SEPSER: A CRISPR-based diagnosis test for causing sepsis bacteria species

ANA CAROLINE SOUSA[1,2]; ANA CRISTINA GALES[3]; NILMAR SILVIO MORETTI[1,2]; SERGIO SCHENKMAN[1]

[1]Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, UNIFESP;
[2]Laboratório de Biologia Molecular de Patógenos – LBMP – Escola Paulista de Medicina – UNIFESP;
[3]Departamento de Infectologia – Escola Paulista de Medicina – UNIFESP

Infectious diseases are a global problem affecting billions of people. The rise in multidrug-resistant pathogens and infections linked to sepsis and / or septic shock, caused by bacteria from different genera, has also been a cause for concern as they are strongly associated with factors such as morbidity and mortality in hospitalized individuals. In this scenario, rapid diagnostic test results for appropriate antibiotic therapy are essential to reduce the risk of patient death. Here, we began the implementation and optimization of the CRISPR-Cas12 methodology for the molecular laboratory diagnosis of infections caused by pathogenic bacteria circulating in hospital environments, such as the Intensive Care Unit, in order to streamline, increase sensitivity and reduce diagnosis time for four species of bacteria: *Acinetobacter baumannii*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus capitis.* To validate the diagnostic assay, genome sequences of each species were cloned into the plasmid pBluescript II (SK)+ for subsequent validation steps. We also obtained Cas12a proteins from three bacterial species, *Acidaminococcus sp.* BV3L6 (AsCas12a), *Francisella tularensis* (FnCas12a), *Lachnospiraceae bacterium* ND2006 (LbCas12a), through heterologous expression using *E. coli* BL21 (DE3), followed by purification using Ni-NTA agarose columns. DNA fragments for synthesizing sgRNAs of the different target genes were obtained through PCR PCR assays, and *in vitro* transcription of the sgRNAs using T7 RNA Polymerase. In the following steps, we carried out in vitro cleavage assays with the generated components (plasmid with specific target gene, Cas12a enzymes, and sgRNA). The reactions were incubated at 37ºC and monitored for 2 hours. We found that in the experiments carried out with AsCas12a, FnCas12a and LbCas12a, we succeeded in detecting the target DNA for *A. baumannii*, *E. coli*, *S. aureus* and *S. capitis* in a species-specific manner, as observed in the cleavage pattern of the plasmid containing the sequence of each pathogen. We also validated this detection using the non-specific activity of Cas12a, by coupling in the reaction fluorescent molecules. The results obtained show the potential of the CRISPR-Cas12a methodology for the detection of different bacteria and that, in the future, it could be extended to the diagnosis of other infectious diseases of medical importance.

Key-words: Infectious bacteria, CRISPR, Cas12a, infectious diseases, diagnosis

Financial agency: FAPESP