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Rapid Polymyxin NP test for the detection of polymyxin resistance mediated by the *mcr-1*/*mcr-2* genes

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ABSTRACT

The Rapid Polymyxin NP test has been recently developed to rapidly detect polymyxin resistance in Enterobacteriaceae. Here we evaluated this test for detecting MCR-1/MCR-2-producing Enterobacteriaceae using a collection of 70 non-redundant strains either recovered from the environment, animals, or humans. Sensitivity and specificity were found to be 100%.

Polymyxins are becoming the last-resort antibiotics against multidrug resistant Enterobacteriaceae (Poirel et al., 2017). In addition to chromosome encoded-mechanisms of resistance to polymyxins, two transferable polymyxin resistance genes, *mcr-1* and *mcr-2* have been identified mostly in *Escherichia coli* since November 2015 among humans, animals, retail meat, and environment (Schwarz and Johnson, 2016; Xavier et al., 2016). Antibiotic susceptibility techniques for determining polymyxin resistance such as E-test and disc diffusion are not reliable due mostly to the poor diffusion of polymyxins in agar (Poirel et al., 2017). The broth microdilution method (BMD) is the reference technique recommended by the Clinical Laboratory Standard Institute (CLSI, 2015) in the US and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2016) in Europe. However, the BMD technique is time consuming (24 h) and requires precise weighting of the polymyxin powder that may constitute a source of error. Recently, the Rapid Polymyxin NP test has been developed to detect polymyxin resistance in Enterobacteriaceae. It is based on the detection of glucose metabolization associated with bacterial growth in the presence of a given concentration of polymyxin B or colistin. When growth occurs (resistant strain), formation of acid metabolism in less than 2 hours is evidenced by a color change of a pH indicator, red phenol (Nordmann et al., 2016). (See Fig. 1.)

Our aim was to evaluate this Rapid Polymyxin NP test for detecting MCR-1/MCR-2-producing Enterobacteriaceae showing resistance to polymyxins, using a collection of enterobacterial strains either recovered from the environment, animals, or humans. A total of 70 non-duplicate *mcr-1*- and *mcr-2*-positive enterobacterial isolates from different origins, isolated between 2011 and 2016, has been studied (Table 1). Among them, 55% were extended-spectrum ß-lactamase (ESBL) producers and 1.5% were carbapenemase producers. The phylogenetic groups of MCR producers *Escherichia coli* strains have been determined using the Clermont quadruplex PCR (Clermont et al., 2013). This method uses the combination of three genes (*chuA*, an outer membrane hemin receptor; *yjaA*, coding for an unknown protein; *arpA*, coding for the Ankyrin repeat protein) and a DNA fragment called TspE4.C2 to divide *E. coli* strains in A, B1, B2, C, D, E, F phylogenetic groups (Clermont et al., 2000, 2013). *E. coli* harboring the A group colonize various environments (omnivorous mammals, herbivorous mammals, ectothermic and endothermic vertebrates). *E. coli* group B1

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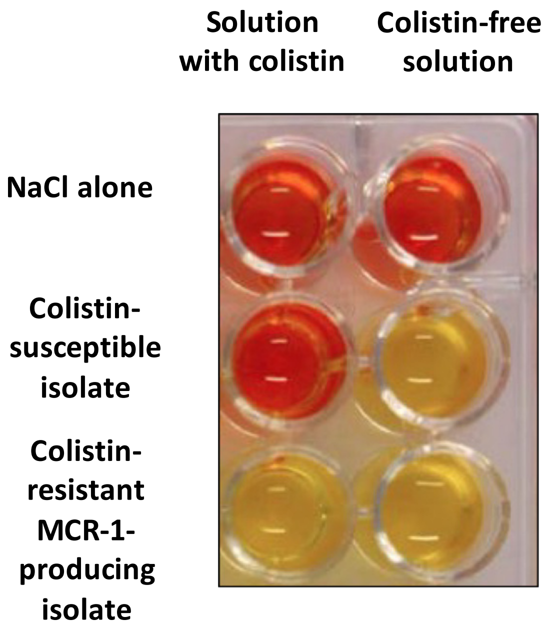

Fig. 1. Representative results of the Rapid Polymyxin NP test. The Rapid Polymyxin NP test was performed with a control (non-inoculated well, first line), a reference colistin-susceptible isolate (second line), and a reference colistin-resistant isolate (third line). The first column is supplemented with colistin, while second column is free of colistin. The photograph was taken after a 1-hour incubation time. The yellow-to-red collar change indicates bacterial growth.

are found in various environments, but seemed to be able to live more easily outside their host, as a secondary habitat (Gordon and Cowling, 2003; Walk et al., 2007). The strains harboring virulent factors and found in extraintestinal infection are usually from the B2 and D phylogenic groups (F sister group to B2) (Clermont et al., 2013; Johnson et al., 2000). In this study, the retail meat and the environmental *E. coli* were almost all from the phylogenetic group A and B1 (Table 1). Intestinal infectious strains seemed more likely to be from the A, B1 and D groups (Pupo et al., 1997), which correlates with origin of the strains tested in this study (Table 1). The MCR producers included five in-vitro obtained MCR-1-positive transconjugants obtained either from *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae* or *Enterobacter aerogenes* as donors, and obtained in a previous study (Dénervaud-Tendon et al., 2017). Those transconjugants permitted to test additional enterobacterial species that produce MCR-1. BMD was performed in cation-adjusted Mueller-Hinton broth (MHB-CA, Bio-Rad, Marnes-La-Coquette, France) to precisely determine the minimal inhibitory concentrations (MIC). Colistin sulfate (Sigma-Aldrich, St. Louis, MO, USA) was tested over a range of concentrations (0.12–256 μg/ml). The MIC breakpoints of polymyxins for Enterobacteriaceae are as follows susceptibility ≤2 μg/ml and resistance >2 μg/ml (The European Committee on Antimicrobial Susceptibility Testing, 2017).

MICs of colistin for MCR-1/MCR-2 producers were variable (4–64 μg/ml), but overall low (Table 1). All resistant strains were detected as being resistant by using the Rapid Polymyxin NP test with a sensitivity of 100% regardless of the species and of the origin of the strains

Table 1
Rapid Polymyxin NP test results for polymyxin-resistant and MCR-producing Enterobacteriaceae and for polymyxin-susceptible Enterobacteriaceae.

Strain	Species	Colistin resistance	ESBL or carbapenemase	Phylogenetic group	MIC of colistin (μg/ml)	Rapid Polymyxin NP test	Origin	Isolation date
Colistin resistant strains								
Human strains								
R2911	*E. coli*	MCR-1	–	A	4	+	Human, Switzerland	July 2016
R2912	*E. coli*	MCR-1	–	F	8	+	Human, Switzerland	July 2016
R2739	*E. coli*	MCR-1	–	A	4	+	Human blood, South Africa	August 2015
R2740	*E. coli*	MCR-1	–	B1	4	+	Human pus, South Africa	August 2015
R2741	*E. coli*	MCR-1	–	F	8	+	Human urine, South Africa	August 2015
R2742	*E. coli*	MCR-1	CTX-M	E	4	+	Human wound, South Africa	August 2015
R2743	*E. coli*	MCR-1	–	B1	8	+	Human urine, South Africa	August 2015
R2744	*E. coli*	MCR-1	–	F	4	+	Human urine, South Africa	August 2015
R2745	*E. coli*	MCR-1	CTX-M	A	4	+	Human urine, South Africa	August 2015
R2746	*E. coli*	MCR-1	–	F	8	+	Human, Switzerland	January 2016
R2747	*E. coli*	MCR-1	TEM-52	B2	16	+	Human, Switzerland	January 2016
R2748	*E. coli*	MCR-1	CTX-M	A	4	+	Human, Switzerland	January 2016
R2749	*E. coli*	MCR-1	CTX-M	A	4	+	Human, Switzerland	January 2016
R2750	*E. coli*	MCR-1	–	D	4	+	Human, France	March 2016
R2751	*E. coli*	MCR-1	–	D	8	+	Human, France	March 2016
R2752	*E. coli*	MCR-1	VIM	A	4	+	Human, Switzerland	November 2015
R2757	*E. coli*	MCR-1	–	A	8	+	Human, France	May 2016
Animal strains								
R2768	*E. coli*	MCR-1	CTX-M	A	4	+	Calf, feces, France	January 2012
R2770	*E. coli*	MCR-1	CTX-M	D	16	+	Calf, feces, France	March 2011
R2771	*E. coli*	MCR-1	CTX-M	D	8	+	Calf, respiratory tract, France	May 2011
R2773	*E. coli*	MCR-1	CTX-M	D	8	+	Calf, feces, France	August 2011
R2776	*E. coli*	MCR-1	CTX-M	A	64	+	Calf, France	December 2012
R2777	*E. coli*	MCR-1	CTX-M	A	8	+	Calf, France	July 2011
R2778	*E. coli*	MCR-1	CTX-M	D	16	+	Calf, feces, France	January 2012
R2782	*E. coli*	MCR-1	CTX-M	D	8	+	Calf, feces, France	February 2011
R2784	*E. coli*	MCR-1	CTX-M	A	16	+	Calf, feces, France	May 2012
R2786	*E. coli*	MCR-1	CTX-M	D	16	+	Calf, feces, France	March 2012
R2790	*E. coli*	MCR-1	CTX-M	D	16	+	Calf, feces, France	February 2012
R2791	*E. coli*	MCR-1	CTX-M	A	16	+	Calf, feces, France	March 2013
R2794	*E. coli*	MCR-1	CTX-M	B1	8	+	Calf, feces, France	May 2012
R2795	*E. coli*	MCR-1	CTX-M	D	8	+	Calf, feces, France	October 2012
R2796	*E. coli*	MCR-1	CTX-M	A	16	+	Calf, feces, France	December 2012
R2797	*E. coli*	MCR-1	CTX-M	A	8	+	Calf, feces, France	March 2012
R2798	*E. coli*	MCR-1	CTX-M	A	16	+	Calf, feces, France	February 2012
R2799	*E. coli*	MCR-1	CTX-M	E	8	+	Calf, intestinal tract, France	May 2012

Table 1 *(continued)*

Strain	Species	Colistin resistance	ESBL or carbapenemase	Phylogenetic group	MIC of colistin (μg/ml)	Rapid Polymyxin NP test	Origin	Isolation date
R2800	*E. coli*	MCR-1	CTX-M	A	8	+	Calf, feces, France	August 2012
R2801	*E. coli*	MCR-1	CTX-M	A	8	+	Calf, feces, France	November 2012
R2803	*E. coli*	MCR-1	CTX-M	D	8	+	Calf, intestinal tract, France	July 2012
R2804	*E. coli*	MCR-1	CTX-M	A	8	+	Calf, intestinal tract, France	October 2012
R2805	*E. coli*	MCR-1	CTX-M	A	16	+	Calf, sepsis, France	July 2012
R2806	*E. coli*	MCR-1	CTX-M	A	8	+	Calf, feces, France	May 2012
R2807	*E. coli*	MCR-1	CTX-M	B1	8	+	Calf, intestinal tract, France	October 2012
R2810	*E. coli*	MCR-1	CTX-M	F	16	+	Calf, sepsis, France	April 2012
R2812	*E. coli*	MCR-2	–	A	8	+	Pig, Belgium	August 2016
R2984	*K. pneumoniae*	MCR-1	–	NA	8	+	Pig, Portugal	July 2016
R2985	*K. pneumoniae*	MCR-1	–	NA	32	+	Pig, Portugal	July 2016
R2986	*K. pneumoniae*	MCR-1	–	NA	64	+	Pig, Portugal	July 2016
Food strains								
R2897	*E. coli*	MCR-1	–	A	8	+	Chicken retail meat, Germany	August 2016
R2898	*E. coli*	MCR-1	–	A	8	+	Chicken retail meat, Germany	August 2016
R2899	*E. coli*	MCR-1	–	B1	8	+	Chicken retail meat, Germany	August 2016
R2900	*E. coli*	MCR-1	–	A	4	+	Chicken retail meat, Italy	August 2016
R2901	*E. coli*	MCR-1	–	B1	4	+	Chicken retail meat, Germany	August 2016
R2902	*E. coli*	MCR-1	–	A	8	+	Chicken retail meat, Germany	August 2016
R2903	*E. coli*	MCR-1	SHV-12	B1	4	+	Chicken retail meat, Germany	July 2015
R2904	*E. coli*	MCR-1	CTX-M	B1	4	+	Chicken retail meat, Italy	July 2016
R2905	*E. coli*	MCR-1	–	B1	4	+	Turkey retail meat, Germany	August 2016
R2906	*E. coli*	MCR-1	–	B1	4	+	Turkey retail meat, Germany	August 2016
R2907	*E. coli*	MCR-1	–	B2	8	+	Turkey retail meat, Germany	August 2016
R2908	*E. coli*	MCR-1	–	A	8	+	Turkey retail meat, Germany	August 2016
R2753	*S. enterica*	MCR-1	–	NA	16	+	Pig retail meat, Portugal	January 2011
R2754	*S. enterica*	MCR-1	CTX-M	NA	8	+	Pig retail meat, Portugal	January 2011
R2755	*S. enterica*	MCR-1	–	NA	8	+	Chicken retail meat, Portugal	January 2011
R2756	*S. enterica*	MCR-1	–	NA	8	+	Calf retail meat, Portugal	January 2012
Environmental strains								
R2910	*E. coli*	MCR-1	CTX-M	A	8	+	Cha-om Plant, Thailand	2014
R2913	*E. coli*	MCR-1	SHV-12	B1	8	+	River water, Switzerland	2012
Transconjugants								
P6–20	*E. aerogenes*	MCR-1	–	NA	4	+	In-vitro obtained	NA
P6–24	*E. cloacae*	MCR-1	–	NA	16	+	In-vitro obtained	NA
P6–27	*K. oxytoca*	MCR-1	–	NA	16	+	In-vitro obtained	NA
P6–30	*K. pneumoniae*	MCR-1	–	NA	64	+	In-vitro obtained	NA
P6–39	*E. coli* J53	MCR-1	–	NA	8	+	In-vitro obtained	NA
Colistin Susceptible strains								
R110	*E. coli* J53	–	–	ND	0.25	–	Human, France	November, 2013
C349	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C352	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C353	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C355	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C358	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C359	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C360	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C361	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C363	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C365	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C368	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C369	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C371	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C372	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C373	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C374	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C375	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C376	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C377	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C378	*E. coli*	–	–	ND	0.12	–	Human, Switzerland	February 2016
C354	*K. pneumoniae*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C362	*K. pneumoniae*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C364	*K. pneumoniae*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C367	*K. pneumoniae*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C370	*K. pneumoniae*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C379	*K. pneumoniae*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C382	*K. pneumoniae*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C351	*K. oxytoca*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C356	*K. oxytoca*	–	–	NA	0.12	–	Human, Switzerland	February 2016
C384	*K. oxytoca*	–	–	NA	0.12	–	Human, Switzerland	February 2016

(continued on next page)

Table 1 *(continued)*

Strain	Species	Colistin resistance	ESBL or carbapenemase	Phylogenetic group	MIC of colistin (µg/ml)	Rapid Polymyxin NP test	Origin	Isolation date
C350	*E. cloacae*	−	−	NA	0.12	−	Human, Switzerland	February 2016
C366	*E. cloacae*	−	−	NA	0.12	−	Human, Switzerland	February 2016
C393	*E. cloacae*	−	−	NA	0.12	−	Human, Switzerland	February 2016
C357	*E. aerogenes*	−	−	NA	0.12	−	Human, Switzerland	February 2016

NA, not applicable; ND, not determined; +, positive; −, negative.

(Table 1). Also, regardless of the phylogenetic group the *E. coli* strains belonged to, all resistant ones were detected by using the Rapid Polymyxin NP test. This test would be useful in many situations including the screening of polymyxin resistant isolates from animal husbandry and the environment. The five *E. coli* transconjugants expressing MCR-1 were also detected by the Rapid Polymyxin NP. The specificity of the Rapid Polymyxin NP was 100% in the present study, although 35 MCR-negative strains had been included (Table 1). The Rapid Polymyxin NP test showed excellent sensibility and specificity toward all kind of Enterobacteriaceae tested and that corresponded to a large collection of MCR producers.

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Declaration of Interest

An international patent form has been filed on behalf of the University of Fribourg, Switzerland corresponding to the Rapid Polymyxin NP test.

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