





Evaluation of a New Commercial Microarray Platform for the Simultaneous Detection of β -Lactamase and *mcr-1* and *mcr-2* Genes in *Enterobacteriaceae*

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The worldwide prevalence of infections due to Gram-negative bacteria co-resistant to extended-spectrum cephalosporins and carbapenems is worrisome. For these organisms, polymyxins are considered the last-resort antibacterials. Thus, the recent emergence of the plasmid-mediated colistin resistance genes *mcr-1*, *mcr-2*, and *mcr-3* has become an alarming public health issue (1, 2) that urges rapid and reliable detection methods (3–6).

In this work, we evaluated the performance of the new commercial CT103XL microarray system (Check-Points). In ~6.5 h (starting from culture strains) and for a cost ranging from 50 to 85€, this platform allows the detection of *mcr-1* (including the variants from *mcr-1.2* to *mcr-1.7*) and *mcr-2* determinants. Along with them, clinically important class A, B, C, and D β -lactamase (*bla*) genes are simultaneously detected, as in the former version (CT103XL) that has demonstrated an overall accuracy of >94% (7).

We tested 106 *Enterobacteriaceae* isolates of human, animal, and food origin consisting of 80 strains of *Escherichia coli*, 14 of *Klebsiella pneumoniae*, and 12 of other species (Table 1). Most isolates were phenotypically (e.g., MICs for colistin) and molecularly (i.e., PCR/sequencing of *mcr-1* and *mcr-2* and *bla* genes or whole-genome sequencing) characterized in previous studies (4, 8–11) (see also Table S1 in the supplemental material). Sixty-one strains (57.5%) were resistant to colistin (12), of which 30 were positive for *mcr-1* (including one positive for *mcr-1.2*) and two were positive for *mcr-2*. This group of 32 isolates (30.2%) included 7 *bla*_{ESBL} and one *bla*_{CMY-2} carrier, as well as 24 without *bla* or possessing only *bla*_{TEM-1-like}. The other 74 *mcr-1*- and *mcr-2*-negative strains included 32 carbapenemase- and 24 extended-spectrum β -lactamase (ESBL)- and/or pAmpC-producing strains (Table S1).

Total bacterial DNA was rapidly extracted from fresh cultured bacteria using Chelex-100 resin (Sigma-Aldrich). In the case of an unclear result, a new extraction was performed using the High Pure PCR template preparation kit (Roche). Then, microarray analyses were performed according to the manufacturer's instructions. The new CT103XL uses a multiplex ligation detection reaction. In brief, each probe consists of two arms containing target gene-specific sequence, a universal primer binding site, and a ZIP code for the hybridization (Fig. S1A). Ligated and amplified probes are hybridized to the microarray, visualized using biotin label incorporated into the primer, and automatically interpreted by software (13).

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TABLE 1 Origin, resistance genes, and accuracy in detecting *mcr-1* and *mcr-2* of the new CT103XL microarray for the 106 tested strains

Species (no. of isolates)	No. (%) of strains				Showing detection of <i>mcr-1</i> and <i>mcr-2</i> by:							
	By origin:				Possessing relevant <i>bla</i> genes ^c :							
	Human ^b	Animal	Food chain	None ^e	pAmpCs	ESBLs	pAmpCs and ESBLs	At least 1 carbapenemase	Colistin resistant ^d	<i>mcr-1</i>	<i>mcr-2</i>	New CT103XL microarray
<i>E. coli</i> (80) ^a	52 (65.0)	21 (26.3)	7 (8.8)	34 (42.5)	9 (11.2)	21 (26.2)	1 (1.2)	15 (18.7)	42 (52.5)	30 (37.5)	2 (2.5)	32 ^f (100)
<i>K. pneumoniae</i> (14)	14 (100)	0	0	0	0	0	0	14 (100)	9 (64.3)	0	0	0
Other <i>Enterobacteriaceae</i> (12) ^h	11 (91.7)	0	1 (8.3)	7 (58.3)	0	2 (16.7)	0	3 (25.0)	10 (83.3)	0	0	0
Total (106)	77 (72.6)	21 (19.8)	8 (7.5)	41 (38.7)	9 (8.5)	23 (21.7)	1 (0.9)	32 (30.2)	61 (57.5)	30 (28.3)	2 (1.9)	32 ^f (100)

^aIncludes the plasmid extract (i.e., plasmid 1 [see Table S1]).^bIncludes control laboratory strains (i.e., ATCC BAA-2452, ATCC 35218, GC 2919, and AH3966 [see Table S1]).^cMost strains were characterized in previous studies implementing PCR/sequencing and/or whole-genome sequencing; for the remaining, the results of the new CT103XL were taken into account. Notably, the majority of strains possess more than one *bla* gene (see Table S1).^dMIC values for colistin were obtained with different methods (i.e., Vitek, microdilution Trek panels, and Etest). Strains were defined as resistant with MICs of >2 µg/ml. Notably, only broth microdilution is the reference method for testing colistin susceptibility (12).^ePCR/sequencing or whole-genome sequencing.^fTwo strains (Table S1) were identified in the second test (i.e., the first test with the new CT103XL failed because of the low DNA concentration).^gIncludes TEM-1-like producers.^hIncludes *Salmonella enterica* serovar Kentucky (*n* = 1), *Serratia marcescens* (*n* = 2), *Proteus mirabilis* (*n* = 2), *Klebsiella oxytoca* (*n* = 1), *Helina alvei* (*n* = 2), *Citrobacter braakii* (*n* = 1), *Citrobacter freundii* (*n* = 1), *Enterobacter asburiae* (*n* = 1), and *Enterobacter cloacae* (*n* = 1). See Table S1.

The *mcr-1* and *mcr-2* genes were correctly detected by the new array system in all 32 isolates (Table 1). In particular, the 30 *mcr-1* carriers displayed positive reactions with all three new hybridization spots (Fig. S1B), corresponding to *mcr-1* detection. On the other hand, both *mcr-2*-harboring strains displayed positive results with two of the three spots, revealing *mcr-2* (Fig. S1C). Two microarray analyses had to be repeated due to inadequate template concentration. The second run, performed with new DNA extracts, yielded correct results (Table S1). Moreover, all 74 strains lacking *mcr-1* and *mcr-2* resulted negative after microarray analyses. Therefore, the new CT103XL platform showed 100% accuracy in identifying *mcr-1* and *mcr-2*. We also noted that all *bla* genes were correctly identified (Table S1), thereby confirming previous validations (7, 14, 15).

This work demonstrated the ability and accuracy of the new CT103XL array to simultaneously identify *mcr-1* and *mcr-2* and clinically important *bla* genes. This strengthened platform represents therefore an essential tool for clinical laboratories, especially for the rapid characterization of large collections of multidrug-resistant Gram-negative bacteria, providing essential epidemiological data. The test is unable to detect the recently described *mcr-3* (only 45% and 47% identity to *mcr-1* and *mcr-2*, respectively) (2), but the flexibility of the microarray allows easy and rapid updating of the platform to include novel and emerging antimicrobial resistance traits.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/JCM.01056-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

SUPPLEMENTAL FILE 2, PDF file, 0.3 MB.

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