Eur J Clin Microbiol Infect Dis (2017) 36:139–146
DOI 10.1007/s10096-016-2782-x

ORIGINAL ARTICLE

Increasing incidence of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Belgian hospitals

M. De Laveleye[1] · T. D. Huang[1] · P. Bogaerts[1] · C. Berhin[1] · C. Bauraing[1] · P. Sacré[1] · A. Noel[1] · Y. Glupczynski[1] · on behalf of the multicenter study group

Received: 26 July 2016 / Accepted: 5 September 2016 / Published online: 17 September 2016

Abstract Carbapenemase-producing Enterobacteriaceae are increasingly reported worldwide. The aim of the study was to determine the incidence and molecular epidemiology of carbapenemase-producing (CP) *Escherichia coli* and *Klebsiella pneumoniae* (CP-E/K) in Belgium. Eleven hospital-based laboratories collected carbapenem non-susceptible (CNS) isolates of *E. coli* and *K. pneumoniae* detected in clinical specimens from January 2013 to December 2014. All CNS strains were tested for carbapenemase production and typed by multilocus sequence typing (MLST) for a 6-month period as part of the European Survey on Carbapenemase-Producing Enterobacteriaceae in Europe (EuSCAPE) structured survey. In addition, an equal number of carbapenem-susceptible isolates collected were preserved as a control group for risk factor analysis. The overall incidence rate of CP-E/K isolates in hospitals increased from 0.124 in 2013 to 0.223 per 1000 admissions in 2014. From November 2013 to April 2014, 30 CP *K. pneumoniae* [OXA-48 ($n = 16$), KPC ($n = 13$), OXA-427 ($n = 1$)] and five CP *E. coli* [OXA-48 ($n = 3$), NDM ($n = 1$), OXA-427 ($n = 1$)] isolates were detected in ten hospitals. The 16 OXA-48-producing *K. pneumoniae* strains were distributed into eight sequence types (STs), while the 13 KPC-producing *K. pneumoniae* clustered into three STs dominated by ST512 ($n = 7$) and ST101 ($n = 5$). Compared to controls, we observed among CP-E/K carriers significantly higher proportion of males, respiratory origins, previous hospitalization, nosocomial setting, and a significantly lower proportion of bloodstream infections. Our study confirms the rapid spread of CP-E/K in Belgian hospitals and the urgent need for a well-structured and coordinated national surveillance plan in order to limit their dissemination.

Introduction

The spread of carbapenemase-producing Enterobacteriaceae (CPE) is alarming and constitutes a major threat to public health worldwide [1, 2]. In most instances, carbapenemases are associated with other β-lactamases and resistance to several other classes of antimicrobials, rendering treatment particularly challenging. Moreover, carbapenemase-encoding genes are easily transferable through plasmids and transposons, and have the propensity to spread amongst Enterobacteriaceae [1].

In Belgium, the number of CPE isolates reported in hospitals has increased dramatically since 2010 [3]. Over the recent years, the epidemiological situation of CPE has worsened in Europe, showing a doubling of the number of countries reporting inter-regional spread or endemic situation for CPE from 2013 to 2015 [4]. However, these data based on voluntary reporting or questionnaires assessment by national experts are not supported by objective epidemiological indicators, which preclude precise comparisons to be made between countries.

The present study, performed as part of the European Survey on Carbapenemase-Producing Enterobacteriaceae in Europe (EuSCAPE), aimed to determine the incidence and the epidemiological characteristics of carbapenemase-

Members of the multicenter study group are listed in the Acknowledgments section.

✉ T. D. Huang
te-din.huang@uclouvain.be

[1] National Reference Laboratory of Antibiotic-Resistant Gram-Negative Bacilli, CHU UCL Namur, Yvoir, Belgium

producing *Escherichia coli* and *Klebsiella pneumoniae* (CP-E/K) in Belgian hospitals.

Materials and methods

Study design

Eleven laboratories serving secondary- and tertiary-care hospitals (median number of 910 beds per hospital) chosen for their representative geographic distribution across Belgium participated in the study. The laboratories were requested to collect carbapenem non-susceptible (CNS) isolates of *E. coli* and *K. pneumoniae* (CNS-E/K) from clinical samples between January 2013 and December 2014. Screening specimens (e.g., stools or rectal swabs) were excluded to limit the impact of variations in screening strategies between hospitals. Carbapenem non-susceptibility was defined as decreased susceptibility to at least one carbapenem drug (meropenem, imipenem, or ertapenem) according to the routine methods performed locally using either Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) interpretative guidelines [5, 6]. From November 2013 to April 2014, for each bacterial isolate, the first successive carbapenem-susceptible isolate of the same species was stored as a control isolate as per the protocol of the EuSCAPE structured survey. All isolates were sent to the reference laboratory, accompanied by the following anonymized data: sample origin, sampling date, type of hospital ward/unit, age and gender of the patient, previous history of hospitalization, recent travel history (last 6 months), clinical significance, and nosocomial context of the sampling (hospital stay for two days or more). Institutional activity data (number of admissions and mean length of stay) of the years 2013 and 2014 were collected individually by each hospital for the calculation of incidence rates.

Characterization of the CNS-E/K isolates

All putative CNS-E/K isolates were verified at the central laboratory for species identification using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Microflex LT, Bruker Daltonics, Bremen, Germany) and were tested for their phenotypic resistance profile by disk diffusion to β-lactams (cefotaxime, ceftazidime, cefepime, aztreonam, piperacillin/tazobactam, temocillin, ertapenem, and meropenem). The production of carbapenemase was sought by the Carba NP test [7] and by multiplex polymerase chain reaction (PCR) targeting $bla_{\text{OXA-48}}$, bla_{KPC}, bla_{VIM}, bla_{NDM}, and bla_{IMP} [8]. In case of phenotypical suspicion of carbapenemase with a negative result for multiplex PCR targeting the five major carbapenemases, additional PCR assays were performed, including the detection of OXA-427, a novel carbapenem-hydrolyzing class D β-lactamase (CHDL) [9]. For confirmed CP-E/K strains, the minimal inhibitory concentrations (MICs) of 14 antibiotics were determined by the broth microdilution method (Sensititre®, TREK Diagnostic Systems, Cleveland, OH, USA), interpreted using EUCAST interpretative criteria [10], except for temocillin [11]. Colistin-resistant CP-E/K isolates were further analyzed by PCR for the presence of plasmid-mediated *mcr-1* encoding gene [12]. In addition, carbapenemase-producing *K. pneumoniae* isolates were characterized by whole-genome sequencing with multilocus sequence typing (MLST) for a 6-month period. The library preparation and the sequencing were performed using the Nextera® XT DNA Library Prep Kit and the Illumina MiSeq® platform (San Diego, CA, USA). The resulting sequence reads were assembled using CLC Genomic workbench (version 8.5.1, Qiagen, Hilden, Germany). Finally, contigs were submitted to the Center for Genomic Epidemiology MLST 1.8 [13] to assign sequence types (STs) based on the closest matches against the MLST database.

Statistical analysis

The statistical analyses were performed with the MedCalc® software (version 16.4.3, Ostend, Belgium). Fisher's exact test and the Wilcoxon–Mann–Whitney rank-sum test were used to compare proportions and age distributions, respectively. A p-value <0.05 was considered statistically significant. Items with missing data were not included in the analysis.

Results

From January 2013 to December 2014, 125 (45.1 %) of the 277 CNS-E/K isolates collected were confirmed as CP-E/K by the reference laboratory. Based on the total yearly number of admissions to the participating hospitals (361,713 in year 2013 and 358,667 in 2014), the incidence rate of clinical CP-E/K increased significantly from 0.124 (95 % confidence interval [CI]: 0.088–0.161) in 2013 to 0.223 (95 % CI: 0.174–0.272) cases per 1000 admissions in 2014 ($p = 0.001$) (Table 1). In 2013, 38 *K. pneumoniae* and seven *E. coli* isolates were detected in nine hospitals, while in 2014, 66 *K. pneumoniae* and 14 *E. coli* were reported by ten hospitals, with OXA-48 as the predominant type of carbapenemase found (Table 2).

During the 6-month EuSCAPE study period, a total of 179,841 admissions and 1,262,758 patient-days were recorded by the 11 participating hospitals. Overall, 35 CP-E/K isolates were found in ten hospitals, resulting in an overall incidence rate of 0.195 (95 % CI: 0.130–0.259) per 1000 admissions and an incidence density rate of 0.028 (95 % CI: 0.019–

Table 1 Number and incidence rates of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* (CP-E/K) isolates ($n = 125$) reported by 11 Belgian hospitals during the years 2013 and 2014

Hospital	No. of isolates		Incidence per 1000 admissions		Incidence per 1000 patient-days	
	2013	2014	2013	2014	2013	2014
A	6	8	0.224	0.294	0.033	0.044
B	15	17	0.521	0.563	0.076	0.086
C	1	1	0.026	0.026	0.004	0.004
D	2	9	0.074	0.326	0.012	0.056
E	5	5	0.14	0.139	0.017	0.017
F	2	4	0.032	0.064	0.004	0.008
G	1	6	0.026	0.187	0.004	0.025
H	8	21	0.198	0.517	0.028	0.075
I	0	5	0	0.183	0	0.031
J	0	0	0	0	0	0
K	5	4	0.258	0.202	0.033	0.024
Total	45	80	0.124	0.223	0.018	0.032

0.037) per 1000 patient-days. The microbiological characteristics of the 30 *K. pneumoniae* and five *E. coli* strains producing a carbapenemase are presented in Table 3. The 13 KPC-producing *K. pneumoniae* strains detected in three hospitals were grouped into three different clonal backgrounds, dominated by ST512 ($n = 7$; two hospitals) and ST101 ($n = 5$; one hospital), all carrying bla_{KPC-3}. The 16 OXA-48-producing *K. pneumoniae* were detected in seven hospitals and belonged to eight different STs, with ST405 being the most represented clone ($n = 6$; three hospitals), followed by ST788 ($n = 3$; one hospital) and ST11 ($n = 2$; two hospitals). The two OXA-427 carbapenemase-producing strains originated from one single hospital.

The antimicrobial susceptibility testing of 80 CP-E/K strains is summarized in Table 4. 100 % and 97.5 % of CP-E/K isolates were found to be resistant to piperacillin/tazobactam and temocillin, respectively, including high-level temocillin resistance for all OXA-48 producers (MIC range: 64 to >256 mg/L), while the non-susceptibility rate for ertapenem and meropenem were 86.3 % and 45.0 %, respectively. The carbapenem MIC_{50} values for OXA-48 *K. pneumoniae* (ertapenem: 4 mg/L, meropenem: 1 mg/L) were lower than those obtained for KPC *K. pneumoniae* (ertapenem: 64 mg/L, meropenem: 32 mg/L). Six *K. pneumoniae* (three OXA-48 and three KPC producers) and one OXA-48-positive *E. coli* isolates were resistant to colistin, but none of these isolates were found to be *mcr-1* producers. Three *K. pneumoniae* (two OXA-48 and one NDM producers) and one OXA-427-producing *E. coli* strains were intermediately resistant to tigecycline (MIC value: 2 mg/L).

Table 2 Distribution of carbapenemase types among CP-E/K isolates ($n = 125$) during the years 2013 and 2014

Species	Carbapenemase no. (%)	2013	2014
K. pneumoniae	OXA-48	23 (60.6)	35 (53.1)
	KPC	13 (34.2)	25 (37.9)
	VIM	1 (2.6)	2 (3.0)
	NDM	0	3 (4.5)
	OXA-427	0	1 (1.5)
	OXA-48+ NDM	1 (2.6)	0
E. coli	OXA-48	6 (85.7)	10 (71.4)
	KPC	0	0
	VIM	0	2 (14.3)
	NDM	1 (14.3)	0
	OXA-427	0	2 (14.3)

When compared to the 56 control patients (Table 5), CP-E/K-positive patients were more likely to be a male, to have been hospitalized in the last 6 months, or to have stayed in hospital for at least two days before sampling. A higher proportion of bacterial isolates from blood cultures was found in control patients, while, conversely, a higher proportion of lower respiratory tract specimens was documented in CP-E/K patients. One NDM-producing *E. coli* was recovered from a patient who had traveled to Pakistan and three OXA-48-positive patients had previously stayed in Spain, Tunisia, and Iran, respectively. Data on possible travel importation were lacking for 34.3 % of CP-E/K patients and for 80.4 % of the control patients.

Discussion

CPE are rapidly disseminating worldwide, including also in Europe [1]. The European Antimicrobial Resistance Surveillance Network (EARS-Net) is the current European surveillance system designed to monitor crude resistance rates to antimicrobial agents (but not resistance mechanism) used in

Table 3 Clonal relatedness of CP-E/K collected during the six-month EuSCAPE study period

Strain number	Species	Carbapenemase	MLST (type)	Hospital code
CNR20130794	*E. coli*	NDM-1		B
CNR20140187	*E. coli*	OXA-427		F
CNR20130827	*E. coli*	OXA-48		B
CNR20130772	*E. coli*	OXA-48		E
CNR20130879	*E. coli*	OXA-48		G
CNR20140140	*K. pneumoniae*	KPC-3	101	B
CNR20130844	*K. pneumoniae*	KPC-3	101	B
CNR20130883	*K. pneumoniae*	KPC-3	101	B
CNR20140093	*K. pneumoniae*	KPC-3	101	B
CNR20140132	*K. pneumoniae*	KPC-3	101	B
CNR20140231	*K. pneumoniae*	KPC-2	258	G
CNR20140232	*K. pneumoniae*	KPC-3	512	G
CNR20140267	*K. pneumoniae*	KPC-3	512	G
CNR20140006	*K. pneumoniae*	KPC-3	512	H
CNR20140008	*K. pneumoniae*	KPC-3	512	H
CNR20140029	*K. pneumoniae*	KPC-3	512	H
CNR20140146	*K. pneumoniae*	KPC-3	512	H
CNR20140230	*K. pneumoniae*	KPC-3	512	G
CNR20140349	*K. pneumoniae*	OXA-427	1942	F
CNR20140270	*K. pneumoniae*	OXA-48	11	D
CNR20140430	*K. pneumoniae*	OXA-48	11	I
CNR20140042	*K. pneumoniae*	OXA-48	15	C
CNR20140314	*K. pneumoniae*	OXA-48	147	H
CNR20140347	*K. pneumoniae*	OXA-48	268	K
CNR20140033	*K. pneumoniae*	OXA-48	405	A
CNR20140247	*K. pneumoniae*	OXA-48	405	A
CNR20140106	*K. pneumoniae*	OXA-48	405	E
CNR20140269	*K. pneumoniae*	OXA-48	405	E
CNR20140182	*K. pneumoniae*	OXA-48	405	I
CNR20140429	*K. pneumoniae*	OXA-48	405	I
CNR20140428	*K. pneumoniae*	OXA-48	416	I
CNR20140064	*K. pneumoniae*	OXA-48	788	H
CNR20140065	*K. pneumoniae*	OXA-48	788	H
CNR20140118	*K. pneumoniae*	OXA-48	788	H
CNR20130744	*K. pneumoniae*	OXA-48	972	D

human medicine. Therefore, the EuSCAPE project was launched in 2012 to gain better insight to the epidemiology of CPE in Europe [14]. This initiative aimed to assess the occurrence of CPE among hospitalized patients using a European-wide standardized sampling frame and to compare calculated epidemiological indicators between countries.

In Belgium, a multicentric prevalence survey performed among 24 Belgian hospitals in 2012 demonstrated an estimated prevalence of 0.28 % of CPE isolates among 4564 screened Enterobacteriaceae isolates (screening samples included in this study). *Klebsiella pneumoniae* was the most frequent CPE species and OXA-48 the most often encountered carbapenemase [15].

The present study highlighted the significant increase in incidence of CP-E/K in Belgium from 2013 to 2014. A similar rising trend in incidence rates of CNS-E/K was also observed in the National Surveillance report of Health-care-associated infections (NSIH) [16]. Indeed, the overall average incidences of meropenem-I/R *E. coli* and *K. pneumoniae* increased from 0.03 and 0.19 cases per 1000 admissions, respectively, in 2013, to 0.15 and 0.36 cases per 1000 admissions in 2014, respectively. However, the results from this particular surveillance program included bacterial isolates from both clinical and screening specimens reported by 120 hospitals country-wide and, like EARS-Net, address mainly carbapenem resistance (intermediately resistant and resistant considered

Table 4 Susceptibility testing and minimum inhibitory concentration (MIC) results of CP-E/K isolates detected in 2014 ($n = 80$)

Antibiotic	Range (mg/L)	*K. pneumoniae* ($n = 66$)			*E. coli* ($n = 14$)		
		MIC_{50} (mg/L)	MIC_{90} (mg/L)	Number of S (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Number of S (%)
Piperacillin/tazobactam	32, >128	>128	>128	0 (0)	128	>128	0 (0)
Temocillin	16, >256	128	>256	1 (1.5)	>256	>256	1 (7.1)
Cefotaxime	<=0.5, >64	64	>64	8 (12.1)	32	>64	5 (35.7)
Ceftazidime	<=0.5, >64	>64	>64	9 (13.6)	4	>64	6 (42.9)
Cefepime	<=0.5, >64	32	>64	9 (13.6)	4	32	6 (42.9)
Aztreonam	<=0.5, >64	>64	>64	9 (13.6)	0.5	64	8 (57.1)
Ertapenem	<=0.25, >32	8	>32	2 (3.0)	0.5	4	9 (64.3)
Meropenem	<=0.25, >32	4	>32	31 (47.0)	<=0.25	1	13 (92.9)
Gentamicin	<=1, >8	>8	>8	24 (36.4)	<=1	>8	10 (71.4)
Tobramycin	<=1, >8	>8	>8	9 (13.6)	0.5	>8	11 (78.6)
Amikacin	<=4, >32	8	>32	37 (56.1)	<=4	<=4	13 (92.9)
Ciprofloxacin	<=0.25, >2	>2	>2	13 (19.7)	>2	>2	5 (35.7)
Colistin	<= 0.25, >8	0.5	1	60 (90.9)	<=0.25	1	13 (92.9)
Tigecycline	<=0.12, 2	0.5	1	63 (95.4)	0.25	0.5	13 (92.9)

S Susceptible strains

together) and not specifically the carbapenemase resistance mechanism.

In comparison to other EuSCAPE participating countries, the incidence rate of CP-E/K (0.195 per 1000 admissions) in Belgium was higher than in Germany (0.047 CP-E/K per 1000 admissions), but showed a similar distribution of carbapenemase types, mainly represented by OXA-48 (54.3 %) and KPC (37.1 %) [17]. In Italy, nearly all (97 %)

Table 5 Comparison of clinical characteristics of control and CP-E/K-carrying patients

Variable	Control patients ($n = 56$)		CP-E/K patients ($n = 35$)		*p*-Value
	Available data	Missing data or unknown	Available data	Missing data or unknown	
Age range (years)	0 to 96		20 to 91		
Median age (years)	71		71		0.9188
Gender (% male)	23 (41.0)		27 (77.1)		0.0011
No. (%) of specimen sources					
Urine	19 (33.9)		10 (28.6)		0.6494
Blood	22 (39.3)		2 (5.7)		0.0004
LRT	7 (12.5)		14 (40.0)		0.0043
Puncture fluid	1 (1.8)		3 (8.6)		0.1567
Wound	3 (5.4)		6 (17.1)		0.0821
Other	4 (7.1)		0 (0)		0.2942
No. (%) of infections	43 (76.8)	3 (5.4)	16 (45.7)	13 (37.1)	0.5370
No. (%) of patients with:					
Stay on ICU	11 (19.6)		12 (34.3)	5 (14.3)	0.0719
Stay on general ward	37 (66.1)		17 (48.6)	5 (14.3)	0.4838
Presentation as outpatient	8 (14.3)		1 (2.9)	5 (14.3)	0.1525
Previous hospitalization*	23 (41.1)	11 (19.6)	19 (54.3)	10 (28.6)	0.0470
Previous travel abroad*	1 (1.8)	45 (80.4)	4 (11.4)	12 (34.3)	1.0000
Nosocomial context**	32 (57.1)		28 (80.0)	3 (8.6)	0.0040

*Within the last 6 months

**At the time of sample, had the patient stayed two days or more in the hospital where the sample was taken?

of the 187 CP-E/K isolates carried bla_{KPC}, confirming the endemic situation of KPC in this country [18], while in Romania, OXA-48 producers represented a large majority (78.5 %) of the 65 CP-E/K isolates [19]. In the Czech Republic, only two CP-E/K isolates were detected [20]. However, for these three countries, no incidence rates were provided by the authors.

Molecular typing of CP *K. pneumoniae* strains showed for KPC producers the clonal dissemination of a small number of clusters (mostly ST512 and ST101) in three participating hospitals. The ST512 clone, a single-locus allelic variant of the pandemic ST258 clone [19], and ST101 have been reported as KPC-producing *K. pneumoniae* causing outbreaks in Italy [21,22]. Our data confirmed the almost complete replacement of ST258 by ST512 and ST101 clones among KPC producers observed since 2011 in Belgium [23].

On the other hand, a more diversified picture was observed for OXA-48-producing *K. pneumoniae*, which were distributed into eight different STs, supporting the horizontal transfer of the *Inc*L/M-type pOXA-48 conjugative plasmid previously described [24]. Nevertheless, three major STs (ST405, ST788, and ST11) associated with OXA-48 were identified in our study. ST405 is a successful clone of OXA-48 *K. pneumoniae* already described in Spain [25] and France [26], while ST11 has been reported in Spain [25] and Greece [27]. Further, the clustering of three OXA-48-producing strains belonging to ST788 at one single hospital strongly suggested the local dissemination of this clone.

OXA-427 is a newly described plasmid-mediated CHDL unrelated to OXA-48 (26 % amino acid homology) that displays a broad resistance profile to β-lactams, including expanded-spectrum cephalosporins and carbapenems [9]. The encoding $bla_{OXA-427}$ gene is carried on an *Inc*A/C plasmid that was detected sporadically in various Enterobacteriaceae species isolates at one Belgian hospital from 2013 onwards.

Antimicrobial susceptibility testing confirmed resistance to piperacillin/tazobactam and temocillin in nearly all CP-E/K isolates, while less than half of the CP-E/K were non-susceptible to meropenem, with lower carbapenem MIC values for OXA-48 producers than those expressing KPC, in line with previous reports [28]. The resistance rate for colistin was 8.7 %, most probably due to chromosomal mutations, as no *mcr-1*-positive isolates were identified among the seven strains tested. This resistance proportion was much lower than that found in Germany (33.3 %) [17] or Italy (43 %) [18]. The non-susceptibility rate for tigecycline was still low, with only four intermediately resistant strains (5.0 %).

Concerning the risk factor analysis, no clear explanation can be provided for the higher proportion of male subjects among patients with CP-E/K, although the same association was reported in the German study [17]. The higher proportion of bacteremic isolates in control patients could probably be reflected by the tendency of laboratories to store preferentially in collections bacterial isolates recovered from deep-seated infection sites. Recent hospitalization was also identified as a predictor for CPE carriage in Israel [29]. Travel data were missing for the majority of patients included in the study. It is, therefore, difficult to draw conclusions, although the proportion of patients with travel history was higher in the CP-E/K group, similar to the German data [17]. A previous publication further highlighted that the proportion of travel-imported CPE cases could be largely underestimated due to the lack of active investigation in most cases [30].

We acknowledge the fact that our study might have suffered from several limitations. In addition to the incompleteness of the clinical data collected, the EuSCAPE study protocol addressed only *K. pneumoniae* and *E. coli* species. In Belgium, at least 20 % of the confirmed CPE isolates belong to Enterobacteriaceae species other than *K. pneumoniae* and *E. coli* [16,31]. Therefore, the EuSCAPE structured survey may not give a complete picture of the incidence of clinical CPE. Secondly, the limited participation in this survey could not allow the extrapolation of the results to the whole country. However, the increasing number of CPE reported by hospitals to the national reference laboratory [31] is in line with the trend observed in this survey. Finally, it is difficult to evaluate the impact of independent outbreaks on the rising incidence of CP-E/K between 2013 and 2014. However, the incidence increase in 7 out of the 11 participating hospitals (and only one hospital had decreased incidence) strongly suggests a true global increase in the incidence of CP-E/K.

In summary, this survey highlighted the rapid diffusion of CP-E/K from clinical specimens of CPE in Belgian hospitals between 2013 and 2014, yet the overall incidence remained low (0.195 per 1000 admissions). OXA-48 and KPC are the two major carbapenemases found in Belgium, with noticeable differences in their mode of transmission, mostly clonal for KPC and preferentially through horizontal plasmid transfer for OXA-48 producers.

Acknowledgments We acknowledge the participation of the following colleagues in the multicentric study group:

Kristien Van Vaerenbergh (OLV Aalst); Margareta Ieven (UZ Antwerpen); Anne Dediste (CHU St-Pierre, Brussels); Hector Rodriguez-Villalobos (CU St-Luc UCL, Brussels); Denis Pierard (UZ Brussel, Brussels); Geert Claeys (UZ Gent); Veroniek Saegeman (UZ Leuven); Pierre Gavage (CHR Citadelle, Liege); Pierrette Melin (CHU Sart-Tilman, Liege); Anne-Marie Verbruggen (AZ Nikolaas); Youri Glupczynski (CHU Dinant-Godinne, Yvoir).

Compliance with ethical standards

Funding The national reference center is partially supported by the Belgian Ministry of Social Affairs through a fund within the health insurance system.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

Informed consent Not applicable.

References

1. Nordmann P, Poirel L (2014) The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect 20(9):821–830. doi:10.1111/1469-0691.12719
2. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL (2012) Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 25(4):682–707. doi:10.1128/CMR.05035-11
3. Huang TD, Bogaerts P, Berhin C, Jans B, Deplano A, Denis O, Glupczynski Y (2011) Rapid emergence of carbapenemase-producing Enterobacteriaceae isolates in Belgium. Euro Surveill 16(26):pii=19900. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19900
4. Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL; European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working group (2015) Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill 20(45). doi:10.2807/1560-7917.ES.2015.20.45.30062
5. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) (2013) Clinical breakpoints version 3.0. European Committee on Antimicrobial Susceptibility Testing (EUCAST). http://www.eucast.org. Accessed 1 Feb 2013
6. Clinical and Laboratory Standards Institute (CLSI) (2013) Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement. CLSI document M100-S23. CLSI, Wayne
7. Nordmann P, Poirel L, Dortet L (2012) Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 18(9):1503–1507. doi:10.3201/eid1809.120355
8. Bogaerts P, Rezende de Castro R, de Mendonça R, Huang TD, Denis O, Glupczynski Y (2013) Validation of carbapenemase and extended-spectrum beta-lactamase multiplex endpoint PCR assays according to ISO 15189. J Antimicrob Chemother 68(7):1576–1582. doi:10.1093/jac/dkt065
9. Bogaerts P, Naas T, Evrard S, Bouchahrouf W, Saegeman V, Lasserre C, Tande D, De Bolle X, Huang TD, Glupczynski Y (2015) OXA-427, a new plasmidic ESBL class D OXA-carbapenemase recovered from Enterobacteriaceae clinical isolates. In: Abstracts of the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen, Denmark, April 2015. Abstract no. P1307
10. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) (2014) Clinical breakpoints version 4.0. European Committee on Antimicrobial Susceptibility Testing (EUCAST). http://www.eucast.org. Accessed 1 Mar 2016
11. Fuchs PC, Barry AL, Thornsberry C, Jones RN (1985) Interpretive criteria for temocillin disk diffusion susceptibility testing. Eur J Clin Microbiol 4(1):30–33
12. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16(2):161–168. doi:10.1016/S1473-3099(15)00424-7
13. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O (2012) Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50(4):1355–1361. doi:10.1128/JCM.06094-11
14. Glasner C, Albiger B, Buist G, Tambić Andrasević A, Canton R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Donker T, Monnet DL, Grundmann H; European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group (2013) Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. Euro Surveill 18(28):pii=20525
15. Huang TD, Berhin C, Bogaerts P, Glupczynski Y; multicentre study group (2013) Prevalence and mechanisms of resistance to carbapenems in Enterobacteriaceae isolates from 24 hospitals in Belgium. J Antimicrob Chemother 68(8):1832–1837. doi:10.1093/jac/dkt096
16. Scientific Institute of Public Health (2015) Surveillance of antimicrobial resistance in Belgian hospitals. 2014 annual report. Available online at: http://www.nsih.be/download/MRSA/MRSA_ESBL_CPE_Y2014/RAPPORT_AMR_Y2014_FR.pdf. Accessed 1 Jan 2016
17. Kaase M, Schimanski S, Schiller R, Beyreiß B, Thürmer A, Steinmann J, Kempf VA, Hess C, Sobottka I, Fenner I, Ziesing S, Burckhardt I, von Müller L, Hamprecht A, Tammer I, Wantia N, Becker K, Holzmann T, Furitsch M, Volmer G, Gatermann SG (2016) Multicentre investigation of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in German hospitals. Int J Med Microbiol. doi:10.1016/j.ijmm.2016.05.009
18. Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S; Network EuSCAPE-Italy, Grundmann H, Pantosti A, Rossolini GM (2014) Colistin resistance superimposed to endemic carbapenem-resistant Klebsiella pneumoniae: a rapidly evolving problem in Italy, November 2013 to April 2014. Euro Surveill 19(42):pii=20939
19. Lixandru BE, Cotar AI, Straut M, Usein CR, Cristea D, Ciontea S, Tatu-Chitoiu D, Codita I, Rafila A, Nica M, Buzea M, Baicus A, Ghita MC, Nistor I, Tuchiluş C, Indreas M, Antohe F, Glasner C, Grundmann H, Jasir A, Damian M (2015) Carbapenemase-producing Klebsiella pneumoniae in Romania: a six-month survey. PLoS One 10(11):e0143214. doi:10.1371/journal.pone.0143214
20. Hrabák J, Študentová V, Jakubů V, Adámková V, Dvořáková L, Balejova M, Bergerová T, Chmelařová E, Ježek P, Kabelíková P, Kolář M, Paterová P, Tejkalová R, Papagiannitsis C, Žemličková H (2015) Prevalence study on carbapenemase-producing Escherichia coli and Klebsiella pneumoniae isolates in Czech hospitals—results from Czech Part of European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE). Epidemiol Mikrobiol Imunol 64(2):87–91
21. Pulcrano G, Iula DV, de Luca C, Roscetto E, Vollaro A, Rossano F, Catania MR (2014) Clonal dissemination of Klebsiella pneumoniae ST512 carrying blaKPC-3 in a hospital in southern Italy. APMIS 122(1):42–46. doi:10.1111/apm.12097
22. Del Franco M, Paone L, Novati R, Giacomazzi CG, Bagattini M, Galotto C, Montanera PG, Triassi M, Zarrilli R (2015) Molecular epidemiology of carbapenem resistant Enterobacteriaceae in Valle d'Aosta region, Italy, shows the emergence of KPC-2 producing Klebsiella pneumoniae clonal complex 101 (ST101 and ST1789). BMC Microbiol 15(1):260. doi:10.1186/s12866-015-0597-z
23. Bauraing C, Bogaerts P, Deplano A, Bouchahrouf W, Huang TD, Jans B, Denis O, Glupczynski Y (2014) Molecular characterisation of KPC-producing Klebsiella pneumoniae from Belgium. In:

Abstracts of the 24th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Barcelona, Spain, May 2014. Abstract no. P1051

24. Potron A, Poirel L, Nordmann P (2014) Derepressed transfer properties leading to the efficient spread of the plasmid encoding carbapenemase OXA-48. Antimicrob Agents Chemother 58(1): 467–471. doi:10.1128/AAC.01344-13
25. Oteo J, Ortega A, Bartolomé R, Bou G, Conejo C, Fernández-Martínez M, González-López JJ, Martínez-García L, Martínez-Martínez L, Merino M, Miró E, Mora M, Navarro F, Oliver A, Pascual Á, Rodríguez-Baño J, Ruiz-Carrascoso G, Ruiz-Garbajosa P, Zamorano L, Bautista V, Pérez-Vázquez M, Campos J; GEIH-GEMARA (SEIMC) and REIPI (2015) Prospective multicenter study of carbapenemase-producing Enterobacteriaceae from 83 hospitals in Spain reveals high in vitro susceptibility to colistin and meropenem. Antimicrob Agents Chemother 59(6): 3406–3412. doi:10.1128/AAC.00086-15
26. Liapis E, Pantel A, Robert J, Nicolas-Chanoine MH, Cavalié L, van der Mee-Marquet N, de Champs C, Aissa N, Eloy C, Blanc V, Guyeux C, Hocquet D, Lavigne JP, Bertrand X; ONERBA (2014) Molecular epidemiology of OXA-48-producing Klebsiella pneumoniae in France. Clin Microbiol Infect 20(12):O1121–O1123. doi:10.1111/1469-0691.12727
27. Voulgari E, Zarkotou O, Ranellou K, Karageorgopoulos DE, Vrioni G, Mamali V, Themeli-Digalaki K, Tsakris A (2013) Outbreak of OXA-48 carbapenemase-producing Klebsiella pneumoniae in Greece involving an ST11 clone. J Antimicrob Chemother 68(1): 84–88. doi:10.1093/jac/dks356
28. Huang TD, Poirel L, Bogaerts P, Berhin C, Nordmann P, Glupczynski Y (2014) Temocillin and piperacillin/tazobactam resistance by disc diffusion as antimicrobial surrogate markers for the detection of carbapenemase-producing Enterobacteriaceae in geographical areas with a high prevalence of OXA-48 producers. J Antimicrob Chemother 69(2): 445–450. doi:10.1093/jac/dkt367
29. Schechner V, Kotlovsky T, Tarabeia J, Kazma M, Schwartz D, Navon-Venezia S, Carmeli Y (2011) Predictors of rectal carriage of carbapenem-resistant Enterobacteriaceae (CRE) among patients with known CRE carriage at their next hospital encounter. Infect Control Hosp Epidemiol 32(5):497–503. doi:10.1086/659762
30. Jans B, Huang TD, Bauraing C, Berhin C, Bogaerts P, Deplano A, Denis O, Catry B, Glupczynski Y (2015) Infection due to travel-related carbapenemase-producing Enterobacteriaceae, a largely underestimated phenomenon in Belgium. Acta Clin Belg 70(3): 181–187. doi:10.1179/2295333715Y.0000000001
31. Huang TD, Bogaerts P, Berhin C, Jans B, Denis O, Glupczynski Y (2015) National survey data of carbapenemase-producing Enterobacteriaceae in Belgium. In: Abstracts of the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen, Denmark, April 2015. Abstract no. O374