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2 OXA-48-like carbapenemases in the UK: an analysis of isolates and cases from 2007 - 2014

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13 Running heading: OXA-48-like carbapenemases in the UK

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23 **Abstract**

24 **Objectives:** OXA-48-like carbapenemases have spread worldwide since 2001. We analysed
25 patient and microbiological data for UK isolates with these enzymes as confirmed by the
26 national reference laboratory from November 2007 - December 2014.

27 **Methods:** MICs were determined using BSAC agar dilution. Isolates with reduced
28 susceptibility or resistance to ≥ 1 carbapenem and high-level resistance to both
29 piperacillin/tazobactam (MIC > 64 mg/L) and temocillin (MICs ≥ 128 mg/L) were screened by
30 PCR for *bla*_{OXA-48-like} genes. The genomes of around half of the isolates were sequenced,
31 with MLST types, resistance genes and plasmid replicon types inferred. Patient data
32 provided by sending laboratories were reviewed.

33 **Results:** Isolates (n=741) with OXA-48-like carbapenemases were submitted from 111 UK
34 laboratories, representing 536 patients. Almost all (99%; 736/741) were Enterobacteriaceae,
35 predominantly *Klebsiella pneumoniae* (55%; 408), and most (80%; 595) were from
36 inpatients. WGS of 351 non-duplicate isolates identified *bla*_{OXA-48} as the most common
37 variant, found in two-thirds (235/351) of isolates, followed by *bla*_{OXA-181} (68), *bla*_{OXA-232} (32),
38 *bla*_{OXA-244} (10), *bla*_{OXA-484} (5) and *bla*_{OXA-245} (1). Among *K. pneumoniae* (163/351), *E.coli*
39 (114/351), and *E. cloacae* (42/351), 119 STs were identified. Mapping analyses revealed
40 that 63% (222/351) of isolates harboured plasmids that shared >99% identity to one of four
41 known plasmids; pOXA-48a (44%; 154/351), pOXA-232 (10%; 34/351), pOXA181 (9%;
42 30/351), and pKP3-A (1%; 4/351); the remaining 37% of isolates harboured *bla*_{OXA-48-like} in
43 unknown environments.

44 **Conclusions:** OXA-48-like carbapenemases are an increasing problem in the UK. This
45 study highlights both the role of successful plasmids and polyclonal nature of their
46 dissemination.

47 Introduction

48 OXA-48-type carbapenemases were first identified in 2001 in a carbapenem-resistant
49 *Klebsiella pneumoniae* isolate from the urine of a patient hospitalised in Istanbul, Turkey.¹
50 Since then, reports of infections caused by OXA-48-producing Enterobacteriaceae have
51 escalated - particularly in Europe, Asia and Africa, and less so in the Americas.²⁻⁵ There are
52 six carbapenem-hydrolysing class D beta-lactamase (CHDL) subgroups that are clinically
53 significant; OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, and OXA-143.⁶ All except the
54 OXA-48 group are predominantly found in *Acinetobacter* spp. isolates,⁶ whereas OXA-48
55 enzymes are usually found in Enterobacteriaceae.⁵ To date, 14 OXA-48-like variants have
56 been described (OXA-48, -162, -163, -181, -199, -204, -232, -244, -245, -247, -370, -405, -
57 436, and -484) of which 11 are CHDLs. These vary in sequence by one to five amino acids
58 from the 'classical' OXA-48 variant and hydrolyse penicillins and carbapenems but not
59 extended-spectrum cephalosporins (e.g. cefepime and ceftazidime).¹ In contrast to their
60 CHDL counterparts, OXAs -163, -247 and -405 also differ from OXA-48 by one or two amino
61 acids but additionally have a four-amino-acid deletion in the active site region: as a result
62 they lack significant carbapenemase activity but do exhibit increased activity toward
63 extended-spectrum cephalosporins.⁷⁻⁹

64 *bla*_{OXA-48} has only been found in Enterobacteriaceae, and has been associated with
65 outbreaks in Turkey, the Middle East and North Africa.^{5, 10} Other variants have different
66 geographical associations: in particular *bla*_{OXA-181} and *bla*_{OXA-232} have been epidemiologically
67 linked to the Indian subcontinent and are often co-harboured with NDM enzymes.^{11, 12}

68 The proliferation of OXA-48-like enzymes has been attributed both to successful clones and
69 to plasmid spread. In 2011, OXA-48-positive *K. pneumoniae* ST395 was identified in patients
70 in Morocco, the Netherlands and France, suggesting the country-to-country transfer of this
71 clone,¹³ with patient-linked transfer of OXA-48-producing *Enterobacter cloacae* from
72 Morocco to France also documented.¹⁴ On the other hand, pOXA-48a, a 61.9 kb self-

73 conjugative IncL/M plasmid, was shown to be the primary vehicle for dissemination of *bla*_{OXA-}
74 ₄₈ in several outbreaks.^{5, 15} This broad-host-range plasmid harbours *bla*_{OXA-48} within Tn1999
75 and occurs across several enterobacterial species.^{5, 15}

76 This study describes the epidemiology of OXA-48-like carbapenemase producers submitted
77 to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI)
78 Reference Unit between 2007 and 2014.

79

80 **Materials and Methods**

81 **Bacterial isolates, identification and susceptibility testing**

82 Isolates were submitted to PHE's AMRHAI Reference Unit from clinical laboratories across
83 the UK between November 2007 and December 2014 for investigation of unusual
84 resistance, including to carbapenems.

85 Bacterial identification was carried out using chromogenic agars [CHROMagar™ Orientation
86 (CHROMagar, Paris, France) and Brilliance UTI (Oxoid, Basingstoke, UK)] together with
87 API-20E tests (bioMérieux SA, Marcy-l'Étoile, France) or, since August 2012, by matrix-
88 assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS;
89 Bruker Microflex LT, Bruker Daltonik GmbH, Bremen, Germany).

90 Antibiotic susceptibilities (MICs) were determined by BSAC agar dilution¹⁶ using AMRHAI's
91 standard Gram-negative antibiotic panel, which includes ertapenem, meropenem and
92 imipenem (the latter with/without 320 mg/L EDTA to detect metallo-carbapenemases), and
93 interpreted using EUCAST breakpoints.¹⁷

94

95 **Screening for carbapenemase genes**

96 Isolates displaying high-level resistance to piperacillin/tazobactam (MICs ≥ 64 mg/L) and
97 temocillin (MICs ≥ 128 mg/L), as well as reduced susceptibility or resistance to any
98 carbapenem were tested for *bla*_{OXA-48-like} genes by in-house PCR¹ and/or with a commercial
99 microarray (Check-MDR CT102; Check-Points, Wageningen, The Netherlands).¹⁸ In
100 instances where imipenem potentiation by EDTA was observed, isolates were also tested by
101 PCR for the presence of metallo-carbapenemases (*bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{GIM}, *bla*_{SIM} and
102 *bla*_{SPM}) by in-house PCRs.¹⁹

103

104 **Whole Genome Sequencing (WGS) and analyses**

105 Three hundred and seventy isolates, temporally and geographically distributed throughout
106 the study, were selected for WGS. Genomes were sequenced using the Nextera sample
107 preparation method with the standard 2 × 100-base sequencing protocols on a HiSeq
108 instrument (Illumina, San Diego, CA, USA). Data were analysed using an in-house
109 bioinformatics pipeline as previously described.²⁰ Sequence types (STs) were inferred from
110 WGS data where MLST schemes exist.

111 For plasmid analysis, sequencing reads were mapped against known OXA-48 plasmids,
112 namely pOXA-48a (NC_019154), pOXA-232 (JX423831), pKP3-A (NC_019160), and
113 pOXA181 (KP400525).

114

115 **Analysis of patient demographic information**

116 Patient data were obtained from the request forms sent with submissions from referring
117 laboratories. A patient was categorized as 'new' if they were found to have OXA-48-like-
118 positive isolates detected by AMRHAI for the first time and 'known' if any OXA-48-like-
119 positive isolate, irrespective of species, had previously been identified from the patient by
120 AMRHAI.

121 **Results**

122 **Demographics of patients affected and distribution**

123 During the study period, AMRHAI confirmed 741 OXA-48-like positive isolates from 111
124 laboratories throughout the UK and obtained from 536 patients. Figure 1 illustrates the
125 temporal distribution of these isolates among 'new' and 'known' patients, and among
126 submitting laboratories. The first OXA-48-like positive isolate was a *K. pneumoniae*,
127 submitted to AMRHAI in November 2007 and obtained from the urine of a patient previously
128 hospitalised in Turkey.¹⁰

129 Isolates with OXA-48-like carbapenemases were submitted from laboratories across all UK
130 regions. The national distribution of affected patients was as follows: England (n=514),
131 Scotland (n=13), Northern Ireland (n=6), and Wales (n=3). The greatest number of affected
132 patients was in the London region (n=203), followed by the North West (n=143).

133 Most source patients were hospitalized (79%; 421/536) but a few were outpatients (7%;
134 38/536), in primary care (8%; 41/536) or in unknown settings (7%; 36/536). The mean
135 patient age was 59.5 years and 54% (289/536) were male.

136 A travel history was reported for 130/536 (24%) patients. Of these, 55 patients had
137 documented foreign travel to the following destinations; India (12), Turkey (9), Pakistan (5),
138 Egypt (4), Libya (4), Spain (4), Kuwait (3), Malta (3), Sri Lanka (2), Tunisia (2) and single
139 patients had travelled to Cyprus, Kenya, Morocco, Russia, Saudi Arabia, Singapore, and
140 Syria. Twenty patients were known to have been hospitalised whilst abroad in Egypt (4),
141 Libya (4), Turkey (4), India (3), Pakistan (2), Cyprus (1), Spain (1) and Sri Lanka (1).

142 Single OXA-48-like-positive isolates were referred from 408/536 (76%) patients and multiple
143 isolates from the remaining 128 (24%). Amongst the patients with multiple OXA-48-like-
144 positive isolates, 43/128 (34%) yielded isolates of different species or genera and 63/128
145 (49%) had isolates referred from different anatomical sites. The OXA-48-like-positive isolates

146 were referred over a period of <14 days in 65/128 (51%) instances, for 17/128 over a period
147 of 14 to 28 days, 38/128 over a period of 1 to 6 months and over a period >6 months from 8
148 patients. Seventeen percent (127/741) of isolates were submitted from a single laboratory
149 (laboratory 1), which serves 3 hospitals (to be subsequently known as hospitals A, B and C),
150 over a 2-year period. Further details of the isolates from this laboratory are given below.

151

152 **Microbiology**

153 Most (99%; 736/741) submitted isolates were Enterobacteriaceae, comprising: *K.*
154 *pneumoniae* (55%; 408/741), *E. coli* (29%; 218/741), *Enterobacter* spp. (9%; 68/741),
155 *Klebsiella oxytoca* (3%; 24/741), *Citrobacter* spp. (2%; 14/741), *Serratia marcescens* (>1%;
156 3/741) and one *Raoutella ornithinolytica*. There were also five non-Enterobacteriaceae
157 isolates comprising: *Pseudomonas aeruginosa* (n=3; all from one patient)²¹ and *Shewanella*
158 spp. (n=2; OXA-48-like enzymes are intrinsic in this genus).

159 If samples, rather than patients were considered as the denominator, most were taken in
160 hospitals (89%; 656/741), but some were general practice urines (6%; 45/741) and a few
161 from unknown settings (5%; 40/741). The most frequently reported specimen type was urine
162 (29%; 215/741), followed by faeces/rectal swabs (27%; 202/741). Fifteen percent (110/741)
163 of isolates were obtained from tissue and fluid samples, 11% (83/741) from blood cultures
164 and line tips, 9% (68/741) from screening swabs, 5% (40/741) from respiratory samples, and
165 3% (23/741) were unknown specimen types (Table 1).

166

167 **Carbapenemase alleles and typing of the isolates**

168 WGS was undertaken for 351 non-duplicate isolates from single patients and their STs
169 (where MLST schemes exist) and carbapenemase alleles were defined. These comprised:
170 *K. pneumoniae* (n=163), *E. coli* (n=114), *E. cloacae* (n=42), *K. oxytoca* (n=13), *Citrobacter*

171 spp. (n=11), other *Enterobacter* spp. (n=5), *S. marcescens* (n=2), and *P. aeruginosa* (n=1).
172 Their carbapenemase genes comprised: *bla*_{OXA-48} (66%; 230/351), *bla*_{OXA-181} (18%; 62/351),
173 *bla*_{OXA-232} (7%; 24/351), *bla*_{OXA-244} (3%; 10/351), *bla*_{OXA-245} (<1%; 1/351), *bla*_{OXA-484} (1%;
174 5/351), *bla*_{OXA-48} + *bla*_{NDM-1} (1%; 5/351), *bla*_{OXA-181} + *bla*_{NDM-1} (2%; 6/351) and *bla*_{OXA-232} +
175 *bla*_{NDM-1} (2%; 8/351). The carbapenemase variants and allele combinations OXA-48, OXA-
176 181, OXA-48 + NDM-1, and OXA-181 + NDM-1, were found in multiple species. OXA-232,
177 OXA-245, OXA-484, and OXA-232 + NDM-1 were found only in *K. pneumoniae* isolates,
178 whilst OXA-244 was found only in *E. coli* isolates. Numerous other genes encoding
179 resistance to several other classes of antibiotics were also detected throughout all species
180 (Table 2). Overall, 19/351 (5%) isolates had OXA-48-like enzymes together with another
181 carbapenemase, 266/351 (76%) also harboured a predicted extended-spectrum beta-
182 lactamase or plasmid-encoded AmpC, and 85/351 (24%) had OXA-48-like enzymes without
183 any of these additional beta-lactamase types.

184

185 *K. pneumoniae*

186 One hundred and sixty-three non-duplicate *K. pneumoniae* isolates, from 56 laboratories
187 across 10 UK regions, were sequenced. Individual laboratories submitted between 1 and 15
188 isolates. Forty-nine STs were identified, the most frequent being ST14 (27 isolates from 17
189 centres over 16-months), followed by ST231 (18 from 14 centres over 15-months), ST147
190 (17 from 13 centres over 7-years), ST101 (13 from 10 centres over 4-years), ST11 (10 from
191 7 centres over 16-months) and ST16 (6 from 5 centres over 3-months). The remaining 43
192 STs were each represented by five isolates or fewer. Five OXA-48-like variants were
193 identified: *bla*_{OXA-48} (n=86), *bla*_{OXA-181} (n=31), *bla*_{OXA-232} (n=24), *bla*_{OXA-484} (n=5) and *bla*_{OXA-245}
194 (n=1), and 16 isolates produced more than one carbapenemase: *bla*_{OXA-48} + *bla*_{NDM-1} (n=3),
195 *bla*_{OXA-181} + *bla*_{NDM-1} (n=5), and *bla*_{OXA-232} + *bla*_{NDM-1} (n=8).

196 Multiple plasmid replicon types were identified including IncL/M, IncA/C, several IncF
197 variants, IncHI2, IncX3 and ColE-like replicons. Plasmid mapping revealed that 70/86 (81%)
198 of isolates with OXA-48-enzymes and one with OXA-245 had plasmids exhibiting >99%
199 sequence identity to pOXA-48a, whereas among the 31 with OXA-181-enzymes 8 (26%)
200 and 5 (16%) had plasmids with >99% sequence identity to pOXA181 and pKP3-A,
201 respectively. All 32 isolates with OXA-232 enzymes had plasmids exhibiting >99%
202 sequence identity to pOXA-232. pOXA-48a sequences were found in 37 STs, most
203 frequently ST11 (n=8) and ST101 (n=9) whereas pOXA181 sequences were found in 5 STs
204 (ST11, ST61, ST25, ST307, and ST709), each with 1-3 representatives; whilst pKP3-A
205 sequences were found in 2 STs with one (ST395) and four representatives (ST147)
206 respectively. pOXA-232 sequences were found in 7 STs (ST14, ST15, ST16, ST147, ST231,
207 ST307 and ST395), most often ST14 (15 isolates from 13 centres), ST231 (11 from 8
208 centres), and ST147 (3 from 2 centres).

209 The earliest UK isolate with an OXA-48-like enzyme dated from 2007 and was from a patient
210 who had previously been hospitalised in Turkey.¹⁰ It was shown here to belong to ST147 and
211 to harbour a pOXA-48a-like plasmid.

212 The most frequent submitter, laboratory 1, sent 15 isolates that were subjected to WGS over
213 22-months – 13 of which were obtained from hospital A. These fifteen represented 11 STs
214 and all harboured plasmids with >99% sequence identity to pOXA-48a.

215

216 *E. coli*

217 One hundred and fourteen non-duplicate *E. coli* isolates, from 49 laboratories across 7 UK
218 regions, were sequenced. Laboratories submitted between 1 and 16 isolates and 37 STs
219 were identified. The most frequent of these were ST38 (53 isolates from 34 laboratories over
220 42-months) and ST410 (12 from nine laboratories over 20-months). The remaining 35 STs

221 were each represented by four isolates or fewer. Three OXA-48-like variants were identified;
222 *bla*_{OXA-48} (n=74), *bla*_{OXA-181} (n=30), and *bla*_{OXA-244} (n=10).

223 Multiple plasmid replicon types were identified including IncL/M, several IncF replicons,
224 IncB/O, IncHI2, IncK and IncX3. Plasmid analyses revealed that 22/74 (30%) of *E. coli* with
225 OXA-48-enzymes had plasmids exhibiting >99% sequence identity to pOXA-48a, and 22/30
226 (73%) of those with OXA-181 had plasmids that exhibited >99% sequence identity to
227 pOXA181. pOXA-48a sequences were found in 17 STs, each with 1-4 representatives.
228 pOXA181 sequences were found in 10 STs, predominantly ST410 (n=11) which were
229 submitted from 8 laboratories across 5 regions. No plasmid could be identified in 70 isolates.
230 Of these, 50 belonged to ST38, 41 of them harbouring *bla*_{OXA-48} and nine harbouring *bla*_{OXA-}
231 ₂₄₄. The most frequent submitter, laboratory 1, sent 16 isolates that were subject to WGS
232 over a 21-month period - 14 of which were obtained from hospital A; these represented 14
233 STs, all had OXA-48 and 12/16 (75%) harboured pOXA-48a sequences.

234

235 *Enterobacter* spp.

236 Forty-seven non-duplicate *Enterobacter* spp. isolates, sent from 15 laboratories across 7 UK
237 regions, were sequenced. These comprised; *E. cloacae* (n=42), *E. aerogenes* (n=4) and *E.*
238 *hormaechei* (n=1). Individual laboratories submitted between 1 and 27 isolates. Forty-five
239 isolates harboured *bla*_{OXA-48} and the remaining 2 isolates harboured *bla*_{OXA-48} + *bla*_{NDM-1}.
240 Thirteen STs were identified among the *E. cloacae* isolates, each with between 1 and 17
241 representatives. The most frequently obtained ST was ST108 (17 isolates from 2 centres
242 over 15-months), the remaining 12 STs were represented by 5 isolates or fewer. Multiple
243 plasmid replicon types were identified including IncL/M, IncN, IncHI1 and several IncF
244 replicons. Plasmid mapping revealed that 42/47 (89%) of isolates had DNA exhibiting >99%
245 sequence identity to pOXA-48a. These comprised 40 *E. cloacae*, and single isolates of *E.*
246 *aerogenes* and *E. hormaechei*. Thirteen STs were identified among the 40 *E. cloacae*

247 isolates but predominantly ST108 with 17 representatives. Most were submitted (16/17) from
248 laboratory 1 and 14/16 were obtained from hospital A, over a 3-month period. Fifteen of
249 these 16 (94%) harboured pOXA-48a sequences. In total, over half (27/48) of the
250 *Enterobacter* spp. isolates with OXA-48-like enzymes were submitted by laboratory 1 over a
251 15-month period – 25 of which were obtained from hospital A, with these comprising 25 *E.*
252 *cloacae* and one *E. hormaechei*. All 26 isolates produced OXA-48 and the 25 *E. cloacae*
253 isolates represented 5 STs.

254

255 *K. oxytoca*

256 Thirteen non-duplicate *K. oxytoca* isolates, sent from 7 laboratories across 4 UK regions,
257 were sequenced, with individual laboratories submitting between 1 and 4 isolates. All
258 harboured the classical *bla*_{OXA-48} variant. Five STs were identified, namely ST176 (6 isolates
259 from 5 centres in four regions), ST27 (4 from one centre in one month), along with single
260 representatives of ST36, ST95 and ST168. Multiple plasmid replicons were identified
261 including IncL/M, IncHI2 and several IncF replicons. Plasmid mapping analyses revealed
262 that all isolates shared >99% sequence identity with plasmid pOXA-48a.

263

264 Other species

265 The remaining 14 isolates that were sent for WGS comprised: *Citrobacter freundii* (n=9),
266 *Citrobacter koseri* (n=2), *S. marcescens* (n=2) and *P. aeruginosa* (n=1). All harboured
267 classical *bla*_{OXA-48} except for one *C. freundii* isolate, which harboured *bla*_{OXA-181} + *bla*_{NDM-1},
268 and the *P. aeruginosa* isolate that had *bla*_{OXA-181}. The *P. aeruginosa* isolate was previously
269 found to belong to ST773 with *bla*_{OXA-181} encoded within Tn2013.²¹ Plasmid replicon types
270 included IncL/M, several IncF replicons and IncA/C. Within the 9 *C. freundii* isolates 8 STs
271 were identified. Five of these were obtained from inpatients in hospital A, 3 of which

272 harboured pOXA-48a sequences. In the remaining isolates 2 *C. koseri* isolates which
273 produced OXA-48 shared >99% identity to plasmid pOXA-48a, and one OXA-181 producing
274 *C. freundii* isolate shared >99% identity to pKP3-A.

275

276 **Antibiotic susceptibility**

277 MIC distributions for OXA-48-like-positive isolates are shown in Table 3. Ninety-nine percent
278 (724/728) of isolates with available susceptibility data were resistant or non-susceptible to
279 ertapenem; however 42% (312/735) and 54% (400/735) remained susceptible to imipenem
280 and meropenem, respectively, based on EUCAST breakpoints. In all but 8 cases, MICs of
281 meropenem were above the EUCAST screening cut-off concentration of 0.125 mg/L.²² For
282 the remaining 8 cases the imipenem MICs were also below the EUCAST screening cut-off of
283 1 mg/L but were above the ertapenem MIC cut-off of 0.125 mg/L. All 8 isolates were *E. coli*
284 and analysis of 7/8 by WGS indicated that 6 different STs and 2 OXA-48-like variants
285 (*bla*_{OXA-48} (5) and *bla*_{OXA181} (2)) were represented. Piperacillin/tazobactam resistance (MIC
286 >16 mg/L) was observed in 732/734 of tested isolates (data not shown). All the isolates that
287 co-produced either NDM or VIM enzymes were non-susceptible to all three carbapenems.
288 Non-susceptibility to ceftazidime and cefotaxime was observed in 69% and 89% of isolates.
289 Non-susceptibility to the aminoglycosides amikacin, gentamicin and tobramycin was
290 observed in 26%, 55% and 61% of isolates, respectively. Almost all (28/34) isolates that co-
291 produced another carbapenemase were non-susceptible to all 3 aminoglycosides and all
292 were resistant to ciprofloxacin. Most (91%; 659/722) isolates were susceptible to colistin.

293 Colistin resistance was observed in 63 isolates, submitted from 32 laboratories over 6 years,
294 the majority (54/63) of which were *K. pneumoniae* and had MICs in the range to 4->32 mg/L.
295 Sequencing of 21/54 colistin resistant *K. pneumoniae* isolates identified 9 STs, 6 of which
296 were represented by a single isolate and the 4 remaining STs were as follows: ST14 (6),
297 ST101 (4), ST147 (2), and ST231 (4). These 21 were submitted from 14 laboratories across

298 5 regions. For 10 isolates colistin MICs were >32mg/L; these comprised 7 *K. pneumoniae*
299 and one *E. coli* along with two *S. marcescens* with inherent resistance. These were referred
300 from 10 laboratories across 4 regions. Non-susceptibility to ciprofloxacin and tigecycline was
301 observed in 63% and 32% of isolates, respectively.

302

303 **Discussion**

304 This report reviews all isolates producing an OXA-48-like carbapenemase and referred to
305 PHE's AMRHA1 Reference Unit from laboratories across the UK between November 2007
306 and December 2014. Over this study period 741 OXA-48-like-positive isolates were obtained
307 from 536 patients across all UK regions.

308 The majority of isolates were from clinical specimens, predominantly urines. All isolates were
309 resistant to ≥ 2 classes of antibiotics and most were non-susceptible at EUCAST breakpoints
310 to at least one of the three carbapenems tested. A high rate of susceptibility was maintained
311 only to colistin (91%), with amikacin (74% susceptible) and tigecycline (68%) next in rank
312 order. High levels of resistance to the third-generation cephalosporins, ceftazidime and
313 cefotaxime, could be attributed to the co-carriage of ESBL/AmpC enzymes in 76% of
314 sequenced isolates. There was huge variation in susceptibility to other antibiotics tested in
315 this study, attributable to the presence of a plethora of other resistance genes, as identified
316 in the WGS analyses (Table 2), sometimes including other carbapenemase genes - 5%
317 (34/741) of isolates with OXA-48-like enzymes also harboured either a *bla*_{NDM} (33/741) or
318 *bla*_{VIM} (1/741) allele. It follows that there can be no 'standard' antibiotic regimen for the
319 treatment of infections caused by OXA-48-like producers without additional susceptibility
320 testing and/or resistance gene data, although ceftazidime-avibactam shows promise based
321 on *in vitro* data.²³ Although colistin, tigecycline and amikacin retained the highest levels of
322 susceptibility their individual indications may make them unsuitable for the treatment of some

323 infections. For example tigecycline cannot be used for the treatment of urinary tract
324 infections²⁴ and colistin use has been associated with both neuro- and nephrotoxicity.²⁵

325 At the time of writing there are 14 known OXA-48-like variants, 11 of them CHDLs, and WGS
326 analysis of 351 non-duplicate UK isolates identified five of the CHDL types. OXA-48 was by
327 far the most common variant, found in two-thirds (235/351) of isolates. The earliest OXA-48-
328 positive isolate identified in the UK was obtained in 2007 from a patient who had recently
329 been hospitalised in Turkey; this isolate was shown here to be an ST147 *K. pneumoniae*
330 carrying a plasmid with >99% identity to pOXA-48a. pOXA-48a has been implicated in early
331 OXA-48 dissemination in Turkey.^{15, 26}

332 A travel history was available for only 24% of patients, of whom 10% had documented travel
333 to 18 different countries/islands, several of which have previously reported outbreaks
334 involving bacteria with OXA-48-like enzymes.^{2, 4, 5, 27} All five patients with reported travel to
335 Turkey and 6/7 with travel to other Middle Eastern or North African countries whose isolates
336 were analysed by WGS were found to carry organisms with *bla*_{OXA-48}, as repeatedly found in
337 Turkey.^{26, 27} By contrast, both OXA-181 and OXA-232 have been associated with the Indian
338 subcontinent,^{12, 28} and were found in 12/13 sequenced isolates from patients reporting travel
339 to India, Pakistan or Sri Lanka. These data further underscore the role that international
340 travel may play in carbapenemase dissemination. Notably four *K. pneumoniae* with OXA-48-
341 like enzymes were from patients transferred to the UK for intensive care treatment of injuries
342 received during the Libyan 'Emergency' of 2011.

343 Forty-four percent (154/351) of sequenced isolates, and 153/235 of those with classical
344 *bla*_{OXA-48} possessed DNA with >99% sequence identity to pOXA-48a, an approx. 62 kb
345 IncL/M plasmid first associated with *bla*_{OXA-48}, in Turkey and North Africa and now with
346 multiple polyclonal and cross species outbreaks.¹⁵ pOXA-48a-like sequences were found
347 here in multiple species and sequence types. Except for one isolate with *bla*_{OXA-245} these all
348 carried *bla*_{OXA-48}. The demonstration of both the broad range and success of this plasmid

349 supports an earlier and much smaller analysis where we found IncL/M OXA-48 plasmids
350 amongst several enterobacterial species and STs.¹⁰ Of the remaining 197 isolates
351 sequenced, 68 carried DNA with >99% identity to one of three plasmids: pOXA-232 (32/68),
352 pOXA181 (30/68) or pKP3-A (6/68). pOXA-232 and pKP3-A are ColE-like plasmids, of
353 approx. 6 kb and 7.6 kb respectively, originally discovered in *E. coli* and *K. pneumoniae*
354 isolates obtained from patients following hospitalisation in India.^{28, 29} All of the 32 sequenced
355 isolates harbouring *bla*_{OXA-232} and six of 69 isolates with *bla*_{OXA-181} contained sequences with
356 >99% sequence identity to pOXA-232 and pKP3-A respectively. Thirty further isolates with
357 OXA-181 enzymes were shown to carry DNA with >99% sequence identity to pOXA181, an
358 IncX3 plasmid of approx. 51.5 kb that was first found in an *E. coli* ST410 isolate obtained
359 from the blood sample of a patient in China who had no history of travel to the Indian
360 subcontinent.³⁰

361 We found *bla*_{OXA-48-like} genes in multiple species and STs and also that there were some
362 clones that were particularly successful as a vehicle for *bla*_{OXA-48-like} dissemination. In *K.*
363 *pneumoniae* some STs (such as ST14 and ST147) were associated with multiple OXA-48-
364 like plasmids, indicating the success of the ST but not indicating expansion of a specific
365 clone/plasmid pairing. Although *K. pneumoniae* ST395 has been associated with outbreaks
366 in Europe and Morocco,¹³ we found only two representatives among those sequenced and
367 these harboured different OXA-48-like variants, *bla*_{OXA-48} and *bla*_{OXA-181}, in different genetic
368 environments; one in a pKP3-A sequence and the other in an unidentified environment.

369 Rather alarmingly, 16% of isolates were submitted from a single hospital, hospital A,
370 representing at least six species and 33 STs. Within the sixty-one isolates from hospital A
371 that were sequenced most (54/61) harboured pOXA-48a sequences. This suggests the local
372 spread of pOXA-48a amongst different genera, species and STs within this hospital and is
373 indicative of the success of pOXA-48a in dissemination.

374 In contrast to the ST diversity among *K. pneumoniae* isolates with OXA-48-like enzymes
375 ST38 accounted for almost half of all the sequenced *E. coli* isolates with OXA-48-like
376 enzymes (53/114). ST38 has previously been associated with *bla*_{CTX-M} carriage in multiple
377 countries.³¹ Plasmid mapping could not establish a location for *bla*_{OXA-48} in most (50/53) of
378 these isolates. In a previous study¹⁰ the authors failed to obtain any plasmid transformants
379 from ST38 isolates and, more recently, it was reported that *bla*_{OXA-48} and *bla*_{OXA-244} can be
380 chromosomally-encoded in ST38 isolates.³² This may apply here, but establishing this would
381 require utilisation of longer read sequencing techniques (e.g. PacBio and MinION).

382 In summary, this study has shown an increase in OXA-48-like enzymes in the UK over a
383 seven-year period. We suggest that the accumulation of OXA-48-like carbapenemases
384 within the UK is due to repeated import, coupled with both the dissemination of successful
385 plasmids, notably pOXA-48a and the spread of successful clones (e.g. *E. coli* ST38); the
386 linkage to plasmid spread was especially strong.

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422 & Dohme Corp, Perkin Elmer, Pfizer amounting to <10% of portfolio value.

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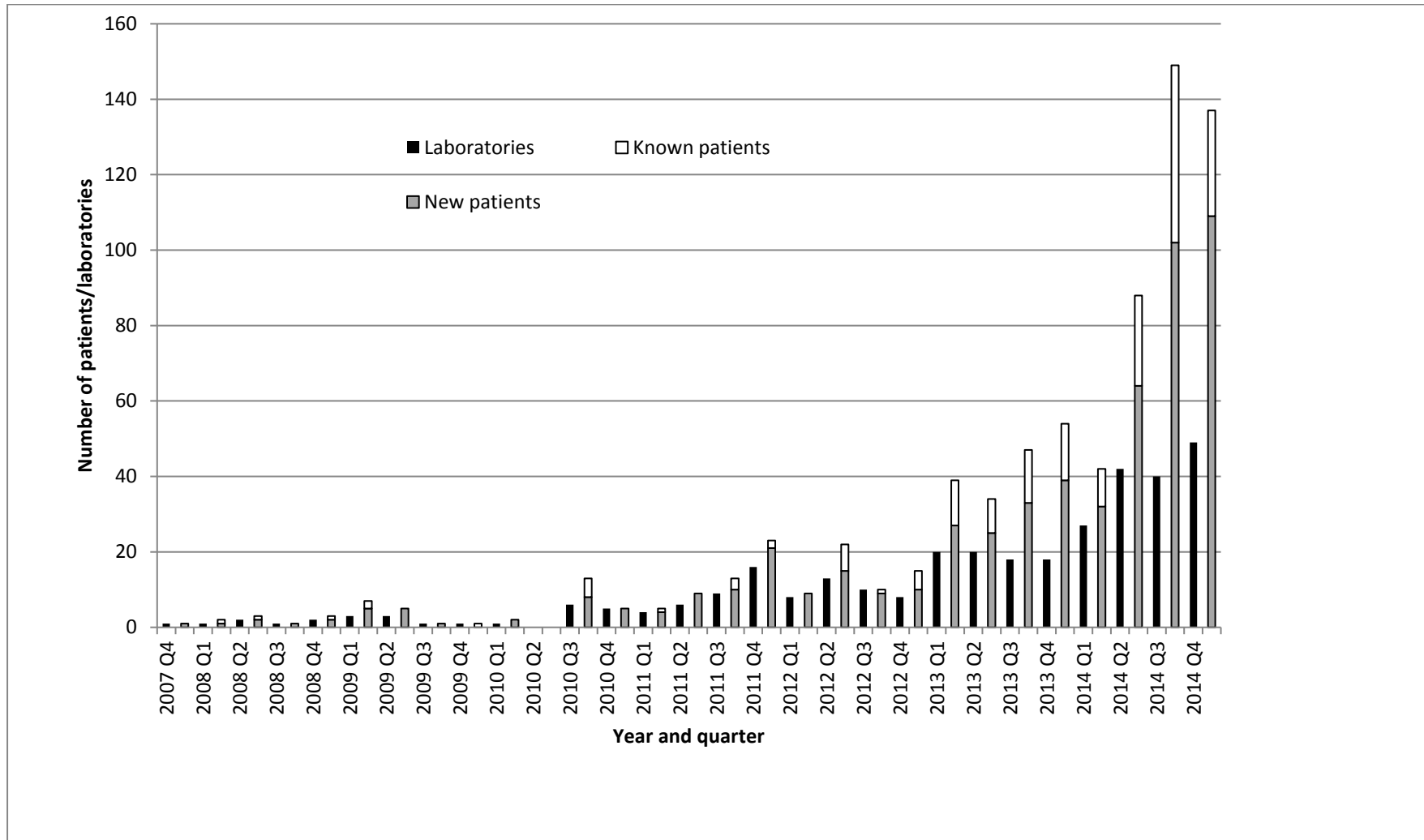
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519 Figure 1. Numbers of new and known affected patients and laboratories sending OXA-48-positive isolates per quarter during the study period.



521 Table 1. Sources of OXA-48-like-positive isolates.

Species	Hospital setting							GP urines	Total
	Urines	Screening swabs	Blood cultures and line tips	Respiratory	Tissue and fluid	Faeces/Rectal swabs	Not known		
<i>K. pneumoniae</i>	91	27	48	28	58	110	8	15	385
<i>E. coli</i>	47	22	18	7	28	58	2	25	207
<i>Enterobacter</i> spp.	6	15	9	1	9	16	10	1	67
<i>K. oxytoca</i>	2	2	7	0	2	6	0	4	23
<i>Citrobacter</i> spp.	5	2	1	0	0	4	0	0	12
<i>S. marcescens</i>	0	0	0	0	1	0	0	0	1
<i>R. ornithinolytica</i>	0	0	0	0	0	1	0	0	1
other spp. ^a	0	0	0	2	2	1	0	0	5
Total	151	68	83	38	100	196	20	45	701

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Species	Unknown setting							Total
	Urines	Screening swabs	Blood cultures and line tips	Respiratory	Tissue and fluid	Faeces/Rectal swabs	Not known	
<i>K. pneumoniae</i>	9	0	0	1	7	4	2	23
<i>E. coli</i>	8	0	0	0	2	1	0	11
<i>Enterobacter</i> spp.	1	0	0	0	0	0	0	1
<i>K. oxytoca</i>	0	0	0	1	0	0	0	1
<i>Citrobacter</i> spp.	1	0	0	0	0	1	0	2
<i>S. marcescens</i>	0	0	0	0	1	0	1	2
Total	19	0	0	2	10	6	3	40

523 ^a- other species comprise *P. aeruginosa* (n=3) and *Shewanella* spp. (n=2).

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526 Table 2. Characteristics of 351 non-duplicate isolates that were subjected to WGS.

Species	No. of isolates	Carbapenemases			Replicons	Other resistance genes		
		OXA-48-like alleles (no.)	OXA + NDM alleles (n=13)	STs (no. if >1)		Beta-lactamases (variants)	Aminoglycoside resistance genes	Others
<i>K. pneumoniae</i>	163	OXA-48(86), OXA-181(31), OXA-232(24), OXA-245(1), OXA-484(5)	OXA-48+NDM-1(3), OXA-181+NDM-1(5), OXA-232+NDM-1(8)	11(10), 14(27), 15(4), 16(6), 17, 25(2), 35, 36, 39, 43, 45(4), 48(2), 101(13), 104, 105, 111, 133(2), 147(17), 152, 187(2), 231(18), 253, 294, 299, 307(7), 323, 327, 336(3), 359, 392(3), 395(2), 405(2), 461(2), 659, 685, 709, 831(2), 922, 985, 1141, 1164, 1473(5), 1680(2), 1819, 1821, 1825, 1827, 1834, 2205	A/C, ColKP3, FIA, FIB, FII, HI2, L/M, X3	<i>bla</i> _{TEM-1} , <i>bla</i> _{SHV} (1,11,28,33,39,75,76,100,103,159), <i>bla</i> _{CTX-M} (14,15,16), <i>bla</i> _{OXA} (1,9), <i>bla</i> _{DHA-1} , <i>bla</i> _{CMY-4}	<i>strA</i> , <i>strB</i> , <i>aadA1</i> , <i>aadA2</i> , <i>aadA3</i> , <i>aadA5</i> , <i>armA</i> , <i>aac(3)-IIa</i> , <i>aac(3)-IIId</i> , <i>aac(6')Ib-cr</i> , <i>rmtF</i>	<i>oqxA</i> , <i>oqxB</i> , <i>qnrB1</i> , <i>qnrS1</i> , <i>qnrB66</i> , <i>arr-2</i> , <i>arr-3</i> , <i>sul1</i> , <i>sul2</i> , <i>fosA</i> , <i>mphA</i> , <i>msrE</i> , <i>ereA</i> , <i>ereB</i> , <i>ereC</i> , <i>ermB</i> , <i>mdfA</i> , <i>dfrA1</i> , <i>dfrA5</i> , <i>dfrA7</i> , <i>dfrA12</i> , <i>dfrA14</i> , <i>catA1</i> , <i>catB3</i> , <i>cmlA1</i> , <i>sat2</i> , <i>tetA</i> , <i>tetD</i>
<i>E. coli</i>	114	OXA-48(74), OXA-181(30), OXA-244(10)		10(4), 28, 38(53), 58, 59, 69, 73, 83, 95, 127, 131, 167, 205(2), 224, 227(2), 354(2), 361, 399(3), 401, 405(4), 410(12), 428, 648, 681, 940(3), 1170, 1284(2), 1431, 1722, 2139, 2164, 2179, 3221, 3541, 6328, 6329, 6330	B/O, FIA, FIB, FII, HI2, K, L/M, X3	<i>bla</i> _{TEM} (1,33,169,190), <i>bla</i> _{CTX-M} (14,15,24,27,82), <i>bla</i> _{OXA} (1,10), <i>bla</i> _{CMY} (2,42,44,54,59,61)	<i>strA</i> , <i>strB</i> , <i>aadA1</i> , <i>aadA2</i> , <i>aadA3</i> , <i>aadA5</i> , <i>aadA23</i> , <i>aac(3)-IIId</i> , <i>aac(3)-IIa</i> , <i>aph(6)-Id</i> , <i>aac(6')Ib-cr</i> , <i>rmtB</i>	<i>qnrB1</i> , <i>qnrS1</i> , <i>qepA</i> , <i>mdfA</i> , <i>mphA</i> , <i>ermB</i> , <i>msrE</i> , <i>dfrA1</i> , <i>dfrA5</i> , <i>dfrA7</i> , <i>dfrA12</i> , <i>dfrA14</i> , <i>dfrA17</i> , <i>fosA</i> , <i>sul1</i> , <i>sul2</i> , <i>catA1</i> , <i>cmlA1</i> , <i>floR</i> , <i>sat2</i> , <i>tetA</i> , <i>tetD</i>
<i>E. cloacae</i> complex	42	OXA-48(40)	OXA-48+NDM-1(2)		FIB, FII, HI1, L/M	<i>bla</i> _{TEM-1} , <i>bla</i> _{OXA-1} , <i>bla</i> _{SHV} (5,12), <i>bla</i> _{CTX-M} (9,15,82), <i>bla</i> _{ACT} (7,14,15,16)	<i>strA</i> , <i>strB</i> , <i>aadA2</i> , <i>aadA3</i> , <i>aadA12</i> , <i>aac(3)-IIa</i> , <i>ant(2'')</i> - <i>IIa</i> , <i>aac(6')-IIc</i> , <i>aac(6')Ib-cr</i>	<i>qnrA1</i> , <i>qnrB1</i> , <i>qnrS1</i> , <i>ereA</i> , <i>mphA</i> , <i>dfrA12</i> , <i>dfrA14</i> , <i>dfrA16</i> , <i>dfrA18</i> , <i>fosA</i> , <i>sul1</i> , <i>sul2</i> , <i>catA1</i> , <i>catA2</i> , <i>tetA</i> , <i>tetD</i>
<i>K. oxytoca</i>	13	OXA-48(13)		27(4), 36, 95, 168, 176(6)	FII, HI2, L/M	<i>bla</i> _{TEM-1} , <i>bla</i> _{OXY} (1,2,5,6)	<i>strA</i> , <i>strB</i> , <i>aac(3)-IIa</i>	<i>qnrA1</i> , <i>mphA</i> , <i>dfrA18</i> , <i>sul1</i> , <i>tetD</i> , <i>tetK</i>
<i>C. freundii</i>	11	OXA-48(10)	OXA-181+NDM-	ND	A/C, FIB,	<i>bla</i> _{TEM-1} , <i>bla</i> _{CMY-48} ,		<i>qnrB12</i> , <i>dfrA7</i> , <i>sul1</i> ,

Other <i>Enterobacter</i> spp.	5	OXA-48(5)	1(1)	45(3), 51, 66(5), 90, 93(3), 104(4), 106, 108(17), 135, 145, 268, 269, 279(3)	FII, L/M H, L/M, N	<i>bla</i> _{MAL-1} <i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-14} , <i>bla</i> _{ACT-37} , <i>bla</i> _{SHV-12}	<i>strA</i> , <i>strB</i> , <i>aadA1</i> , <i>aadA2</i> , <i>aph(6)-I_d</i> , <i>aac(6')-IIc</i> , <i>aph(3')-Vib</i>	<i>sul2</i> , <i>tetD</i> <i>qnrA1</i> , <i>ereA</i> , <i>dfrA18</i> , <i>floR</i> , <i>tetA</i> , <i>sul1</i> , <i>sul2</i> , <i>sul3</i>
<i>S. marcescens</i>	2	OXA-48(2)		ND	L/M	<i>bla</i> _{CTX-M} (14,82)	<i>strA</i> , <i>strB</i> , <i>aph(3')-Vib</i>	
<i>P. aeruginosa</i>	1	OXA-181(1)		773		<i>bla</i> _{PAO} , <i>bla</i> _{OXA-50}	<i>aac(3)-Ie</i> , <i>aph(3')-IIb</i> , <i>aadA1</i>	<i>catB7</i> , <i>dfrB5</i> , <i>sul1</i>

528 Table 3. MIC distributions for OXA-48-like-positive isolates (n=741).

Carbapenemase gene(s)	Isolates	Antibiotic (range tested, mg/L)	EUCAST breakpoints $\leq S / > R$	Number of isolates with MIC (mg/L)												NA	%S
				≤ 0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128			
OXA-48-like	Enterobacteriaceae	Ertapenem (0.125-16)	$\leq 0.5 / > 1$			4	15	53	124	147	351 ^a			7	<1		
	other spp. ^c													6	NA		
NDM + OXA-48-like	Enterobacteriaceae										33 ^a				0		
VIM + OXA-48-like	Enterobacteriaceae										1 ^a				0		
OXA-48-like	Enterobacteriaceae	Imipenem (0.06-128)	$\leq 2 / > 8$	5	28	92	186	180	98	47	22	20	17 ^a	6	45		
	other spp. ^c				1				1		1	1	1	2 ^a		17	
NDM + OXA-48-like	Enterobacteriaceae							1	4	6	3	7	12 ^a		0		
VIM + OXA-48-like	Enterobacteriaceae										1 ^a				0		
OXA-48-like	Enterobacteriaceae	Meropenem (0.06-32)	$\leq 2 / > 8$	7 ^b	38	105	138	110	69	35	81	112 ^a		6	57		
	other spp. ^c			1 ^b			1					1	3 ^a			33	
NDM + OXA-48-like	Enterobacteriaceae								1	4	28 ^a				0		
VIM + OXA-48-like	Enterobacteriaceae										1 ^a				0		
OXA-48-like	Enterobacteriaceae	Ceftazidime (0.125-256)	$\leq 1 / > 4$	14 ^b	57	69	86	31	28	26	23	47	83	231 ^a	6	33	
	other spp. ^c			2 ^b				2	2								33
NDM + OXA-48-like	Enterobacteriaceae													33 ^a		0	
VIM + OXA-48-like	Enterobacteriaceae													1 ^a		0	
OXA-48-like	Enterobacteriaceae	Cefotaxime (0.125-256)	$\leq 1 / > 2$	1 ^b	3	28	48	40	45	42	23	22	33	405 ^a	11	12	
	other spp. ^c			1 ^b												5	100
NDM + OXA-48-like	Enterobacteriaceae													33 ^a		0	
VIM + OXA-48-like	Enterobacteriaceae													1 ^a		0	
OXA-48-like	Enterobacteriaceae	Amikacin (0.5-64)	$\leq 8 / > 16$			26 ^b	138	186	124	58	47	30	86 ^a	6	77		
	other spp. ^c				2			1				1	2 ^a			50	
NDM + OXA-48-like	Enterobacteriaceae							2	2	2			27 ^a		18		
VIM + OXA-48-like	Enterobacteriaceae												1 ^a		0		
OXA-48-like	Enterobacteriaceae	Gentamicin (0.125-32)	$\leq 2 / > 4$	3 ^b	55	175	85	10	11	10	22	324 ^a			6	47	
	other spp. ^c				1				1	1	2	1					33
NDM + OXA-48-like	Enterobacteriaceae					1						32 ^a				3	
VIM + OXA-48-like	Enterobacteriaceae											1 ^a				0	
OXA-48-like	Enterobacteriaceae	Tobramycin (0.125-32)	$\leq 2 / > 4$	1 ^b	17	119	114	29	36	59	87	230 ^a			9	40	
	other spp. ^c						1	1			1		3 ^a				33
NDM + OXA-48-like	Enterobacteriaceae									5	28 ^a					0	
VIM + OXA-48-like	Enterobacteriaceae										1 ^a				0		

OXA-48-like	Enterobacteriaceae	Ciprofloxacin	≤0.5/>1	191 ^b	45	33	24	11	37	343 ^a	17	39			
	other spp. ^c	(0.125-8)		1 ^b			1			4 ^a		17			
NDM + OXA-48-like	Enterobacteriaceae									31 ^a	2	0			
VIM + OXA-48-like	Enterobacteriaceae									1 ^a		0			
OXA-48-like	Enterobacteriaceae	Colistin	≤2/>2				397 ^b	204	20	11	16	13	21 ^a	19	91
	other spp. ^c	(0.5-32)					2 ^b	2	1	1					83
NDM + OXA-48-like	Enterobacteriaceae						19 ^b	12	1	1					97
VIM + OXA-48-like	Enterobacteriaceae						1								100
OXA-48-like	Enterobacteriaceae	Tigecycline	≤1/>2				185 ^b	140	144	118	65	24	6 ^a	19	69
	other spp. ^c	(0.25-16)					1 ^b			1				4	50
NDM + OXA-48-like	Enterobacteriaceae						8 ^b	3	6	12	3		1 ^a		52
VIM + OXA-48-like	Enterobacteriaceae										1				0

529 S, susceptible; R, resistant; NA, not available.

530 The dotted vertical lines indicate intermediate breakpoints and the solid vertical lines indicate resistant breakpoints.

531 ^aMIC greater than or equal to indicated value.

532 ^bMIC less than or equal to indicated value.

533 ^cother spp. comprise *Pseudomonas aeruginosa* and *Shewanella* spp..

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