

# First report of blaOXA-24 carbapenemase gene, armA methyltransferase and aac(6')-Ib-cr among multidrug-resistant clinical isolates of *Proteus mirabilis* in Algeria

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1 **First report of *bla*<sub>OXA-24</sub> carbapenemase-encoding gene, *armA* Methyltransferase and**  
2 ***aac(6)-Ib-cr* producing multidrug-resistant clinical isolates of *Proteus mirabilis* in**  
3 **Algeria.**

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21 **Abstract**

22 **Background:** Carbapenemase-producing, or carbapenem-resistant *Enterobacteriaceae*, are  
23 an emerging threat to human and animal health because they are resistant to many of the last-  
24 line antimicrobials available for disease treatment. The aim of this study was to analyze  
25 antimicrobial resistance patterns and their encoding genes of *P. mirabilis* isolated in  
26 Constantine, Algeria.

27 **Methods:** A total of 106 *PMP* (*Proteus- Morganella- Providencia*) strains were isolated from  
28 a large variety of clinical specimens, at University Hospital of Constantine in Algeria, and  
29 identified by the API 20E system and Bruker MALDI Biotyper 2.0 (MALDI-TOF/MS)  
30 platforms for microbial identification. Diagnostic accuracy was determined by independent  
31 comparison of each method to phylogenetic analysis based on the 16S rRNA gene  
32 sequencing. Antimicrobial susceptibility was determined using disc diffusion and E-test  
33 methods. The presence of antibiotic resistance genes was screened for by PCR amplification  
34 and sequencing.

35 **Results:** a total of 72 *PMP* strains were multidrug- resistant. Among them, one isolate was  
36 resistant to imipenem with minimum inhibitory concentration  $\geq 12 \mu\text{g/ml}$ . PCR and  
37 sequencing showed the presence of different antibiotic resistance encoding genes: *bla*<sub>CTX-M-15</sub>,  
38 *bla*<sub>TEM-1</sub>, *bla*<sub>TEM-2</sub>, *bla*<sub>PER-1</sub>, *bla*<sub>SHV-11</sub>, *aadA1*, *aadA2*, *armA*, *aac(6')-Ib*, *aac(6')-Ib-cr*, *aac(3)-Ia*,  
39 *ant(2'')-I*, and forming different profiles. Moreover, the *bla*<sub>OXA-24</sub> gene was detected in the  
40 imipenem-resistant strain.

41 **Conclusion:** in this study, we found for the first time in Algeria a multidrug resistant  
42 *P. mirabilis* isolates harboring *bla*<sub>OXA-24</sub>, *armA* 16S rRNA methylase and *aac(6)-Ib-cr* gene.

43 **Keywords:** Multidrug-resistant; *bla*<sub>OXA-24</sub>; *arma* methyltransferase; *aac(6)-ib-cr*, *Proteus*,  
44 *Morganella* and *Providencia*.

45           **1. Introduction**

46           Members of the three genera *Proteus*, *Morganella* and *Providencia* (*PMP*) are  
47 components of the normal bacterial flora of the intestinal tracts of humans and animals and  
48 are widespread in the environment (1). Owing to their varied habitats, members of the *PMP*  
49 genera have many possible routes of human infection. The modes of transmission may  
50 include nosocomial sources, such as hospital food and equipment, intravenous solutions and  
51 human contact through contaminated skin surfaces (2), causing primary and secondary  
52 infections (1). Interest in the species comprising these genera has occurred mainly that most  
53 infections are associated with prolonged hospitalization and in the case of *Proteus* and  
54 *Morganella spp.*, colonization of indwelling catheters and associated urinary tract infections  
55 (2). These organisms are intrinsically resistant to nitrofurantoin and tetracycline, but are  
56 naturally susceptible to  $\beta$ -lactams, aminoglycosides, fluoroquinolones, and trimethoprim-  
57 sulfamethoxazole (1;2). However, drug resistance has been increasingly reported for these  
58 species, and the diffusion of resistance to extended-spectrum cephalosporins due to the  
59 production of extended-spectrum  $\beta$ -lactamases (ESBLs) has become of great concern (3)  
60 since ESBL production in *P. mirabilis* was first documented in 1987 (4). Carbapenems are  
61 now employed frequently in the treatment of serious nosocomial infections caused by Gram-  
62 negative bacteria, including ESBL-producing *Enterobacteriaceae* (5). However, the  
63 emergence of clinical strains of various species producing Class D carbapenemases include  
64 oxacillin-hydrolyzing, or OXA-type enzymes has been reported (6). These class D  
65 carbapenemases have so far been associated with imipenem-resistant *A. baumannii* strains (7).  
66 However, the first and only detection of a clinical *P. mirabilis* strain producing class D  
67 carbapenemase was in France in 2002, producing an OXA-23 enzyme (6).

68           The purpose of the present study was (i) to determine the rate of antibiotic resistance of  
69 a large series of clinical isolates of the *PMP* group, from University Hospital of Constantine,

70 Algeria, against molecules usually prescribed first intention and (ii) to detect for the first time  
71 the carbapenem-resistant *P. mirabilis* carrying the *bla*<sub>OXA-24</sub>.

## 72 **2. Materials and methods**

### 73 **2.1. Bacterial isolates**

74 A total of 106 clinical isolates belonging to the *Proteus-Morganella-Providencia* group  
75 were isolated from outpatient and patients hospitalized between January and December 2011  
76 in the University Hospital of Constantine, Algeria. A large variety of clinical specimens were  
77 issued from pus (n = 60), urine (n = 38), sonde (n = 5), catheter (n = 1), biological fluids (n =  
78 3) and blood cultures (n = 1) with sex ratio=1. Strains were cultured on TSA (Trypticase Soja)  
79 agar plates at 37°C for 18 to 24 hours. Species identification was performed by standard  
80 biochemical tests using API20E system (BioMerieux, Marcy l'Etoile, France) and by use of  
81 the matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-  
82 TOF MS) method (Microflex; Bruker Daltonics) as previously described (8). Additionally,  
83 species identification was confirmed by sequencing of the 16S ribosomal RNA gene.

### 84 **2.2. Antibiotic susceptibility testing**

85 Antimicrobial susceptibility was tested with Mueller-Hinton agar by standard disk  
86 diffusion procedure as described by the Antibiogram Committee of the French society for  
87 microbiology (CA-SFM) ([www.sfm.asso.fr](http://www.sfm.asso.fr)). The following antibiotics were tested:  
88 Amoxicillin (25 µg), Amoxicillin/Clavulanic acid (20/10 µg), Cefotaxim (30 µg), Ceftazidim  
89 (30 µg), Ceftriaxon (30 µg), Aztreonam (30 µg), Imipenem (10 µg), Gentamicin (15 µg (10  
90 UI), Kanamicin (30 UI), Tobramicin(10 µg), Amikacine (30 µg), Pefloxacin (5 µg),  
91 Ciprofloxacin (5 µg), Ofloxacin (5 µg), Triméthoprim/sulfamethoxazol (1,25/23,75 µg) and  
92 Colistin (50 µg).

93 Strains producing ESBL were detected by the test of synergy between a central disk of  
94 amoxicillin/clavulanic acid distant 30mm discs of cefotaxime, ceftriaxone, ceftazidime or  
95 aztreonam. The presence of ESBL was suspected in an appearance in "champagne cork".

96 The minimum inhibitory concentrations (MICs) for imipenem were determined using  
97 Etest method (AB Biodisk). Interpretations were made according to CA-SFM breakpoints.

### 98 **2.3. PCR amplification of resistance-encoding genes**

99 Detection of antimicrobial resistance genes was performed by Conventional PCR using  
100 the forward and reverse primers for the ESBL genes (*bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, *bla<sub>CTX-M</sub>*, *bla<sub>VEB</sub>*, *bla<sub>PER</sub>*  
101 and *bla<sub>GES</sub>*) (9) , Fluoroquinolone resistance genes (*qnrA* and *qnrB*) (10), aminoglycoside-  
102 modifying enzymes (AMEs) (*armA*, *aac3*, *aac(6')*, *ant(2'')*, *aph(3')*, *aad*, and *rmtA*) (11) and  
103 carbapenemases, for the strain resistant to imipenem, (*bla<sub>VIM</sub>*, *bla<sub>IPM</sub>*, *bla<sub>KPC</sub>*, *bla<sub>NDM-1</sub>*, *bla<sub>OXA23</sub>*,  
104 *bla<sub>OXA24</sub>*). Positive PCR products obtained were sequenced using the Big Dye<sup>®</sup> terminator  
105 chemistry on an automated ABI 3730 sequencer (Applied Biosystems, Foster City, California,  
106 United States). The sequences obtained were analyzed using BlastN and BlastP against the  
107 NCBI database (<http://www.ncbi.nlm.nih.gov/blast>) for characterization.

### 108 **2.4. Conjugation test**

109 Conjugation experiments were carried out between *P. mirabilis* donor imipenem  
110 resistant and the azide-resistant recipient strain *E. coli J53* on TSA plates. Transconjugants  
111 were selected on TSA plates supplemented with 2 µg ceftazidime (CAZ) ml<sup>-1</sup> or 2 µg  
112 cefotaxime (CTX) ml<sup>-1</sup> and 100 µg sodium azide ml<sup>-1</sup>.

## 113 **3. Results**

### 114 **3.1. *Proteus*, *Morganella* and *Providencia* isolates**

115 During the study period, a total of 106 isolates were identified for 3 genera, *Proteus*,  
116 *Morganella* and *Providencia* both by the API20E identification system, by MALDI-TOF MS

117 and by sequencing of the 16S rRNA gene. Four species were recovered, *Proteus mirabilis* is  
118 the species most frequently isolated and represents 89.62 % or 95 strains of all *PMP* isolated,  
119 followed by *Proteus vulgaris* and *Morganella morganii* with a frequency 4.72% (5 strains) for  
120 each one, and *Providencia stuartii* represent 0.94 % (1 strain). Among these strains, 84  
121 isolates (79%) were isolated from hospitalized patient, and 22 isolates (21%) were isolated  
122 from outpatient

### 123 **3.2. Antimicrobial susceptibility**

124 Results of antibiotic susceptibility testing for the 106 isolates are summarized in table 1.  
125 A review of the antimicrobial resistance profile of isolates from the different clinical  
126 specimens showed that amoxicillin, amoxicillin/clavulanic acid, cefotaxim, tobramycin,  
127 ciprofloxacin, pefloxacin and trimethoprim/sulfamethoxazol were the most active antibiotics.  
128 Antibiotics with the lowest activities on all four species were aztreonam, gentamicin and  
129 amikacin. All strains were resistant to colistin, but just one strain of *P. mirabilis* was found to  
130 be resistant to imipenem (MIC  $\geq$  12  $\mu$ g/ml, confirmed by E test).

### 131 **3.3. Resistance gene determination**

132 All 106 strains were checked for the presence of ESBL, resistance to fluoroquinolones  
133 and aminoglycoside-modifying enzymes encoding genes using the PCR methods described  
134 above. 72 strains were positive for different genes including *bla<sub>CTX-M-15</sub>*, *bla<sub>TEM-1</sub>*,  
135 *bla<sub>TEM-2</sub>*, *bla<sub>PER-1</sub>*, *bla<sub>SHV-11</sub>*, *aadA1*, *aadA2*, *armA*, *aac(6')-Ib*, *aac(6')-Ib-cr*, *aac(3)-Ia*,  
136 *ant(2'')-I*, and forming different profiles that it has been shown in table 2. Moreover, the  
137 sequencing amplification products confirmed the presence of the *bla<sub>OXA-24</sub>* gene in the  
138 imipenem-resistant strain.

139

140 Conjugal studies between *E. coli J53* and the imipenem-resistant *P. mirabilis* isolate  
141 was successful with the transfer of *bla*<sub>TEM-1</sub>, *aadA-2* and *armA* genes. However, it has failed  
142 to transfer carbapenemase resistance to *E. coli J53* by conjugation.

#### 143 **4. Discussion**

144 The genera *Proteus*, *Morganella* and *Providencia* are considered as one of the most  
145 important human pathogens that often cause serious infections in hospitalized patients and  
146 immunocompromised persons. Different methods of isolation and identification have been  
147 developed for most *PMP* species; however, the treatment of infected patients is often  
148 problematic due to the development of antibiotic resistance. The occurrence of MDR and pan  
149 drug-resistant *PMP* is a growing concern. In this study, we investigated the molecular  
150 mechanism of antibiotic resistance in *PMP* clinical isolates recovered from University  
151 hospital of Constantine in Algeria. Our data revealed genetic diversity of genes that encode  
152 ESBL with the emergence of new genes.

153 Epidemiological data regarding ESBLs available for Algeria report the presence of  
154 different genes such as *bla*<sub>CTX-M-3</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>TEM-110</sub>, *bla*<sub>SHV-1</sub>, *bla*<sub>SHV-12</sub>,  
155 *bla*<sub>SHV-28</sub>, *bla*<sub>PER1</sub> and *bla*<sub>VEB-1</sub> in various species of Gram-negative bacteria (12). In our  
156 series of *PMP* isolates, the main molecular support explaining the resistance to ESBL was the  
157 presence of *bla*<sub>CTX-M-15</sub>, *bla*<sub>TEM-1</sub>, *bla*<sub>TEM-2</sub>, *bla*<sub>PER-1</sub> and *bla*<sub>SHV-11</sub> ESBL encoding genes along  
158 with the coexistence of *bla*<sub>OXA-24</sub> carbapenemase encoding gene for one strain *P. mirabilis*  
159 imipenem-resistant.

160 The presence of *bla*<sub>SHV-11</sub> gene has been observed in only one strain of *P. vulgaris*. This  
161 gene is different from Gln - Leu substitution at amino acid 35 to the *bla*<sub>SHV-1</sub> gene, and it's  
162 differing only at position 1 of codon 238 and 240 (13). This gene was previously described in  
163 *K. pneumonia* (14) but never in a strain belonging to the *PMP* group.

164



165           Currently, carbapenems are the most potent antimicrobial agents used in the treatment  
166 of serious infections caused by multidrug-resistant gram-negative bacteria. especially in the  
167 current context of massive diffusion of bla<sub>CTX-M</sub> type ESBLs, but are antibiotics which it is  
168 necessary to preserve (15) . This especially that there is no current perspective to placing on  
169 the market of new antibiotics. These antibiotics have good activity against the *PMP* group.  
170 Unfortunately, there has been in recent years the emergence and spread of resistant strains to  
171 carbapenems (16). This resistance is due, mainly, to the production of carbapenemases  
172 essentially of class D (oxacillinases) sometimes class B (metallo  $\beta$ -lactamases).

173           According to the literature, genes encoding carbapenemases have widely been detected  
174 in many bacterial groups in different countries. However, reports on *Proteus spp.* producing  
175 carbapenemases are rare. Bonnet et al. first reported chromosome-encoded class D beta-  
176 lactamase *OXA-23* in *P. mirabilis* in 2002 in France, which was exclusively found  
177 in *Acinetobacter spp* (6). Different studies reported the *P. mirabilis* isolates producing a *VIM-*  
178 *1* molecular class B metallo- $\beta$ -lactamase resulting in carbapenem resistance (5;17). In 2008,  
179 Tibbetts et al. first reported a single isolate of *P. mirabilis* harboring *bla<sub>KPC</sub>* in USA (18). Hu  
180 et al. reported for the first time emergence of *P. mirabilis* producing *bla<sub>KPC-2</sub>* and *qnrD* in the  
181 same strain in a Chinese hospital (19), and Cicek et al. reported the first identification of  
182 *bla<sub>OXA-320</sub> -aadA1* gene cassette, a novel variant of Class D  $\beta$ -lactamase, in *P. mirabilis* from  
183 Turkey (20). Recently, Girlich et al. describe for the first time the *bla<sub>NDM-1</sub>* gene in a *Proteus*  
184 *mirabilis* clinical isolate (21), in addition, the production of *bla<sub>OXA-58</sub>* in *P. mirabilis* has been  
185 reported from France (22) and Germany (23). Another recent study has demonstrated the  
186 presence of *bla<sub>OXA-58</sub>* in Multidrug-Resistant *Proteus mirabilis* Strain from Gaza, Palestine  
187 (24).

188

189 In our study, the main molecular support of resistance to carbapenems is the presence of  
190 *bla<sub>OXA-24</sub>* gene encoding a class of carbapenemase D, gene typically present in *A. baumannii*.  
191 This gene was identified in isolates in 1997, which were part of an outbreak in Spain, and  
192 since, it has never been detected in strains other than *A. baumannii* (25). In Algeria, this gene  
193 is still rare, with only a very few reports, it was previously reported in 6 strains of *A.*  
194 *baumannii* isolated in 2011 in different hospitals in Tlemcen, Setif, Sidi Bel Abbes, Oran and  
195 Tizi Ouzou (26) and in 17 other strains of *A. baumannii* isolated in three different hospitals in  
196 the west of Algeria (Tlemcen, Oran and Sidi Bel Abbes) from 2008 to 2012 (27). However,  
197 the existence of *P. mirabilis* isolate resistant to carbapenems with a class D carbapenemase  
198 *bla<sub>OXA24</sub>* type has never been described before. In this report, we describe what we believe to  
199 be the first reported case of infection caused by a strain of carbapenem resistant *Proteus*  
200 *mirabilis* positive for the *bla<sub>OXA-24</sub>* gene. It is a disturbing trend, given the relatively recent  
201 discovery of this family of carbapenemase. While extended-spectrum  $\beta$ -lactamase and  
202 carbapenemase activities have previously been documented in *Proteus* species (5;6;17-20),  
203 the addition of *bla<sub>OXA-24</sub>* to the spectrum of resistance factors carried by an organism that  
204 traditionally has been placed in the low-level endogenous resistance category is equally  
205 troubling.

206 Conjugating experiments reveal the association of genes *bla<sub>TEM-1</sub>*, *aadA-2* and *armA* on  
207 a same genetic structure, since they were found in the transconjugant after transfer of a single  
208 plasmid. But the carbapenemase resistance of *P. mirabilis* encoded by *bla<sub>OXA-24</sub>* is mainly  
209 referred to the chromosomal gene rather than plasmid-mediated factors. According to the  
210 literature, the *bla<sub>OXA-23</sub>* and *bla<sub>OXA-58</sub>* genes are mostly found on plasmids, whereas the *bla<sub>OXA-</sub>*  
211 *24* genes have been identified as chromosomally encoded (28). It is tempting to speculate that  
212 genes encoding *bla<sub>OXA-24</sub>* enzymes could belong to a subspecies of *P. mirabilis* that had

213 acquired this type of gene in the distant past. The reservoir (natural producer) of these genes  
214 is unknown, as is the location of the genetic exchange.

215 Aminoglycosides are highly potent, broad spectrum antibiotics with many desirable  
216 properties for the treatment of human infections caused by both Gram-positive  
217 (*Staphylococcus spp.*, *Enterococcus spp.*) and Gram-negative, including *Proteus spp.* strains.  
218 In the past decade, these antibiotics are no longer used because of the spread of AMEs  
219 worldwide (34). In this research we observed a high rates of AME among multidrug resistant  
220 isolates (30,55%). Acquisition of new resistance mechanisms by strains already resistant to  
221 particular antimicrobials creates serious concern, due to the propagation of multidrug  
222 resistant. In our survey, the most prevalent gene encoding AME was *aac(6)-Ib*, present in  
223 59.09% of AME-positive isolates. These results are consistent with the literatures data in  
224 which *aac(6')-Ib* is considered as the most common variant of AME among Gram-negatives,  
225 as well as Gram-positives (29), but resistance of *Proteus* to aminoglycosides still remains low  
226 over other bacteria (30) . In Algeria similar results are reported in previous studies on  
227 aminoglycoside resistance mechanisms among different clinical strains Gram-negative  
228 (12;27;31), except that none of these reports has studied resistance to aminoglycosides in  
229 *PMP* strains. From these results it is suggested that during the period from 2007 to 2013,  
230 genes coding for AME have become endemic in Algeria and have spread among different  
231 species of Gram-negative bacteria.

232 Despite the existence of strains resistant to fluoroquinolones, no strain carries neither  
233 the gene *qnrA* nor *qnrB* gene. The results of resistance to ciprofloxacin, observed in our  
234 strains, suggest that the main mechanism of fluoroquinolone resistance is probably  
235 due to the mutations in genes encoding topoisomerases or gyrases which express high levels  
236 of resistance (32). However, one strain of *P. mirabilis* is found carrier gene *aac(6')-Ib-cr*.

237 This variant is an acetyltransferase which is part of aminoglycoside modifying enzymes  
238 (AME)-(33).

239 Fluoroquinolone resistance genes are recent identification in Algeria. The first study  
240 reported the presence of genes *qnr* in *E. cloacae* strains was published in 2008 (34).  
241 Thereafter, several studies have identified different variants determinants *qnr* often in  
242 combination with gene *aac(6')-Ib-cr* among *Enterobacteriaceae* strains (12;27;31). This  
243 determinant was discovered for the first time in 2006 in a strain of *E. coli qnrA* positive in  
244 China (33). In Algeria, the gene *aac(6')-Ib-cr* was detected for the first time in 2009 in a strain  
245 of *E. cloacae* (31). Since then, two other studies have reported the presence of this gene in  
246 both hospital and community (27;35). But this gene has not been reported in Algeria in strains  
247 belonging to the *PMP* group, although it is recently reported in North Africa in clinical strains  
248 of *P. mirabilis* and *M. morgani* isolated in Tunisia (36). Our study is the first description in  
249 Algeria of *aac(6')-Ib-cr* determinant in clinical strains of *P. mirabilis*, this suggesting that  
250 there is a spread of this gene between bacterial groups and clonal spread within *PMP* strains  
251 in North Africa. This plasmid mechanism of quinolone resistance confers a low level of  
252 resistance to fluoroquinolones, but their presence could further encourage the move to a  
253 higher level of resistance by mutation selection in the target of these molecules (31).

254 In conclusion, The acquisition of resistance to carbapenems in *P. mirabilis* may be of  
255 significant concern for physicians because this organism is usually resistant to colistin and is  
256 poorly susceptible to tigecycline, which represents an important option for treating infections  
257 caused by multi-drug-resistant Gram-negative bacilli.

258 This study is the first report describing imipenem-resistant *P. mirabilis* isolated from  
259 patients in Algeria. We report for the first time the emergence of *bla<sub>OXA-24</sub>*, and the  
260 cooccurrence of 16S rRNA methylase *armA* with *bla<sub>OXA-24</sub>* in Eastern Algeria. We also report

261 the first identification of multidrug-resistant *PMP* isolates harboring *bla<sub>SHV-11</sub>* and  
262 *aac(6)-Ib-cr* genes in Algeria.

263 The emergence of a combination of resistance genes in *PMP* group could pose a public  
264 health problem, thus substantially restricting the therapeutic alternatives. Based on this  
265 finding, it would be prudent to systematically review all clinically relevant Enterobacteriaceae  
266 isolates for resistance to carbapenem, even in circumstances where the use of this class of  
267 drug for the treatment of the infection would be less likely , i.e., uncomplicated urinary tract  
268 infection.

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- 290 (1) O'HARA CM, BRENNER FW, MILLER JM. Classification, identification, and  
291 clinical significance of *Proteus*, *Providencia*, and *Morganella*. Clin Microbiol Rev  
292 2000; 13:534-546.
- 293 (2) Manos J. and Belas R. The Genera *Proteus* , *Providencia* , and *Morganella*  
294 The Prokaryotes. In: Dworkin, Martin, Falkow, Stanley, Rosenberg, Eugene, Schleifer, Karl  
295 Heinz, and Stackebrandt, Erko. Springer New York, 2006: 245-269.
- 296 (3) STURENBURG E, MACK D. Extended-spectrum beta-lactamases: implications for  
297 the clinical microbiology laboratory, therapy, and infection control. J Infect 2003;  
298 47:273-295.
- 299 (4) KIM JY, PARK YJ, KIM SI, KANG MW, LEE SO, LEE KY. Nosocomial outbreak  
300 by *Proteus mirabilis* producing extended-spectrum beta-lactamase VEB-1 in a Korean  
301 university hospital. J Antimicrob Chemother 2004; 54:1144-1147.
- 302 (5) VOURLI S, TSORLINI H, KATSIFA H et al. Emergence of *Proteus mirabilis*  
303 carrying the bla metallo-beta-lactamase gene. Clin Microbiol Infect 2006; 12:691-694.
- 304 (6) BONNET R, MARCHANDIN H, CHANAL C et al. Chromosome-encoded class D  
305 beta-lactamase OXA-23 in *Proteus mirabilis*. Antimicrob Agents Chemother 2002;  
306 46:2004-2006.
- 307 (7) AFZAL-SHAH M, WOODFORD N, LIVERMORE DM. Characterization of OXA-  
308 25, OXA-26, and OXA-27, molecular class D beta-lactamases associated with  
309 carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. Antimicrob  
310 Agents Chemother 2001; 45:583-588.

- 311 (8) SENG P, DRANCOURT M, GOURIET F et al. Ongoing revolution in bacteriology:  
312 routine identification of bacteria by matrix-assisted laser desorption ionization time-  
313 of-flight mass spectrometry. *Clin Infect Dis* 2009; 49:543-551.
- 314 (9) KIRATISIN P, APISARNTHANARAK A, LAESRIPA C, SAIFON P. Molecular  
315 characterization and epidemiology of extended-spectrum-beta-lactamase-producing  
316 *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated  
317 infection in Thailand, where the CTX-M family is endemic. *Antimicrob Agents*  
318 *Chemother* 2008; 52:2818-2824.
- 319 (10) ROBICSEK A, STRAHILEVITZ J, SAHM DF, JACOBY GA, HOOPER DC. qnr  
320 prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United  
321 States. *Antimicrob Agents Chemother* 2006; 50:2872-2874.
- 322 (11) WANG-SHENG Z, ZU-HUANG M, XING-BEI W. Emergence of five kinds of  
323 aminoglycoside-modifying enzyme genes simultaneously in a strain of multidrug-  
324 resistant *Escherichia coli* in China. *Clin Microbiol Infect* 2012; 18:E11-E12.
- 325 (12) BABA AHMED-KAZI TANI Z., ARLET G. News of antibiotic resistance among  
326 Gram-negative bacilli in Algeria. *Pathol Biol (Paris)* 2014; 62:169-178.
- 327 (13) HOWARD C, VAN DA, KELLY G, SCHOONEVELDT J, NIMMO G, GIFFARD  
328 PM. Identification and minisequencing-based discrimination of SHV beta-lactamases  
329 in nosocomial infection-associated *Klebsiella pneumoniae* in Brisbane, Australia.  
330 *Antimicrob Agents Chemother* 2002; 46:659-664.
- 331 (14) BERRAZEG M, DRISSI M, MEDJAHED L, ROLAIN JM. Hierarchical clustering as  
332 a rapid tool for surveillance of emerging antibiotic-resistance phenotypes in *Klebsiella*  
333 *pneumoniae* strains. *J Med Microbiol* 2013; 62:864-874.



- 334 (15) PATERSON DL. Recommendation for treatment of severe infections caused by  
335 Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). Clin  
336 Microbiol Infect 2000; 6:460-463.
- 337 (16) TSAI HY, CHEN YH, TANG HJ et al. Carbapenems and piperacillin/tazobactam for  
338 the treatment of bacteremia caused by extended-spectrum beta-lactamase-producing  
339 *Proteus mirabilis*. Diagn Microbiol Infect Dis 2014; 80:222-226.
- 340 (17) SCHNEIDER I, MARKOVSKA R, MARTEVA-PROEVSKA Y, MITOV I,  
341 MARKOVA B, BAUERNFEIND A. Detection of CMY-99, a novel acquired AmpC-  
342 type beta-lactamase, and VIM-1 in *Proteus mirabilis* isolates in Bulgaria. Antimicrob  
343 Agents Chemother 2013.
- 344 (18) TIBBETTS R, FRYE JG, MARSCHALL J, WARREN D, DUNNE W. Detection of  
345 KPC-2 in a clinical isolate of *Proteus mirabilis* and first reported description of  
346 carbapenemase resistance caused by a KPC beta-lactamase in *P. mirabilis*. J Clin  
347 Microbiol 2008; 46:3080-3083.
- 348 (19) HU YY, CAI JC, ZHANG R, ZHOU HW, SUN Q, CHEN GX. Emergence of *Proteus*  
349 *mirabilis* harboring blaKPC-2 and qnrD in a Chinese Hospital. Antimicrob Agents  
350 Chemother 2012; 56:2278-2282.
- 351 (20) CICEK AC, DUZGUN AO, SARAL A, SANDALLI C. Determination of a novel  
352 integron-located variant (bla ) of Class D beta-lactamase in *Proteus mirabilis*. J Basic  
353 Microbiol 2013.
- 354 (21) GIRLICH D, DORTET L, POIREL L, NORDMANN P. Integration of the blaNDM-1  
355 carbapenemase gene into *Proteus* genomic island 1 (PGI1-PmPEL) in a *Proteus*  
356 *mirabilis* clinical isolate. J Antimicrob Chemother 2015; 70:98-102.

- 357 (22) GIRLICH D, BONNIN RA, BOGAERTS P et al. Chromosomal Amplification of the  
358 blaOXA-58 Carbapenemase Gene in a *Proteus mirabilis* Clinical Isolate. *Antimicrob*  
359 *Agents Chemother* 2017; 61.
- 360 (23) LANGE F, PFENNIGWERTH N, GERIGK S et al. Dissemination of blaOXA-58 in  
361 *Proteus mirabilis* isolates from Germany. *J Antimicrob Chemother* 2017; 72:1334-  
362 1339.
- 363 (24) CHEN L, AL LN, CHAVDA KD et al. First report of an OXA-48-producing  
364 multidrug-resistant *Proteus mirabilis* strain from Gaza, Palestine. *Antimicrob Agents*  
365 *Chemother* 2015; 59:4305-4307.
- 366 (25) EVANS BA, AMYES SG. OXA beta-lactamases. *Clin Microbiol Rev* 2014; 27:241-  
367 263.
- 368 (26) KEMPF M, BAKOUR S, FLAUDROPS C et al. Rapid detection of carbapenem  
369 resistance in *Acinetobacter baumannii* using matrix-assisted laser desorption  
370 ionization-time of flight mass spectrometry. *PLoS One* 2012; 7:e31676.
- 371 (27) MESLI E, BERRAZEG M, DRISSI M, BEKKHOUCHA SN, ROLAIN JM.  
372 Prevalence of carbapenemase-encoding genes including New Delhi metallo-beta-  
373 lactamase in *Acinetobacter* species, Algeria. *Int J Infect Dis* 2013; 17:e739-e743.
- 374 (28) POIREL L., NORDMANN P. Carbapenem resistance in *Acinetobacter baumannii*:  
375 mechanisms and epidemiology. *Clin Microbiol Infect* 2006; 12:826-836.
- 376 (29) VANHOOF R, NYSSSEN HJ, VAN BE, HANNECART-POKORNI E.  
377 Aminoglycoside resistance in Gram-negative blood isolates from various hospitals in

- 378 Belgium and the Grand Duchy of Luxembourg. Aminoglycoside Resistance Study  
379 Group. J Antimicrob Chemother 1999; 44:483-488.
- 380 (30) WIECZOREK P, SACHA P, HAUSCHILD T et al. The aac(6')Ib gene in *Proteus*  
381 *mirabilis* strains resistant to aminoglycosides. Folia Histochem Cytobiol 2008;  
382 46:531-533.
- 383 (31) MERADI L, DJAHOUDI A, ABDI A, BOUCHAKOUR M, PERRIER GROS  
384 CLAUDE JD, TIMINOUNI M. Qnr and aac (6')-Ib-cr types quinolone resistance  
385 among *Enterobacteriaceae* isolated in Annaba, Algeria. Pathol Biol (Paris) 2011;  
386 59:e73-e78.
- 387 (32) HAWKEY PM. The growing burden of antimicrobial resistance. J Antimicrob  
388 Chemother 2008; 62 Suppl 1:i1-i9.
- 389 (33) ROBICSEK A, STRAHILEVITZ J, JACOBY GA et al. Fluoroquinolone-modifying  
390 enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med  
391 2006; 12:83-88.
- 392 (34) IABADENE H., MESSAI Y., AMMARI H. et al. Dissemination of ESBL and Qnr  
393 determinants in *Enterobacter cloacae* in Algeria. J Antimicrob Chemother 2008;  
394 62:133-136.
- 395 (35) BABA AHMED-KAZI TZ, DECRE D, GENEL N, BOUCHERIT-OTMANI Z,  
396 ARLET G, DRISSI M. Molecular and epidemiological characterization of  
397 enterobacterial multidrug-resistant strains in Tlemcen Hospital (Algeria) (2008-2010).  
398 Microb Drug Resist 2013; 19:185-190.

399 (36) MAHROUKI S, PERILLI M, BOUROUIS A et al. Prevalence of quinolone resistance  
400 determinant qnrA6 among broad- and extended-spectrum beta-lactam-resistant  
401 *Proteus mirabilis* and *Morganella morganii* clinical isolates with sul1-type class 1  
402 integron association in a Tunisian Hospital. Scand J Infect Dis 2013; 45:600-605.

Table1. Phenotypic antimicrobial resistance of 106 *PMP* isolates

	AMX	AMC	CTX	CAZ	CRO	ATM	IMP	GN	K	TOB	AK	PEF	CIP	OFX	STX	CT
<i>P.mirabilis</i>	65,62	25	10,42	4,16	9,38	1,04	1,04	10,42	15,63	54,16	9,38	36,46	31,25	36,46	56,25	100
<i>P.vulgaris</i>	100	16,66	50	16,67	33,33	16,67	0	0	16,66	33,33	0	16,67	16,67	16,67	33,33	100
<i>M.morganii</i>	80	80	20	20	0	0	0	0	20	0	0	40	40	80	80	100
<i>P.stuartii</i>	0	0	0	0	0	0	0	0	0	0	0	100	100	0	100	100

AMX: amoxicillin; AMC : amoxicillin/clavulanic acid; CTX : cefotaxim; CAZ : ceftazidim; CRO : ceftriaxon; ATM : Aztreonam; IMP : imipenem; GN : gentamicine; K : kanamicin; TOB : tobramicin; AK : amikacin; PEF : pefloxacin; CIP : ciprofloxacin; OFX : ofloxacin; SXT : triméthoprim/sulfaméthoxazol; CT : colistine.

Table 2. Genotypic profiles of antimicrobial resistance of *PMP* isolates

Species	Groups	Genes	Nb of Strains	Rate of isolates (%)
<i>P. mirabilis</i>	1	<i>bla</i> <sub>CTX-M-15</sub>	6	5.45
	2	<i>bla</i> <sub>CTX-M-15</sub> + <i>bla</i> <sub>TEM-1</sub>	3	2.73
	3	<i>bla</i> <sub>CTX-M-15</sub> + <i>bla</i> <sub>TEM-2</sub>	1	0.91
	4	<i>bla</i> <sub>TEM-1</sub>	29	26.36
	5	<i>bla</i> <sub>TEM-1</sub> + <i>aadA2</i>	3	2.73
	6	<i>bla</i> <sub>TEM-1</sub> + <i>bla</i> <sub>OXA-24</sub> + <i>aadA2</i> + <i>armA</i>	1	0.91
	7	<i>bla</i> <sub>TEM-1</sub> + <i>aadA1</i> + <i>aac(3)-Ia</i>	1	0.91
	8	<i>bla</i> <sub>TEM-1</sub> + <i>aac(6')-Ib</i>	1	0.91
	9	<i>bla</i> <sub>TEM-2</sub>	9	8.18
	10	<i>bla</i> <sub>TEM-2</sub> + <i>aac(6')-Ib-cr</i>	1	0.91
	11	<i>aac(6')-Ib</i>	6	5.45
	12	<i>aac(6')-Ib</i> + <i>ant(2'')-I</i>	1	0.91
	13	<i>aac(3)-Ia</i>	1	0.91
<i>P. vulgaris</i>	1	<i>bla</i> <sub>TEM-1</sub>	1	0.91
	2	<i>bla</i> <sub>TEM-2</sub> + <i>bla</i> <sub>PER-1</sub> + <i>bla</i> <sub>SHV-11</sub> + <i>aadA1</i> + <i>aac(6')-Ib</i> + <i>ant(2'')-I</i>	1	0.91
	3	<i>bla</i> <sub>TEM-2</sub> + <i>bla</i> <sub>PER-1</sub> + <i>aadA1</i> + <i>aac(6')-Ib</i> + <i>ant(2'')-I</i>	1	0.91
	4	<i>bla</i> <sub>PER-1</sub> + <i>aac(6')-Ib</i>	1	0.91
	5	<i>armA</i> , <i>aadA2</i>	1	0.91
<i>M. morgani</i>	1	<i>bla</i> <sub>TEM-1</sub>	1	0.91
	2	<i>aac(6')-Ib</i>	1	0.91
	3	<i>aac(3)-Ia</i> + <i>aadA2</i> + <i>ant(2'')-I</i>	1	0.91
<i>P. stuartii</i>	1	<i>aadA1</i>	1	0.91
Total			72	67.92