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Evaluation of a Loop-Mediated Isothermal Amplification-Based Assay for the Rapid Detection of Plasmid-Encoded Colistin Resistance Gene *mcr-1* in *Enterobacteriaceae* Isolates

Can Imirzalioglu,[a] Linda Falgenhauer,[a] Judith Schmiedel,[a] Said-Elias Waezsada,[a] Konrad Gwozdzinski,[a] Nicole Roschanski,[b] Uwe Roesler,[b] Lothar Kreienbrock,[c] Arthur P. Schiffmann,[d] Alexandra Irrgang,[e] Annemarie Käsbohrer,[e,f] Rolf Bauerfeind,[g] Eugen Domann,[a] Trinad Chakraborty[a]

Justus Liebig University, Institute of Medical Microbiology, and German Center for Infection Research, Partner Site Giessen-Marburg-Langen, Giessen, Germany[a]; Freie Universitaet Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany[b]; University of Veterinary Medicine Hannover, Institute for Biometrics, Epidemiology and Information Processing, Hannover, Germany[c]; Hessian State Laboratory (LHL), Giessen, Germany[d]; Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany[e]; Veterinary University Vienna, Institute for Veterinary Public Health, Vienna, Austria[f]; Justus Liebig University Giessen, Institute of Hygiene and Infectious Diseases of Animals, Giessen, Germany[g]

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Colistin, which belongs to the class of polypeptide antibiotics known as polymyxins, is a "last-resort" antibiotic often used for the treatment of patients infected with extensively multidrug-resistant (MDR) bacteria such as carbapenem-resistant members of the family *Enterobacteriaceae*. The surge of MDR bacteria worldwide has resulted in an increased use of colistin in human medicine and the emergence of colistin resistance due to chromosomal mutations and spread of these bacteria by vertical transmission (1). This situation has recently changed with the discovery of a transmissible colistin resistance gene, *mcr-1*, present in various bacterial species and on different classes of plasmids (2, 3). MDR isolates harboring *mcr-1* have been detected mostly in livestock and livestock products but also in humans worldwide (4). Infections with MDR bacteria treatable only with colistin could become untreatable if *mcr-1* is acquired through horizontal gene transfer. It is therefore of great relevance to be able to discriminate between transferable and intrinsic colistin resistance by using rapid genotyping methods.

Here we evaluated a commercially available loop-mediated isothermal amplification (LAMP) system developed for the rapid detection of *mcr-1* (eazyplex SuperBug *mcr-1* kit; Amplex Biosystems GmbH, Giessen, Germany). To avoid loss of the *mcr-1*-encoding plasmid, colistin-resistant isolates were grown on LB plates supplemented with colistin sulfate (2 mg/liter). Sensitive isolates were grown in the absence of colistin. In total, 67 *mcr-1*-positive and 37 *mcr-1*-negative *Enterobacteriaceae* isolates, including 9 intrinsically colistin-resistant isolates, were included in the evaluation (see Table S1 in the supplemental material). The presence or absence of *mcr-1* was tested for by using the PCR assay of Liu et al. (2) and whole-genome sequencing.

Overall, for the 104 isolates tested, the LAMP test matched the results obtained by PCR/whole-genome sequencing, yielding 100.0% sensitivity and specificity (Table S1). The test clearly distinguished *mcr-1*-bearing isolates from intrinsically colistin-resistant isolates. The great advantages of the rapid molecular test were the short hands-on time

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Address correspondence to Trinad Chakraborty, Trinad.Chakraborty@mikrobio.med.uni-giessen.de.

C.I. and L.F. contributed equally to this article.

for each sample (2 min), the simplicity of sample processing, and the short total turnaround time to results (~20 min). The Genie II instrument (OptiGen Limited, Horsham, United Kingdom) is mobile and can be used for at least 4 h without a power supply. Therefore, direct use of this system for diagnostic purposes with samples derived from livestock farms, food-processing plants, or human health care settings is possible and would have a great impact on the assessment of the incidence of *mcr-1* and surveillance. This is all the more important, as incidental genotypic detection of *mcr-1* has been reported (5) and because current phenotypic tests for colistin resistance can be inconclusive, thereby masking its true incidence. The use of a rapid assay such as the eazyplex SuperBug *mcr-1* kit or other genotype-based tests, e.g., those based on real-time PCR (6–8), overcomes this limitation. A drawback of the system is that the number of samples being processed on a single machine is limited (six samples/h), and the cost of scaling up is not known. Another limitation of the test is that, according to the manufacturer's information, the eazyplex SuperBug *mcr-1* kit is only suitable for the detection of *mcr-1* and not *mcr-2*. Also, direct sampling without preculture has yet to be assessed.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02326-16.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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