Clinical validation of a real-time polymerase chain reaction assay for rapid detection of *Acinetobacter baumannii* colonization

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Keywords: *Acinetobacter baumannii*, Active surveillance, Real-time PCR, Colonization

Summary

Real-time polymerase chain reaction (PCR)-based approaches have not been assessed in terms of their ability to detect patients colonized by *Acinetobacter baumannii* during active surveillance. This prospective, double-blind study demonstrated that a real-time PCR assay had high sensitivity (100%) and specificity (91.2%) compared with conventional culture for detecting *A. baumannii* in 397 active surveillance samples, and provided results within 3 h. Receiver-operator curve analyses demonstrated that the technique has diagnostic accuracy of 97.7% (95% confidence interval 96.0e99.3%). This method could facilitate the rapid implementation of infection control measures for preventing the transmission of *A. baumannii*.

Introduction

Hospital-associated infections caused by *Acinetobacter baumannii*, especially those caused by multi-drug-resistant strains, continue to represent an important problem.[1,2] The Healthcare Infection Control Practices Advisory Committee of the Centers for Disease Control and Prevention and the European Society of Clinical Microbiology and Infectious Diseases recommend the use of active surveillance cultures in the control of outbreaks caused by multi-drug-resistant Gramnegative bacteria, including *A. baumannii*.[3,4] In addition, several studies have reported the use of active surveillance cultures in combination with additional measures, such as contact precautions, in the successful control of *A. baumannii* transmission in both outbreak[5,6] and endemic[7,8] settings. Active surveillance for *A. baumannii* colonization commonly involves culturing samples collected from multiple patient anatomical sites, and this process typically requires between 24 and 72 h.[5-11] In this context, it has been suggested that detection methods which provide more immediate results could facilitate the rapid implementation of appropriate infection control measures, and contribute to reducing the transmission of *A. baumannii*.[3] The detection of *A. baumannii* colonization has also been limited by low sensitivity of the conventional microbiological methods used for active surveillance.[9-11] However, in spite of these limitations, to the authors' knowledge, no study to date has compared real-time polymerase chain reaction (PCR)-based approaches with conventional culture for the detection of *A. baumannii* colonization during active surveillance. As such, this prospective double-blind study compared a real-time PCR assay with conventional culture methods for the detection of *A. baumannii* in active surveillance samples.

Methods

This study was conducted in the intensive care units (ICUs) at the University Hospital Virgen del Rocío, a tertiary care centre with 62 ICU beds, over a five-week period between October and November 2013. The study was performed in the context of an eradication programme aimed at reducing infection/colonization by endemic, carbapenem-resistant *A. baumannii*. Surveillance samples were collected weekly from

ICU patients admitted at least 48 h prior to sample collection, and from whom a positive clinical sample had not been obtained previously. Pharyngeal and perianal swabs were obtained from all patients included in the study for testing by both methods, except when the attending physician deemed that sample collection was contraindicated. Sterile swabs were used for sample collection from the oropharynx and the perianal region, and were stored at 4 °C until analysis. All swabs were processed within 24 h of sample collection. The real-time PCR technique and conventional culture were performed by different members of the research team, and results were compared at the end of the study. Patients were considered to be colonized if *A. baumannii* was detected by conventional culture or if the real-time PCR demonstrated the presence of *A. baumannii* genomic DNA in surveillance samples. Microbiological evaluation of clinical samples from suspected sites of infection were used to identify *A. baumannii* infection episodes. The clinical characteristics of patients whose samples gave discordant results were extracted from medical records. This study was approved by the University Hospital Virgen del Rocío Committee on Ethics in Research.

Surveillance samples were cultured on MacConkey agar, and micro-organisms were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker, Billerica, MA, USA). Susceptibility testing for colistin was performed on all *A. baumannii* isolates using E-test strips (AB Biodisk, Solna, Sweden).

A non-commercial real-time PCR that was developed previously by the authors' group was employed in this study.[12] This assay employs TaqMan chemistry to amplify a highly conserved region (nucleotides 774e859) of the *A. baumannii* ompA gene (accession no. AY485227). This assay is specific for *A. baumannii*, has a limit of detection of seven genome copies, and has previously been shown to have intra-and interassay variabilities of 0.8% and 1.3%, respectively, for quantifying *A. baumannii* genome copies.[12] Surveillance swabs were placed in 1 mL of physiological saline, vortexed, and incubated for 15 min to liberate associated bacteria. DNA was extracted from 200 mL and eluted in 200 mL of sterile water using the Qiamp DNA Minikit (Qiagen, Hilden, Germany). Real-time PCR was performed as described previously using 10 mL of the eluted DNA in a 50-mL reaction containing 25 mL of TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA, USA), the primers (final concentration 300 nm each) and probe (final concentration 100 nm).[12]

DNA was amplified using a Stratagene Mx3000P as follows: 50°C for 2 min, 95°C for 10 min, and then 38 cycles at 95°C for 30 s and 62°C for 1 min. Negative controls containing 10 mL of sterile water were performed in duplicate with each run. A. baumannii genome copies in each sample were quantified by extrapolating from reactions with known concentrations of a plasmid containing the ompA Gene.[12]

Genome copy numbers were compared between groups using the Mann-Whitney U-test. Receiver-operator curve (ROC) analyses were performed using Statistical Package for the Social Sciences Version 15.0 (IBM Corp., Armonk, NY, USA).

Results

In total, 397 samples consisting of 202 perianal (50.9%) and 195 pharyngeal (49.1%) swabs were collected (Table I). One hundred and forty-five patients were included in the study, with an average of 1.39 perianal and 1.34 pharyngeal samples taken from each patient. Conventional culture detected *A. baumannii* in 11 samples (eight perianal and three pharyngeal) that corresponded to eight colonization episodes in eight patients, whereas real-time PCR detected *A. baumannii* in 45 samples (31 perianal and 14 pharyngeal), corresponding to 39 colonization episodes in 35 patients. The real-time PCR detected *A. baumannii* in all 11 samples that were positive by conventional culture (sensitivity 100%), and of the 386 samples negative by conventional culture, 352 were negative by real-time PCR (specificity 91.2%). Positive samples were considered as colonization events for each method (Table I). Importantly, the real-time PCR provided results within 3 h.

Quantification of *A. baumannii* genome copies in samples positive by real-time PCR demonstrated no significant difference between perianal and pharyngeal samples {1.3 x 10^5 copies [interquartile range (IQR) 2.3 x 10^4 - 3.1 x 10^6 copies] vs 4.8 x 10^6 copies (IQR 7.0 x 10^4 - 2.0 x 10^7 copies); P = 0.21}. Of the samples positive by real-time PCR, there were significantly more genome copies in samples positive by conventional culture compared with those that were negative [3.1 x 10^6 copies (IQR 1.6 x 10^5 - 6.0 x 10^7 copies) vs 1.1 x 10^5 copies (IQR 2.2 x 10^4 - 1.0 x 10^7 copies); P = 0.018]. The number of genome copies in real-time PCR positive samples from patients who subsequently developed *A. baumannii* infection (N = 14 samples) was higher than in

samples from patients who did not develop infection (N = 31 samples) [2.6 x 10^6 copies (IQR 1.4 x 10^5 - 3.0 x 10^7 copies) vs 1.2 x 10^5 copies (IQR 2.0 x 10^5 - 9.0 x 10^6 copies);P = 0.042].

An ROC was elaborated in order to determine how the application of a threshold number of copies for classifying samples as positive or negative affected the diagnostic accuracy of the real-time PCR (Figure 1). A threshold of 1.7 x 10^4 genome copies increased specificity to 92.7% while sensitivity remained at 100%, and a threshold of 1.4 x 10^5 genome copies increased specificity to 96.4% while decreasing sensitivity to 90.9%, indicating that applying a threshold could reduce the false-positive rate effectively while maintaining high sensitivity. The diagnostic accuracy of the real-time PCR was estimated to be 97.7% [95% confidence interval (CI) 96.0 - 99.3%] by calculating the area under the ROC.

Factors potentially leading to discordant results between methods were assessed by reviewing the medical records of the 28 patients from whom discordant samples were collected. Three of the patients were receiving colistin at the time of sample collection. Given that all *A. baumannii* isolates were susceptible to colistin (minimum inhibitory concentration <2 mg/L), a potential cause of discordance is that only non-viable A. baumannii was present due to the activity of colistin, and that the DNA from these non-viable cells was detected by real-time PCR but not by culture. Four patients from whom samples with discordant results were collected had clinical samples positive for *A. baumannii* by conventional culture within seven days of surveillance sample collection (one sputum sample, one bronchial aspirate, one peritoneal fluid sample and one ulcer sample).

Discussion

This prospective, double-blind study of 397 active surveillance samples demonstrated that a real-time PCR assay showed high sensitivity and specificity compared with conventional culture for the detection of *A. baumannii* colonization. The availability of assay results within 3 h has the potential to facilitate the rapid implementation of infection control measures for reducing the transmission of *A. baumannii*. In addition, this technique is not affected by resistance mechanisms present in *A. baumannii*. It should be noted, however, that the real-time PCR assay required more hands-on time

than conventional culture due to the need to perform DNA extraction; however, the increasing automation of both DNA extraction and real-time PCR assays may help to overcome these additional requirements.[13] PCR-based techniques are being used increasingly to diagnose infection by *A. baumannii* in clinical samples taken from patients with suspected infection.[14,15] In addition, a previous report described the use of conventional PCR for the detection of *A. baumannii* in endotracheal aspirates from intubated patients receiving mechanical ventilation.[16] However, to the authors' knowledge, the present study represents the first controlled trial to compare real-time PCR with conventional culture for the detection of *A. baumannii* colonization in surveillance cultures.

Multiple studies have described the low sensitivity of conventional methods for detecting *A. baumannii* colonization, with reports of sensitivities between 13.5% and 85% depending on the number of anatomical sites tested.[9e11] In the present study, in addition to detecting *A. baumannii* in the 11 samples that were positive by culture methods, the real-time PCR was positive for 34 additional samples collected from 28 patients. Interestingly, clinical samples positive for *A. baumannii* were obtained from four of these patients in the seven days following surveillance sample collection, raising the possibility that the real-time PCR detected colonization events not detected by conventional culture. The sensitivity of the method used for detecting *A. baumannii* colonization is of critical importance given a recent study concluding that the use of methods with sensitivity of 55% for active surveillance of *A. baumannii* colonization would reduce *A. baumannii* transmission, infection and mortality by only 48%, whereas a method with 90% sensitivity would reduce these rates by 78%.[17] Importantly, this study also concluded that active surveillance for *A. baumannii* colonization was cost-effective (a 19-53% reduction in per-patient hospital cost) and would reduce transmission and mortality in both endemic and outbreak settings.[17]

Although this study included 397 samples and was performed in a prospective double-blind manner, its limitations include the short time interval over which the study was undertaken, the single centre design, and the application of real-time PCR in an endemic (vs outbreak) setting alone. However, the results presented here provide microbiological validation of the use of real-time PCR for active surveillance of *A.*

baumannii colonization, and provide a basis for the use of this method to facilitate the rapid implementation of infection control measures for preventing *A. baumannii* transmission. The use of this approach thus has potential to contribute to antimicrobial stewardship initiatives, as it may aid in preventing the spread and transmission of antibiotic resistance.

Conflict of interest statement

MJM and PPR are listed as inventors on a patent describing the real-time PCR method used for detection and quantification of *A. baumannii* genomes. The other authors declare no conflict of interests.

Funding

This work was supported by the Ministerio de Economı´ay Competitividad, Instituto de Salud Carlos III, co-financed by the European Development Regional Fund 'A way to achieve Europe' ERDF, Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008/0000]) and a grant from the European Community's 7th Programme Framework (MagicBullet; Grant Agreement No. 278232). MJM is supported by the Subprograma Miguel Servet from the Ministerio de Economı´a y Competitividad of Spain (CP11/00314).

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Table I.

Identification of *Acinetobacter baumannii* colonization in active surveillance samples using conventional culture methods and real-time polymerase chain reaction (RT-PCR)

	Week 1	Week 2	Week 3	Week 4	Week 5	Total
Patient screenings[a,b]	42 (20.6)	47 (23.0)	45 (22.1)	35 (17.2)	35 (17.2)	204 (100)[c]
Samples	83 (20.9)	93 (23.4)	85 (21.4)	69 (17.4)	67 (16.9)	397 (100)
Pharyngeal	41 (49.4)	48 (51.6)	42 (49.4)	32 (46.4)	32 (47.8)	195 (49.1)
Perianal	42 (50.6)	45 (48.4)	43 (50.6)	37 (53.6)	35 (52.2)	202 (50.9)
Culture						
Positive	3 (3.6)	4 (4.3)	3 (3.5)	0 (0.0)	1 (1.5)	11 (2.8)
Negative	80 (96.4)	89 (95.7)	82 (96.5)	69 (100)	66 (98.5)	386 (97.2)
Colonization[d]	2 (4.8)	3 (6.4)	2 (4.4)	0 (0.0)	1 (2.9)	8 (5.4)[e]
RT-PCR						
Positive	8 (9.6)	7 (7.5)	22 (25.9)	4 (5.8)	4 (6.0)	45 (11.3)
Negative	75 (90.4)	86 (92.5)	63 (74.1)	65 (94.2)	63 (94.0)	352 (88.7)
Colonization[d]	7 (16.7)	6 (12.8)	19 (42.2)	3 (8.7)	4 (11.4)	35 (17.2)[e]

[a] Data for all variables are presented as N (%).
[b] Number of patients screened.
[c] The total represents the total number of patient screenings performed (N¼204) on 145 patients over the five-week inclusion period.
[d] Number of colonized patients detected using this technique (% of patient screenings performed).
[e] Number of unique patients identified as colonized over the five-week inclusion period.

Figure 1.

Receiver-operator analysis of real-time polymerase chain reaction (PCR) for detection of colonization. Receiver-operator curve (blue) showing the relationship between sensitivity and specificity of real-time PCR for detection of *Acinetobacter baumannii* in surveillance samples when different threshold values for number of genome copies that must be present in order to classify a sample as positive are applied. The green reference line represents values for a test that is unable to discriminate between positive and negative samples. CI, 95% confidence interval; AUC, area under the curve.