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Protocols Article

Detection of plasmid-mediated colistin-resistant and carbapenem-resistant genes by multiplex PCR

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A B S T R A C T

A multiplex PCR was described to simultaneously detect *mcr-1* and frequently occurring carbapenem-resistant genes including bla_{KPC}, bla_{NDM}, bla_{IMP}, and $bla_{OXA-48-like}$ in a single reaction. The PCR product sizes of these 4 carbapenem-resistant genes were 232 bp, 438 bp, 621 bp, and 798 bp for bla_{IMP},$bla_{OXA-48-like}$, bla_{NDM}, and bla_{KPC}, respectively, whereas *mcr-1* revealed 1126 bp of PCR product. This protocol accurately detected those resistant genes in agreement with the reference strains, 127 local carbapenem-resistant Enterobacteriaceae, 8 *mcr-1* carrying Enterobacteriaceae, and 62 carbapenem-susceptible Enterobacteriaceae. This method will be useful for laboratory application and surveillance of carbapenem and/or colistin-resistant bacteria.

A R T I C L E I N F O

Protocol name: mPCR for *mcr-1* and carbapenem-resistant genes
Keywords: PCR, *mcr-1*, bla_{NDM}, bla_{KPC}, bla_{IMP}, $bla_{OXA-48-like}$
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Specifications Table

Subject area	Immunology and Microbiology
More specific subject area	Clinical Bacteriology
Protocol name	mPCR for *mcr-1* and carbapenem-resistant genes
Reagents/tools	1. JumpStart™ REDTaq® ReadyMix™ PCR Reaction Mix (Sigma-Aldrich, USA) 2. Primers IMP-F = 5'-GGAATAGAGTGGCTTAAYTCTC-3' IMP-R = 5'-GGTTTAAYAAAACAACCACC-3' OXA48-like-F = 5'-GCGTGGTTAAGGATGAACAC-3' OXA48-like-R = 5'-CATCAAGTTCAACCCAACCG-3' NDM-F = 5'-GGTTTGGCGATCTGGTTTTC-3' NDM-R = 5'-CGGAATGGCTCATCACGATC-3' KPC-F = 5'-CGTCTAGTTCTGCTGTCTTG-3' KPC-R = 5'-CTTGTCATCCTTGTTAGGCG-3' MCR1-F = 5'- GGGTGTGCTACCAAGTTTGC -3' MCR1-R = 5'- CATTGGCGTGATGCCAGTTT -3'
Experimental design	We modified a multiplex PCR for detection of acquired carbapenemase genes described by Poirel et al. [1] and added the primer to detect *mcr-1* in the same PCR reaction. This method can simultaneously detect 4 prevalent carbapenem-resistant genes (bla_{IMP}, $bla_{OXA-48-like}$, bla_{NDM}, and bla_{KPC}) and a colistin-resistant gene (*mcr-1*) in a single reaction and revealed different PCR product sizes that are easy to interpret.
Trial registration	None
Ethics	None

Value of the protocol

- Simultaneous detection of four frequent clinically relevant carbapenem-resistant genes and *mcr-1* by multiplex PCR in a single reaction.
- Rapid, simple, and reliable for detection of frequently clinically relevant carbapenem and colistin-resistant genes (*mcr-1*) from pure culture.
- Useful for laboratory application and surveillance of carbapenem-resistant and/or colistin-resistant bacteria.
- Useful for detection of isolates co-carry *mcr-1* and carbapenemase genes such as *mcr-1* and bla_{NDM}.

Description of protocol

Carbapenem-resistant organisms such as bla_{KPC}, bla_{NDM}, bla_{IMP}, $bla_{OXA48-like}$, and the emergence of the *mcr-1* gene, a plasmid-mediated gene that confers colistin resistance in *Enterobacteriaceae,* have both been increasingly recognized worldwide. The spread of *mcr-1*-encoding plasmids into carbapenem-resistant *Enterobacteriaceae* raises concerns about the emergence of untreatable bacteria and it poses a serious threat to public health worldwide.

Many PCR techniques have been described to detect these resistant genes; however, no PCR (especially multiplex PCR) procedure has been described for detecting both *mcr-1* and carbapenem-resistant genes in a single reaction. This study describes a protocol to simultaneously detect *mcr-1* and frequently occurring carbapenem-resistant genes (bla_{KPC}, bla_{NDM}, bla_{IMP}, $bla_{OXA48-like}$) as well as to detect co-existence of *mcr-1* and carbapenem-resistant genes in a single reaction from Gram-negative bacteria.

Major equipment and supplies for PCR assay

- PCR thermal cycler (Takara, Japan or equivalent)
- PCR tubes (Nest Scientific, USA or equivalent)
- Sterile Eppendorf style microcentrifuge tubes (Nest Scientific, USA or equivalent)