Comparison of two molecular methods for bartonella detection in blood donors from University of Campinas Blood Bank / SP

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Abstract

Bartonella spp. are a worldwide bacteria that can cause chronic infection. In order to identify a better molecular diagnosis we analyzed 100 blood donor samples. We performed conventional and nested PCRs from DNA extracted from blood and liquid culture. We detected bloodstream infection in eight samples using nested PCR and only in three by conventional PCR from liquid culture. None blood samples were positive. More sensitive diagnostic methods should be used in studying this infection.

Key words: *Bartonella*, Blood Transfusion, Blood Donor

Introduction

Bartonella spp. are fastidious gram-negative bacilli. They are re-emerging and neglected zoonotic agents. These bacteria can cause several human diseases as Peruvian bartonellosis, trench fever, cat scratch disease, bacillary angiomatosis, endocarditis.¹ *Bartonella* sp. infection can be fatal, especially in immunocompromised patients. These bacteria can survive for over a month in stored blood.² Its prevalence among blood donors is unknown, and screening of blood supplies for this pathogen is not routinely performed.¹ There is no gold standard diagnosis to detect these agents. In order to identify a specific and sensible *Bartonella* sp. molecular diagnosis we analyzed 100 blood donors samples using conventional and nested PCRs from DNA extracted from blood and liquid culture.

Results and Discussion

We used samples from a previous study with 500 blood donors. DNA from 100 whole blood and liquid culture samples was extracted using QIAmp DNA Mini Kit (Qiagen®). DNA samples were tested by genus specific conventional PCR targeting 16S-23S ribosomal RNA intergenic spacer (ITS)³. We also performed *Bartonella henselae* species specific nested PCR targeting *FtsZ* region⁴. We tested all DNA samples using conventional PCR targeting the GAPDH gene in order to verify the extracted DNA quality and absence of amplification inhibitors. All amplicons from conventional PCR were sequenced. An overview of the diagnostic procedures performed in this study is presented in the Figure 1.

Figure 1. Flowchart PCR-based procedures

We detected *Bartonella* sp. DNA in 8/100 samples using nested PCR (*FtsZ*) and only in 3/100 by conventional PCR (ITS) from liquid culture. *Bartonella* sp. DNA was not detected in any blood samples. We detected GAPDH amplicons in all DNA extracted. The sequencing of ITS positive samples showed 100% of similarity with *B. henselae* strain Houston-I complete genome (BX.897699.1). In this study, we used samples from asymptomatic subjects (blood donors). The low bacteremia was only found using nested PCR that is admittedly more sensitive than conventional PCR and after liquid culture.

Conclusions

Bartonella sp. bacteremia occurs in asymptomatic blood donors. The use of liquid culture and nested PCR improved the chances to detect the bacterium. Our findings support further evaluation of *Bartonella* sp. transmission through blood transfusions. Low bacteremia and fastidious characteristic are the challenge to be overcome in the field of bartonellosis diagnosis.

Acknowledgement

We thank São Paulo Research Foundation (FAPESP) grant #2013/14211-3 for financial support.

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² Magalhães, R.F. et al.. *Transfus Med.* **2008**, 18, 287.

³ Diniz, P.P. et al.. *Vet Res.* **2007**, 38, 697.

⁴ Kawasato, K.H. et al.. *Rev Inst Med Trop Sao Paulo.* **2013**, 55, 1.