Bacteriology

Rapid multiplex polymerase chain reaction for detection of mcr-1 to mcr-5 genes

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A rapid (total time <2 h) and reliable multiplex polymerase chain reaction for screening of mcr-1 to mcr-5 genes conferring resistance to colistin has been developed. This technique has been tested on a collection of isolates previously identified as bearing mcr-1, mcr-2, and 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 mcr-1 and mcr-5 genes.

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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 mutations in genes involved in modification of the lipopolysaccharide (LPS) (Poirel et al., 2017). Recently, the plasmid-mediated polymyxin resistance determinant MCR-1, responsible for acquired resistance to polymyxins, has been reported from Escherichia coli and Klebsiella pneumoniae isolates in China (Liu et al., 2016). Then, the mcr-1 gene has been reported worldwide in various enterobacterial species including Enterobacter, Salmonella, and Shigella and from various animal and environmental origins (Poirel et al., 2017). The encoded MCR-1 protein is a phosphoethanolamine transferase that adds a phosphoethanolamine group to the lipid A, a portion of the LPS, leading to a more cationic LPS structure and consequently to resistance to polymyxins (Liu et al., 2016). Epidemiologic surveys show that the livestock is the most important reservoir of MCR producers (Irgang et al., 2016; Liu et al., 2016; Perrin-Guyomard et al., 2016). A recent study showed a very high prevalence (99%) of MCR-1-producing Enterobacteriaceae among pigs receiving colistin from 2 farms in Portugal (Kieffer et al., 2017). A recent large epidemiologic study in Chinese individuals showed a prevalence of 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 (mcr-2, mcr-3, mcr-4, and 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 MCR-like proteins shows a low level of identity (ca. 30%–50%), except MCR-1 and MCR-2 that share ca. 80% amino-acid identity (Table 1). Searching the mcr genes progenitors among Moraxella sp. strains led to the identification of the progenitor of mcr-2 gene (Poirel et al., 2017) being Moraxella pluranaitalium, and other Moraxella species (commensal of the tracheal flora) were considered as potential sources of other mcr-like genes (Kieffer et al., 2017). Multiple variants have been reported within the past 2 years, to date, 5 major groups, namely, mcr-1, mcr-2, mcr-3, mcr-4, and mcr-5 each possessing 13, 2, 10, 2 and 2 variants, respectively (Table S1). Although mcr-2 and mcr-4 have been only reported in Europe, mcr-1 and 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 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 isolate by eletroporating 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 bands of the 16S rDNA gene) was cloned by the manufacturer RD-Biotech (http://www.rd-biotech.com). An easy interpretation (avoiding similar-in-size bands) was achieved by designing primers (Table S2). We detected that one isolate, the positive control (used separately and in mix of 2 DNA) (Fig. 1) and the progenitor of mcr-2 gene, M. plumalium, presented the expected bands of mcr-1 to mcr-5 gene fragments. The M. portii 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.

Table 1
Amino acid identity of MCR polymyxin resistance determinants.

<table>
<thead>
<tr>
<th>MCR determinant</th>
<th>Amino acid identity level</th>
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<tbody>
<tr>
<td>MCR-3</td>
<td>80.7</td>
</tr>
<tr>
<td>MCR-4</td>
<td>32.5</td>
</tr>
<tr>
<td>MCR-5</td>
<td>34.0</td>
</tr>
<tr>
<td>MCR-1</td>
<td>36.1</td>
</tr>
</tbody>
</table>

| MCR-2 | 31.7 |
| MCR-3 | 35.0 |
| MCR-4 | 34.0 |
| MCR-5 | 33.7 |

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 isolate by eletroporating 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 bands of the 16S rDNA gene) was achieved by designing primers (Table S2). We detected that one isolate, the positive control (used separately and in mix of 2 DNA) (Fig. 1) and the progenitor of mcr-2 gene, M. plumalium, presented the expected bands of mcr-1 to mcr-5 gene fragments. The M. portii 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., 2017).

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.
et al., 2017), and in these studies, several mcr genes were separately detected (El Garch et al., 2018; Mavrici et al., 2017). A microarray detection method of several genes including mcr-1 and mcr-2 genes was also developed (Bernasconi et al., 2017). Additionally, a multiplex SYBR green real-time PCR detecting mcr-1, mcr-2, and mcr-3 genes was developed (Li et al., 2017). However, although these methods are sensitive, they cannot be applied in numerous laboratories because they need specific and expensive equipment. A multiplex method detecting the 5 mcr genes together was recently developed (Rebelo et al., 2018); however, this method uses a traditional PCR program, consequently last in time 3 h (amplification and gel electrophoresis) and does not include an internal control.

Here we developed a rapid (2 h; amplification and gel electrophoresis) and reliable multiplex PCR for a rapid screening of the mcr-1 to mcr-5 genes with an internal control. This technique can be adapted easily to any laboratory that possesses a PCR amplification machine. It could allow for the determination of the prevalence of these colistin resistance genes in other clinical collections such as human, animal, and environmental collections.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2018.04.010.

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Conflict of Interest

All authors declare that they have no conflict of interest.

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