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

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

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

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

Summary

Background

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

Methods

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

Findings

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

Interpretation

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

Funding

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

Research in context

Evidence before this study

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

Added value of this study

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

Implications of all the available evidence

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

Introduction

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

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

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

Methods

Capacity building and proficiency testing

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

Structured survey

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

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

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

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

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

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

Data analysis

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

Role of the funding source

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Results

Summary statistics and incidence estimates

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

During the six-month period 2,301 *K. pneumoniae* and 402 *E. coli* isolates were collected (Table 1). . Most (86·1%) index isolates submitted were *K. pneumoniae* (1,203 isolates vs. 194 *E. coli*). Proportions of index and comparator isolates did not differ in terms of anatomical origin or specimen type except for blood stream infections caused by *E. coli,* where carbapenem-susceptible isolates contributed significantly more infections (Supplementary Table 1). It therefore seems that the ability to cause infections is not contingent on the resistance traits under study. Of all isolates submitted by the NELs as carbapenem non-susceptible, PCR tests confirmed the presence of KPC, NDM, OXA-48-like, and VIM type genes for 850 (70·7%) of *K. pneumoniae* and 77 (39·7%) of *E. coli.* Among the 927 carbapenemase-producers, the ratio between *K. pneumoniae* and *E. coli* was 11:1.

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

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

KPC enzymes detected in 393 isolates of all 927 CPE isolates (42·4%) represented the most frequent carbapenemases. OXA-48-like enzymes were the second most frequent (353 isolates, 38·1%) and were the most prominent class of carbapenemases in eight countries. NDM genes were detected in 113 (12·2%) and VIM in 68 *K. pneumoniae* isolates (7.3%).

Likewise, among *K. pneumoniae*, the most frequently detected carbapenemases were KPC enzymes (379 isolates, 44·6%), followed by OXA-48-like (310 isolates, 36·5%), NDM (93 isolates, 10·9%) and VIM (68 isolates, 8·0%). In *E. coli* the most frequently detected carbapenemases were OXA-48-like enzymes (43 isolates, 55·8%) followed by NDM (20 isolates, 26·0%) and KPC (14 isolates, 18·2%), albeit with substantial country-to country variation in relative prevalence (Table 2a and 2b).

At country level, high proportions of KPC-positive *K. pneumoniae* among carbapenem-non-susceptible isolates were found in Italy (187 isolates, 95·9%), Israel (31 isolates, 79·5%), Greece (56 isolates, 65·1 %)

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

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

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

Phenotypic drug resistance

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

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

Risk factors

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

important and unbiased sample, representative of the local susceptible population and served as an appropriate 'control' group. Univariate analysis identified six risk factors that were positively associated with carbapenemase-producing *K. pneumoniae* or *E. coli*, and two factors that were negatively associated (Supplementary Table 2). Four of these remained significantly and independently associated with carbapenemase-producing *K. pneumoniae* or *E. coli* in the multivariable model which included intensive 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), hospital-acquisition (OR=2·6, 1·9 – 3·7) and travel outside the country of residence in the previous six months (OR=3·0, 1·6 – 5·7).

Discussion

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

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

However, as with all sampling frameworks for bacteria and epidemiological data, important caveats remain. Despite decisions to minimise workload by concentrating on the two clinically most relevant species and reducing the amount of additional information, nine countries failed to recruit their quota of sentinel sites and an another eight countries did not provide crucial denominator data. In some cases this was because of financial constraints and because the workload could not be accommodated by some of the hospital laboratories that had initially agreed to participate. Some NELs with established routines could not manage to test additional antibiotics. As with other international surveillance systems (EARS-Net) this study relied on routinely available data. For these reasons, the precision of some of the estimates on the occurrence and risk factors of CPE in the European region could still be improved. For example, some countries reported very low numbers of index CPE isolates whereas, judging from existing publications and high endemicity in neighbouring countries, much higher rates would have been expected. It is possible that in these countries diagnostic habits result in a lower sampling density or that the recruited sentinel sites were less able to reliably identify carbapenem-non-susceptible isolates despite testing proficiency of the NELs, concealing the true incidence of CPE through these types of ascertainment bias. Moreover, 353 (29·3%) of the *K. pneumoniae* isolates and 117 (60·3%) of the *E. coli* isolates that were submitted by the sentinel hospital laboratories as suspected carbapenem-non-susceptible had none of the four major carbapenemases (KPC, NDM, OXA-48-like and VIM) and were reported as “Other”. This lack in specificity could be the result of a carbapenemase not included in the test panel, or alternative mechanisms such as reduced permeability. At the same time, sentinel laboratories relied on their local routine antibiotic susceptibility tests which may also be the source of potential misclassification. Nevertheless, these first data on CPE generated in a comprehensive manner will serve as a benchmark against which future initiatives and trends will be measured.

Hospital incidence of carbapenemase-producing *K. pneumoniae* and *E. coli* per 10,000 admissions ranged from six in Italy to 0·02 in Norway with an average of 1·3. The incidence density per 100,000 hospital patient-days ranged from 17·3 in Greece to 0·09 in Lithuania, an average of 2·5 across all countries. These values will underestimate total CPE incidence, because carbapenemases also occur in other

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

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

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

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

Only 12 countries tested the complete panel of antibiotics recommended by the study protocol. This makes it difficult to determine the extent with which extensively drug-resistant (XDR) or pandrug-resistant (PDR) *Enterobacteriaceae* phenotypes prevail in European hospitals.[22] Clinically more important than these epithets are, however, the proportions of carbapenemase-producing isolates that are also resistant to last-line antibiotics such as colistin, fosfomycin and tigecycline. We generally observed that high-CPE-incidence countries saw more resistance also to these last-line antibiotics, perhaps reflecting greater use and selection pressure. However, there were exceptions. Germany, which has a moderate CPE incidence, reported much higher rates of colistin and fosfomycin resistance than other moderate incidence countries. More worrying is the fact that the overall proportions of fosfomycin resistance (54%) and colistin resistance (28·3%) have become so high among carbapenemase-producing *K. pneumoniae* that even the 'colistin-plus' treatment regimens favoured for infections due to CPE are increasingly jeopardized, leaving ever so little choice in many cases.[23,24]

Conclusions

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

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[§]This designation is without prejudice to positions on status, and is in line with UNSCR 1244/1999 and the ICJ Opinion on the Kosovo declaration of independence**.**

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

Conflict of interest

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References

1 Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 2011; **17**: 1791–8.

2 Monaco M, Giani T, Raffone M, *et al.* Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. *Euro Surveill* 2014; **19**: pii:20939.

3 Livermore DM, Warner M, Jamrozy D, *et al. In vitro* selection of ceftazidime-avibactam resistance in *Enterobacteriaceae* with KPC-3 carbapenemase. *Antimicrob Agents Chemother* 2015; **59**: 5324–30.

4 Alm RA, Johnstone MR, Lahiri SD. Characterization of *Escherichia coli* NDM isolates with decreased susceptibility to aztreonam/avibactam: role of a novel insertion in PBP3. *J Antimicrob Chemother* 2015; **70**: 1420–8.

5 Glasner C, Albiger B, Buist G, *et al.* Carbapenemase-producing *Enterobacteriaceae* in Europe: a survey among national experts from 39 countries, February 2013. *Euro Surveill* 2013; **18**: pii:20525.

6 European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance (version 1.0, December 2013).

7 Nordmann P, Poirel L, Dortet L. Rapid Detection of Carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2012; **18**: 1503–7.

8 Decision number 2119/98/EC of the European Parliament and of the Council of 24 September 1998: setting up a network for the epidemiological surveillance and control of communicable diseases in the Community. 1998; : 1–6.

9 Commission decision of 22 December 1999 on the communicable diseases to be progressively covered by the Community network under Decision No 2119/98/EC of the European Parliament and of the Council (2000/96/EC). 1998.

10 Giakoupi P, Maltezou H, Polemis M, *et al.* KPC-2-producing *Klebsiella pneumoniae* infections in Greek hospitals are mainly due to a hyperepidemic clone. *Euro Surveill* 2009; **14**: pii:19218.

11 Schwaber MJ, Lev B, Israeli A, *et al.* Containment of a country-wide outbreak of carbapenem-resistant *Klebsiella pneumoniae i*n Israeli hospitals via a nationally implemented intervention. *Clin Infect Dis* 2011; **52**: 848–55.

12 Hasan CM, Turlej-Rogacka A, Vatopoulos AC, Giakkoupi P, Maâtallah M, Giske CG. Dissemination of blaVIM in Greece at the peak of the epidemic of 2005–2006: clonal expansion of *Klebsiella pneumoniae* clonal complex 147. *Clin Microbiol Infect* 2014; **20**: 34–7.

13 Samra Z, Ofir O, Lishtzinsky Y, Madar-Shapiro L, Bishara J. Outbreak of carbapenem-resistant *Klebsiella pneumoniae* producing KPC-3 in a tertiary medical centre in Israel. *Int J Antimicrob Agents* 2007; **30**: 525–9.

14 Sisto A, D'Ancona F, Meledandri M, *et al.* Carbapenem non-susceptible *Klebsiella pneumoniae* from Micronet network hospitals, Italy, 2009 to 2012. *Euro Surveill* 2012; **17**: pii:20247.

15 Oteo J, Ortega A, Bartolomé R, *et al.* Prospective multicenter study of carbapenemase-producing Enterobacteriaceae from 83 hospitals in Spain reveals high in vitro susceptibility to colistin and meropenem. *Antimicrob Agents Chemother* 2015; **59**: 3406–12.

16 Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL, the European Survey of Carbapenemase-

Producing Enterobacteriaceae (EuSCAPE) working group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. *Euro Surveill* 2015; **20**: 30062–18.

17 Izdebski R, Baraniak A, Herda M, *et al.* MLST reveals potentially high-risk international clones of *Enterobacter cloacae*. *J Antimicrob Chemother* 2015; **70**: 48–56.

18 Giakkoupi P, Xanthaki A, Kanelopoulou M, *et al.* VIM-1 Metallo-beta-lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals. *J Clin Microbiol* 2003; **41**: 3893–6.

19 Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. *Trends Microbiol* 2011; **19**: 588–95.

20 Lixandru BE, Cotar AI, Straut M, *et al.* Carbapenemase-Producing *Klebsiella pneumoniae* in Romania: A Six-Month Survey. *PLoS ONE* 2015; **10**: e0143214–10.

21 Meletis G, Oustas E, Bagkeri M. Carbapenemase reports from the Balkans: a systematic review. *Infez Med* 2014; **22**: 85–106.

22 Magiorakos AP, Srinivasan A, Carey RB, *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2014; **18**: 268–81.

23 Lee GC, Burgess DS. Treatment of *Klebsiella pneumoniae* carbapenemase (KPC) infections: a review of published case series and case reports. *Ann Clin Microbiol Antimicrob* 2012; **11**: 1–9.

24 Tumbarello M, Viale P, Viscoli C, *et al.* Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. *Clin Infect Dis* 2012; **55**: 943–50.

Figure and Table Legends

Figure 1. Locations of participating sentinel hospitals.

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

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

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

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

Supplementary Material

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

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

The EuSCAPE Working Group. Affiliations of authors.

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

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