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The commercial Rapid Polymyxin NP test was evaluated to detect colistin-resistant Enterobacteriaceae. A total of 223 enterobacterial isolates corresponding to 136 resistant (including 38 MCR-like producers), 19 heteroresistant, and 78 colistin-susceptible isolates were tested. The test was performed according to the manufacturer's instruction, and the color of the wells was read after 2 and 3 hours of incubation. The results were compared with those of the homemade Rapid Polymyxin NP test, and manual broth microdilution according to EUCAST guidelines was used as the reference method to determine the performance of the test. Excellent performance of the commercial Rapid Polymyxin NP test was found with a very major error rate, a major error rate, a sensitivity, and a specificity of 1.9%, 5.1%, 98.1%, and 94.9%, respectively. The performance of the homemade Polymyxin NP test was similar, with a slightly better value for the very major error (1.2 %).

Polymyxins represent one of the few remaining treatment options for treating carbapenemase-producing Enterobacteriaceae. The increasing use of polymyxins is now associated with increasing isolation of colistin-resistant isolates worldwide (Poirel et al., 2017). Since the identification of the plasmid-mediated colistin resistance gene *mcr-1*, and then more recently of other MCR-like encoding genes, controlling the dissemination of colistin resistance has now become a major concern (Poirel et al., 2017). Indeed, the fear is to isolate carbapenem- and polymyxin-resistant isolates, therefore exhibiting pandrug resistance. It becomes now mandatory to possess reliable and rapid diagnostic techniques for determining polymyxin susceptibility.

The broth microdilution (BMD) technique that is time-consuming (18 h) has been retained as the reference method for evaluating polymyxin susceptibility. A rapid test (Rapid Polymyxin NP test) has been recently developed to detect colistin resistance in Enterobacteriaceae within 2 hours (Nordmann et al., 2016a,b). This test is based on the detection of the glucose metabolism of Enterobacteriaceae upon culture and differentiates colistin-susceptible from colistin-resistant isolates by supplementing the growth medium with a given concentration of colistin. An industrial version of this test is now available (ELITechGroup, Puteaux, France). The objective of this study was to evaluate the performance of this industrial Rapid Polymyxin NP test to detect colistin resistance and heteroresistance in Enterobacteriaceae.

A collection of 233 enterobacterial isolates was tested using the homemade and the industrial Rapid Polymyxin NP test. It included 136 colistin-resistant, 19 colistin-heteroresistant (Guerin et al., 2016; Hjort et al., 2016), and 78 colistin-susceptible isolates, belonging to various enterobacterial species. Among the 136 colistin-resistant isolates, 10 belonged to bacterial species intrinsically resistant to colistin, 120 had been characterized for their acquired mechanisms of resistance (chromosomally or plasmid encoded), and 6 isolates presented an unknown mechanism of resistance (Table 1). Overall, resistance mechanisms corresponded to substitutions or truncations in the PmrA/PmrB proteins, the PhoP/PhoQ regulatory proteins, and the MgrB protein. In addition, 38 of the resistant isolates produced the plasmid-mediated MCR-1, MCR-2, MCR-3, or MCR-4 determinants.

The homemade and industrial Polymyxin NP tests were both performed by using a 3–3.5 McFarland bacterial suspension into 0.9% NaCl as recommended (Nordmann et al., 2016a,b, and manufacturer guidelines). Results were interpreted by 2 independent readers after 2 and 3 hours of incubation at 37 °C. A positive test corresponded to an orange-to-yellow color change, whereas an orange color corresponded to a negative test.

Table 1
Features of the tested strain collection and comparative results between the homemade and commercialized Rapid Polymyxin NP tests.

Strains	Species	Homemade Rapid Polymyxin NP[a]	Industrial Rapid Polymyxin NP[a]	MIC of colistin (μg/mL)	Resistance mechanisms	Reference
Resistant isolates						
R1583	*P. vulgaris*	+	+	>16	Intrinsic	This study
R2260	*P. mirabilis*	+	+	>16	Intrinsic	This study
R2556	*P. mirabilis*	+	+	>16	Intrinsic	This study
R1473	*M. morganii*	+	+	>16	Intrinsic	This study
R1474	*M. morganii*	+	+	>16	Intrinsic	This study
R1607	*P. stuartii*	+	+	>16	Intrinsic	This study
R2593	*P. rettgeri*	+	+	>16	Intrinsic	This study
R2594	*P. rettgeri*	+	+	>16	Intrinsic	This study
R1278	*S. marcescens*	+	+	>16	Intrinsic	This study
R2480	*S. marcescens*	+	+	>16	Intrinsic	This study
FR-06	*K. pneumoniae*	+	+	32	PmrA G53C	(Nordmann et al., 2016a)
FR-07	*K. pneumoniae*	+	+	32	PmrA G53S	(Nordmann et al., 2016a)
FR-08	*K. pneumoniae*	+	+	128	PmrA G53S	(Nordmann et al., 2016a)
FR-09	*K. pneumoniae*	+	+	32	PmrB L17Q	(Nordmann et al., 2016a)
FR-10	*K. pneumoniae*	+	+	16	PmrB T157P	(Nordmann et al., 2016a)
FR-11	*K. pneumoniae*	+	+	32	PmrB T157P	(Nordmann et al., 2016a)
FR-12	*K. pneumoniae*	+	+	16	PmrB T157P	(Nordmann et al., 2016a)
FR-13	*K. pneumoniae*	+	+	32	PmrB T157P	(Nordmann et al., 2016a)
FR-14	*K. pneumoniae*	+	+	32	PmrB T157P	(Nordmann et al., 2016a)
FR-15	*K. pneumoniae*	+	+	16	PmrB T157P	(Nordmann et al., 2016a)
FR-16	*K. pneumoniae*	+	+	128	PhoP D191Y and deletion of 25 nt	(Nordmann et al., 2016a)
FR-17	*K. pneumoniae*	+	+	>128	PhoQ R16C	(Nordmann et al., 2016a)
FR-18	*K. pneumoniae*	+	+	32	MgrB W20R	(Nordmann et al., 2016a)
FR-19	*K. pneumoniae*	+	+	32	MgrB M27K	(Nordmann et al., 2016a)
FR-20	*K. pneumoniae*	+	+	64	MgrB C39Y	(Nordmann et al., 2016a)
FR-22	*K. pneumoniae*	+	+	64	MgrB I45T	(Nordmann et al., 2016a)
FR-23	*K. pneumoniae*	+	+	64	MgrB P46S	(Nordmann et al., 2016a)
FR-24	*K. pneumoniae*	+	+	4	MgrB W47R	(Nordmann et al., 2016a)
FR-25	*K. pneumoniae*	+	+	128	MgrB truncated (2 aa)	(Nordmann et al., 2016a)
FR-26	*K. pneumoniae*	+	+	128	MgrB truncated (2 aa)	(Nordmann et al., 2016a)
FR-27	*K. pneumoniae*	+	+	64	MgrB truncated (2 aa)	(Nordmann et al., 2016a)
FR-28	*K. pneumoniae*	+	+	128	MgrB truncated (27 aa)	(Nordmann et al., 2016a)
FR-31	*K. pneumoniae*	+	+	64	MgrB truncated (29 aa)	(Nordmann et al., 2016a)
FR-32	*K. pneumoniae*	+	+	64	MgrB truncated (29 aa)	(Nordmann et al., 2016a)
FR-33	*K. pneumoniae*	+	+	32	MgrB truncated (29 aa)	(Nordmann et al., 2016a)
FR-34	*K. pneumoniae*	+	+	64	MgrB truncated (29 aa)	(Nordmann et al., 2016a)
FR-35	*K. pneumoniae*	+	+	128	MgrB truncated (29 aa)	(Nordmann et al., 2016a)
FR-36	*K. pneumoniae*	+	+	128	MgrB truncated (29 aa)	(Nordmann et al., 2016a)
FR-37	*K. pneumoniae*	+	+	32	MgrB truncated (32 aa)	(Nordmann et al., 2016a)
FR-38	*K. pneumoniae*	+	+	32	MgrB truncated (46 aa)	(Nordmann et al., 2016a)
FR-39	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-40	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*Ecp1*/$bla_{CTX-M-15}$	(Nordmann et al., 2016a)
FR-41	*K. pneumoniae*	+	+	32	*mgrB* truncated by IS*102*-like	(Nordmann et al., 2016a)
FR-42	*K. pneumoniae*	+	+	>128	*mgrB* truncated by IS*102*-like	(Nordmann et al., 2016a)
FR-43	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*903b*	(Nordmann et al., 2016a)
FR-44	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*2*	(Nordmann et al., 2016a)
FR-45	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-46	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-47	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*903b*-like	(Nordmann et al., 2016a)
FR-48	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*903*-like	(Nordmann et al., 2016a)
FR-49	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*5*-like	(Nordmann et al., 2016a)
FR-50	*K. pneumoniae*	+	+	16	*mgrB* truncated by IS*5*-like	(Nordmann et al., 2016a)
FR-51	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*5*-like	(Nordmann et al., 2016a)
FR-52	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*5*-like	(Nordmann et al., 2016a)
FR-54	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*Kpn13*	(Nordmann et al., 2016a)
FR-56	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*Kpn26*-like	(Nordmann et al., 2016a)
FR-58	*K. pneumoniae*	+	+	32	*mgrB* truncated by IS*Kpn26*-like	(Nordmann et al., 2016a)
FR-62	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*903b*	(Nordmann et al., 2016a)
FR-63	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*1R*-like	(Nordmann et al., 2016a)
FR-67	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*Kpn26*-like	(Nordmann et al., 2016a)
FR-68	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*Kpn14*	(Nordmann et al., 2016a)
FR-69	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-81	*K. pneumoniae*	+	+	64	Full *mgrB* gene deletion	(Nordmann et al., 2016a)
FR-84	*K. pneumoniae*	+	+	128	Deletion nt 70 *mgrB* and substitution nt 73	(Nordmann et al., 2016a)
FR-89	*K. pneumoniae*	+	+	>128	Deletion nt 23 to 33 *mgrB*	(Nordmann et al., 2016a)
FR-90	*K. pneumoniae*	+	+	64	Deletion nt 30 et 31 *mgrB*	(Nordmann et al., 2016a)
FR-87	*K. pneumoniae*	+	+	16	Deletion nt 100 *mgrB*	(Nordmann et al., 2016a)
FR-30	*K. pneumoniae*	+	+	>128	MgrB truncated (27 aa)	(Nordmann et al., 2016a)
FR-53	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*5*-like	(Nordmann et al., 2016a)
FR-55	*K. pneumoniae*	+	+	> 128	*mgrB* truncated by IS*Kpn26*-like	(Nordmann et al., 2016a)
FR-57	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*Kpn26*-like	(Nordmann et al., 2016a)
FR-59	*K. pneumoniae*	+	+	32	*mgrB* truncated by IS*903B*	(Nordmann et al., 2016a)

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Table 1 (*continued*)

Strains	Species	Homemade Rapid Polymyxin NP[a]	Industrial Rapid Polymyxin NP[a]	MIC of colistin (μg/mL)	Resistance mechanisms	Reference
FR-60	*K. pneumoniae*	+	+	128	*mgrB* IS*Kpn14* between +77 and +78	(Nordmann et al., 2016a)
FR-61	*K. pneumoniae*	+	+	128	*mgrB* duplication 19 nt between +84 and +85	(Nordmann et al., 2016a)
FR-64	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*903b*-like	(Nordmann et al., 2016a)
FR-65	*K. pneumoniae*	+	+	8	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-66	*K. pneumoniae*	+	+	32	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-70	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*10R*	(Nordmann et al., 2016a)
FR-71	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*Kpn14*	(Nordmann et al., 2016a)
FR-72	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*Kpn14*	(Nordmann et al., 2016a)
FR-73	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-74	*K. pneumoniae*	+	+	128	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-75	*K. pneumoniae*	+	+	64	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-76	*K. pneumoniae*	+	+	32	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-77	*K. pneumoniae*	+	+	32	*mgrB* truncated by IS*Kpn14*-like	(Nordmann et al., 2016a)
FR-78	*K. pneumoniae*	+	+	32	*mgrB* truncated by IS*1R*	(Nordmann et al., 2016a)
FR-79	*K. pneumoniae*	+	+	16	Full *mgrB* gene deletion	(Nordmann et al., 2016a)
FR-80	*K. pneumoniae*	+	+	> 128	Full *mgrB* gene deletion	(Nordmann et al., 2016a)
FR-82	*K. pneumoniae*	+	+	64	Full *mgrB* gene deletion	(Nordmann et al., 2016a)
FR-83	*K. pneumoniae*	+	+	32	Deletion nt 23 *mgrB*	(Nordmann et al., 2016a)
FR-85	*K. pneumoniae*	+	+	64	Deletion nt 74 *mgrB*	(Nordmann et al., 2016a)
FR-86	*K. pneumoniae*	+	+	64	Deletion nt 100 *mgrB*	(Nordmann et al., 2016a)
FR-93	*E. coli*	+	+	4	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
FR-94	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
FR-95	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
FR-96	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
FR-97	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
FR-98	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
FR-99	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
Af48	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	Poirel et al., 2016
CDF1	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
CDF2	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016a)
CDF6	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017b)
CDF8	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017b)
AGU	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	This study
DJE	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	This study
S115	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Nordmann et al., 2016b)
P4	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
P26	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B4	*E. coli*	+	+	4	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B8	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B9	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B12	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B15	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B18	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B19	*E. coli*	+	+	16	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B20	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B22	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B47	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B50	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
C2729	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
C2730	*E. coli*	+	+	8	Plasmid-mediated *mcr-1* gene	(Kieffer et al., 2017a)
B31K	*K. pneumoniae*	+	+	16	Plasmid-mediated *mcr-1* gene	This study
P26	*K. pneumoniae*	+	+	8	Plasmid-mediated *mcr-1* gene	This study
C2728	*K. pneumoniae*	+	+	16	Plasmid-mediated *mcr-1* gene	This study
C2731	*S. enterica*	+	+	8	Plasmid-mediated *mcr-1* gene	This study
C2732	*S. enterica*	+	+	16	Plasmid-mediated *mcr-1* gene	This study
R2812	*E. coli*	+	+	4	Plasmid-mediated *mcr-2 gene*	(Xavier et al., 2016)
R3181	*E. coli*	+	+	4	Plasmid-mediated *mcr-3–like gene*	This study
S136	*S. enterica*	+	+	4	Plasmid-mediated *mcr-4–like gene*	This study
FR-119	*E. coli*	−	−	8	Unknown	This study
FR-109	*K. pneumoniae*	+	+	64	Unknown	This study
FR-115	*K. pneumoniae*	+	+	64	Unknown	This study
FR-103	*K. pneumoniae*	+	+	4	Unknown	This study
FR-118	*K. pneumoniae*	+	+	4	Unknown	This study
FR-121	*E. coli*	+	+	4	Unknown	This study
Heteroresistant isolates						
DA27007	*S. enterica*	+	+	4	Chromosomal amplification of *pmrD* gene	(Hjort et al., 2016)
DA33039	*S. enterica*	+	+	4	Chromosomal amplification of *pmrD* gene	(Hjort et al., 2016)
R2914	*E. cloacae*	+	+	>256	Cluster XI	(Guerin et al., 2016)
R2915	*E. cloacae*	+	+	>256	Cluster I	(Guerin et al., 2016)
R2917	*E. cloacae*	+	+ (3h)	>256	Cluster I	(Guerin et al., 2016)
R2919	*E. cloacae*	+	+	256	Cluster II	(Guerin et al., 2016)
R2922	*E. cloacae*	+	−	16	Cluster IV	(Guerin et al., 2016)
R2923	*E. cloacae*	−	−	16	Cluster IV	(Guerin et al., 2016)
R2928	*E. cloacae*	+	+	64	Cluster VII	(Guerin et al., 2016)
R2929	*E. cloacae*	+	+	256	Cluster VII	(Guerin et al., 2016)

Table 1 (*continued*)

Strains	Species	Homemade Rapid Polymyxin NP[a]	Industrial Rapid Polymyxin NP[a]	MIC of colistin (μg/mL)	Resistance mechanisms	Reference
R2932	*E. cloacae*	+	+ (3 h)	256	Cluster IX	(Guerin et al., 2016)
R2933	*E. cloacae*	+	+ (3 h)	64	Cluster IX	(Guerin et al., 2016)
R2936	*E. cloacae*	+	+	128	Cluster XI	(Guerin et al., 2016)
R2938	*E. cloacae*	+	+	>256	Cluster XII	(Guerin et al., 2016)
R2939	*E. cloacae*	+	+	128	Cluster XII	(Guerin et al., 2016)
R2945	*E. cloacae*	+	+	256	Cluster XI	(Guerin et al., 2016)
R2946	*E. cloacae*	+	+	16	Cluster XI	(Guerin et al., 2016)
R2947	*E. cloacae*	+	+	256	Cluster XI	(Guerin et al., 2016)
R2950	*E. cloacae*	+	+	16	Cluster XI	(Guerin et al., 2016)
Susceptible isolates						
ATCC 25922	*E. coli*	−	−	0.125	Wild-type	Reference strain
FR-180	*K. pneumoniae*	+	+	1	NA	(Nordmann et al., 2016a)
FR-181	*K. pneumoniae*	+	+	2	NA	(Nordmann et al., 2016a)
FR-182	*K. pneumoniae*	+	+	2	NA	(Nordmann et al., 2016a)
C451	*E. coli*	−	−	1	NA	This study
C889	*K. pneumoniae*	−	−	2	NA	This study
C861	*K. pneumoniae*	+	+	2	NA	This study
FR-165	*K. pneumoniae*	−	−	1	NA	(Nordmann et al., 2016a)
C349	*K. pneumoniae*	−	−	<1	NA	This study
C352	*E. coli*	−	−	<1	NA	This study
C353	*E. coli*	−	−	<1	NA	This study
C355	*E. coli*	−	−	<1	NA	This study
C358	*E. coli*	−	−	<1	NA	This study
C360	*E. coli*	−	−	<1	NA	This study
C361	*E. coli*	−	−	<1	NA	This study
C468	*E. coli*	−	−	<1	NA	This study
C469	*E. coli*	−	−	<1	NA	This study
C470	*C. koseri*	−	−	<1	NA	This study
C472	*E. aerogenes*	−	−	0.25	NA	This study
C474	*C. koseri*	−	−	<1	NA	This study
C481	*K. oxytoca*	−	−	<1	NA	This study
C490	*K. pneumoniae*	−	−	<1	NA	This study
C508	*K. pneumoniae*	−	−	<1	NA	This study
C509	*K. pneumoniae*	−	−	<1	NA	This study
C512	*E. aerogenes*	−	−	<1	NA	This study
C519	*C. koseri*	−	−	<1	NA	This study
C524	*K. pneumoniae*	−	−	<1	NA	This study
C533	*K. pneumoniae*	−	−	<1	NA	This study
C539	*C. koseri*	−	−	<1	NA	This study
C548	*E. aerogenes*	−	−	<1	NA	This study
C554	*K. pneumoniae*	−	−	<1	NA	This study
C597	*K. pneumoniae*	−	−	<1	NA	This study
C607	*K. pneumoniae*	−	−	<1	NA	This study
C632	*E. aerogenes*	−	−	<1	NA	This study
C635	*C. koseri*	−	−	<1	NA	This study
C867	*C. koseri*	−	−	<1	NA	This study
C901	*K. pneumoniae*	−	−	0.5	NA	This study
C914	*K. pneumoniae*	−	−	0.125	NA	This study
C937	*C. koseri*	−	−	<1	NA	This study
C1029	*K. oxytoca*	−	−	<1	NA	This study
C1033	*E. cloacae*	−	−	<1	NA	This study
C1066	*E. coli*	−	−	<1	NA	This study
C1077	*E. cloacae*	−	−	<1	NA	This study
C1082	*K. oxytoca*	−	−	<1	NA	This study
C1085	*C. koseri*	−	−	<1	NA	This study
C1087	*K. oxytoca*	−	−	<1	NA	This study
C1216	*E. cloacae*	−	−	<1	NA	This study
C1221	*E. aerogenes*	−	−	<1	NA	This study
C1231	*K. oxytoca*	−	−	<1	NA	This study
C1234	*K. oxytoca*	−	−	<1	NA	This study
C1285	*E. aerogenes*	−	−	<1	NA	This study
C1288	*E. aerogenes*	−	−	<1	NA	This study
C1293	*E. aerogenes*	−	−	<1	NA	This study
C1397	*K. oxytoca*	−	−	<1	NA	This study
C1591	*C. freundii*	−	−	<1	NA	This study
C1637	*E. cloacae*	−	−	<1	NA	This study
C1664	*K. oxytoca*	−	−	<1	NA	This study
C1671	*C. freundii*	−	−	<1	NA	This study
C1703	*E. cloacae*	−	−	<1	NA	This study
C1753	*E. cloacae*	−	−	<1	NA	This study
C1877	*C. freundii*	−	−	<1	NA	This study
C2155	*C. freundii*	−	−	<1	NA	This study
C2635	*Salmonella* spp.	−	−	<1	NA	This study
C3690	*C. freundii*	−	−	<1	NA	This study

(*continued on next page*)

Table 1 (*continued*)

Strains	Species	Homemade Rapid Polymyxin NP[a]	Industrial Rapid Polymyxin NP[a]	MIC of colistin (μg/mL)	Resistance mechanisms	Reference
R281	*E. asburiae*	—	—	<1	NA	This study
R1414	*C. freundii*	—	—	<1	NA	This study
R1415	*C. freundii*	—	—	<1	NA	This study
R1416	*C. freundii*	—	—	<1	NA	This study
R1527	*S. Concord*	—	—	<1	NA	This study
R1528	*Salmonella* spp.	—	—	<1	NA	This study
R1529	*S. Isangi*	—	—	<1	NA	This study
C362	*K. pneumoniae*	—	—	0.125	NA	This study
C367	*K. pneumoniae*	—	—	0.125	NA	This study
C370	*K. pneumoniae*	—	—	0.125	NA	This study
C382	*K. pneumoniae*	—	—	0.125	NA	This study
C383	*K. pneumoniae*	—	—	0.125	NA	This study
C609	*K. pneumoniae*	—	—	0.125	NA	This study
C643	*K. pneumoniae*	—	—	0.125	NA	This study

[a] Isolates with discordant results are shaded.

MICs of colistin were determined in parallel by BMD using cation-adjusted Muller Hinton broth (Bio-Rad, Cressier, Switzerland), and *Escherichia coli* ATCC 25922 was used as control strain. In case of "skip wells" (likely indicating heteroresistance), MICs were determined by considering the well with the highest concentration in which growth was observed, regardless the clear wells (Guerin et al., 2016). Isolates with MICs of colistin >2 μg/mL were considered as resistant, whereas isolates showing a MIC ≤2 μg/mL were considered as susceptible. Results of the 2 Polymyxin NP tests read after 3 hours of incubation were compared with those of the BMD. Errors were ranked as follows: very major error (VME) defined by isolates categorized as susceptible using the marketed panel but resistant by the BMD reference method (false-susceptibility result), and major error (ME) defined by isolates categorized as resistant using the marketed panel but susceptible by the BMD reference method (false-resistant result).

Among the susceptible isolates tested (n = 78), 4 ME (5.1%) were detected with both tests giving a specificity of 94.9% (74/78). Those false-positive results corresponded to isolates having MICs of colistin close to the breakpoint value (i.e., 1–2 μg/mL).

Among the resistant isolates (n = 155), 2 VME (1.2%) and 3 VME (1.9%) were detected with both the homemade and of the industrial Rapid Polymyxin NP tests, giving sensitivity rates of 98.7% (153/155) and 98.1% (152/155), respectively. Experiments were reproduced twice with distinct reading persons and results showed perfect agreement. A single *E. coli* with an unknown mechanism of resistance was not detected by using both tests. A single and 2 heteroresistant *Enterobacter cloacae* isolates were not detected with the homemade and of the industrial Rapid Polymyxin NP tests, respectively. Noteworthy, 3 heteroresistant *E. cloacae* isolates were detected after 2 hours of incubation with the homemade test, whereas they were detected after 3 hours of incubation with the industrial test. The performance of the homemade test was therefore quite equivalent to the industrial test for detection of colistin heteroresistance. Such detection is challenging because most of the routine methods (Etest, Vitek-2 automated system) give false-susceptible results for such isolates (Poirel et al., 2017). Recently, an evaluation of the homemade Rapid Polymyxin NP test for detection of colistin heteroresistance in *Enterobacter* spp. was performed, using a collection of 25 isolates, and a low sensitivity (25%) was actually found (Simar et al., 2017). Such difference of results remains unexplained, but it is worth highlighting that the strains tested were different. Further studies performed in different laboratories will be needed to evaluate the reliability of the Polymyxin NP tests to detect colistin heteroresistance.

Noteworthy, all the 38 isolates producing a plasmid-mediated colistin resistance determinant (MCR-1 to MCR-4) were perfectly detected by the 2 methods within 2 hours.

To conclude, the homemade and the industrial Polymyxin NP tests showed high sensitivity (98.1%–98.7%) and specificity (94.9%) for detection of colistin resistance in Enterobacteriaceae, regardless of the resistance mechanisms. Those tests therefore possess the potential to be reliable tools for screening colistin-resistant Enterobacteriaceae in clinical laboratories.

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Conflict of Interest

None to declare.

References

Guerin F, Isnard C, Sinel C, Morand P, Dhalluin A, Cattoir V, et al. Cluster-dependent colistin hetero-resistance in *Enterobacter cloacae* complex. J Antimicrob Chemother 2016. https://doi.org/10.1093/jac/dkw260. [dkw260 [pii]].

Hjort K, Nicoloff H, Andersson DI. Unstable tandem gene amplification generates heteroresistance (variation in resistance within a population) to colistin in *Salmonella enterica*. Mol Microbiol 2016;102:274–89. https://doi.org/10.1111/mmi.13459.

Kieffer N, Aires-de-Sousa M, Nordmann P, Poirel L. High rate of MCR-1-producing Escherichia coli and Klebsiella pneumoniae among pigs, Portugal. Emerg Infect Dis 2017a;23:2023–9. https://doi.org/10.3201/eid2312.170883.

Kieffer N, Poirel L, Clerc O, Lienhard R, Nordmann P. Co-production of MCR-1 and extended-spectrum β-lactamase in *Escherichia coli* recovered from urinary tract infections in Switzerland. Infection 2017b. https://doi.org/10.1007/s15010-017-1080-6.

Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in *Enterobacteriaceae*. Emerg Infect Dis 2016;22:1038–43. https://doi.org/10.3201/eid2206.151840.

Nordmann P, Lienhard R, Kieffer N, Clerc O, Poirel L. Plasmid-mediated colistin-resistant *Escherichia coli* in bacteremia in Switzerland. Clin Infect Dis 2016;62:1322–3. https://doi.org/10.1093/cid/ciw124. [ciw124 [pii]].

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. https://doi.org/10.1128/CMR.00064-16.

Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P. Genetic features of MCR-1–producing colistin-resistant *Escherichia coli* isolates, South Africa. Antimicrob Agents Chemother 2016. https://doi.org/10.1128/AAC.00444-16. [AAC.00444-16 [pii]].

Simar S, Sibley D, Ashcraft D, Pankey G. Evaluation of the Rapid Polymyxin NP test for Polymyxin B resistance detection using *Enterobacter cloacae* and *Enterobacter aerogenes* isolates. J Clin Microbiol 2017;55:3016–20. https://doi.org/10.1128/JCM.00934-17.

Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Euro Surveill 2016;21. https://doi.org/10.2807/1560-7917.ES.2016.21.27.30280. [30280 [pii]].