Gram-negative bacteria, such as enterobacteria or non-fermenting bacteria, are responsible for a wide variety of different infections in humans, such as surgical, abdominal or urinary tract infections, ventilator-associated pneumonias and sepsis. They often harbor different resistance and virulence mechanisms. In recent years, the treatment of these infections got more complicated due to the emergence of Gram-negative bacteria which are resistant to a wide range of antibiotics.

An early identification and confirmation of suspicious pathogens beside its potential antimicrobial resistance is crucial for infection control, prevention, rapid confirmation of other diagnostic test procedures, treatment and epidemiological purposes. Furthermore, it may have a significant impact on the selection of an appropriate initial treatment and could therefore be of great benefit for ICU patients.

Molecular identification methods could become a suitable tool for this task, especially in point-of-care settings, because these methods combine high speed with excellent sensitivity and specificity. For this purpose a novel multiplex real-time PCR was designed, developed and validated to detect different species markers of four clinically relevant Gram-negative bacteria, simultaneously. This included gsd (glutamate decarboxylase) for Escherichia coli, ecfX (extracytoplasmic function sigma factor) for Pseudomonas aeruginosa, basC (acinetobactin biosynthesis) for Acinetobacter baumannii and khe (klebsolysin) for Klebsiella pneumoniae.

Multiplex and multi-color channel real-time PCR provides very accurate and reproducible quantitation of gene copy numbers from a single sample. The method measures PCR product accumulation through dual-labeled TaqMan probes.

Singleplex real-time PCR

Multiplex real-time PCR

Colony PCR

Table 1: a) sensitivity (true positive rate) tests; b) specificity (true negative rate) tests. Table contains the genotyping data obtained by unit sequencing and multiplex real-time PCR, as well as the data of the phenotypical species determination tests (VITEK 2 system and/or Biotyper BioMérieux, Germany) and/or MALDI-TOF (Bruker) systems.

Conclusion

The genes gsd, basC, khe and ecfX can reliably be detected via multiplex colony PCR. The entire assay is time and cost effective. Colonies can be used directly from agar plates saving DNA preparation. The assay does not require post-PCR sample handling, preventing carry-over contaminations of amplicons. This multiplex PCR could be used as rapid test to confirm or identify the target species, e.g., isolated from selective media or blood cultures or to detect them in native samples possibly combined with other PCR targets, e.g., carbapenemase genes (Weiss et al., 2017).