Simple and Inexpensive Methods for Routine Detection of Colistin-Resistant MCR-1-Positive *Escherichia coli* in Human and Veterinary Diagnostic Laboratories

Fernanda Esposito,^a Miriam R. Fernandes,^a Ralf Lopes,^b Maria E. Muñoz,^a Caetano P. Sabino,^a Marcos P. Cunha,^c Rodrigo Cayô,^d Willames M. Martins,^d Ana C. Gales,^d and Nilton Lincopan^{a,b}

Department of Clinical Analysis, School of Pharmacy, University de São Paulo, São Paulo, Brazil^a; Department of Microbiology, Institute of Biomedical Sciences, Universidade de São Paulo, São Paulo, Brazil^b; School of Veterinary Medicine, Universidade de São Paulo, São Paulo, Brazil^c; Division of Infectious Diseases, Department of Internal Medicine, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil^d

Background: The emergence and rapid dissemination of colistin-resistant Escherichia *coli* carrying the plasmid-mediated *mcr-1* gene has created an urgent need to develop specific screening methods. Methods: In this study, we evaluate four assays based on the inhibition of the MCR-1 activity by EDTA: i) a combined disk test (CDT) comparing the inhibition zones of colistin (10-µg) and colistin-plus-EDTA (10-plus-100 mM); ii) reduction of colistin MIC (CMR) in the presence of EDTA (80 µg/mL); iii) a modified rapid polymyxin Nordmann/Poirel test (MPNP) and; iv) alteration of Zeta potential $(\Delta ZP = ZP_{+EDTA}/ZP_{-EDTA})$. **Results:** We obtained accurate and reliable results for detection of MCR-1 in *E. coli* isolates recovered from human, food, and animal samples, using the following assay parameters: ≥ 3 mm difference in the inhibition zones between colistin disks without and with EDTA; \geq 2-fold colistin MIC decrease in the presence of EDTA; $\Delta ZP \ge 1.5$; and absence of metabolic activity and proliferation, indicated by unchanged color of phenol red, in the presence of colistin/EDTA, in the MPNP test. In this regard, the CDT, CMR, ΔZP and MPNP assays exhibited sensitivities of 98.3% and specificities of 100, 95, 100, and 100%, respectively, for detecting MCR-1-positive E. coli. Conclusions: Our results demonstrate that inhibition by EDTA and Zeta potential assays may provide simple and inexpensive methods for detecting MCR-1-producing E. *coli* in human and veterinary diagnostic laboratories.