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► **To cite this version:**

Tania Nawfal Dagher, Charbel Al-Bayssari, Selma Chabou, Sophie Baron, Linda Hadjadj, et al..
Intestinal Carriage of Colistin Resistant Enterobacteriaceae at Saint Georges Hospital in Lebanon.
Journal of Global Antimicrobial Resistance, 2019, 10.1016/j.jgar.2019.12.001 . hal-02454459

HAL Id: hal-02454459

<https://amu.hal.science/hal-02454459>

Submitted on 22 Aug 2022

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1 **Intestinal Carriage of Colistin Resistant Enterobacteriaceae at Saint Georges Hospital in**
2 **Lebanon**

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17 **Submission:** Journal of Global Antimicrobial Resistance; **article type** Original article

18 Abstract word count = 247

19 Text word count = 2618

20 Number of references = 24

21 Number of Tables = 2

22 **Keywords:** Colistin resistance, Gram-negative bacteria, *pmrAB*, *phoPQ*, *mgrB*, Lebanon.

23 **Running title:** Detection of colistin-resistant Gram-negative bacteria in Lebanese patients.

24 **Abstract:**

25 **Objectives**

26 The increase in resistance to antibiotics has led to the revival of colistin, as the last option for
27 treatment, which automatically led to an increase of colistin-resistant Gram-negative bacteria. In
28 this study, we report the presence of clinical colistin-resistant *Enterobacteriaceae* isolated from a
29 Lebanese hospital.

30 **Method**

31 From twenty-three rectal swabs, eight colistin-resistant clinical strains (5 *Escherichia coli*, 2
32 *Enterobacter cloacae*, and 1 *Klebsiella pneumoniae*) were isolated. Antibiotic susceptibility
33 testing was performed using the disk diffusion method and E-test. Broth microdilution method
34 was performed to determine colistin susceptibility. RT-PCR, standard PCR, and sequencing were
35 used to investigate genes encoding for extended-spectrum β -lactamases, carbapenemases, and
36 colistin resistance. Genotyping of these isolates was conducted by MLST.

37 **Results**

38 Results of antibiotic susceptibility testing revealed that all isolates were resistant to colistin. They
39 have MICs for colistin that ranged from 8 mg/L to 32 mg/L. Real-time PCR results showed that
40 5 strains harbored *bla*_{TEM-1} and one strain harbored *bla*_{TEM-163}. Moreover, 4 strains were positive
41 for *bla*_{CTX-M-15}, *bla*_{CTX-M-103}, and *bla*_{CTX-M-189}, and *K. pneumoniae* harbored *bla*_{SHV-1}. Observed
42 colistin resistance was linked to amino acid substitutions into protein sequences of *pmrA/B*,
43 *phoP/Q*, and *mgrB*. Interestingly, we report here mutation in the *mgrB* regulator and *pmrA/B*,
44 *phoP/Q* in colistin resistant *E. cloacae* and *E. coli* clinical isolates for the first time in Lebanon.

45 **Conclusion**

46 This study highlights the presence of colistin-resistant Gram-negative bacteria in a Lebanese
47 hospital, which is worrisome. An urgent strategy needs to be adopted in order to avoid the spread
48 of such bacteria.

49 **1. Introduction**

50 Resistance to antibiotics in Gram-negative bacteria such as *Acinetobacter baumannii*,
51 *Pseudomonas aeruginosa* and *Enterobacteriaceae* has become a field of interest over the last
52 decade. Those bacteria develop high resistance to most available antibiotics such as beta-lactams,
53 aminoglycosides, and fluoroquinolones [1]. This growing infection caused by multidrug-resistant
54 (MDR) Gram-negative bacteria has led to the renewal of colistin as a last-resort treatment option
55 especially for patients in intensive care units (ICUs) [2]. Unfortunately, the resistance to
56 polymyxins has been increasingly reported worldwide [1]. Polymyxin E (colistin) and polymyxin
57 B have been used in humans and are bactericidal toward Gram-negative bacteria except for the
58 bacteria that are intrinsically resistant to colistin such as *Burkholderia*, *Edwardsiella*, *Proteus*,
59 *Providencia*, *Morganella* and *Serratia*,. From 1959, colistin was available to treat infections
60 caused by Gram-negative bacteria. Conversely, its utilization is limited due to the daily dose that
61 cannot be increased because it will lead to nephrotoxicity [3]. Between the late 1990s and early
62 2000s, colistin was re-introduced into clinical medicine due to the emergence of resistance to
63 beta-lactams and carbapenems. This led to the emergence of colistin-resistant bacteria among
64 patients treated with this compound [1]. The bacterial cell membrane is the main target for the
65 antimicrobial activity of colistin: colistin binds to the anionic lipid A moiety of
66 lipopolysaccharide (LPS) and disrupts the cell membrane [1,3]. Several strategies are used by
67 Gram-negative bacteria to escape from polymyxin. The major mechanism of resistance occurs by
68 the alteration of the negatively charged lipopolysaccharide (LPS), by the addition of
69 phosphoethanolamine (PEtN) or 4-amino-4-deoxy-L-arabinose (L-Ara4N), to the lipid A moiety
70 of the LPS. This can be accomplished by specific mutations of the two-component systems
71 (TCSs) (*pmrA/pmrB*, *phoP/phoQ*), or its negative regulator *mgrB*. The modified LPS with this

72 positive charge reduces its binding to polymyxins and produces this resistance [1]. Moreover, it
73 has been demonstrated that resistance to colistin can also be due to the loss of LPS production
74 due to mutations in *lpxACD* genes [4], in addition to the use of efflux pumps, the capsules
75 formation, and overexpression of OprH, which are all efficiently regulated at the molecular level
76 [1]. Finally, colistin resistance can also be due to the presence of a plasmid-mediated *mcr* gene
77 that encodes for the phosphoethanolamine transferase enzyme which is capable of modifying the
78 lipid A moiety of LPS with the addition of phosphoethanolamine [5]. Finally, the increased use
79 of colistin as a last-resort therapeutic medication to treat patients infected with multidrug-
80 resistant (MDR) Gram-negative bacteria, has been followed by an increase in the number of
81 Gram-negative bacteria resistant to colistin [1]. The present study aims to investigate the
82 prevalence of colistin-resistant Enterobacteriaceae isolated from rectal swabs of patients
83 remaining at ICU of Saint-George Hospital in Beirut, Lebanon for more than one week and who
84 were treated with carbapenem and colistin combination therapy.

85 **2. Materials and methods**

86 **2.1 Microbiology procedure:**

87 2.1. Study Design

88 Between October 2016 and February 2017, a rectal swab was collected for each patient
89 hospitalized in the ICU of Saint-George Hospital in Beirut who received colistin and carbapenem
90 during their stay in ICU for more than one week. These patients were treated with combination
91 therapy because it was given as a precaution for patients to prevent their infections by
92 carbapenem-resistant bacteria that were spreading in the hospital. Only patients of the ICU
93 treated with combination therapy were selected because we believe that patients receiving
94 colistin have a bigger chance to have an intestinal carriage of colistin-resistant isolates. The
95 rectal swab was collected after 7 days of therapy for the analysis of colistin and carbapenem-
96 resistant bacteria. They were kept at -80°C before being transported to the laboratory in
97 Marseille, France.

98 **2.2 Microbiological procedures**

99 **2.2.1 Screening of colistin-resistant enterobacteria**

100 Rectal swabs were transferred to an enrichment Tryptic Soy Broth (TSB) medium and
101 incubated overnight at 37°C. After incubation, 100 µL of the enrichment medium were cultivated
102 for 24 hours at 37°C on the selective medium LBJMR for the screening of colistin-resistant
103 organisms, and on MacConkey agar plates supplemented with Ertapenem (2 µg/ml) for the
104 screening of carbapenem-resistant organisms. Species intrinsically resistant to colistin were
105 excluded from this study. Bacterial identification at the species level was performed using the

106 matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF)
107 method (Microflex; Bruker Daltonics).

108 **2.2.2 Antibiotic susceptibility testing**

109 The standard disk diffusion method on Mueller-Hinton agar was performed to determine
110 the antibiotic susceptibility testing of the strains as recommended by the European Committee of
111 Antimicrobial Susceptibility Testing (EUCAST) 2017 ([http://www.sfm-](http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM%20V2_0_Mai2017.pdf)
112 [microbiologie.org/UserFiles/files/casfm/CASFM%20V2_0_Mai2017.pdf](http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM%20V2_0_Mai2017.pdf)). The minimal
113 inhibitory concentration (MIC) of colistin was determined using the broth microdilution method
114 according to EUCAST 2017. Each strain with a MIC > 2 mg/L for colistin was considered as
115 resistant to this antibiotic.

116 **2.2.3 DNA extraction**

117 The automatic robot EZ1 (Qiagen BioRobot EZ1-, Tokyo, Japan) was performed to
118 extract the DNA of the bacteria, with the extraction kit (EZ1 DNA, Qiagen, Hilden, Germany),
119 according to the manufacturer's guidelines and eluted in 200 µL of elution buffer and stored at -
120 20°C.

121 **2.2.4 Multilocus sequence typing (MLST)**

122 To determine the genetic relationship between the clinical isolates, genotyping analysis
123 was done using seven housekeeping genes for *E. coli*, *K. pneumoniae* and *E. cloacae*, as
124 described on Institute Pasteur's MLST Web site (www.pasteur.fr/mlst).

125 **2.2.5 Screening of isolates by real-time PCR, standard PCR, and sequencing of the** 126 **antibiotic resistance genes**

127 To detect the presence of carbapenemase encoding genes, a real-time PCR assay was
128 done, using specific primers for *bla*_{OXA-48}, *bla*_{OXA-58}, *bla*_{KPC}, *bla*_{NDM}, and *bla*_{VIM} genes. All
129 colistin-resistant isolates were also screened for the presence of extended-spectrum β -lactamase
130 (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M},) genes and for the *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5* genes.
131 Probes and primers used were described previously [6,7]. Positive PCR amplification targeting
132 antibiotic resistance genes were sequenced using BigDye terminator chemistry on an automated
133 ABI 3130 sequencer (PE Applied Biosystems, Foster City, CA). ARG-ANNOT database was
134 used to analyze the sequenced genes and was compared to other genes using the BlastN and
135 BlastP analysis. The described colistin-resistant genes including *pmrA*, *pmrB*, *phoP*, *phoQ*, and
136 *mgrB* were amplified and sequenced [2,8–10]. Sequenced genes were compared with those of the
137 reference strains including *E. coli* K-12 MG 1655 (NCBI GenBank accession no.**CP000647**), *K.*
138 *pneumoniae* MGH 78578 (NCBI GenBank accession no.**CP000647**) and *E. cloacae* ATCC
139 13047 (NCBI GenBank accession no.**CP000647**) using the nps alignment software
140 (<https://prabi.ibcp.fr/htm/site/web/home>). We used PROVEAN (Protein Variation Effect
141 Analyser) software (http://provean.jcvi.org/seq_submit.php), to predict whether the identified
142 amino acids substitutions that result from missense mutations would affect the function of the
143 proteins. If the protein variant has a score below or equal to a predefined threshold (-2.5), it is
144 predicted to have a "deleterious" effect. If it is above this threshold, it is predicted to have a
145 "neutral" effect.

146 3. Results

147 23 rectal swabs were collected from 23 different patients treated with colistin and
148 carbapenem combination therapy. No carbapenem-resistant *Enterobacteriaceae* have been
149 isolated from MacConkey agar medium supplemented with Ertapenem. Of the 23 patients, 12
150 patients have no colistin-resistant *Enterobacteriaceae* (ColiRE) whereas 11 patients carried at
151 least one ColiRE. The mean carriage of ColiRE was 1 /sample except one patient carries
152 2ColiREs/sample. In summary, twelve ColiRE were collected with the LBJMR medium. Among
153 the isolates, 8 colistin-resistant strains were identified as: (5 *Escherichia coli*, 2 strains as
154 *Enterobacter cloacae*, and 1 strain as *Klebsiella pneumoniae*). In addition, 4 isolates that were
155 intrinsically resistant to colistin were also isolated (1 *Proteus mirabilis* and 3 *Morganella*
156 *morganii*) but were excluded from this study. The antibiotic resistance profile of all the isolates
157 is presented in Table 1. Results showed that all isolates were resistant to amoxicillin,
158 amoxicillin/clavulanic acid, cefalotin, doxycycline, and colistin. Colistin MICs ranged from 8 to
159 32 mg/L. Inversely, all isolates were susceptible to ertapenem, imipenem, amikacin, gentamicin,
160 and nitrofurantoin. In addition, six strains were resistant to the third generation cephalosporins.
161 Real-time PCR and standard PCR results showed that *E. coli* (EC-5, EC-10, and EC-23), *E.*
162 *cloacae* (Eclo-14A and Eclo-14B), and *K. pneumoniae* (KP-16), were positive for *bla*_{TEM} gene.
163 Sequence analysis of detected penicillinase identifies *bla*_{TEM-1} gene in positive strains except for
164 *E. coli* EC-23 that was identified as *bla*_{TEM-163}. Moreover, 4 out of the 5 *E. coli* strains harbored
165 *bla*_{CTX-M-15}, *bla*_{CTX-M-103}, and *bla*_{CTX-M-189} (Table 1). Single *K. pneumoniae* KP-16 harbored
166 *bla*_{SHV-1} gene. None of the strains harbored mobile colistin resistance genes (from *mcr-1* to *mcr-*
167 5). MLST analysis reveals that five *E. coli* isolates belonged to five different sequence types
168 (STs) including ST131, ST6174, ST405, ST162 and ST1451. *K. pneumoniae* has ST45, and the

169 two *E. cloacae* have new sequence types ST924 and ST925 (Table 1). Due to the absence of *mcr*
170 genes, the associated colistin resistance genes including *mgrB*, *pmrA*, *pmrB*, *phoP*, and *phoQ*
171 were amplified and sequenced. As shown in Table 2, sequence analysis of these latter revealed
172 that the two *E. cloacae* isolates exhibited non-synonymous mutations in *mgrB*, *pmrA*, *pmrB*, and
173 *phoP* genes leading to amino acid changes. These mutations in *mgrB* were detected for the first
174 time in Lebanon and were considered as deleterious by the PROVEAN software. Moreover,
175 sequence analysis of *E. coli* strains revealed that colistin resistance of *E. coli* EC-5 was due to
176 different missense mutations in *pmrAB* operon resulting in amino acid substitutions. For *E. coli*
177 EC-10, the missense mutations occurred in *phoPQ* proteins. *E. coli* EC-12 exhibited missense
178 and deletion mutations in *pmrB* and *phoPQ*. *E. coli* EC-21 showed *pmrA* and *phoP* genes
179 affected by missense mutations, and for *E. coli* EC-23 colistin resistance was due to missense
180 and deletion mutations in *pmrA* and *phoPQ*. Analysis of *K. pneumoniae* KP-16 revealed that
181 there were two genetic changes linked to colistin resistance: firstly, missense mutations leading
182 to a premature stop codon of the *mgrB* and secondly, a missense in the *pmrB* gene that results in
183 amino acid substitutions (Table 2). All these mutations were predicted as deleterious by the
184 PROVEAN software.

185 4. Discussion

186 The increase of multidrug-resistant Gram-negative bacteria, especially carbapenem-
187 resistant bacteria, is a worldwide clinical problem. Indeed, this has led to the re-introduction of
188 colistin into clinical treatment against infections caused by MDR bacteria [5]. This study
189 describes the presence of intestinal carriage of colistin-resistant enterobacteria in patients who
190 received colistin and carbapenem for treatment. We found that these patients carried only
191 colistin-resistant bacteria but no carbapenem-resistant bacteria, suggesting that the use of these
192 molecules may select colistin resistance but not carbapenem resistance. One limitation of our
193 study is that no sample was collected before the instauration of the treatment. It is possible that
194 these patients already carried these colistin-resistant bacteria, before the use of colistin. Different
195 studies, in different countries such as France, Columbia, Spain, Laos, Thailand, USA, Nigeria,
196 and Saudi Arabia have described the presence of colistin-resistant enterobacteria in humans
197 without prior colistin exposure [11]. Moreover, a study done by Nakayama T et al. showed that
198 even a short-term trip to some countries may result in the spread of *strains* that harbored *mcr-1*
199 and that are carried by international travelers where their numbers continue to increase, thereby
200 increasing the risk of spreading of *colistin-resistant* to developed countries [12].

201 Genotyping results showed that our strains belonged to different sequence types (ST)
202 suggesting, therefore, no link between strains isolated in the same hospital. It has been shown
203 that the clone ST131 is a globally important pathogen among multidrug resistance *E. coli* and is
204 linked to nosocomial and acquired infections [13]. Moreover, the clones of other *E. coli* ST405,
205 ST162, ST1451, and *K. pneumoniae* ST45 have been reported in hospitalized patients in
206 different counties, such as Brazil, Spain, Sweden and Uruguay [14–17]. In addition, *E. coli*
207 ST405 has also been reported in animals in Algeria [18].

208 In addition, all strains in this study were resistant to amoxicillin, amoxicillin-clavulanic
209 acid, and cephalosporins including cefalotin, cefepime, and ceftriaxone except the *E. cloacae*
210 isolates, which are sensitive to ceftriaxone and cefepime. All isolates harbored β -lactamase genes
211 such as TEM, SHV, and CTX-M. *bla*_{TEM-163}, *bla*_{CTX-M-103}, *bla*_{CTX-M-189}, and *bla*_{SHV-1} detected in
212 this present study have never been previously reported in Lebanon.

213 Interestingly, many different gene mutations including missenses and deletions have been
214 identified in genes associated with colistin resistance in our clinical isolates. However, some of
215 these mutations have been reported earlier such as R81S found in PmrA of a colistin-resistant
216 strain isolated from animal samples under the Spanish Surveillance Network of Antimicrobial
217 Resistance in Bacteria [9,19]. R81S is also similar to the missense mutation R81H which was
218 found in PmrA of *S. Typhimurium* [20]. The D150W mutation in PhoQ of our *E. coli* EC-10
219 isolate was previously reported in Taiwan in a *K. pneumoniae* isolate collected from patient at
220 the Taipei Veterans General Hospital [21]. Mutation T92H found in *pmrB* of *E. coli* EC-12
221 isolate, were similar to T92A described in PmrB of *S. Typhimurium* [4,20]. D191Y mutation in
222 PhoP of *E. coli* EC-21 was reported in South Africa in clinical *K. pneumoniae* isolate [22].
223 Olaitan et al. have also identified mutation L96P in *K. pneumoniae* isolated from patients and
224 healthy individuals in Nigeria, Thailand, Lao PDR, and France [2] which is similar to L96S
225 found in PhoQ of our study. Regarding colistin-resistant *K. pneumoniae* KP-16 isolate, different
226 missense mutations have been found, such as G37M, C39P and N42Y that were similar to the
227 mutations identified in the *mgrB* of *K. pneumoniae* described by Poirel et al. In Lebanon, there is
228 no report describing mechanism of colistin resistance of *Enterobacteriaceae* clinical isolates
229 except a study reported recently by one of our group that describe three colistin-resistant *K.*
230 *pneumoniae* isolated from Lebanese Hospital in Beirut in 2015. The resistance of those strains

231 was due to mutations of *mgrB*, *pmrA*, *pmrB*, and *phoQ* genes [5]. Those strains were isolated
232 from patients without previous use of colistin. On the other hand, two studies were done by
233 Dandachi et al., which described the mechanism of colistin resistance in animals. In these studies,
234 colistin resistance was due to the presence of a plasmid-mediated *mcr-1* gene isolated from *E.*
235 *coli* strain from poultry in Lebanon [23] and from 23 *E. coli* strains isolated from Lebanese
236 swine farms [23]. In fact, carriage of *mcr-1* in farms could constitute a potential key for the
237 introduction of this gene into the community as well as to clinical settings in Lebanon through
238 horizontal transfer from animals to humans. Due to the spread of carbapenemase producers in
239 hospitals in Lebanon, it is expected that once *mcr-1* would be introduced, this latter will be
240 selected by the frequent use of colistin.

241 Few studies have described the mechanism of colistin resistance in *E. cloacae*. It seems
242 that the PhoP/PhoQ two-component system may play a role in this colistin resistance. The *mgrB*
243 gene acts as a repressor of this two-component system and inactivation of this gene leads to
244 colistin resistance in this species. However, a previous study on colistin-resistant *E. cloacae* has
245 detected no alteration of the *mgrB* gene, leading the mechanism still unknown [10]. On the other
246 hand, a recent study had detected a missense mutation in the *mgrB* gene (I10V), in addition to
247 many missense mutations in the *pmrA/B* genes which may contribute to the colistin resistance
248 [24]. In our study, we have detected for the first time in Lebanon mutations in the two
249 components system *pmrA/B*, *phoP/Q* and *mgrB* genes in *E. cloacae*. Moreover, different
250 missense mutations that have been found, such as C39G, N42S, I45Y, W47V, W47S, *48K and
251 *48Y were similar to the mutation identified in the *mgrB* of *K. pneumoniae* described by Poirel
252 et al [19].

253 In conclusion, this study reports the emergence of colistin resistance in Gram-negative
254 bacteria in Lebanon. The use of polymyxins to treat patients has probably contributed to the
255 emergence of colistin-resistant strains in this hospital since no relationship between our strains
256 has been observed. An urgent strategy must be implemented to prevent the spread of these
257 resistant microorganisms among hospitalized patients.

258

259 **Funding source:**

260 This work was supported by the French Government under the « Investissements d’avenir»
261 (Investments for the Future) program managed by the Agence Nationale de la Recherche (ANR,
262 fr: National Agency for Research), (reference: Méditerranée Infection 10-IAHU-03).

263

264 **Competing interests:**

265 No conflicts of interest or financial disclosure for all authors

266

267 **Ethical approval:**

268 Not required.

269

270 **Acknowledgments**

271 The authors thank CookieTrad for English language corrections.

272

273 **Authors’ affiliations**

274 TND and ED collect samples and isolate strains. TND, CAB, SC, LH, SB, ED, and SMD
275 performed experiments and analyzed the data. TND, SMD, ED, and JMR draft and proofreading

276 the manuscript. JMR and ED conceived the study, participated in its design and coordination. All
277 authors read and approved the final version of the manuscript.

278

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358 **Table 1:** Phenotypic and genotypic features of the eight colistin-resistant strains isolated at the Saint Georges hospital in Lebanon

Strain name	AMX	AMC	TZP	CF	CRO	FEP	ERT	IPM	AK	GEN	CIP	FF	F	DO	SXT	CS	Colistin MIC (µg/ml)	<i>bla</i> _{SHV}	<i>bla</i> _{TEM}	<i>bla</i> _{CTX-M-A}	ST
<i>E. coli</i> EC-5	R	R	S	R	R	R	S	S	S	S	S	S	S	R	S	R	16	-	TEM-1	-	1451
<i>E. coli</i> EC-10	R	R	S	R	R	R	S	S	S	S	R	S	S	R	S	R	16	-	TEM-1	CTX-M-15	131
<i>E. coli</i> EC-12	R	R	S	R	R	R	S	S	S	S	S	S	S	R	S	R	16	-	-	CTX-M-189	6174
<i>E. coli</i> EC-21	R	R	R	R	R	R	S	S	S	S	R	S	S	R	S	R	16	-	-	CTX-M-15	405
<i>E. coli</i> EC-23	R	R	S	R	R	R	S	S	S	S	R	S	S	R	R	R	8	-	TEM-163	CTX-M-103	162
<i>E. cloacae</i> Eclo-14A	R	R	S	R	S	S	S	S	S	S	S	R	S	R	S	R	8	-	TEM-1	-	924
<i>E. cloacae</i> Eclo-14B	R	R	S	R	S	S	S	S	S	S	S	R	S	R	S	R	16	-	TEM-1	-	925
<i>K. pneumoniae</i> KP-16	R	R	R	R	R	R	S	S	S	S	R	S	S	R	R	R	32	SHV-1	TEM-1	-	45

359 amoxicillin (AMX), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), Cefalotin (CF), ceftriaxone (CRO), cefepime (FEP),

360 ertapenem (ERT), imipenem (IPM), amikacin (AK), gentamicin (GEN), ciprofloxacin (CIP), fosfomycin (FF), nitrofurantoin (F), doxycycline

361 (DO), trimethoprim-sulfamethoxazole (SXT), colistin (CS). R, resistant; S, sensitive, sequence type (ST)

362 **Table 2:** Mutations of the associated colistin-resistance proteins

Strain names	MgrB	PmrA	PmrB	PhoP	PhoQ
<i>E. coli</i> EC-5	Not tested	L11Q R81S	A65del	No mutation	No mutation
<i>E. coli</i> EC-10	Not tested	No mutation	No mutation	R163P, N165del	I88T, L95P, P111T, W113R, L114S, S116W, R145W, D150W, H157P, L218F
<i>E. coli</i> EC-12	Not tested	No mutation	V77A, L81P, T92H R93P, L95A, E97G L98A, Q99A, L102S E103W	Q113P, K171R, H198del	L231R, N235I, E246del
<i>E. coli</i> EC-21	Not tested	G15E, R81S D82del, D86R, K87del	No mutation	K171R, R185P, S187I, D191Y, K200R, I210S, V213G, Y218H	L96S, Q99A, I109H, W113M, F119S
<i>E. coli</i> EC-23	Not tested	R81S	No mutation	N119I, E154del	K46I, del ₅₆₋₇₀
<i>K. pneumoniae</i> KP-16	S36G, G37D, I38A, C39A, I41T, N42Y	No mutation	D150N, S205I, G207del	No mutation	No mutation
<i>E. cloacae</i> Eclo-14A	G37_V38insG V38S, C39G, A40K I41M, N42S, K43G, I45Y, P46G	S64C, L216W, E217I	L38S	No mutation	No mutation

W47V, ins48K

<i>E. cloacae</i> Eclo-14B	V38I, W47S, ins 48V	No mutation	No mutation	D46V, I47F, I49F, E140del, F141del, I143D, N144A, del ₁₄₈₋₁₆₃	No mutation
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