1 # Comparison of methods for detection of plasmid-mediated and

2 # chromosomally-encoded colistin resistance in Enterobacteriaceae

3 **Aurélie Jayol,[1,2,3,4] Patrice Nordmann,[3,4,5] Philippe Lehours,[1] Laurent Poirel,[3,4] and**

4 **Véronique Dubois[1,2*]**

5 *[1]Laboratory of Bacteriology, Bordeaux University Hospital, [2]CNRS UMR5234, University of*

6 *Bordeaux, France, [3]Emerging Antibiotic Resistance Unit, Medical and Molecular*

7 *Microbiology, Department of Medicine, University of Fribourg, Switzerland, [4]INSERM*

8 *European Unit (LEA, IAME), Paris, France, and [5]University of Lausanne and University*

9 *hospital Center, Lausanne, Switzerland*

10

13

14 *Corresponding author. Véronique Dubois, MFP Laboratory CNRS UMR-5234. 146 rue Léo

15 Saignat, Batiment 3A, 33076 BORDEAUX Cedex, France. E-mail: veronique.dubois@u-

16 bordeaux.fr

17

18
19
20
21
22
23
24

ABSTRACT

Objectives: Because of the emergence of plasmid-mediated (*mcr-1* and *mcr-2* genes) and chromosomally-encoded colistin resistance, reliable methods for detecting colistin resistance/susceptibility in routine laboratories are required. We evaluated the respective performances of the BD Phoenix automated system, the newly-developed Rapid Polymyxin NP test and the broth microdilution (BMD) reference method to detect colistin resistance in Enterobacteriaceae, and particularly those producing MCR-1 and MCR-2.

Methods: Colistin susceptibility of 123 enterobacterial clinical isolates (40 colistin-susceptible and 83 colistin-resistant isolates) was tested with the Phoenix automated system, the Rapid Polymyxin NP test and the BMD method. Molecular mechanisms responsible for plasmid-mediated and chromosomally-encoded colistin resistance mechanisms were investigated by PCR and sequencing.

Results: Considering BMD as a reference method, the Phoenix system failed to detect ten colistin-resistant isolates (one *Escherichia coli*, one *Klebsiella pneumoniae*, seven *Enterobacter* spp., and one *Salmonella enterica*). The Rapid Polymyxin NP test failed to detect the same single *E. coli* isolate. Those two latter methods detected the sixteen *E. coli, K. pneumoniae* and *S. enterica* isolates producing the plasmid-encoded MCR-1 and MCR-2.

Conclusion: The Phoenix system and the Rapid Polymyxin NP test are reliable techniques for detecting plasmid-mediated MCR-1 and MCR-2-related colistin resistance. However, a high rate of false susceptibility was observed with the Phoenix system, indicating that susceptibility results obtained with that system should be confirmed by BMD method. By contrast, the Rapid Polymyxin NP test showed a good agreement with the BMD method and

48   results were obtained rapidly (within two hours). The BMD method should be performed if

49   MIC values are needed.

50

INTRODUCTION

The increasing use of colistin in human medicine, and the recent discovery of plasmid-mediated polymyxin resistance [1–4], highlight the need for reliable methods for polymyxin susceptibility testing.

The Clinical Laboratory Standard Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recently gathered in a joint subcommittee, chose the broth microdilution (BMD) method as the reference method (www.eucast.org). It must be performed with sulfate salts of polymyxins (colistimethate used in human medicine shall not be used), with cation-adjusted Mueller-Hinton broth, without additive (in particular without polysorbate 80) and without treated polystyrene trays. Other methods such as, agar dilution, disk diffusion and gradient diffusion (E-test) have been ruled out. However, this gold standard BMD method is difficult to performed in routine laboratories since it requires qualified staff, is time-consuming, and requires manual preparation of antibiotic solutions [5].

Automated dilution methods such as those performed by the BD Phoenix system could be an alternative for the screening of colistin resistance for laboratories that cannot perform manual BMD. However, the performance of this automate for colistin susceptibility testing, especially its accuracy for the detection of isolates exhibiting a plasmid-mediated colistin resistance, have never been evaluated. Recently, a rapid colorimetric test, the Rapid Polymyxin NP test, has been developed for detecting polymyxin resistance in *Enterobacteriaceae* within 2 hours [6].

The objective of this study was to evaluate the performance of the BD Phoenix automated system to detect plasmid-mediated and chromosomally-encoded colistin resistance, using a collection of clinical enterobacterial isolates. We also aimed to compare their performances to those of the Rapid Polymyxin NP test and the BMD reference method.

MATERIAL AND METHODS

76   **Bacterial strains.** This study was carried out using 123 non-duplicated clinical

77 isolates of various enterobacterial species. The collection included 40 colistin-susceptible and

78 83 colistin-resistant isolates. Out of the 83 colistin-resistant isolates, sixteen belonged to a

79 genus known to be naturally-resistant to colistin (*Morganella*, *Proteus*, *Providencia*, *Serratia*,

80 *Hafnia*), and 67 isolates belonged to the *Escherichia, Klebsiella, Enterobacter*, or *Salmonella*

81 genus with acquired resistance mechanisms to colistin. Identification of the isolates at the

82 species level was performed using the Microflex bench-top MALDI-TOF mass spectrometer

83 (Brücker, Champs-sur-Marne, France). Isolates were grown on Luria Bertani (LB)

84 (GibcoBRL, Cergy Pontoise, France) or Mueller Hinton (MH) (bioMérieux, Marcy-l'Etoile,

85 France) agar plates at 35±2°C for 18 h. The colistin-susceptible *E. coli* ATCC 25922 strain

86 was included in all experiments as quality control.

87   **Susceptibility testing**

88 *Reference antimicrobial susceptibility testing*

89 The BMD method was performed according to the EUCAST/CLSI joined guidelines

90 (www.eucast.org). Briefly, BMD panels were prepared extemporaneously in 96-wells sterile

91 polystyrene microplates (Sarstedt, Nümbrecht, Germany). Dilutions of colistin (Sigma Aldrich,

92 St Louis, USA) ranging from 0.125 to 128 mg/l were made in cation-adjusted MH broth (Bio-

93 Rad, Marnes-la-Coquette, France), without addition of polysorbate 80 (Tween 80), and with a

94 final concentration of 5×10^5 CFU/ml of bacteria in each well. This procedure was performed

95 in triplicate in separate experiments and the Minimum Inhibitory Concentrations (MICs) were

96 read after 16 to 20 h of incubation at 35±2°C in ambient air. Results were interpreted

97 according to the EUCAST breakpoints [7], i.e. isolates with MICs of colistin ≤ 2 mg/l were

98 categorized as susceptible although those with MICs > 2 mg/l were resistant.

BD Phoenix automated system

100   Colistin susceptibility testing was assessed using the Phoenix automated system (BD Phoenix

101   100, BD Diagnostic systems, Le Pont de Claix, France), which performs automated BMD

102   method. The panel selected to perform this evaluation was the Gram-negative panel NMIC-

103   93, using the BMD method for colistin concentrations ranging from 0.5 to 4 mg/l in order to

104   cover the EUCAST breakpoints [7]. The bacterial suspension and the panel inoculation were

105   performed according to the manufacturer's guidelines. Panels were incubated up to 16 h at

106   $35\pm2°C$ under ambient air, and results were interpreted with the BD EpiCenter software.

107   *Rapid Polymyxin NP test*

108   The Rapid Polymyxin NP test is based on the detection of the glucose metabolism related to

109   bacterial growth in presence of a fixed concentration of colistin (3.75 mg/l) in cation-adjusted

110   MH broth medium [6]. Formation of acid metabolites consecutive to the glucose metabolism

111   is evidenced by a color change (orange to yellow) of the pH indicator (red phenol). The test is

112   positive (colistin resistance) if a strain grows in presence of colistin, whereas it is negative

113   (colistin susceptibility) if a strain does not grow in presence of colistin. Results of the Rapid

114   Polymyxin NP test were read at 2 h of incubation at $35\pm2°C$ in ambient air.

115   **Molecular characterization of the colistin resistance.** Molecular mechanisms

116   responsible for plasmid-mediated (*mcr-1* and *mcr-2* genes) and chromosomally-encoded

117   (*pmrA*, *pmrB*, *phoP*, *phoQ*, *mgrB*, and *crrB* alterations) colistin resistance were determined as

118   described previously [1,2,8–12].

119   **Results analysis.** The results obtained with the BD Phoenix system and the Rapid

120   Polymyxin NP test were compared to those obtained with the reference BMD method.

121   Discrepancies were determined for each method in order to assess their performance to detect

122   colistin resistance. For strains for which discrepant susceptibility results were obtained, the

123   isolates were retested with the three methods. Unsolved discrepancies were then maintained in

124   the database for performance evaluation. Errors were ranked as follows: a very major error

125   (VME) was defined when isolates were categorized as susceptible using the Phoenix system

126   or the Rapid Polymyxin NP test but resistant by the BMD method (false-susceptible result),

127   while a major error (ME) was defined when isolates were found resistant using the Phoenix

128   system or the Rapid Polymyxin NP test, but were found susceptible by using the BMD

129   method (false-resistant result). The number of resistant isolates, and the number of susceptible

130   isolates were used as denominators for VME and ME calculations, respectively. Acceptance

131   criteria that provide requirements, and specifications to evaluate performances of

132   antimicrobial susceptibility test devices were those defined by the ISO standards (VME and

133   ME must be ≤3%) [13].

134   **RESULTS**

135   The features of the 123 enterobacterial isolates included in this study to evaluate the

136   performance of the BD Phoenix system and the Polymyxin NP test for determining colistin

137   susceptibility are presented in the Table.

138   Fourty isolates defined as colistin-susceptible according to the results of the BMD

139   method (MICs of colistin ranging from 0.12 to 2 µg/ml) were found susceptible by the BD

140   Phoenix system (Table). While a single susceptible *K. pneumoniae* isolate with an MIC of

141   colistin at 2 mg/l was found resistant using the Rapid Polymyxin NP test. The MIC value of

142   colistin for this same isolate as determined by the BD Phoenix system was underestimated

143   (MIC ≤ 0.5 mg/l) but the isolate was well categorized as susceptible.

144   Out of the 83 colistin-resistant enterobacterial isolates (MICs of colistin ranging from

145   4 to higher than 128 mg/l), the Phoenix system failed to detect colistin resistance for seven

146   *Enterobacter* spp. isolates, a single *K. pneumoniae*, a single *S. enterica*, and a single *E. coli*

147   isolate, whereas the Rapid Polymyxin NP test only failed for detecting a single colistin-

148   resistant *E. coli* isolate. (Table). Identical results were obtained when those strains were

149   repeatedly tested with the Phoenix system and the Rapid Polymyxin NP test indicating a good

150   reproducibility of the methods.

151   Noteworthy, thirteen non clonally-related colistin-resistant *E. coli*, one *K. pneumoniae*,

152   and one *S. enterica* isolate possessing the plasmid-mediated *mcr-1* gene were tested (MICs of

153   colistin ranging from 4 to 64 mg/l using the BMD method) and all were identified as resistant

154   with the BD Phoenix system and the Rapid Polymyxin NP test. Similarly, the *E. coli* isolate

155   possessing the plasmid-mediated *mcr-2* gene (MIC = 4 mg/l) was detected by the two

156   methods.

157   **DISCUSSION**

158   Out of the 40 colistin-susceptible enterobacterial isolates, no ME (i.e. false resistance)

159   was found with the Phoenix system, and only a single susceptible *K. pneumoniae* isolate with

160   an MIC of colistin at 2 mg/l (therefore just below the EUCAST breakpoint value > 2 mg/l)

161   was falsely identified as colistin resistant with the Rapid Polymyxin NP test revealing a ME

162   rate of 2.5%.test

163   Out of the 83 colistin-resistant enterobacterial isolates, the BD Phoenix system and the

164   Rapid Polymyxin NP test showed excellent performances to detect the 13 isolates with

165   plasmid-mediated colistin resistance regardless of the level of resistance. However, ten VME

166   (i.e. false susceptibility) were found with the Phoenix system whereas a single VME was

167   found with the Rapid Polymyxin NP test (Table). A high VME rate of 12% was thus found

168   with the BD Phoenix system, whereas a low VME rate of 1.2% was found with the Rapid

169   Polymyxin NP test. The single colistin-resistant *E. coli* isolate that was not detected with the

170   BD Phoenix system and the Rapid Polymyxin NP test, presented a low level of resistance

171 (MIC of colistin at 8 mg/l). Its mechanism of colistin resistance remains unknown (neither

172 chromosomally-encoded mutations in genes known to be involved in lipopolysaccharide

173 modifications, i.e. *mgrB*, *pmrAB* and *phoPQ* genes, nor plasmid-mediated *mcr-1* and *mcr-2*

174 genes were detected).

175 The *S. enterica* isolate identified as susceptible (MIC = 2 mg/l) with the BD Phoenix system

176 presented a low level of colistin resistance (MIC = 4 mg/l) and its mechanism of resistance

177 remains unknown (neither chromosomal mutations, nor plasmid-mediated resistance).

178 The *K. pneumoniae* resistant isolate and the seven *Enterobacter* spp. resistant isolates not

179 detected with the BD Phoenix system exhibited MIC values of colistin ranging from 16 to

180 higher than 128 mg/l and were identified as colistin resistant with the Rapid Polymyxin NP

181 test. During the determination of MICs by the BMD method, skipped wells (i.e. wells that

182 exhibit no growth although growth does occur at higher concentrations) were observed for

183 88% of those isolates (the *K. pneumoniae* isolate and six *Enterobacter* spp. isolates). This

184 observation suggests that the failure of the BD Phoenix system to detect colistin resistance in

185 those isolates could be related to a heteroresistance phenotype (defined by the presence of two

186 subpopulations exhibiting different susceptibilities to colistin) [14]. The skipped wells

187 observed during the MIC determination of those isolates by the BMD method are mainly for

188 dilutions comprised between 0.125 and 4 mg/l. The Phoenix panel used in this study

189 contained dilutions of colistin ranging from 0.5 to 4 mg/l. It is therefore likely that the failure

190 of detection of heteroresistance for those isolates was linked to the absence of testing at higher

191 colistin concentrations. The low sensitivity to detect colistin heteroresistance has already been

192 described for another automated system, i.e. the bioMérieux Vitek system [15].

193 The limitation of our study could be the absence of testing of non-fermenting Gram negative

194 rods in our collection.

CONCLUSION

This study shows that the BD Phoenix system and the Rapid Polymyxin NP test are reliable tools for detection of plasmid-mediated colistin resistance (*mcr-1* and *mcr-2* genes), which is currently a major concern. However, the BD Phoenix system is not reliable for detection of colistin heteroresistance in enterobacterial isolates. Thus, we recommend the determination of MICs by the BMD method when susceptible results are obtained and if clinical use is required. By contrast, the Rapid Polymyxin NP test showed a good agreement with the BMD method and results were obtained rapidly (within two hours), but BMD method should be performed if determination of MIC values is necessary.

CONFLICT OF INTEREST

An international patent form has been filed on behalf of the University of Fribourg, Switzerland corresponding to the Rapid Polymyxin NP test.

REFERENCES

[1] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a

microbiological and molecular biological study. Lancet Infect Dis 2015;16:161–8. doi:S1473-3099(15)00424-7 [pii] 10.1016/S1473-3099(15)00424-7.

[2] 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. doi:10.2807/1560-7917.ES.2016.21.27.30280 30280 [pii].

[3] Skov RL, Monnet DL. Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. Euro Surveill 2016;21. doi:10.2807/1560-7917.ES.2016.21.9.30155 30155 [pii].

[4] 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. doi:10.1128/CMR.00064-16.

[5] Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis 2009;49:1749–55. doi:10.1086/647952.

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

[7] EUCAST. Breakpoints tables for interpretation of MICs and zone diameters, Version 1.0. EUCAST; 2016; 2016.

[8] Jayol A, Poirel L, Villegas MV, Nordmann P. Modulation of *mgrB* gene expression as a source of colistin resistance in *Klebsiella oxytoca*. Int J Antimicrob Agents 2015;46:108–10. doi:S0924-8579(15)00109-0 [pii] 10.1016/j.ijantimicag.2015.02.015.

[9] Poirel L, Jayol A, Bontron S, Villegas MV, Ozdamar M, Turkoglu S, et al. The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*. J Antimicrob Chemother 2014;70:75–80. doi:dku323 [pii] 10.1093/jac/dku323.

242   [10]   Sun S, Negrea A, Rhen M, Andersson DI. Genetic analysis of colistin resistance in

243   *Salmonella enterica serovar Typhimurium*. Antimicrob Agents Chemother 2009;53:2298–

244   305. doi:AAC.01016-08 [pii] 10.1128/AAC.01016-08.

245   [11]   Quesada A, Porrero MC, Tellez S, Palomo G, Garcia M, Dominguez L. Polymorphism

246   of genes encoding PmrAB in colistin-resistant strains of *Escherichia coli* and *Salmonella*

247   *enterica* isolated from poultry and swine. J Antimicrob Chemother 2015;70:71–4.

248   doi:dku320 [pii] 10.1093/jac/dku320.

249   [12]   Cheng YH, Lin TL, Lin YT, Wang JT. Amino acid substitutions of CrrB responsible

250   for resistance to colistin through CrrC in *Klebsiella pneumoniae*. Antimicrob Agents

251   Chemother 2016 May 236063709-16 2016.

252   [13]   International Standard Organization. Clinical laboratory testing and in vitro diagnostic

253   test systems - Susceptibility testing of infectious agents and evaluation of performance of

254   antimicrobial susceptibility test devices - Part2: evaluation of performance of

255   antimicrobial susceptibility test devices. International Standard ISO 20776-2:2007,

256   Geneva: ISO, 2007.) n.d.

257   [14]   Landman D, Salamera J, Quale J. Irreproducible and uninterpretable polymyxin B

258   MICs for *Enterobacter cloacae* and *Enterobacter aerogenes*. J Clin Microbiol

259   2013;51:4106–11. doi:JCM.02129-13 [pii] 10.1128/JCM.02129-13.

260   [15]   Lo-Ten-Foe JR, de Smet AM, Diederen BM, Kluytmans JA, van Keulen PH.

261   Comparative evaluation of the VITEK 2, disk diffusion, Etest, broth microdilution, and

262   agar dilution susceptibility testing methods for colistin in clinical isolates, including

263   heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* strains. Antimicrob

264   Agents Chemother 2007;51:3726–30. doi:AAC.01406-06 [pii] 10.1128/AAC.01406-06.

265

266

Table. MICs of colistin (mg/l) using the BMD method and the BD Phoenix system and results of the Rapid Polymyxin NP test.

Isolate	Species (number of isolates)	Phenotype	Mechanism of resistance to colistin[a]	BMD MIC colistin	Phoenix MIC colistin	Discrepancies[b]	Rapid Polymyxin NP test Result	Discrepancies[b,c]
Isolates susceptible to colistin								
ATCC25922	*E. coli*	S	NA	0.25	≤0.5	No	-	No
2 to 15	*E. coli* (n= 14)	S	NA	0.12 to 0.5	≤0.5	No	-	No
16 to 26	*K. pneumoniae* (n=11)	S	NA	0.12 to 2	≤0.5	No	-	**Yes, ME (n=1)**
27 to 29	*K. oxytoca* (n=3)	S	NA	0.12 to 0.25	≤0.5	No	-	No
30 to 32	*E. cloacae* (n=3)	S	NA	0.12 to 0.25	≤0.5	No	-	No
33	*E. asburiae*	S	NA	0.12	≤0.5	No	-	No
34	*E. aerogenes*	S	NA	0.12	≤0.5	No	-	No
35 to 37	*C. freundii* (n=3)	S	NA	0.25	≤0.5	No	-	No
38 to 40	*C. koseri* (n=3)	S	NA	0.12 to 0.25	≤0.5	No	-	No
Isolates resistant to colistin								
41	*M. morganii*	R	Intrinsic	>128	>4	No	+	No
42-43	*P. mirabilis* (n=2)	R	Intrinsic	>128	>4	No	+	No
44	*P. vulgaris*	R	Intrinsic	>128	>4	No	+	No
45	*P. stuartii*	R	Intrinsic	>128	>4	No	+	No
46 to 48	*S. marcescens* (n=3)	R	Intrinsic	>128	>4	No	+	No
49 to 52	*H. alvei* (n=4)	R	Intrinsic	8 or 16	4 or >4	No	+	No
53 to 56	*H. paralvei* (n=4)	R	Intrinsic	8	4 or >4	No	+	No
57 to 68	*E. coli* (n= 11)	R	Plasmid-mediated *mcr-1* gene	4 or 8	4 or >4	No	+	No
69	*E. coli*	R	Plasmid-mediated *mcr-1* gene	64	>4	No	+	No
70	*E. coli*	R	Plasmid-mediated *mcr-2* gene	4	4	No	+	No
71	*K. oxytoca*	R	IS*Kpn26* into *mgrB* promotor	64	>4	No	+	No
72	*E. coli*	R	Unknown	8	**≤0.5**	**Yes, VME**	-	**Yes, VME**
73	*E. coli*	R	Unknown	8	>4	No	+	No
74	*E. coli*	R	Unknown	4	4	No	+	No
75	*E. coli*	R	Unknown	16	>4	No	+	No
76	*K. pneumoniae*	R	PmrA G53C	64	>4	No	+	No
77-78	*K. pneumoniae* (n=2)	R	PmrA G53S	16 or 32	>4	No	+	No
79-80	*K. pneumoniae* (n=2)	R	PmrB T157P	16 or 32	>4	No	+	No
81	*K. pneumoniae*	R	PhoP D191Y	128	**≤0.5**	**Yes, VME**	+	No

82	*K. pneumoniae*	R	PhoQ R16C	128	>4	No	+	No
83	*K. pneumoniae*	R	MgrB N42Y et K43I	64	>4	No	+	No
84	*K. pneumoniae*	R	MgrB I45T	64	>4	No	+	No
85 to 87	*K. pneumoniae* (n=3)	R	MgrB truncated	64 or 128	>4	No	+	No
88	*K. pneumoniae*	R	Deletion of 11 nucleotides into *mgrB* gene	>128	>4	No	+	No
89	*K. pneumoniae*	R	bla*CTX-M-15*/IS*Ecp1* into *mgrB*	64	>4	No	+	No
90	*K. pneumoniae*	R	IS*5* into *mgrB* gene	64	>4	No	+	No
91	*K. pneumoniae*	R	IS*102* into *mgrB* gene	>128	>4	No	+	No
92	*K. pneumoniae*	R	IS*Kpn14* into *mgrB* gene	32	>4	No	+	No
93	*K. pneumoniae*	R	IS*Kpn13* into *mgrB* gene	128	>4	No	+	No
94	*K. pneumoniae*	R	IS*Kpn26* into *mgrB* gene	64	>4	No	+	No
95	*K. pneumoniae*	R	IS*903* into *mgrB* gene	128	>4	No	+	No
96	*K. pneumoniae*	R	IS*903b* into *mgrB* gene	64	>4	No	+	No
97	*K. pneumoniae*	R	IS*5* into *mgrB* gene	128	>4	No	+	No
98	*K. pneumoniae*	R	IS*10R* into *mgrB* promotor	128	>4	No	+	No
99	*K. pneumoniae*	R	IS*Kpn14* into *mgrB* promotor	32	>4	No	+	No
100	*K. pneumoniae*	R	CrrB N141Y	>128	>4	No	+	No
101	*K. pneumoniae*	R	CrrB P151L	>128	>4	No	+	No
102	*K. pneumoniae*	R	CrrB G183V	>128	>4	No	+	No
103	*K. pneumoniae*	R	Plasmid mediated *mcr-1* gene	16	4	No	+	No
104	*K. pneumoniae*	R	Unknown	16	>4	No	+	No
105	*K. pneumoniae*	R	Unknown	64	>4	No	+	No
106	*K. pneumoniae*	R	Unknown	32	>4	No	+	No
107	*K. pneumoniae*	R	Unknown	>128	>4	No	+	No
108	*K. pneumoniae*	R	Unknown	64	>4	No	+	No
109	*K. pneumoniae*	R	Unknown	64	>4	No	+	No
110	*K. pneumoniae*	R	Unknown	32	>4	No	+	No
111	*E. cloacae*	R	Unknown	32	>4	No	+	No
112	*E. cloacae*	R	Unknown	>128	>4	No	+	No
113	*E. cloacae*	R	Unknown	32	>4	No	+	No
114	*E. cloacae*	R	Unknown	>128	**1**	**Yes, VME**	+	No
115	*E. cloacae*	R	Unknown	64	**≤0.5**	**Yes, VME**	+	No
116	*E. cloacae*	R	Unknown	>128	**≤0.5**	**Yes, VME**	+	No
117	*E. cloacae*	R	Unknown	16	**≤0.5**	**Yes, VME**	+	No

118	*E. cloacae*	R	Unknown	>128	**≤0.5**	**Yes, VME**	+	No
119	*E. cloacae*	R	Unknown	>128	**≤0.5**	**Yes, VME**	+	No
120	*E. asburiae*	R	Unknown	>128	**≤0.5**	**Yes, VME**	+	No
121	*S. enterica*	R	Plasmid mediated *mcr-1* gene	16	>4	No	+	No
122	*S. enterica*	R	Unknown	4	**2**	**Yes, VME**	+	No
123	*S. enterica*	R	Unknown	4	>4	No	+	No

S, susceptible; R, resistant; NA, not applicable.

[a]Unknown : no mutation in genes known to be involved in colistin resistance (*pmrA*, *pmrB*, *phoP*, *phoQ*, *mgrB* and *crrB* genes)

[b]VME, very major error (false-susceptibility compared to the results obtained by broth microdilution reference method)

[c]ME, major error (false-resistance compared to the results obtained by broth microdilution reference method)