AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

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

Odette J. Bernasconi,[a,b] Luigi Principe,[c] Regula Tinguely,[a] Aneta Karczmarek,[d] Vincent Perreten,[e] Francesco Luzzaro,[c] Andrea Endimiani[a]

Institute for Infectious Diseases, University of Bern, Bern, Switzerland[a]; Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland[b]; Clinical Microbiology and Virology Unit, A. Manzoni Hospital, Lecco, Italy[c]; Check-Points, B.V., Wageningen, The Netherlands[d]; Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland[e]

KEYWORDS colistin, ESBL, *Enterobacteriaceae*, β-lactamases, carbapenemases, detection, diagnostics, *mcr-1*, *mcr-2*, microarrays

The worldwide prevalence of infections due to Gram-negative bacteria coresistant 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).

Accepted manuscript posted online 2 August 2017

Citation Bernasconi OJ, Principe L, Tinguely R, Karczmarek A, Perreten V, Luzzaro F, Endimiani A. 2017. Evaluation of a new commercial microarray platform for the simultaneous detection of β-lactamase and *mcr-1* and *mcr-2* genes in *Enterobacteriaceae*. J Clin Microbiol 55:3138–3141. https://doi.org/10.1128/JCM.01056-17.

Editor Erik Munson, Marquette University

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Andrea Endimiani, aendimiani@gmail.com.

Downloaded from http://jcm.asm.org/ on April 11, 2021 by guest

Downloaded from http://jcm.asm.org/ on April 11, 2021 by guest

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											
	By origin:			Possessing relevant *bla* genes[c]:						Showing detection of *mcr-1* and *mcr-2* by:		
										Standard methods[e]		
	Human[b]	Animal	Food chain	None[g]	pAmpCs	ESBLs	pAmpCs and ESBLs	At least 1 carbapenemase	Colistin resistant[d]	*mcr-1*	*mcr-2*	New CT103XL microarray
E. coli (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)
K. pneumoniae (14)	14 (100)	0	0	0	0	0	0	14 (100)	9 (64.3)	0	0	0
Other *Enterobacteriaceae* (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)

[a]Includes the plasmid extract (i.e., plasmid 1 [see Table S1]).
[b]Includes control laboratory strains (i.e., ATCC BAA-2452, ATCC 35218, GC 2919, and AH3966 [see Table S1]).
[c]Most 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).
[d]MIC 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).
[e]PCR/sequencing or whole-genome sequencing.
[f]Two 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).
[g]Includes TEM-1-like producers.
[h]Includes *Salmonella enterica* serovar Kentucky ($n = 1$), *Serratia marcescens* ($n = 2$), *Proteus mirabilis* ($n = 2$), *Klebsiella oxytoca* ($n = 1$), *Hafnia 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.

ACKNOWLEDGMENTS

This work was supported by the Swiss National Science Foundation (SNF grant no. 153377 to A.E.). Odette J. Bernasconi is a Ph.D. student (2015 to 2018) supported by the Hans Sigrist Foundation (Bern, Switzerland).

A.K. is an employee of Check-Points. The new CT103XL microarrays used in the present study were provided for free by Check-Points to A.E.

We thank Surbhi Malhotra-Kumar (University of Antwerp, Wilrijk, Belgium) for donating the *mcr-2*-positive *E. coli* strain.

REFERENCES

1. Poirel L, Jayol A, Nordmann P. 2017. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 30:557–596. https://doi.org/10.1128/CMR.00064-16.
2. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y. 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. mBio 8:e00543-17. https://doi.org/10.1128/mBio.00543-17.
3. Jayol A, Nordmann P, Lehours P, Poirel L, Dubois V. 2017. Comparison of methods for detection of plasmid-mediated and chromosomally-encoded colistin resistance in *Enterobacteriaceae*. Clin Microbiol Infect https://doi.org/10.1016/j.cmi.2017.06.002.
4. Dona V, Bernasconi OJ, Kasraian S, Tinguely R, Endimiani A. 2017. A SYBR Green-based real-time PCR method for improved detection of *mcr-1*-mediated colistin resistance in human stool samples. J Glob Antimicrob Resist 9:57–60. https://doi.org/10.1016/j.jgar.2017.01.007.
5. Imirzalioglu C, Falgenhauer L, Schmiedel J, Waezsada SE, Gwozdzinski K, Roschanski N, Roesler U, Kreienbrock L, Schiffmann AP, Irrgang A, Kasbohrer A, Bauerfeind R, Domann E, Chakraborty T. 2017. Evaluation of a loop-mediated isothermal amplification-based assay for the rapid detection of plasmid-encoded colistin resistance gene *mcr-1* in *Enterobacteriaceae* isolates. Antimicrob Agents Chemother 61:e02326-16. https://doi.org/10.1128/AAC.02326-16.
6. Chabou S, Leangapichart T, Okdah L, Le Page S, Hadjadj L, Rolain JM. 2016. Real-time quantitative PCR assay with Taqman probe for rapid detection of MCR-1 plasmid-mediated colistin resistance. New Microbes New Infect 13:71–74. https://doi.org/10.1016/j.nmni.2016.06.017.
7. Cunningham SA, Vasoo S, Patel R. 2016. Evaluation of the Check-Points Check MDR CT103 and CT103 XL microarray kits by use of preparatory rapid cell lysis. J Clin Microbiol 54:1368–1371. https://doi.org/10.1128/JCM.03302-15.
8. Pires J, Bernasconi OJ, Hauser C, Tinguely R, Atkinson A, Perreten V, Dona V, Rauch A, Furrer H, Endimiani A. 2017. Erratum to 'Intestinal colonisation with extended-spectrum cephalosporin- and colistin-resistant *Enterobacteriaceae* in HIV-positive individuals in Switzerland: molecular features and risk factors' [International Journal of Antimicrobial Agents 49/4 (2017) 519-521]. Int J Antimicrob Agents 50:276. https://doi.org/10.1016/j.ijantimicag.2017.05.002.
9. Pires J, Bernasconi OJ, Kasraian S, Hilty M, Perreten V, Endimiani A. 2016. Intestinal colonisation with extended-spectrum cephalosporin-resistant *Escherichia coli* in Swiss pets: molecular features, risk factors and transmission with owners. Int J Antimicrob Agents 48:759–760. https://doi.org/10.1016/j.ijantimicag.2016.08.007.
10. Pires J, Kuenzli E, Kasraian S, Tinguely R, Furrer H, Hilty M, Hatz C, Endimiani A. 2016. Polyclonal intestinal colonization with extended-spectrum cephalosporin-resistant *Enterobacteriaceae* upon traveling to India. Front Microbiol 7:1069. https://doi.org/10.3389/fmicb.2016.01069.
11. Bernasconi OJ, Kuenzli E, Pires J, Tinguely R, Carattoli A, Hatz C, Perreten V, Endimiani A. 2016. Travelers can import colistin-resistant *Enterobacteriaceae*, including those possessing the plasmid-mediated *mcr-1* gene. Antimicrob Agents Chemother 60:5080–5084. https://doi.org/10.1128/AAC.00731-16.
12. European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2017. Breakpoint tables for interpretations of MICs and zone diameters, version 7.0. http://www.eucast.org.

Downloaded from http://jcm.asm.org/ on April 11, 2021 by guest

13. Endimiani A, Hujer AM, Hujer KM, Gatta JA, Schriver AC, Jacobs MR, Rice LB, Bonomo RA. 2010. Evaluation of a commercial microarray system for detection of SHV-, TEM-, CTX-M-, and KPC-type β-lactamase genes in Gram-negative isolates. J Clin Microbiol 48:2618–2622. https://doi.org/10.1128/JCM.00568-10.
14. Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P. 2012. Evaluation of a DNA microarray for the rapid detection of extended-spectrum beta-lactamases (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/MOX) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). J Antimicrob Chemother 67:1865–1869. https://doi.org/10.1093/jac/dks156.
15. Naas T, Cuzon G, Truong H, Bernabeu S, Nordmann P. 2010. Evaluation of a DNA microarray, the Check-Points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and KPC carbapenemases. Antimicrob Agents Chemother 54:3086–3092. https://doi.org/10.1128/AAC.01298-09.

Downloaded from http://jcm.asm.org/ on April 11, 2021 by guest