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Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing

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1 European Survey of Carbapenemase-Producing
2 *Enterobacteriaceae* (EuSCAPE): Period Prevalence of
3 Carbapenemase-Producing *Klebsiella pneumoniae* and
4 *Escherichia coli*, November 2013 to April 2014

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57

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59 Material.

60

61 **Key Words:** Carbapenemase; *Klebsiella pneumoniae*; *Escherichia coli*; Epidemiology; Europe; Surveillance;
62 Public Health

63

64 **Running Title:** European Survey of Carbapenemase-Producing *Enterobacteriaceae*

65

66

67 **Summary**

68 **Background**

69 Gaps in the diagnostic capacity and heterogeneity of national surveillance and reporting standards in
70 Europe make it difficult to contain carbapenemase-producing *Enterobacteriaceae*. We here report the
71 development of a consistent sampling framework and the results of the first structured survey on the
72 occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in European hospitals.

73 **Methods**

74 National Expert Laboratories (NELs) recruited hospitals with diagnostic capacities. In Winter 2013/14 these
75 collected the first 10 carbapenem non-susceptible clinical isolates of *K. pneumoniae* or *E. coli* and 10
76 susceptible same-species comparator isolates and pertinent patient and hospital information. Isolates and
77 data were relayed back to NELs, which made laboratory-confirmed information available for central
78 analysis.

79 **Findings**

80 In 36 countries, 455 sentinel hospitals submitted 2,703 clinical isolates. Among the 927 confirmed
81 carbapenemase (KPC, NDM, OXA-48-like, or VIM) producers, the ratio *K. pneumoniae* : *E. coli* was 11:1. For
82 every 10,000 hospital admissions 1-3 patient had positive clinical specimen. Incidence differed greatly, with
83 Mediterranean and Balkan countries showing the highest rates. Carbapenemase-producing *K. pneumoniae*
84 isolates showed high proportions of resistance to last-line antibiotics.

85 **Interpretation**

86 This initiative demonstrates an encouraging commitment, and shows that challenges in the establishment
87 of a continent-wide enhanced sentinel surveillance for CPE can be overcome. Strengthening infection
88 control efforts in hospitals is imperative for controlling spread through local and national healthcare
89 networks.

90 **Funding**

91 The European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) was initiated and
92 funded by the European Centre for Disease Prevention and Control (ECDC) through a framework contract
93 (ECDC/2012/055) following an open call for tender (OJ/25/04/2012-PROC/2012/036).

94

95 **Research in context**

96 **Evidence before this study**

97 On April 1, 2016, we search Pubmed with the terms "carbapenemase-producing *Enterobacteriaceae*" or
98 "carbapenem-resistant *Enterobacteriaceae*", or "*Klebsiella pneumoniae*", "*Escherichia coli*", "Europe" and
99 "surveillance" for reports published between the 1st of January 2000 and the 1st of January 2016, with no
100 language restrictions. This search identified 72 publications. These consisted of larger national surveillance
101 studies, reviews or single case studies. None of the studies showed comprehensive European coverage,
102 standardization of methods or diagnostic quality assessment. Before this study, only anecdotal evidence
103 existed for several countries with high endemicity. Since national reference laboratory structures were
104 often lacking and diagnostic standards differed between laboratories, cases remained unconfirmed leaving
105 wide scope for ascertainment bias.

106

107 **Added value of this study**

108 This study reports data on the occurrence of carbapenemase-producing and last-line resistant *K.*
109 *pneumoniae* and *E. coli* at continental scales using standardized procedures and provide the first
110 comparable and laboratory-confirmed data on the incidence of these difficult-to-treat bacteria across
111 Europe.

112

113 **Implications of all the available evidence**

114 *K. pneumoniae* of nosocomial provenance is the main source of carbapenemase-producing
115 *Enterobacteriaceae* (CPE) infection in Europe. The emergence and spread of antibiotic resistance against
116 last-line antibiotics increasingly erodes the ability to successfully treat patients infected with CPE especially
117 in countries where CPE prevalence in hospitals is high. At a time when novel and effective antibiotic
118 compounds have not become available, containment of CPE is bound to rely on stricter infection control
119 measures in hospitals.

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127 **Introduction**

128 Carbapenemase-producing *Enterobacteriaceae* (CPE) are the most pervasive antibiotic resistance threat to
129 health services worldwide. Because of the dearth of alternative drugs, patients are often left without
130 effective treatment, revealing burgeoning resistance, long concealed by adaptive prescribing when doctors
131 could still choose carbapenems as a last-line drug. Thus, expanding of CPE could be the tipping point when
132 significant morbidity and mortality from antibiotic resistance comes to the fore. ¹

133 Few alternative antibiotics (e.g. colistin, fosfomycin and tigecycline) remain,² and while resistance can
134 extend even to agents still in development or recently approved,^{3,4} public health efforts are beginning to
135 emphasise containment of CPE in populations and healthcare networks. This requires an understanding of
136 the geographical distribution of CPE infections, their population reservoirs, and the risk factors for
137 acquisition. However, there is a lack of internationally comparable data.

138 The European Survey on CPE (EuSCAPE) was initiated with the aim of providing the first comparable and
139 quality-controlled data on the occurrence of the most important CPE (*Klebsiella pneumoniae* and *E. coli*) in
140 Europe and neighbouring countries and to establish a framework for future enhanced sentinel surveillance.
141 It entailed the stepwise build-up of structures through (i) identification of national expert laboratories
142 (NELs)⁵, (ii) a joint agreement on diagnostic standards, (iii) improvement of quality-assessed diagnostic
143 capacity among NELs, and (iv) as a proof of feasibility, a structured survey using a standard sampling
144 protocol in all participating sites. The current manuscript describes the execution and final results of the
145 EuSCAPE structured survey.

146

147 **Methods**

148 *Capacity building and proficiency testing*

149 Technical staff from all national expert laboratories (NEL) was trained to use a set of standard phenotypic
150 and genotypic tests in accordance with EUCAST guidelines.⁶ Subsequently, all NELs were required to take
151 part in an External Quality Assessment (EQA) exercise, which was carried out and analysed by the United
152 Kingdom National External Quality Assessment Service (UK NEQAS). Successful completion was a
153 prerequisite for participation.

154 *Structured survey*

155 A defined number of hospitals with microbiologic diagnostic capacity were recruited by each NEL
156 depending on the country's population; 20 sites for large countries (>15 million population), 10 sites for
157 medium-sized countries (2-15 million population) and one site for small countries (<2 million population).
158 To prevent geographical bias, the NELs were asked to enrol hospitals in a geo-demographical
159 representative manner (Figure 1, see also Step 1 in the structured survey protocol provided as
160 Supplementary Material). In addition, NELs were asked to collect additional information about the
161 participating hospitals for 2013, such as their number of beds, annual number of admissions, total number
162 of patient days, average bed occupancy, and average length of stay and the estimated size of their
163 catchment population.

164 The sampling period was six months, starting on November 1, 2013 and ending on April 30, 2014. During
165 this period, each sentinel site was required to collect the first 10 consecutive primary isolates of *K.*
166 *pneumoniae* or *E. coli* from clinical specimens from individual patients if local routine tests showed non-
167 susceptibility to any carbapenem (imipenem, meropenem or ertapenem). All clinical specimens were
168 accepted, except for stool and surveillance screening samples. Each index isolate (i.e. carbapenem-non-
169 susceptible *K. pneumoniae* or *E. coli*) was matched to the first subsequent carbapenem-susceptible isolate
170 of the same species irrespective of anatomical site serving as a comparator isolate.

171 Isolates were dispatched to the NEL accompanied by additional information such as sample date,
172 anatomical origin of specimen, patient age and gender, clinical relevance of the isolate (colonisation or
173 infection), patient location in the hospital (intensive care unit, normal ward, outpatient/accidents &
174 emergency), and during the preceding six months, previous hospital admission and travel outside their
175 country of residence. Hospital acquisition was inferred when an isolate was sampled from patients after
176 being admitted for more than 48 hours, or community-associated otherwise. Instructions on the collection
177 of isolates, and the ascertainment of clinical and epidemiological data were given by the structured survey
178 protocol (Step 4 and 5, see Supplementary Material), which was translated by NEs into their respective
179 language and distributed to the sentinel hospital laboratories if necessary.

180 The NELs confirmed species and phenotypic susceptibility and used PCR tests for four carbapenemase gene
181 families (KPC, NDM, OXA-48-like, or VIM). Antimicrobial susceptibility tests according to EUCAST guidelines

182 variously included, ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, ceftazidime,
183 cefepime, aztreonam, imipenem, meropenem, ertapenem, ciprofloxacin, trimethoprim/sulfamethoxazole,
184 gentamicin, amikacin, tobramycin, tigecycline, colistin and fosfomycin. Phenotypic confirmation of
185 carbapenemase production consisted of double disk synergy tests (DDSTs), combination disk tests (CDTs),
186 and Carba NP I or II test.⁷ Methodological details for any of these tests are described in the laboratory
187 manual in the Supplementary Material. Carbapenem non-susceptible isolates that were tested PCR-
188 negative were classified as “Other”. Results and epidemiological information were uploaded for central
189 analysis using a password-protected web tool.

190 All data were anonymised and collected in accordance with the European Parliament and Council decisions
191 on the epidemiological surveillance and control of communicable disease in the European Community.^{8,9}
192 Ethical approval and informed consent were thus not required.

193 *Data analysis*

194 Data were analysed with STATA version 13.1 (StataCorp, Texas, USA) using Mantel-Haenzel odds ratios and
195 Pearson chi-square test for univariate risk factor analysis and multiple logistic regression for multivariable
196 analysis with log likelihood ratio tests after fitting interaction terms to identify effect modification. For
197 hospitals that could not provide figures on the total number of patient days in 2013, we estimated this
198 value as the product of the number of admissions and the average length of stay. Country-aggregated
199 incidence estimates were reported as hospital admission incidence i.e. number of patients diagnosed with
200 either confirmed carbapenemase-producing *K. pneumoniae* or *E. coli* per 10,000 hospital admissions and
201 incidence densities as per 100,000 hospital patient-days. Confidence intervals for random errors are not
202 provided due to heterogeneity of sampling density as a result of different diagnostic habits.

203 *Role of the funding source*

204 The study was funded by the European Centre for Disease Prevention and Control (ECDC) through a specific
205 framework service contract (ECDC/2012/055) to the University Medical Center Groningen,
206 Groningen, Netherlands. The decision to submit for publication was taken by the study coordinator (HG) in
207 the Netherlands. ECDC provided comments on the study design, suggested national coordinators, and
208 provided comments on the analysis and the final report.

209

210

211 Results

212 Summary statistics and incidence estimates

213 Between November 1, 2013 and April 30, 2014, 455 sentinel hospitals from 36 countries contributed to the
214 structured survey (Figure 1). Participating countries included 27 European Union (EU) Member States, two
215 European Economic Area (EEA) countries and six EU enlargement countries plus Israel. For the UK, Scotland
216 participated on its own behalf. Albania, Finland, Israel, Latvia, The former Yugoslav Republic of Macedonia,
217 Romania, Slovakia, Turkey and UK-England and Northern Ireland did not reach their quota of participating
218 sentinel hospitals, whilst Belgium, Bulgaria, Croatia, France, Hungary, Italy, Kosovo, Luxembourg, Norway,
219 Poland, Portugal, Serbia, Slovenia and UK-Scotland recruited more hospitals.

220 During the six-month period 2,301 *K. pneumoniae* and 402 *E. coli* isolates were collected (Table 1). . Most
221 (86.1%) index isolates submitted were *K. pneumoniae* (1,203 isolates vs. 194 *E. coli*). Proportions of index
222 and comparator isolates did not differ in terms of anatomical origin or specimen type except for blood
223 stream infections caused by *E. coli*, where carbapenem-susceptible isolates contributed significantly more
224 infections (Supplementary Table 1). It therefore seems that the ability to cause infections is not contingent
225 on the resistance traits under study. Of all isolates submitted by the NELs as carbapenem non-susceptible,
226 PCR tests confirmed the presence of KPC, NDM, OXA-48-like, and VIM type genes for 850 (70.7%) of *K.*
227 *pneumoniae* and 77 (39.7%) of *E. coli*. Among the 927 carbapenemase-producers, the ratio between *K.*
228 *pneumoniae* and *E. coli* was 11:1.

229 Country-aggregated incidence differed greatly between countries. Based on population-weighted averages,
230 1.3 patients per 10,000 hospital admissions and 2.5 patients per 100,000 hospital patient-days were
231 identified with a carbapenemase-producing *K. pneumoniae* or *E. coli*. High incidence countries included
232 Greece, Italy, Montenegro, Spain, and Serbia.

233 Distribution of KPC, NDM, OXA-48, and VIM carbapenemases

234 KPC enzymes detected in 393 isolates of all 927 CPE isolates (42.4%) represented the most frequent
235 carbapenemases. OXA-48-like enzymes were the second most frequent (353 isolates, 38.1%) and were the
236 most prominent class of carbapenemases in eight countries. NDM genes were detected in 113 (12.2%) and
237 VIM in 68 *K. pneumoniae* isolates (7.3%).

238 Likewise, among *K. pneumoniae*, the most frequently detected carbapenemases were KPC enzymes (379
239 isolates, 44.6%), followed by OXA-48-like (310 isolates, 36.5%), NDM (93 isolates, 10.9%) and VIM (68
240 isolates, 8.0%). In *E. coli* the most frequently detected carbapenemases were OXA-48-like enzymes (43
241 isolates, 55.8%) followed by NDM (20 isolates, 26.0%) and KPC (14 isolates, 18.2%), albeit with substantial
242 country-to country variation in relative prevalence (Table 2a and 2b).

243 At country level, high proportions of KPC-positive *K. pneumoniae* among carbapenem-non-susceptible
244 isolates were found in Italy (187 isolates, 95.9%), Israel (31 isolates, 79.5%), Greece (56 isolates, 65.1%)

245 and Portugal (36 isolates, 59·0%). These four countries, plus Cyprus, were the only countries where KPC
246 genes were also detected in *E. coli*, albeit in very small numbers. OXA-48-like enzymes, were frequent in
247 Turkey where 98 of 124 carbapenem-non-susceptible *K. pneumoniae* (79·0%) and 19 of 22 *E. coli* (86·4%)
248 had these enzymes, followed by Romania, where 50 of 68 (73·5%) carbapenem-non-susceptible *K.*
249 *pneumoniae* had OXA-48-like enzymes. These enzymes were also frequent in Spain (81 of 116, 69·8%),
250 Belgium (18 of 48, 37·5%), France (10 of 27, 37·0%) and Germany (12 of 36, 33·3%).

251 NDM was the most frequent carbapenemase in Serbia (33 of 67 isolates, 49·3 %) and in Montenegro, where
252 all ten submitted carbapenem non-susceptible *K. pneumoniae* isolates were NDM-positive. In Greece, NDM
253 was the second most frequent carbapenemase in *K. pneumoniae* (12 of 86, 13·9%). Other countries with
254 notable proportions of NDM-producing *K. pneumoniae* were Romania (5 of 68, 7·4%) and Turkey (9 of 124,
255 7·3%). NDM-producing *K. pneumoniae* were also isolated in another 12 European countries but in small
256 numbers ranging between one and three isolates, though they also made up the majority of
257 carbapenemase-producing *K. pneumoniae* isolates in Bulgaria and Denmark. In the case of *E. coli*, small but
258 significant numbers of NDM-producing isolates were found in Bulgaria (8 of 8, 100%) and Serbia (5 of 5,
259 100%). Single isolates of NDM-producing *E. coli* were identified in another seven countries.

260 VIM carbapenemases only found in *K. pneumoniae*, were the least frequent but represented the majority of
261 carbapenemase-producing isolates in Hungary (26 of 36, 72·2%) and Croatia (5 of 48, 10·4%). Otherwise,
262 only Greece (9 of 86, 10·5%) and Spain (12, 10·3%) had notable numbers of VIM-producing *K. pneumoniae*,
263 whilst these were also found in another seven countries, albeit in low numbers.

264 *Phenotypic drug resistance*

265 Twelve (33·3%) of the NELs tested the full panel of 18 recommended antibiotics. Some NELs found it
266 difficult to obtain particular compounds, whereas others used their routine reference service panel and
267 Denmark did not report any antibiotic susceptibility test results. Last-line antibiotics included colistin,
268 tigecycline and fosfomycin and were tested by 22, 20 and 18 NELs, respectively.

269 For *K. pneumoniae*, the proportion of isolates that were reported resistant to all antibiotics varied between
270 zero and 28·6% (average 9·3%, Table 3). Resistance to colistin was reported for 183 of 646 (28·3%) *K.*
271 *pneumoniae* isolates, fosfomycin resistance in 270 of 500 isolates (54·0%) and tigecycline resistance
272 (according to its current EUCAST recommended breakpoint) in only 29 of 555 (5·2%). High proportions of *K.*
273 *pneumoniae* resistant to last-line antibiotics were found in Italy, Romania, Turkey and Spain (Table 3). Of
274 the 77 *E. coli* confirmed to have carbapenemases, 57 were tested for susceptibility to colistin with three
275 being resistant, 43 to fosfomycin (two isolates resistant) and 48 to tigecycline (1 isolate resistant).

276 *Risk factors*

277 Carbapenem-susceptible comparator isolates of the same species were collected irrespective of anatomical
278 site from clinical material submitted for diagnostic purposes from successive patients. These provided an

279 important and unbiased sample, representative of the local susceptible population and served as an
280 appropriate 'control' group. Univariate analysis identified six risk factors that were positively associated
281 with carbapenemase-producing *K. pneumoniae* or *E. coli*, and two factors that were negatively associated
282 (Supplementary Table 2). Four of these remained significantly and independently associated with
283 carbapenemase-producing *K. pneumoniae* or *E. coli* in the multivariable model which included intensive
284 care therapy (OR=1.9, 95% CI, 1.4 – 2.7), hospital admission in the preceding six months (OR=2.0, 1.5 – 2.7),
285 hospital-acquisition (OR=2.6, 1.9 – 3.7) and travel outside the country of residence in the previous six
286 months (OR=3.0, 1.6 – 5.7).

287

288 **Discussion**

289 Clinicians increasingly depend on carbapenem antibiotics for the treatment of infections due to otherwise
290 multidrug-resistant bacteria. CPE have been implicated in hospital outbreaks and have the propensity to
291 spread (or disseminate their plasmids) rapidly at local, regional and international levels.¹⁰⁻¹⁵

292 We provide comprehensive survey results on the occurrence of carbapenemase-producing *K. pneumoniae*
293 and *E. coli* between November 2013 and April 2014 from 455 hospitals in 34 countries plus Turkey and
294 Israel, altogether serving an estimated catchment of over 270 million citizens out of a total population of
295 600 million. During the course of this investigation, NELs successfully expanded their capacity and adjusted
296 workflows to accommodate new diagnostic tests.¹⁶

297 However, as with all sampling frameworks for bacteria and epidemiological data, important caveats remain.
298 Despite decisions to minimise workload by concentrating on the two clinically most relevant species and
299 reducing the amount of additional information, nine countries failed to recruit their quota of sentinel sites
300 and another eight countries did not provide crucial denominator data. In some cases this was because of
301 financial constraints and because the workload could not be accommodated by some of the hospital
302 laboratories that had initially agreed to participate. Some NELs with established routines could not manage
303 to test additional antibiotics. As with other international surveillance systems (EARS-Net) this study relied
304 on routinely available data. For these reasons, the precision of some of the estimates on the occurrence
305 and risk factors of CPE in the European region could still be improved. For example, some countries
306 reported very low numbers of index CPE isolates whereas, judging from existing publications and high
307 endemicity in neighbouring countries, much higher rates would have been expected. It is possible that in
308 these countries diagnostic habits result in a lower sampling density or that the recruited sentinel sites were
309 less able to reliably identify carbapenem-non-susceptible isolates despite testing proficiency of the NELs,
310 concealing the true incidence of CPE through these types of ascertainment bias. Moreover, 353 (29.3%) of
311 the *K. pneumoniae* isolates and 117 (60.3%) of the *E. coli* isolates that were submitted by the sentinel
312 hospital laboratories as suspected carbapenem-non-susceptible had none of the four major
313 carbapenemases (KPC, NDM, OXA-48-like and VIM) and were reported as “Other”. This lack in specificity
314 could be the result of a carbapenemase not included in the test panel, or alternative mechanisms such as
315 reduced permeability. At the same time, sentinel laboratories relied on their local routine antibiotic
316 susceptibility tests which may also be the source of potential misclassification. Nevertheless, these first
317 data on CPE generated in a comprehensive manner will serve as a benchmark against which future
318 initiatives and trends will be measured.

319 Hospital incidence of carbapenemase-producing *K. pneumoniae* and *E. coli* per 10,000 admissions ranged
320 from six in Italy to 0.02 in Norway with an average of 1.3. The incidence density per 100,000 hospital
321 patient-days ranged from 17.3 in Greece to 0.09 in Lithuania, an average of 2.5 across all countries. These
322 values will underestimate total CPE incidence, because carbapenemases also occur in other

323 *Enterobacteriaceae*, though less frequently than in *Klebsiella* spp.¹⁷ Moreover, the lack of denominator data
324 from eight countries cautions against our ranking of incidence rates. Proportions of carbapenemase-
325 positive bacteria considered in this study varied between countries and between the two species under
326 investigation (Table 2a and Table 2b). This may be the result of the differential success of certain clonal
327 lineages in different countries.^{10,14} Importantly, we found a clear association with healthcare as most
328 isolates were either hospital-acquired, often associated with intensive care treatment, or isolated from
329 patients with previous hospital admission. We also found an association with previous travel outside the
330 country of residence (Supplementary Table 2). But when interpreting this finding one need to consider that
331 many of the highly endemic countries could not provide information on previous travel, which may have
332 led to an inflation of the risk estimate.

333 The highest incidence for carbapenemase-producing *K. pneumoniae* and *E. coli* were reported from
334 southern and south-eastern Europe. In Greece, VIM-positive *K. pneumoniae* started to expand in the mid-
335 2000s,¹⁸ but that changed with the rapid spread of KPC-producing *K. pneumoniae* from around 2007 which
336 subsequently became the dominant CPE.¹⁰ The present observation that NDM is now the second-ranking
337 carbapenemase in Greece is striking and raises the concern that there may be a further replacement event
338 by this more recently expanding carbapenemase.¹⁹

339 There were fewer carbapenemase-producing isolates among *E. coli* than *K. pneumoniae*. KPC enzymes were
340 especially rare in *E. coli* and were only identified in countries with high levels of KPC-producing *K.*
341 *pneumoniae*, where they probably reflect a spill-over of resistance genes from the *K. pneumoniae* reservoir.
342 Significant numbers of *E. coli* with OXA-48-like were found in Belgium, France, Spain, Turkey, and UK and
343 NDM carbapenemases in Bulgaria and Serbia. Penetration into *E. coli* is of concern, because *E. coli* spreads
344 in the community more readily than *K. pneumoniae*, meaning that infection control interventions that
345 mainly focus on hospitals are less likely to be effective. Moreover, *E. coli* from the digestive tract are
346 common vectors for promiscuous plasmids, which could also accelerate epidemic expansion.

347 In Romania, eight of 12 participating hospitals submitted *K. pneumoniae* isolates with OXA-48-like enzymes
348 and the majority were genetically indistinguishable by DNA fingerprinting, indicating countrywide spread of
349 a single clone.²⁰ This may be analogous to the national expansion of *K. pneumoniae* ST258-related clones
350 with KPC-2 or -3 enzymes in *e.g.* Greece, Italy and Israel, though with a different clonal lineage and
351 carbapenemase type. OXA-48-like carbapenemases were frequent in Malta, Spain, France and Belgium,
352 where they appear to be repeatedly introduced from Northern Africa. Genes coding for NDM seem to also
353 be spreading in the Balkan region, with significant numbers in Montenegro, Serbia and Greece but also
354 extending north into Slovenia and Austria. Surprisingly, no NDM-producing isolates were reported from
355 Albania, Kosovo and the former Yugoslav Republic of Macedonia, despite their occurrence in adjacent
356 countries and reports from patients transferred from these countries to other European countries.²¹

357 Only 12 countries tested the complete panel of antibiotics recommended by the study protocol. This makes
358 it difficult to determine the extent with which extensively drug-resistant (XDR) or pandrug-resistant (PDR)
359 *Enterobacteriaceae* phenotypes prevail in European hospitals.²² Clinically more important than these
360 epithets are, however, the proportions of carbapenemase-producing isolates that are also resistant to last-
361 line antibiotics such as colistin, fosfomycin and tigecycline. We generally observed that high-CPE-incidence
362 countries saw more resistance also to these last-line antibiotics, perhaps reflecting greater use and
363 selection pressure. However, there were exceptions. Germany, which has a moderate CPE incidence,
364 reported much higher rates of colistin and fosfomycin resistance than other moderate incidence countries.
365 More worrying is the fact that the overall proportions of fosfomycin resistance (54%) and colistin resistance
366 (28.3%) have become so high among carbapenemase-producing *K. pneumoniae* that even the 'colistin-plus'
367 treatment regimens favoured for infections due to CPE are increasingly jeopardized, leaving ever so little
368 choice in many cases.^{23,24}

369 **Conclusions**

370 As exemplified with this structured survey, the EuSCAPE project documented an encouraging degree of
371 commitment from NELs, and shows that the political and logistical challenges of establishing a framework
372 of enhanced sentinel surveillance for CPE can be overcome in Europe, Turkey and Israel. There were large
373 variations across Europe with respect to the distribution of the four major types of carbapenemases among
374 clinical isolates of *K. pneumoniae* and *E. coli*. Clinicians should pay attention to antibiotic susceptibility
375 testing results and be alerted when isolates show any degree of carbapenem non-susceptibility, which
376 would require confirmation of carbapenemase production. For the majority of isolates, there were still
377 alternative options for patient treatment; however, resistance to all tested antibiotics was also reported,
378 which is another reminder of the urgent need for prevention and control of CPE in Europe and emphasizes
379 the need for novel antibacterial agents that are active against carbapenem-resistant bacteria.

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414 ICJ Opinion on the Kosovo declaration of independence.

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416 Designed the study: HG, DLM. Modified the sampling frame and defined diagnostic procedures: HG, CG, BA,
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418 the survey protocol: HG, CG. Recruited sentinel sites and collected isolates and epidemiological data and
419 carried out diagnostic procedures: all members of the EuSCAPE Working Group. Supervised and
420 coordinated the survey: HG, CG, all members of the EuSCAPE Working Group. Developed tools for data
421 collection: DMA, CTT, CG. Managed data and isolate collection: CG. Analysed the data: HG, CG. Wrote the
422 first draft manuscript: HG, CG. Provided feedback, contributed with comments, reviewed and edited the
423 manuscript: DML, DLM, NW, BA, AAT, RC, YC, AWF, CGG, YG, MG, PN, LP, GMR, HS, AV, TM, and the
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520 **Figure and Table Legends**

521

522 **Figure 1.** Locations of participating sentinel hospitals.

523 **Table 1.** Summary overview of the numbers of clinical *K. pneumoniae* and *E. coli* isolates submitted by
 524 country, and combined incidence estimates in European hospitals.

525 **Table 2a.** *K. pneumoniae*: Summary overview of clinical isolates submitted as non-susceptible to
 526 carbapenems, confirmed as producing a carbapenemase and type of carbapenemase, by country.

527 **Table 2b.** *E. coli*: Summary overview of clinical isolates submitted as non-susceptible to carbapenems,
 528 confirmed as producing a carbapenemase and type of carbapenemase, by country.

529 **Table 3.** Resistance of confirmed carbapenemase-producing *K. pneumoniae* to last-line antibiotics and to all
 530 tested antibiotics.

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534 **Supplementary Material**

535

536 **Supplementary Table 1.** Overview of *K. pneumoniae* and *E. coli* isolates according to carbapenem
537 susceptibility and specimen type.

538

539 **Supplementary Table 2.** Risk factors for confirmed carbapenemase-producing *K. pneumoniae* or *E. coli*
540 infection. Univariate analysis.

541

542 **The EuSCAPE Working Group.** Affiliations of authors.

543

544 **The EuSCAPE Laboratory Manual.** Identification and confirmation of carbapenemase-producing
545 *Enterobacteriaceae*.

546

547 **The EuSCAPE Structured Survey Protocol.** A stepwise workflow through the structured survey performed
548 on a country by country basis.

549