Rapid multiplex polymerase chain reaction for detection of \textit{mcr-1} to \textit{mcr-5} genes

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A rapid (total time \(<2\,h\)) and reliable multiplex polymerase chain reaction for screening of \textit{mcr-1} to \textit{mcr-5} genes conferring resistance to colistin has been developed. This technique has been tested on a collection of isolates previously identified as bearing \textit{mcr-1}, \textit{mcr-2}, and \textit{mcr-like} genes and had a sensitivity and a specificity of 100\%. Using this method, we were also able to identify a single isolate possessing both \textit{mcr-1} and \textit{mcr-5} genes.

\textbf{Keywords:}
Polymyxin resistance
\textit{mcr} genes
Multiplex PCR

Infections due to multidrug-resistant bacteria are increasing in health care facilities, which lead physicians to reintroduce the old antibiotic polymyxins (colistin, polymyxin B) as possible therapeutic options (Vincent et al., 2009; Wright et al., 2017). However, resistance to polymyxins in Gram-negative bacteria is now increasingly described and is mostly due in Enterobacteriaceae to chromosomal resistance to polymyxins (Wright et al., 2017). However, resistant antibiotic polymyxins (colistin, polymyxin B) as possible therapeutic options (Vincent et al., 2009; Wright et al., 2017). Recently, epidemiologic study in Chinese individuals showed a prevalence of \textit{mcr-1}–positive Enterobacteriaceae isolates close to 1% in clinical samples, and comprised between 0.5% and 3% in carriage samples from volunteers and patients (Wang et al., 2017). In parallel, other mobile colistin resistance genes have been identified recently (\textit{mcr-2}, \textit{mcr-3}, \textit{mcr-4}, and \textit{mcr-5}) mostly from single isolate (Borowiak et al., 2017; Carattoli et al., 2017; Xavier et al., 2016; Yin et al., 2017). Comparison of protein sequences of \textit{mcr-like} proteins shows a low level of identity (ca. 30\%–50\%), except \textit{MCR-1} and \textit{MCR-2} that share ca. 80\% amino acid identity (Table 1). Searching the \textit{mcr} genes progenitors among \textit{Moraxella} strains led to the identification of the progenitor of \textit{mcr-2} gene (Poirel et al., 2017) being \textit{Moraxella pluranimalium}, and other \textit{Moraxella} species (commensal of the tracheal flora) were considered as potential sources of other \textit{mcr-like} genes (Kieffer et al., 2017). Multiple variants have been reported within the past 2 years, to date, 5 major groups, namely, \textit{mcr-1}, \textit{mcr-2}, \textit{mcr-3}, \textit{mcr-4}, and \textit{mcr-5} each possessing 13, 2, 10, 2 and 2 variants, respectively (Table S1). Although \textit{mcr-2} and \textit{mcr-4} have been only reported in Europe, \textit{mcr-1} and \textit{mcr-3} are identified worldwide (Di Pilato et al., 2016; Liu et al., 2017; Lu et al., 2017; Teo et al., 2017; Zhao et al., 2017). Most of the bacteria carry a single \textit{mcr} gene, but a single
isolate possessing 2 mcr genes has been reported (Liu et al., 2017). More studies including the detection of mcr genes are needed to evaluate the emergence of these colistin resistance genes with high transferability potency.

The aim of this work was to design an easy-to-perform polymerase chain reaction (PCR) technique to detect all of mcr-1 to mcr-5 genes in a single mix. For this purpose, a PCR-based method was designed and has the advantage to give result in less than 2 h (amplification and electrophoresis).

For optimization of the multiplex PCR, 5 control strains harboring the mcr-1 to mcr-5 genes were used as positive controls, being all enterobacterial isolates. We obtained the positive mcr-5 gene isolate by electroporating in the E. coli strain TOP10 a plasmid in which the mcr-5 gene was cloned by the manufacturer RD-Biotech (http://www.rd-biotech.com). An easy interpretation (avoiding similar-in-size amplicons for 1 given PCR tube) of the PCR results was obtained. We also tested mixes of all couples of DNA of the 5 positive controls (used separately and in mix of 2 DNA) (Fig. 1) and the positive controls (used separately and in mix of 2 DNA) (Fig. 1) and the progenitor of mcr-2 gene, M. plurimalium, presented the expected bands of mcr-1 to mcr-5 gene fragments. The M. porci isolate and the 2 E. coli recombinant strains harboring the mcr-like genes from the M. osloensis and M. lincolnii isolates did not give a positive result and confirmed the specificity of the PCR primers used in this multiplex approach.

Then, a total of 43 isolates of enterobacterial species (38 E. coli and 2 K. pneumoniae isolates and 2 Salmonella enterica) bearing the mcr-1 gene and a single mcr-4 positive S. enterica isolate from our collections were tested (Table S2). We detected that one isolate, the S. enterica named FR-290, previously identified as mcr-1 gene-positive isolate, was also positive for mcr-5 gene. All other isolates yielded the expected band for mcr-1 or mcr-4 amplicons and no amplification of the other mcr genes as attempted (sensitivity and specificity of 100%).

PCR, real-time PCR, or loop-mediated isothermal amplification-based methods have previously been developed to assess the detection of colistin resistance genes. However, for most of these techniques, only a single mcr gene is detected (mcr-1 gene) (Bontron et al., 2016; Donà et al., 2017; Liu et al., 2016; Xavier et al., 2016; Zou et al., 2017). In a single test, both the mcr-1 and mcr-2 genes could be detected (Mavrici et al., 2016).

### Table 1

<table>
<thead>
<tr>
<th>MCR determinant</th>
<th>Amino acid identity level</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>MCR-2</td>
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<td>MCR-4</td>
<td>34.0</td>
</tr>
<tr>
<td>MCR-5</td>
<td>36.1</td>
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</table>

Fig. 1. Agarose gel electrophoresis (2.5%) used for the separation of multiplex PCR products. Lanes: 1, negative control (susceptible E. coli isolate); 2, mcr-1-positive isolate; 3, mcr-2-positive isolate; 4, mcr-3-positive isolate; 5, mcr-4-positive isolate; 6, mcr-5-positive isolate; 7, mix of DNA of mcr-1 and mcr-2-positive isolates; 8, mix of DNA of mcr-1 and mcr-3-positive isolates; 9, mix of DNA of mcr-1 and mcr-4-positive isolates; 10, mix of DNA of mcr-1 and mcr-5 isolates; 11, mix of DNA of mcr-2 and mcr-3-positive isolates; 12, mix of DNA of mcr-2 and mcr-4-positive isolates; 13, mix of DNA of mcr-2 and mcr-5-positive isolates; 14, mix of DNA of mcr-3 and mcr-4-positive isolates; 15, mix of DNA of mcr-3 and mcr-5-positive isolates; 16, mix of DNA of mcr-4 and mcr-5-positive isolates; and 17, negative control (water). M = molecular size marker (GeneRuler™, 100-bp DNA Ladder Plus; Thermo Fisher Scientific, USA). The size of each PCR product is indicated in base pairs.
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Conflict of interest

All authors declare that they have no conflict of interest.

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References


