Published in "Diagnostic Microbiology and Infectious Disease 90 (1): 7–10, 2018"
which should be cited to refer to this work.

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

Laurent Poirel [a,b,c], Yu Larpin [a,b], Jan Dobias [a], Roger Stephan [d], Jean-Winoc Decousser [e,f], Jean-Yves Madec [g], Patrice Nordmann [a,b,c,h,*]

[a] *Emerging Antibiotic Resistance, Medical and Molecular Microbiology Unit, Department of Medicine, University of Fribourg, Fribourg, Switzerland*
[b] *INSERM European Unit (IAME, France), University of Fribourg, Fribourg, Switzerland*
[c] *Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Switzerland*
[d] *Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland*
[e] *Department of Virology, Bacteriology - Infection Control, Parasitology - Mycology, Assistance Publique - Hôpitaux de Paris, University Hospital Henri Mondor, Créteil, France*
[f] *IAME, UMR 1137, INSERM, Paris, France*
[g] *Agence Nationale de Sécurité Sanitaire (Anses), Unité Antibiorésistance et Virulence Bactériennes, Lyon, France*
[h] *Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland*

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%.

*Keywords:*
Polymyxin
MCR
Resistance
Enterobacteriaceae
Rapid Polymyxin NP test



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

* Corresponding author. Tel.: +41 26 300 9581.
*E-mail address:* patrice.nordmann@unifr.ch (P. Nordmann).

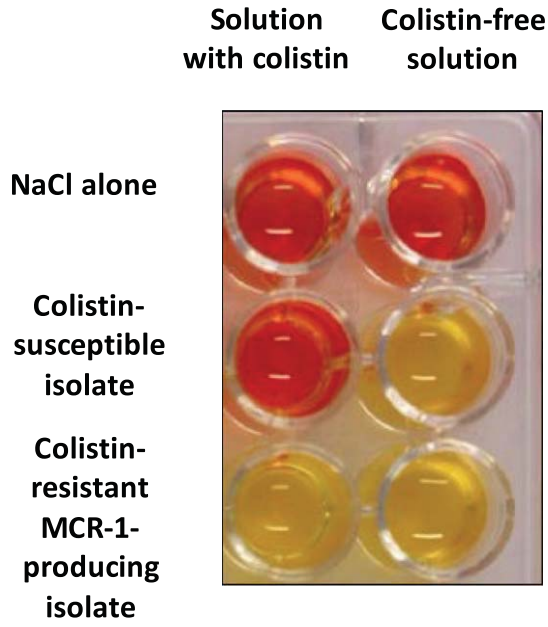


**Fig. 1.** Representative results of the Rapid Polymyxin NP test. The Rapid Polymyxin NP test was performed with a control (non-inoculated well, first line), a reference colistin-susceptible isolate (second line), and a reference colistin-resistant isolate (third line). The first column is supplemented 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.


## Funding

This work has been funded by the University of Fribourg, by grants from the ANIWHA ERA-NET project, Switzerland, by the OFSP, Bern, Switzerland (grant n°16,009,294), and by the Novartis Foundation for medical-biological Research.


## 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.


## Acknowledgments

We are grateful to S. Kumar-Malhotra for the gift of the MCR-2-producing *E. coli* strain, and G. Jorge da Silva for the gift of the MCR-1-producing *Salmonella* isolates.


## References


Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 2000;66:4555–8.

Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylotyping method revisited : improvement of specificity and detection of new phylogroups. Environ Microbiol Rep 2013;5:58–65.

Clinical and Laboratory Strandards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard. Document M07-A10. Tenth edition. Wayne (PA): The Institute; 2015.

Dénervaud-Tendon V, Poirel L, Nordmann P. Transferability of the *mcr-1* colistin resistance gene. Microb Drug Resist 2017;23:813–4.

Gordon DM, Cowling A. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. Microbiology 2003;149:3575–86.

Johnson JR, Delavari P, Kuskowski M, Stell AL. Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. J Infect Dis 2000;183:78–88.

Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in *Enterobacteriaceae*. Emerg Infect Dis 2016;22:1038–43.

Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 2017;30:557–96.

Pupo GM, Karaolis DKR, Lan R, Reeves PR. Evolutionary relationships among pathogenic and nonpathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and *mdh* sequence studies. Infect Immun 1997;65:2685–92.

Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother 2016;71:2066–70.

The European Committee on Antimicrobial Susceptibility Testing. Recommendations for MIC determination of colistin (polymyxin E) As recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. www.eucast.org, 2016.

The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters Version 7.1. http://www.eucast.org, 2017.

Walk ST, Alm EW, Calhoun LM, Mladonicky JM, Whittam TS. Genetic diversity and population structure of *Escherichia coli* isolated from freshwater beaches. Environ Microbiol 2007;9:2274–88.

Xavier BB, Lammens C, Ruhal R, Butaye P, Goossens H. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Euro Surveill 2016;21:1–5.