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PCR Assay Based on the *gyr*B Gene for Rapid Identification of *Acinetobacter baumannii-calcoaceticus* Complex at Specie Level

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Background: The genus *Acinetobacter* sp. comprises more than 50 species, and four are closely related and difficult to be distinguished by either phenotypic or genotypic methods: the *Acinetobacter calcoaceticus-baumannii* complex (ABC). The correct identification at species level is necessary mainly due to the epidemiological aspects. *Methods:* We evaluated a multiplex PCR for *gyr*B gene to identify the species of the ABC using the sequencing of the ITS 16S-23S fragment as a gold standard. Isolates identified as *Acinetobacter calcoaceticus-baumannii* from three hospitals at southern Brazil in 2011 were included in this study. *Results:* A total of 117 isolates were obtained and 106 (90.6%) were confirmed as *A. baumannii*, 6 (5.1%) as *A. nosocomialis* and 4 (3.4%) as *A. pittii* by PCR for *gyr*B gene. Only one isolate did not present a product of the PCR for the *gyr*B gene; this isolate was identified as *Acinetobacter* genospecie 10 by sequencing of ITS. We also noted that the non-*A. baumannii* isolates were recovered from respiratory tract (8/72.7%), blood (2/18.2%) and urine (1/9.1%), suggesting that these species can cause serious infection. *Conclusion:* These findings evidenced that the multiplex PCR of the *gyr*B is a feasible and simple method to identify isolates of the ABC at the species level. J. Clin. Lab. Anal. 31:e22046, 2017. © 2016 Wiley Periodicals, Inc.

Key words: *A. baumannii*; *A. nosocomialis*; *Acinetobacter pitti*; *Acinetobacter* sp; *gyr*B; multiplex PCR

INTRODUCTION

Originally, only two species were described in the genus *Acinetobacter: A. calcoaceticus* and *A. lwoffii*. Later, Bouvet and Grimont defined 12 genospecies based in DNA-DNA hybridization. Thereafter, new species have been described and, currently, the genus comprises 51 species with valid published names that were delineated by DNA-DNA hybridization, 16S-rRNA and whole genome sequencing among others (1–5).

Despite the fact that many identification methods have been described, there are four closely related species which are difficult to be distinguished by either phenotypic or genotypic methods. These were referred as *Acinetobacter calcoaceticus- baumannii* complex (ABC complex) and comprises groups 1, 2, 3 e 13 of Bouvet and Grimont (*A. calcoaceticus, A. baumannii, A. genospecie 3* and *A. genospecie 13TU*, respectively) (2, 3). More recently, *A. genospecie* 3 and *A. genospecie* 13TU were named *Acinetobacter pittii* and *Acinetobacter nosocomialis,* respectively, according to a detailed study of their genotypic characteristics (4).

It is important to identify the species of the ABC complex as they may present distinct epidemiological and clinical contexts. The species *A. baumannii,*

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A. pittii and *A. nosocomialis* are found more frequently in clinical specimens and are related to nosocomial infections. *A. baumannii* is by far the specie most commonly involved in nosocomial infections and usually present resistance to multiple antibiotics (MDR). On the other hand, *A. calcoaceticus* is commonly obtained from soil and water and not observed in reports of diseases (5–7).

A. baumannii has already been widely described as a cause of nosocomial infections and is associated with high level of mortality and a poor outcome mainly because *A. baumannii* isolates from outbreaks usually are carbapenems resistant. In contrast, only a few reports have described carbapenem resistance among *A. pittii* and *A. nosocomialis* (8–10).

Several methodologies have been described to identify the ABC species. Commercial methods (automated and semi-automated) of identification present low performance to distinguish the species as well as other phenotypic methods. Methodologies based in nucleic acids analysis (such as DNA-DNA hybridization, amplified rRNA gene restriction analysis—ARDRA, tRNA spacer fingerprinting, partial sequencing of the *rpo*B gene and the 16S-23S rRNA gene spacer region—ITS) have improved the identification of these species but these methods are rarely used in the routine of microbiology laboratories as they are too expensive (4, 11–14).

The *gyr*B gene corresponds to the fragment encoding the β subunit of the enzyme DNA gyrase and along with the multilocus analysis studies it proved to be heterogeneous interspecies. Therefore, analysis of the *gyr*B gene is a promising tool to differentiate species of ABC complex due to its high specificity and ease of implementation. PCR products of the gyrB gene are of different sizes for each species, allowing the distinction among them. Higgins et al. (2010) proposed a multiplex PCR method with 7 primers targeting this gene which does not need DNA sequencing and, therefore, is a fast and less laborious method which would allow differentiation among the *A. calcoaceticus* and *A. pittii* (*A. genomic* species 3) (15).

In this study, we evaluated the multiplex PCR for *gyr*B gene to identify the species of the ABC complex using the 16S-23S rRNA gene spacer region—ITS sequencing as the gold standard.

MATERIALS AND METHODS

Bacterial isolates

A total of 118 isolates obtained from patients at three different hospitals in southern Brazil, during 2011, were included in this study. These isolates were identified initially as *Acinetobacter calcoaceticus-baumannii* by Vitek 2 system® (bioMeriéux, Marcy l'Etoile, France).

DNA preparation

The boiling method described by Vaneechoutte et al. (1995) was used to extract the DNA from the isolates. DNA was stored at −20°C until use.

Amplification 16S-23S rRNA gene spacer (ITS) and sequencing

The PCR reaction was performed as described previously by Chang et al. (2005) with a few modifications. The total reaction volume was 25 μl and consisted of 1.0 μM of each primer, 1.5 mM of $MgCl_2$, 1X buffer, 0.2 mM of each DNTP, and 2U of Taq DNA polymerase. The PCR was performed as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation (94°C for 1 min), annealing (57°C for 1 min), and extension (72°C for 1.5 min), with a final extension step at 72°C for 7 min.

PCR products were purified with EXOSAP-IT® enzyme USB and were sequenced using the ABI 3500 Genetic Analyzer with 50 cm capillaries and POP7 polymer (Applied Biosystems, Foster City, CA).

Multiplex PCR for *gyr*B gene

The isolates were submitted to *gyr*B multiplex PCR as described by Higgins et al. (2010) with few modifications. The total reaction volume was 25 μl consisting of 0.2 μM of each primer, 1.5 mM of $MgCl_2$, 1X buffer, 0.2 mM of each DNTP, and 1 U of *Taq* DNA polymerase. The PCR was run as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation (94°C for 1 min), annealing (56°C for 30 sec), and extension (72°C for 1 min), with a final extension at 72°C for 10 min. PCR products were analyzed on agarose 1.5% w/ v gels, stained with ethidium bromide, and visualized under UV.

Minimal Inhibitory Concentration (MIC) was performed by broth microdilution for Imipenem, Polymyxin B, and Tigeciycline according to criteria of the Clinical and Laboratory Standards Institute (CLSI). *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 were used as quality control.

RESULTS AND DISCUSSION

The multiplex PCR for the *gyr*B gene, identified the majority (106/117–90.6%) of isolates as *A. baumannii*. 6 (5.1%) and 4 (3.4%) isolates were identified as *A. nosocomialis* and *A. pittii*, respectively. Only one

isolate did not present a product of the PCR for the *gyr*B gene; this isolate was identified as *Acinetobacter* genospecie 10 by sequencing of ITS (Table 1).

There was 100% of concordance of the *gyr*B PCR with sequencing of the ITS. This technique proved to be able to identify all species of ABC since, only one isolate which was not identified in the multiplex PCR for *gyr*B gene proved to be *A*. genospecie 10 when analyzed by sequencing ITS, a specie that does not belong to the ABC complex. Although the sequencing of the ITS could be considered the gold standard to identify species of ABC, the multiplex PCR of the *gyr*B is a more feasible method to be used in the routine laboratory as a rapid alternative to identification at specie level.

Other methods based on DNA analysis such as hybridization, the 16S rDNA and whole genome sequencing require at least two steps of accomplishment becoming more laborious and expensive to be implemented in the routine laboratory. Moreover, requires sophisticated technologies and equipment that, usually, are restricted to reference laboratories (4, 14). According to our experience, the multiplex PCR for the *gyr*B could be performed in nearly 3 hr and requires less sophisticated equipment and analysis of the results.

As shown in the literature, the *A. baumannii* is involved in serious and difficult to treat infections due to high rates of resistance, particularly to carbapenems (16, 17). For other species of ABC complex, non-*A. baumannii*, there are still few reports of susceptibility profile and clinical impact (8–10). In this study, the non-*A. baumannii* isolates were recovered from respiratory tract (8/11; 72.7%), blood (2/11; 18.2%), urine (1/11; 9.1%) and 4 of these (36.4%) were resistant to imipenem. In addition to causing infections, ABC may be colonizing of patients with some comorbidity or who are hospitalized and receiving antibiotics. This fact confirmed non-*A. baumannii* species may be associated with severe infections and present phenotypes of multidrug resistant as *A. baumannii* (18, 19) highlighting the importance of correct identification at the species level for a better understanding of the clinical and epidemiological aspects of these infections.

TABLE 1. Comparison of 16S-23S ITS and *gyr*B Methods to Identify *Acinetobacter Calcoaceticus-baumannii* Complex Species

Isolate number	ITS	*gyr*B	Clinical specimen
1nb	*A. pittii*	*A. pittii*	Tracheal aspirate
2nb	*A. nosocomialis*	*A. nosocomialis*	Blood culture
3nb	*A. pittii*	*A. pittii*	Sputum catheter
4nb	*A. pittii*	*A. pittii*	Urine
5nb	*A. genomic specie 10*	*Non-identified**	Sputum catheter
6nb	*A. nosocomialis*	*A. nosocomialis*	Tracheal aspirate
7nb	*A. nosocomialis*	*A. nosocomialis*	Tracheal aspirate
8nb	*A. nosocomialis*	*A. nosocomialis*	Blood culture
9nb	*A. pittii*	*A. pittii*	Tracheal aspirate
10nb	*A. nosocomialis*	*A. nosocomialis*	Tracheal aspirate
11nb	*A. nosocomialis*	*A. nosocomialis*	Tracheal aspirate

*There is not product amplified on PCR to *gyr*B.

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CONFLICT OF INTEREST

The authors declare have not conflict of interests.

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