic.2009.07.012.
6. **Munoz-Price LS, Arheart K, Nordmann P, Boulanger AE, Cleary T, Alvarez R, Pizano L, Namias N, Kett DH, Poirel L.** 2013. Eighteen years of experience with *Acinetobacter baumannii* in a tertiary care hospital. Crit Care Med **41:**2733–2742. http://dx.doi.org/10.1097/CCM.0b013e318298a541.
7. **Munoz-Price LS, Fajardo-Aquino Y, Arheart KL, Cleary T, DePascale D, Pizano L, Namias N, Rivera JI, O'Hara JA, Doi Y.** 2013. Aerosolization of *Acinetobacter baumannii* in a trauma ICU*. Crit Care Med **41:**1915–1918. http://dx.doi.org/10.1097/CCM.0b013e31828a39c0.
8. **Perez F, Endimiani A, Ray AJ, Decker BK, Wallace CJ, Hujer KM, Ecker DJ, Adams MD, Toltzis P, Dul MJ, Windau A, Bajaksouzian S, Jacobs MR, Salata RA, Bonomo RA.** 2010. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of postacute care facilities on dissemination. J Antimicrob Chemother **65:**1807–1818. http://dx.doi.org/10.1093/jac/dkq191.
9. **Rock C, Harris AD, Johnson JK, Bischoff WE, Thom KA.** 2015. Infrequent air contamination with *Acinetobacter baumannii* surrounding known colonized or infected critical care patients. Infect Control Hosp Epidemiol **2015:** 1–3. http://dx.doi.org/10.1017/ice.2015.68.
10. **Munoz-Price LS.** 2015. *Acinetobacter* in the air: did Maryland get it wrong? Infect Control Hosp Epidemiol http://dx.doi.org/10.1017/ice.2015.85.