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Detecção de contaminação bacteriana em bolsas de plaquetas por método de amplificação molecular

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RESUMO

Entre os casos de transmissão de agentes infecciosos por transfusão, a contaminação bacteriana ocupa o primeiro lugar no número de eventos, morbidade e mortalidade. Isto ocorre principalmente em transfusões de concentrados de plaquetas, que são armazenados à temperatura ambiente e sob agitação constante, condições propícias ao crescimento bacteriano. A cultura automatizada é adotada por alguns bancos de sangue para detecção de contaminação bacteriana, mas a um custo elevado, e oferecendo tempo inadequado na realização frente ao curto período de validade dos concentrados de plaquetas, de apenas 5 dias. Recentemente, alguns grupos vem avaliando a utilização de métodos de amplificação molecular, como um ensaio de PCR em tempo real baseado no gene de RNA ribossômico (16S RNAr), um método prático, uma vez que um par de iniciadores/sonda permite a detecção da mais ampla gama de bactérias. O objetivo do nosso trabalho foi estabelecer um método semi-automatizado de amplificação de DNA para a triagem universal de bactérias em concentrados de plaquetas. Concentrados de plaquetas foram contaminados com suspensões de cinco bactérias diferentes, *Staphylococcus aureus*, *Staphylococcus hominis*, *Staphylococcus epidermidis*, *Enterobacter cloacae* e *Serratia marcescens,* em 1 e 10 unidades formadoras de colônia (UFC)/mL, simulando a contaminação que ocorre durante a doação de sangue. Os concentrados de plaquetas foram armazenados à temperatura ambiente sob agitação durante 5 dias e alíquotas de 1 mL foram colhidas a cada 24 horas. A presença de bactérias foi investigada por PCR em tempo real e pelo ensaio *eBDS* (*Enhanced Bacterial Detection System, PALL*) como um método de referência. O DNA foi extraído no sistema automatizado *MagNA Pure 96* (Roche). A amplificação por PCR em tempo real foi realizada com um conjunto de iniciadores universais e sonda alvo do gene de 16S RNAr em paralelo a um alvo de DNA mitocondrial como controle interno. A mistura de amplificação foi tratada com etídio-monoazida (EMA) seguido por fotoativação, para eliminar a contaminação com DNA bacteriano em reagentes. Com o PCR em tempo real foi possível detectar a presença de todas as espécies bacterianas testadas com uma concentração inicial de 10 UFC/mL 24 horas após a contaminação, com exceção de *Staphylococcus hominis*. Além disso, detectou-se a presença das bactérias *Staphylococcus aureus*, *Serratia marcescens* e *Enterobacter cloacae* com uma concentração inicial de 1 UFC/mL. Durante o período de estudo foram realizados 26.728 testes eBDS, com 9 resultados positivos. 800 amostras foram testadas simultaneamente pelo eBDS e PCR em tempo real, apenas uma das amostras contaminadas estava entre as testadas por ambos os testes. A incidência da contaminação bacteriana de CPs foi de 1:2.969, semelhante a outros trabalhos. Os resultados do teste molecular podem ser obtidos em 4 horas. O ensaio de PCR em tempo real pode ser uma boa alternativa aos métodos convencionais de cultura na triagem de contaminação bacteriana em concentrados de plaquetas, permitindo a detecção de bactérias, mesmo com uma quantidade inicial baixa de microrganismos, apresentando boa sensibilidade e resultados rápidos.

Descritores: Contaminação. Bacteremia. Reação em cadeia por polimerase. Bancos de sangue.

ABSTRACT

Among cases of transfusion transmission of infectious agents, bacterial contamination ranks first in the number of events, morbidity and mortality. This occurs mainly by transfused platelets, which are stored at room temperature and under constant agitation what favors bacterial growth. Automated culture is adopted by some blood banks for screening of bacterial contamination, but this is expensive and has a relatively long turnaround time. Recently, some groups have evaluated the use of molecular amplification methods such as real-time PCR based on the highly conserved 16S rRNA gene; this allows a single pair of 'universal' primers/probe to detect a very broad range of bacteria. The aim of our work was to establish a semi-automated high-throughput DNA amplification method for universal screening of bacteria in platelet concentrates. Platelet concentrates were spiked with suspensions of *Staphylococcus aureus, Staphylococcus hominis, Staphylococcus epidermidis, Enterobacter cloacae* and *Serratia marcescens* to 1 and 10 colony-forming units (CFU)/mL, to simulate contamination occurring during blood donation or processing. The platelet concentrates were stored at room temperature under agitation for 5 days and 1 mL aliquots were drawn every 24 hours. The presence of bacteria was investigated by real-time PCR and by the eBDS assay (*Enhanced Bacterial Detection System, PALL*) as a reference method. DNA was extracted by using a Large Volume kit in the MagNA Pure 96 (Roche) system. Real-time PCR amplification was performed with a set of universal primers and probe targeting the 16S rRNA gene. Co-amplification of human mitochondrial DNA served as an internal control. The amplification mixture was treated with ethidium monoazide (EMA) followed by photoactivation to eliminate contamination with spurious bacterial DNA in reagents. By the real-time PCR it was possible to detect the presence of all bacterial species tested with an initial concentration of 10 CFU/mL 24 hours after contamination of platelet concentrates, except for *Staphylococcus hominis*. The PCR assay also detected the presence of bacteria *Serratia marcescens* and *Enterobacter cloacae* with an initial concentration of 1 CFU/mL. During the study period, 26.728 eBDS tests were performed, with 9 positive results. Eight hundred samples were tested simultaneously by eBDS and real-time PCR, only one of the contaminated samples was among those evaluated by both methods. The incidence of bacterial contamination on platelets concentrates was 1: 2.969, similar to other reports. The results of the molecular test could be obtained in 4 hours. The real-time PCR assay may be a good alternative to conventional culture methods in the screening of bacterial contamination of platelet concentrates, enabling bacterial detection even with a low initial concentration of microorganisms, whilst offering good sensitivity and a fast turnaround time.

Descriptors: Contamination. Bacteremia. Polymerase chain reaction. Blood banks.

CONCLUSÕES

O teste em formato de PCR em tempo real para triagem de contaminação bacteriana conseguiu detectar inóculos experimentais com 1 e 10 UFC/mL, obtendo boa sensibilidade. Além disso, todos os resultados positivos obtidos na triagem de rotina com o eBDS também foram positivos com PCR, mostrando a eficácia do método utilizados em detectar diferentes espécies bacterianas.

O ensaio de PCR em tempo real pode ser uma boa alternativa aos métodos convencionais de cultura na triagem de contaminação bacteriana em concentrados de plaquetas, principalmente quando é desejável a análise de 100% destes hemocomponentes, permitindo a detecção de bactérias, mesmo com uma quantidade inicial baixa de microrganismos, apresentando boa sensibilidade e resultados mais rápidos. Além disso, pode ser realizado em outros componentes e situações que necessitam de uma triagem microbiológica rápida.

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