Quantitative Real-Time PCR for Detection of *Acinetobacter baumannii* Colonization in the Hospital Environment

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A real-time PCR assay was developed for detecting the presence of *Acinetobacter baumannii* on hospital equipment and compared to conventional bacterial culture using 100 hospital environmental samples. The real-time PCR detected contaminated surfaces in 4 h with high sensitivity (100%) compared to conventional culture. Thirty-eight percent of samples were positive by real-time PCR and negative by bacterial culture (false positives), possibly indicating the widespread presence of bacterial DNA that is not associated with viable bacteria.

Nosocomial infections caused by drug-resistant bacteria represent an important clinical challenge. *Acinetobacter baumannii* has become one of the most problematic causative agents of nosocomial infections due to its remarkable ability to survive on hospital surfaces and acquire antibiotic resistance, resulting in the global emergence of multidrug-resistant strains with resistance to multiple antibiotic classes (5). *A. baumannii* has been especially problematic in critically ill patients in the intensive care setting, as it is an important cause of ventilator-associated pneumonia and bacteremia. In this context, patients are exposed to *A. baumannii* via contact with contaminated hospital equipment or by contact with hospital personnel carrying the bacteria. A number of studies have demonstrated widespread contamination with *A. baumannii* on hospital environmental surfaces, most notably in intensive care units (ICUs) (1, 4, 8, 9).

Environmental surveillance protocols have been employed for the identification of hospital equipment colonized by *A. baumannii* so that appropriate decontamination procedures can be carried out (1, 4, 8, 9). Since these surveillance methods employ

34 conventional bacterial culture to determine the presence of *A. baumannii*, definitive
35 species identification can require between 24 and 48 h. Nucleic acid-based tests, such as
36 real-time PCR, have been employed for the identification of numerous bacterial
37 pathogens (2); however, to our knowledge this technique has not been applied to
38 identifying contaminated hospital equipment. The objective of the present study was to
39 develop a real-time PCR for identifying hospital surfaces colonized by *A. baumannii*. A
40 real-time PCR assay was developed using TaqMan chemistry for the amplification of
41 nucleotides 774 to 859 of the outer membrane protein A gene (*ompA*; accession number
42 AY485227). The *ompA* gene was chosen because it is present in all sequenced genomes
43 of *A. baumannii* available in the public domain (as of March 2010), and the sequences
44 chosen for the primers and probe correspond to regions highly conserved between
45 published *A. baumannii ompA* sequences (100% sequence identity). The primers OmpA
46 Forward (5=-TCTTGGTGGTCACTTGAAGC-3=) and Ompa Reverse (5=-
47 ACTCTTGTGGTTGTGGAGCA-3=) and the probe (5=-
48 AAGTTGCTCCAGTTGAACCAACTCCA-3=), 5= labelled with 6-carboxyfluorescein
49 and the 3= labeled with 6-carboxytetramethylrhodamine, were used. A quantification
50 standard, pGEM-ompA, was constructed by inserting the *ompA* gene from the ATCC
51 19606 strain of *A. baumannii* into the pGEM-T Easy vector (Promega) after amplification
52 with the primers 5=-ACAGGATCCATGAAATTGAGTCGTATT-3= and 5=-
53 ACAGGGCCCTTATTGAGCTGCTGCA-3=. Each 50-µl reaction mix consisted of 25
54 µl of the 2x TaqMan Universal PCR master mix (Applied Biosystems), 10 µl of DNA
55 (sample or quantification control), OmpA Forward and OmpA Reverse primers at a
56 concentration of 300nMeach, and the probe at a concentration of 100 nM. PCR
57 parameters were 50°C for 2 min, 95°C for 10 min, and then 38 cycles at 95°C for 30 s
58 and 62°C for 1 min. All assays were carried out on a Stratagene Mx3005P thermal cycler.
59 Assay characteristics, including reaction efficiency, dynamic range, intraand interassay
60 variability, and limit of detection, were determined as described previously (7). The
61 sensitivity of the assay for detecting genomic DNA from diverse *A. baumannii* strains
62 was determined using purified genomic DNA (QIAamp DNA minikit; Qiagen) from 20
63 clonally distinct clinical isolates, as determined by pulsed-field gel electrophoresis or
64 repetitive element PCR (REP-PCR). The specificity of the assay for *A. baumannii* was
65 determined using genomic DNA from a clinical isolate of each of the following species:
66 *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, and
67 *Escherichia coli*. In all assays, three concentrations of the quantification standard were

68 used (6.2 x 10^6, 6.2 x 10^3, and 6.2 x 10^1) to determine the number of genome copies
69 present in unknown samples.
70 A total of 100 environmental samples were collected from the general ICU (n = 50) and
71 the trauma/neurosurgical ICU (n = 50) at the Virgen del Rocío University Hospital on
72 separate days in June 2011. Each surface was sampled in duplicate using sterile swabs
73 moistened with physiologic saline. One swab from each surface was used to detect *A.*
74 *baumannii* using conventional culture methods previously described by our group (6).
75 Briefly, the swab was placed in 1 ml of Luria-Bertani medium and incubated for 24 h at
76 37°C to enrich bacteria present in the sample. One hundred microliters from each
77 enrichment culture was plated on Leeds *Acinetobacter* medium (LAM; Hardy
78 Diagnostics) (3) and incubated at 37°C for 24 h to select for *A. baumannii*. The definitive
79 identification of bacteria that grew on LAM plates was made by matrix-assisted laser
80 desorption ionization–time-of-flight (MALDI-TOF) analysis using a MALDI biotyper
81 (Bruker Daltonics). To detect and quantify *A. baumannii* genomic DNA using real-time
82 PCR, the second swab from each surface was placed in 1 ml physiologic saline and
83 vortexed vigorously, and the DNA from 200 µl of the sample was extracted using the
84 QIAamp DNA minikit and eluted in 200 µl water. Ten microliters of the extracted
85 DNAwas used in the real-time assay as described above. The number of genome copies
86 present in the sample was determined by extrapolation from the quantification standards
87 performed in parallel. Negative controls consisted of testing the eluant from unused swabs
88 treated using the DNA isolation procedure described above. Samples were considered
89 positive if a threshold cycle was reached during the 38 cycles. The analytical
90 characteristics of the *ompA* real-time PCR were determined using purified genomic DNA
91 from the ATCC 19606 strain and the pGEM-ompA plasmid. Amplification was linear
92 over 9 log dilutions of the plasmid pGEM-ompa (r^2 = 0.991; slope = -3.58), and the
93 amplification efficiency was 0.90. Intraassay and interassay variabilities using 7,200
94 copies of genomic DNA were 0.8 and 1.32%, respectively. The limit of detection of the
95 assay was 6.8 copies of genomic DNA, as this quantity could reproducibly be amplified.
96 The assay was able to amplify 20 clonally distinct clinical isolates of *A. baumannii*,
97 indicating that diverse *A. baumannii* strains could be detected. The real-time assay was
98 negative when genomic DNA from clinical isolates of *Pseudomonas aeruginosa*,
99 *Klebsiella pneumoniae*, *Moraxella catarrhalis*, and *Escherichia coli* was used in the
100 assay. Taken together, these results indicate that the *ompA* real-time assay is sensitive and
101 specific for detecting and quantifying *A. baumannii* genomic DNA. Results from

102 environmental samples in the ICUs demonstrated that *A. baumannii* was identified on
103 39% of surfaces using bacterial culture (Table 1), a prevalence similar to those of previous
104 reports describing the presence of *A. baumannii* on environmental surfaces in intensive
105 care settings in which this species is endemic (1, 8). Duplicate samples tested using real-
106 time PCR showed the presence of *A. baumannii* on 77% of surfaces, a significantly higher
107 level than the results obtained with bacterial culture ($P < 0.0001$ by chi-squared test).
108 Data from the general ICU demonstrated 27 culture-positive and 43 PCR-positive
109 samples, whereas data from the trauma/neurosurgical ICU demonstrated 11 culture-
110 positive and 34 PCR-positive samples. The quantification of the number of genome
111 copies present in samples taken from environmental surfaces demonstrated a wide range,
112 with between 744 and 189,131 copies present and a median of 19,696. Samples from
113 surfaces positive for *A. baumannii* ($n = 39$) by bacterial culture had significantly more
114 genome copies (median [interquartile range], 27,851 [12,950 to 63,324]) than samples
115 from the 38 negative surfaces (13,798 [2,185 to 25,092]; $P = 0.002$ by Mann-Whitney U
116 test). Importantly, definitive results could be obtained in 4 h using the real-time PCR
117 assay versus 48 h using bacterial culture. Compared to conventional culture, the realtime
118 PCR assay demonstrated a sensitivity of 100%, a specificity of 37.7%, a positive
119 predictive value of 50.6%, and a negative predictive value of 100%. Our results indicate
120 that surfaces colonized by *A. baumannii* can be rapidly identified using real-time PCR
121 with high sensitivity (100%). However, we observed a high frequency of samples that are
122 negative by conventional bacterial culture but positive by realtime PCR. One possibility
123 is that some *Acinetobacter* strains that are detected by real-time PCR are not able to grow
124 on Leeds *Acinetobacter* medium. A second possibility explaining this difference is that
125 bacterial culture detects viable bacteria, whereas the realtime PCR measures the presence
126 of genomic DNA in the sample. We show that 38% of surfaces contained *A. baumannii*
127 DNA in the absence of detectable viable *A. baumannii*, suggesting the widespread
128 presence of genomic DNA that is not associated with viable bacteria. We hypothesize that
129 the presence of this DNA results from decontamination procedures that effectively kill
130 viable bacteria but do not completely remove bacterial remains from the decontaminated
131 surface. Interestingly, although a number of studies have detected the presence of viable
132 *A. baumannii* in the hospital setting (1, 8, 9), no study has characterized the presence of
133 free bacterial genomic DNA in this environment. Further study is required to determine
134 if the presence of free bacterial genomic DNA in the hospital environment is of clinical
135 importance.

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172 **TABLE 1** Presence of *Acinetobacter baumannii* on intensive care unit environmental surfaces using bacterial culture and real-
173 time PCR
174

Surface	No. of samples	No. (%) with growth on LAM[a]	No. (%) positive by real-time PCR	Copies of genomic DNA (median [range])
Bed rail	13	9 (69.2)	12 (92.3)	29,110 (9,794–123,531)
Bedside table	13	4 (30.8)	9 (69.2)	19,583 (10,172–98,010)
AHRD[b]	12	6 (50.0)	10 (83.3)	20,031 (6,351–66,375)
Intravenous (i.v.) pole/i.v. valves	12	3 (25.0)	9 (75.0)	32,157 (1,616–55,969)
Bedside chairs	7	5 (71.4)	7 (100.0)	30,137 (1,176–126,423)
Equipment carts	6	1 (16.7)	4 (66.7)	41,267 (816–48,314)
Infusion pumps	5	3 (60.0)	4 (80.0)	27,890 (3,346–65,835)
Folders	5	2 (40.0)	3 (60.0)	1,842 (1,781–10,814)
Doorknobs	5	1 (20.0)	4 (80.0)	18,377 (6,847–24,300)
Keyboards	4	2 (50.0)	4 (100.0)	7,249 (2,191–189,131)
Storage cabinets	3	1 (33.3)	2 (66.7)	7,314 (3,005–11,622)
Nurses' station	3	1 (33.3)	1 (33.3)	17,775
Sink	3	0 (0.0)	2 (66.7)	2,372 (1,792–2,951)
Light switch	2	0 (0.0)	1 (50.0)	1,261
Air conditioning grate	2	0 (0.0)	2 (100.0)	3,965 (1,858–6,071)
Ambubag	2	0 (0.0)	0 (0.0)	
Dialysis module	1	1 (100.0)	1 (100.0)	93,920
Phone	1	0 (0.0)	1 (100.0)	744
Ultrasound equipment	1	0 (0.0)	1 (100.0)	17,597
Total	100	39 (39.0)	77 (77.0)	19,696 (744–189,131)

175 [a] Leeds *Acinetobacter* medium.
176 [b] Alcohol-based hand rub dispensers.