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Letter to the Editor

Direct detection of *bla*_{OXA-23} gene from endotracheal aspirates by real time PCR

Dear Editor,

Acinetobacter baumannii has emerged worldwide as an important nosocomial pathogen.¹ The carbapenems are highly active against *A. baumannii*, but their activity has been threatened by the widespread of Class D carbapenemases, mainly OXA-23, which is highly prevalent in Brazil.²

Treatment of patients infected by carbapenem-resistant *A. baumannii* (CRAB) has been very difficult and therapy is usually based on polymyxins.³ The use of polymyxins has been guided by the isolation of the organism and detection of carbapenem resistance. However, final results by conventional microbiological methods usually take two to three days, which consequently results in a delay in commencing appropriate therapy for CRAB, which may potentially contribute to adverse outcomes in patients with severe infections such as ventilator-associated pneumonia (VAP).⁴

In this study, we aimed to develop a SYBR green-based real time PCR (qPCR) to detect the *bla*_{OXA-23} gene directly from respiratory tract specimens in patients under mechanical ventilation, potentially serving as a surrogate marker for the presence of CRAB in those patients.

Twenty samples of endotracheal aspirates (ETAs), one specimen per patient, obtained from August to December 2010 from patients under mechanical ventilation at Hospital São Lucas, Porto Alegre, Brazil, were analyzed. All samples were routinely cultivated and only the growth of $\geq 10^5$ CFU/mL of *A. baumannii* was considered positive in ETA culture.

DNA extraction from 200 μ L of the bacterial suspension (0.5 McFarland standard) and from ~ 400 μ L of ETA samples were carried out using the Invitex RTP DNA/RNA Virus Mini Kit according to the protocol suggested by the manufacturer.

Two μ L of the extracted DNA were used in a total volume of 25 μ L of PCR mix containing SYBR Green II (Invitrogen). For primer design, multiple nucleotide sequences were aligned to search for conserved regions within the *bla*_{OXA-23} gene. The primers used to amplify the *bla*_{OXA-23} gene were (F: 5'-AAA GAA GTA AAA CGT ATT GGT TTC G-3' and R: 5'-CCC AAC CAG TCT TTC CAA AA-3'), which correspond to nucleotides 518–442 (sense) and 724–743 from *A. baumannii* (accession number AY795964.1). qPCR was performed using the LightCycler platform (Roche, Germany) and confirmation of the amplicon was

done by melting curve analysis (TM $\sim 80^\circ$ C). The specificities of the primers for the detection of *bla*_{OXA-23} gene were evaluated by the BLAST search program. No matches to the primers were found other than *bla*_{OXA-23}. Negative (0.9% sodium chloride) and positive controls (an OXA-23 producing *A. baumannii* strain confirmed by direct sequencing analysis) were used in each qPCR.

Of 20 ETAs, CRAB isolates were recovered in 10, carbapenem-susceptible *A. baumannii* (CSAB) in three, and seven cultures were negative to *A. baumannii*. The results of *bla*_{OXA-23} qPCR in both colony and ETA are shown in Table 1.

The number of samples analyzed did not allow for a thorough sensitivity and specificity evaluation of the qPCR in ETA, but concordant result rates between both culture and qPCR of ETA and qPCR of the colony and qPCR of ETA were considered satisfactory. Nonetheless, a few disagreements between results were observed. One culture positive specimen for CRAB presented negative results for *bla*_{OXA-23} in both qPCR of the colony and ETA. It is possible that carbapenem-resistance in such isolate was due to other mechanisms (other carbapenemases or by a combination of non-carbapenemases enzymes plus porin modification and/or efflux pump hyperexpression). A thorough investigation of other resistance mechanisms to carbapenems was beyond the scope of our study. One CRAB and one CSAB presented negative result for *bla*_{OXA-23} in qPCR of the colony, but showed positive qPCR in ETA. Although these may be considered false-positive results, we cannot rule out the real presence of a subpopulation of *A. baumannii* harboring the *bla*_{OXA-23} gene in the samples, which could not be detected by the phenotypic method used to isolate this pathogen from ETA. Although the presence of CRAB in lower numbers may be simply considered colonization, not considering growth $< 10^5$ CFU/mL in our study impaired the interpretation of these discordant results.

Overall, we believe that our findings support the potential use of qPCR to detect the presence of *bla*_{OXA-23} gene directly from ETAs, and possibly the detection of other carbapenemase-encoding genes, such as *bla*_{OXA-143} gene for example, which has been shown to be highly prevalent in some Brazilian Southeastern cities,⁵ as surrogate marker for the presence or absence of CRAB in patients under mechanical ventilation. The final results can be available in a much

Table 1 – Results of quantitative culture, detection of *bla*_{OXA-23} gene by real time PCR (qPCR) from colony and endotracheal aspirate.

| Sample | Quantitative culture | Carbapenem susceptibility | Colony qPCR | ETA qPCR |
|--------|----------------------|---------------------------|-------------|----------|
| 1 | <i>A. baumannii</i> | R | Positive | Positive |
| 2 | <i>A. baumannii</i> | R | Positive | Positive |
| 3 | <i>A. baumannii</i> | R | Positive | Positive |
| 4 | <i>A. baumannii</i> | R | Positive | Positive |
| 5 | <i>A. baumannii</i> | R | Positive | Positive |
| 6 | <i>A. baumannii</i> | R | Positive | Positive |
| 7 | <i>A. baumannii</i> | R | Positive | Positive |
| 8 | <i>A. baumannii</i> | R | Positive | Positive |
| 9 | <i>A. baumannii</i> | R | Negative | Negative |
| 10 | <i>A. baumannii</i> | R | Negative | Negative |
| 11 | <i>A. baumannii</i> | S | Negative | Negative |
| 12 | <i>A. baumannii</i> | S | Negative | Negative |
| 13 | <i>A. baumannii</i> | S | Negative | Positive |
| 14 | Negative | – | – | Negative |
| 15 | Negative | – | – | Negative |
| 16 | Negative | – | – | Negative |
| 17 | Negative | – | – | Negative |
| 18 | Negative | – | – | Negative |
| 19 | Negative | – | – | Negative |
| 20 | Negative | – | – | Negative |

ETA, endotracheal aspirate; R, resistant; S, susceptible.

shorter period (one working day) when compared to conventional bacterial culture and susceptibility tests results (may take up to 72 h or more), consequently saving time to identify CRAB in patients with proved or suspected VAP, and potentially shortening the time for initiating appropriate therapy for this difficult to treat infections.

Conflict of interest

A. P. Z. is research fellow of CNPq and has received consultancy fees from Pfizer and Eurofarma. All other authors have nothing to disclose.

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