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# SYBRGreen-based real-time PCR for the detection of mcr-1-mediated colistin resistance in human stool samples

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**Background:** Transferable *mcr-1*-mediated colistin resistance is a major public health concern. Highly sensitive molecular methods to promptly detect the presence of this life-threatening antimicrobial resistance determinant are urgently needed. The aim of our study was to design a rapid and cheap real-time PCR method for *mcr-1* gene detection in human fecal samples.

**Material/methods:** Stools (n=88) from 38 healthy volunteers (29 people provided more than one sample in different periods) were screened for the presence of colistin-resistant *Enterobacteriaceae*. After enriching overnight ~20 µg of stools in LB broth containing 2 mg/L colistin, 100 µL were plated on CHROMagar Orientation plates with colistin (4 mg/L). Based on this approach, 3 unique volunteers were colonized with *mcr-1*-harboring *E. coli* (*mcr-1-Ec*) isolates.
In the efforts to rapidly detect positive fecal specimens, we designed a SYBRGreen-based real-time PCR (Quantstudio7 Flex, Applied Biosystems) targeting the *mcr-1*. For method validation, total DNA was extracted (QiaAMP DNA Mini kit, Qiagen) from 8 *mcr-1*-negative and the 3 *mcr-1-Ec*, as well as from the 88 stools, both native (100 mg extracted with the QiaAMP Stool Mini kit, Qiagen, and eluted in 100 µL) and after enrichment in LB plus colistin (200 µL extracted with the QiaAMP DNA Mini kit, Qiagen, and eluted in 100 µL).

**Results:** For culture isolates, our real-time PCR method exhibited a limit of detection of 10 genomic copies/reaction in 40 cycles. It correctly identified all *mcr-1-Ec* showing both sensitivity and specificity of 100%.

Nevertheless, when using DNA extracted from native stools, only two out of the three *mcr-1-Ec*-positive specimens were correctly identified (in 37 and 34 cycles, respectively) indicating that direct screening of stool samples using our real-time PCR may lead to false-negative results.
On the other hand, after enrichment in LB containing colistin and DNA extraction, the real-time PCR was strongly positive for all 3 samples. The average cycle threshold was 22, granting rapid and confident detection of positive specimens within 30 cycles. No false-positive results were observed for the remaining 85 *mcr-1*-negative specimens.

**Conclusions:** We developed a real-time PCR for the detection of *mcr-1* from stool specimens. Since the sensitivity for native stools was relatively low for several potential reasons (e.g., PCR inhibitors, low amounts of *mcr-1*-carrying bacteria), we increased the detection rate by the implementation of a previous selective broth enrichment, which also displayed the major advantage of simple concomitant isolation of the *mcr-1*-harboring strains for further antimicrobial susceptibility and genetic tests.