Real-time quantitative PCR assay with Taqman® probe for rapid detection of MCR-1 plasmid-mediated colistin resistance

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Abstract

Here we report the development of two rapid real-time quantitative PCR assays with TaqMan® probes to detect the MCR-1 plasmid-mediated colistin resistance gene from bacterial isolates and faecal samples from chickens. Specificity and sensitivity of the assay were 100% on bacterial isolates including 18 colistin-resistant isolates carrying the *mcr-1* gene (six *Klebsiella pneumoniae* and 12 *Escherichia coli*) with a calibration curve that was linear from 10^1 to 10^8 DNA copies. Five out of 833 faecal samples from chickens from Algeria were positive, from which three *E. coli* strains were isolated and confirmed to harbour the *mcr-1* gene by standard PCR and sequencing.

Keywords: Antibiotic resistance surveillance, colistin resistance, *mcr-1* gene, PCR detection, Taqman probe

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Introduction

The increasing prevalence of infections caused by multidrug-resistant Gram-negative bacteria combined with few antimicrobial agents being in development has led to a resurgence in interest in colistin as a last-line therapy with the inevitable risk of emerging resistance [1–3]. MCR-1 plasmid-mediated colistin resistance is a member of the phosphoethanolamine transferase enzyme family, with expression in *Escherichia coli* resulting in the addition of phosphoethanolamine to lipid A and resistance to colistin [4]. This plasmid-mediated colistin resistance is an emerging concern that has already spread worldwide [5] in *E. coli* and *Klebsiella pneumoniae* from pigs, chicken, retail meat (pork, chicken), humans [4]. In animal health, colistin is used to prevent infections from *E. coli* isolates that are known to cause serious adverse effects such as diarrhoea, sepsis and colibacillosis, which result in huge economic losses [6]. The extensive use of antibiotics in food-animal production has been shown to increase the risk of transferring resistant bacteria to humans [7].

There is a need to screen for colistin resistance even in patients without a history of colistin usage for the timely detection and isolation of patients harbouring such resistant strains to prevent clonal transmission [8]. For this reason the aim of this study was to develop rapid real-time quantitative PCR (qPCR) to detect the MCR-1 plasmid-mediated colistin resistance and to evaluate its sensitivity and specificity both from strains and stool samples.

Materials and methods

Specific primers and probes design

Primers and probes design. We designed specific primers and probes to develop two real-time qPCR assays (PE1 and PE2) for the detection of MCR-1-encoding gene (Table 1). Specificity of the primers and probes were verified *in silico* by BLASTN analysis on the National Center for Biotechnology Information (NCBI) database.

Sample collection

Bacterial strains. A total of 100 strains from humans and animals were used in this study including 18 colistin-resistant isolates carrying the *mcr-1* gene (six *K. pneumoniae* and 12 *E. coli*).

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TABLE 1. Primers and probe designed to target the plasmid-mediated colistin resistance (MCR-1)

Primer/probe name	Sequence	PCR product size (bp)	References
Real-time PCR			
PE_F1	GCAGCATACTTCTGTGTGGTAC	145	This study
PE_R1	ACAAAGCCGAGATTGTCCGCG		
PE_Probe 1	6 FAM –GACCGCGACCGCCAATCTTACC-TAMRA		
PE_F2	GGGTGTGCTACCAAGTTTGCTT		
PE_R3	TATGCACGCGAAAGAAACTGGC		
PE_Probe	6 FAM –GCGCTGATTTTACTGCCTGTGGTG-TAMRA		
Standard PCR			
PE_F1	GCAGCATACTTCTGTGTGGTAC	554	This study
PE_R3	TATGCACGCGAAAGAAACTGGC		
CLR5-F	5′-CGGTCAGTCCGTTTGTTC-3′		[4]
CLR5-R	5′-CTTGGTCGGTCTGTA GGG-3′		

TABLE 2. Presentation of strains of the study with the genes specificity

Species	Presence of gene MCR-1	COL MIC (mg/L)	Genes specificity	CT value with PE1 system	CT value with PE2 system	Origins	References
Escherichia coli (*n* = 25)	+ (*n* = 12) − (*n* = 13)	4–16 <1–16	None	18–25 0	19–25 0	Thailand, Laos, Algeria, France, Nigeria.	[5,7] unpublished data
Klebsiella pneumoniae (*n* = 33)	+ (*n* = 6) − (*n* = 27)	4–32 <1–32	*mgrB** (*n* = 2) pmrB*(*n* = 1) *mgrB** (12)	18–24 0	19–25 0	Thailand, Laos, France, Nigeria, Algeria	[9] unpublished data
Klebsiella oxytoca (*n* = 2)	–	6–12	*mgrB** (*n* = 1)	0	0		[9]
Salmonella enterica subsp. *enterica* (*n* = 5)	–	0.125–16	pmrB* (*n* = 2) $bla_{CTX-M-2}$ (*n* = 5)	0	0	France	[10]
Pseudomonas aeruginosa (*n* = 10)	–	<1	bla_{VIM-2} (*n* = 10)	0	0	Lebanon	[11]
Acinetobacter baumannii (*n* = 10)	–	<1	bla_{OXA23} (*n* = 2), bla_{OXA24} (*n* = 2), bla_{OXA58} (*n* = 1), bla_{VEB} (*n* = 1)	0	0	Algeria	[12–14]
Providencia rettgeri (*n* = 2)	–	>256	bla_{NDM-1} (*n* = 1)	0	0	Israel	[15]
Morganella morganii (*n* = 2)	–	>256	bla_{NDM-1} (*n* = 1)	0	0	Israel	[16]
Enterobacter cloacae (*n* = 5)	–	<1	None	0	0	Laos, Nigeria.	unpublished data
Proteus mirabilis (*n* = 2)	–	>256	None	0	0	Algeria	unpublished data
Proteus vulgaris (*n* = 2)	–	>256	None	0	0	Algeria	unpublished data
Serratia marcescens (*n* = 2)	–	>256	None	0	0	Algeria	unpublished data

* Mutation; +, positive; −, negative.
COL MIC, minimum inhibitory concentration of colistin.

Phenotypic and genotypic features of these strains are summarized in Table 2.

Chicken stool collection. A total of 833 faeces samples from broilers were collected between August and February 2015 from eight regions in Algeria (El Tarf, Souk Ahras, Skikda, Setif, Jijel, Algiers, Biskra and Ourgla; *n* = 503) and in three slaughterhouses in Marseille (*n* = 330). All the extracted DNA from the 833 faeces of broilers was tested using our qPCR assay and positive samples were inoculated on agar for isolation of positive *mcr-1* isolates.

Molecular analysis

Strategy for PCR amplification and sequencing. Standard PCR amplification and sequencing of the MCR-1-encoding gene was used as the gold standard and performed as previously described [4]. Quantification of the MCR-1-encoding gene using our two sets of primers and probes was performed using a quantitative CFX96™ Real Time system C1000™ Touch thermal cycler (Bio-Rad, Singapore). The qPCR conditions were as follows: the reaction mixtures were kept at 95°C for 15 min and subsequently put through 35 cycles of 95°C for 30 s and 60°C for 1 min.

Specificity and reproducibility of the new system of real-time PCR. The specificity of the primers and probes were verified *in vitro* using our local collection of 100 strains (Table 2). The sensitivity of our assays was determined using ten-fold serial dilutions (between 10^8 and 10^1 DNA copies) of *E. coli* strain P10 by triplicate amplification, the number of *mcr-1* in each sample was calculated based on the DNA copy numbers. The obtained Ct values were used to generate the calibration curves compared with the number of bacteria quantified by standard bacterial count on agar plates. The standard curve was