

Prevalence and mechanisms of resistance to carbapenems in Enterobacteriaceae isolates from 24 hospitals in Belgium

Te-Din Huang*, Catherine Berhin, Pierre Bogaerts and Youri Glupczynski on behalf of a multicentre study group†

National Reference Laboratory for Monitoring of Antimicrobial Resistance in Gram-negative Bacteria, CHU Mont-Godinne, Université Catholique de Louvain (UCL), 1 Avenue Docteur Gaston Therasse, 5530 Yvoir, Belgium

*Corresponding author. Tel: +32-81-423212; Fax: +32-81-423204; E-mail: te-din.huang@uclouvain.be

†Members of the multicentre study group are listed in the Acknowledgements section.

Received 25 November 2012; returned 1 January 2013; revised 11 February 2013; accepted 24 February 2013

Objectives: To determine the point prevalence of carbapenem-non-susceptible Enterobacteriaceae (CNSE) and carbapenemase-producing Enterobacteriaceae (CPE) isolates among hospitalized patients in Belgium.

Methods: Twenty-four hospital-based laboratories prospectively collected 200 non-duplicated Enterobacteriaceae isolates from clinical specimens of hospitalized patients over a 2 month period. All isolates were screened locally for decreased susceptibility to carbapenem drugs using a disc diffusion method according to CLSI interpretative criteria. CNSE strains were referred centrally for confirmation of carbapenemase by phenotypic and molecular testing.

Results: From February to April 2012, 158 of the 4564 screened Enterobacteriaceae isolates were categorized as non-susceptible to carbapenems, resulting in a point prevalence of CNSE of 3.5% (95% CI: 2.9%–4.2%; range per centre: 0.5%–8.5%). Of the 125 referred CNSE isolates, 11 *Klebsiella pneumoniae* isolates [OXA-48 ($n=7$), KPC type ($n=3$) and NDM type ($n=1$)], 1 OXA-48-positive *Escherichia coli* isolate and 1 KPC-positive *Klebsiella oxytoca* isolate were detected in eight hospitals. None of the 72 carbapenem-non-susceptible *Enterobacter* spp. isolates were confirmed as CPE. The minimal estimated point prevalence of CPE isolates was 0.28% (13/4564; 95% CI: 0.13%–0.44%) overall (range per centre: 0%–1.5%).

Conclusions: Despite the overall low prevalence of CNSE found in this study, the detection of CPE isolates in one-third of the participating centres raises concerns and highly suggests the spread and establishment of CPE in Belgian hospitals.

Keywords: epidemiology, carbapenem resistance, carbapenemases

Introduction

Acquired carbapenemases in Enterobacteriaceae have been reported extensively worldwide.¹ Asymptomatic carriage and infection caused by carbapenemase-producing Enterobacteriaceae (CPE) isolates currently raise major public health concerns for individual therapeutic management and collective infection-control issues.² The prevalence of carbapenem resistance and the types of carbapenemases found in Europe vary between countries.^{3,4} In Belgium, a rapid increase in the number of CPE has been observed since 2010.⁵

The present cross-sectional survey aimed to estimate the prevalence of carbapenem-non-susceptible Enterobacteriaceae (CNSE) isolates and CPE isolates collected in Belgian hospitals.

Methods

Study design, inclusion criteria and testing at participating centres

Twenty-four hospital-based laboratories (Table S1, available as Supplementary data at JAC Online), representing ~25% of all acute hospitals and equally distributed in the different geographical regions of Belgium, were requested to collect consecutively 200 Enterobacteriaceae isolates over a period of 2 months from hospitalized patients. Only the first isolate of the same species per patient was included. Sample collection, culture and bacterial identification were performed according to local guidelines. None of the participating hospitals had been confronted with a CPE outbreak at the time of the study.

All isolates were tested locally for susceptibility to meropenem (10 µg), imipenem (10 µg) and ertapenem (10 µg) by a disc diffusion

method according to CLSI guidelines.⁶ All testing materials purchased from the same manufacturer (Oxoid, Basingstoke, UK) had the same manufacture batch number, in order to avoid interlot variation. *Escherichia coli* ATCC 25922 was tested as a quality-control strain in each centre during the survey.

Enterobacteriaceae isolates showing a decreased susceptibility to any of the three carbapenems were defined as CNSE and had to be referred to the reference laboratory. For *Proteus*, *Morganella* and *Providencia* spp. intrinsically less susceptible to imipenem, only meropenem and/or ertapenem zone sizes were considered.

Characterization of resistance mechanisms and data analysis

All putative CNSE isolates sent to the reference laboratory were tested for carbapenemase production by the imipenem hydrolysis-based Carba NP test⁷ and underwent multiplex PCR targeting *bla_{VIM}*, *bla_{IMP}*, *bla_{NDM}*, *bla_{KPC}* and *bla_{OXA-48}* for the detection of carbapenemase-encoding genes.⁸ For confirmed CPE isolates, the MIC values of 14 antimicrobial agents, including carbapenems, were determined by a broth microdilution method (Sensititre[®], Trek Diagnostic Systems, Cleveland, OH, USA). The MIC value of temocillin was determined by an Etest method (bioMérieux, Marcy l'Étoile, France). Susceptibility categorization was interpreted according to CLSI interpretative criteria⁶ for all antimicrobial agents except tigecycline (2012 EUCAST clinical breakpoints v2.0)⁹ and temocillin (breakpoints according to Fuchs *et al.*¹⁰). In carbapenemase-negative CNSE isolates, extended-spectrum β -lactamase (ESBL) production was sought by a combined double-disc test with cephalosporin indicators according to CLSI guidelines⁶ and AmpC cephalosporinase by a boronic acid-based disc potentiation test.¹¹

Susceptibility rates for the three carbapenems tested and the prevalence of CNSE isolates and CPE isolates were calculated globally, for each centre and also by species or genus.

Results and discussion

In total, 4564 isolates were screened by the 24 participating laboratories from February to April 2012. A mean number of 190 isolates per laboratory were tested and only one centre screened <150 isolates. Roughly one-third each of the 158 CNSE isolates were isolated from urine ($n=53$) and from lower respiratory tract samples ($n=49$). Medical care and intensive care units (ICUs) were the two wards with the highest number of CNSE isolates (59 and 51, respectively). The highest proportion of CNSE isolates was found in ICUs (6.9%).

The number of tested and referred isolates per species and the proportion of carbapenem non-susceptibility are reported in Table 1. The global point prevalence of CNSE isolates was 3.5% (158/4564; 95% CI: 2.9%–4.2%) and it ranged per centre from 0.5% to 8.5%. The rate of imipenem non-susceptibility found in *Klebsiella pneumoniae* (3.5%; 16/451) is much higher than the rates reported by European Antimicrobial Resistance Surveillance Network (EARS-Net) data in 2011 for Belgium (0.4%) and its neighbouring countries (0.1% in France, 0.2% in Germany and 0.3% in the Netherlands).¹² However, EARS-Net is a passive surveillance programme for which there is great variability between countries regarding data contribution and methodology used. Therefore, we believe that active national or regional epidemiological studies using standardized protocols are warranted to measure more accurately the burden of carbapenem resistance.

The strength of our study was the particular attention to the use of identical materials and testing methods, to minimize variation in the results due to technical interlaboratory variability.

Overall, *Enterobacter* spp. together accounted for more than half of the referred CNSE isolates (72/125), but none of these isolates could be confirmed as CPE. These results confirm the poor positive predictive value of ertapenem (15% in our study) in a low-prevalence setting for the detection of CPE isolates mostly in *Enterobacter* spp. in which carbapenem resistance usually reflects the combination of hyperproduced AmpC with impermeability caused by porin loss.¹³ On the other hand, two-thirds of the carbapenem-non-susceptible *K. pneumoniae* isolates (11/17; 65%) were eventually confirmed as CPE [OXA-48 ($n=7$), KPC type ($n=3$) and NDM type ($n=1$)]. These results clearly confirm the trends observed in the national surveillance programme showing that *K. pneumoniae* was by far the most encountered CPE species and OXA-48 the most frequent carbapenemase in Belgium.^{4,5} All PCR-negative CNSE isolates did not hydrolyse imipenem by the Carba NP assay, thus excluding the expression of carbapenemases not targeted (or not detected) by our multiplex PCR assay. Resistance to β -lactams, including carbapenems, among these non-CPE strains presumably resulted from AmpC hyperproduction ($n=85$, including 8 ESBL positive), ESBL production ($n=17$) and other β -lactamases ($n=10$) in association with impaired permeability due to porin deficiency.

All but 1 of the 13 confirmed CPE isolates showed intermediate resistance or resistance to all three carbapenems discs tested (Table S2, available as Supplementary data at JAC Online). One OXA-48 *K. pneumoniae* isolate had an inhibition zone to meropenem of 23 mm and was therefore categorized as susceptible according to the CLSI breakpoints. Three other OXA-48 *K. pneumoniae* isolates had a 22 mm zone to meropenem (intermediately resistant by CLSI criteria), but these would have been categorized as susceptible according to the EUCAST breakpoints.⁹

On the whole, the minimal estimated point prevalence of CPE was 0.28% (13/4564; 95% CI: 0.13%–0.44%) and ranged per centre from 0% (for 16 centres) to 1.5%. It is, however, of concern that one-third (8/24) of the participating laboratories had isolated at least one CPE isolate in their institution during the study period and that in only two cases (one KPC positive and one NDM positive) had the patient travelled abroad, suggesting the probable acquisition and importation of CPE from foreign countries. In a prospective multicentre study in 35 Spanish hospitals in 2009, Miró *et al.*¹⁴ reported a prevalence rate of CPE of 0.04%, but only the presence of metallo- β -lactamases was sought in this survey. Another survey in 25 Italian laboratories in 2011 found a point prevalence of CNSE among inpatients (3.5%) similar to our results. However, most CNSE (87%) were *K. pneumoniae* (largely confirmed as KPC producers), in contrast to the predominance of *Enterobacter* spp. among CNSE in our survey.¹⁵

The MIC results of the 13 confirmed CPE isolates are detailed in Table 2. On the basis of CLSI interpretative criteria, all CPE isolates were found intermediately or fully resistant to ertapenem and piperacillin/tazobactam, while five OXA-48-positive *K. pneumoniae* isolates had imipenem and/or meropenem MICs

Table 1. Species/genus distribution and proportion of carbapenem non-susceptibility in Enterobacteriaceae isolates screened in Belgian hospitals

Species or genus	Number of isolates screened	Meropenem I/R ^a		Imipenem I/R ^a		Ertapenem I/R ^a		CNSE in total		CNSE referred to the NRC		CPE	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
<i>E. coli</i>	2580	7	0.3	13	0.5	9	0.3	18	0.7	13	0.5	1	0.04
<i>K. pneumoniae</i>	451	16	3.5	16	3.5	17	3.8	21	4.7	17	3.8	11	2.44
<i>K. oxytoca</i>	211	1	0.5	2	0.9	1	0.5	2	0.9	2	0.9	1	0.47
<i>Enterobacter</i> spp. ^b	406	14	3.4	52	12.8	67	16.5	90	22.2	72	17.7		
<i>Citrobacter</i> spp. ^c	157	1	0.6	12	7.6			12	7.6	12	7.6		
<i>Serratia</i> spp. ^d	146	2	1.4	8	5.5	4	2.7	9	6.2	5	3.4		
<i>Proteus</i> spp. ^e	386	1	0.3	NA ^f				1	0.3				
<i>Morganella morganii</i>	151	3	2.0	NA ^f		1	0.7	3	2.0	2	1.3		
<i>Providencia</i> spp. ^g	25			NA ^f									
Others ^h	51	1	2.0	1	2.0			2	3.9	2	3.9		
Total	4564	46	1.0	104	2.6	99	2.2	158	3.5	125	2.7	13	0.28

NA, not applicable; NRC, National Reference Centre.

^aI/R, intermediate/resistant using 2012 CLSI interpretive criteria.⁶

^b*Enterobacter cloacae* (n=259), *Enterobacter aerogenes* (n=130) and other *Enterobacter* spp. (n=17).

^c*Citrobacter freundii* (n=75), *Citrobacter koseri* (n=68) and other *Citrobacter* spp. (n=14).

^d*Serratia marcescens* (n=133), *Serratia liquefaciens* (n=10) and other *Serratia* spp. (n=3).

^e*Proteus mirabilis* (n=348), *Proteus vulgaris* (n=36) and other *Proteus* spp. (n=2).

^fSusceptibility rates to imipenem were calculated only for Enterobacteriaceae species other than *Proteus* spp., *M. morganii* and *Providencia* spp.

^g*Providencia stuartii* (n=22) and *Providencia rettgeri* (n=3).

^h*Hafnia alvei* (n=27), *Salmonella* spp. (n=10), *Aeromonas hydrophila* (n=1) and unidentified *Klebsiella* species (n=13).

Table 2. Clinical data and broth microdilution MICs (mg/L) of 15 antimicrobials for confirmed CPE isolates

Centre no.	Origin	Ward	Patient travel history to foreign country	Bacterial species	Carbapenemase	TMC ^a	CTX	CAZ	FEP	TZP	ETP	IPM	MEM	ATM	GEN	AMK	TOB	CIP	TGC	CST
C03	urine	medicine	no	<i>K. pneumoniae</i>	OXA-48	>1024	4	≤0.5	2	>128	4	2	1	≤0.5	≤1	≤4	≤1	≤0.25	0.25	0.5
C10	urine	surgery	no	<i>K. pneumoniae</i>	OXA-48	512	>64	16	16	>128	2	0.5	0.5	32	≤1	≤4	8	>2	0.5	1
C10	urine	surgery	no	<i>K. pneumoniae</i>	OXA-48	1024	>64	16	16	128	2	0.5	≤0.25	32	≤1	≤4	8	>2	0.25	1
C12	respiratory	ICU	no	<i>K. oxytoca</i>	KPC	16	32	>64	32	>128	16	4	8	>64	2	16	>8	>2	1	0.5
C12	pus	medicine	Vietnam	<i>K. pneumoniae</i>	NDM	48	>64	>64	>64	>128	4	4	2	>64	>8	≤4	8	>2	0.5	>8
C12	respiratory	medicine	no	<i>K. pneumoniae</i>	KPC	48	>64	>64	32	>128	16	2	2	>64	>8	>32	>8	>2	1	0.5
C13	respiratory	ICU	no	<i>E. coli</i>	OXA-48	768	>64	2	4	128	4	4	8	8	>8	≤4	4	0.5	≤0.12	0.5
C15	urine	other	no	<i>K. pneumoniae</i>	OXA-48	1024	>64	16	32	>128	1	2	0.5	32	>8	≤4	>8	>2	0.5	2
C17	screening	ICU	no	<i>K. pneumoniae</i>	OXA-48	>1024	>64	>64	>64	>128	>32	4	16	>64	>8	≤4	>8	>2	2	1
C17	respiratory	ICU	no	<i>K. pneumoniae</i>	OXA-48	>1024	2	≤0.5	≤0.5	128	4	1	2	≤0.5	≤1	≤4	≤1	≤0.25	2	1
C17	screening	ICU	no	<i>K. pneumoniae</i>	OXA-48	>1024	>64	32	>64	>128	>32	8	16	64	>8	≤4	>8	>2	0.5	0.5
C19	urine	medicine	no	<i>K. pneumoniae</i>	KPC	64	>64	>64	>64	>128	>32	>32	>32	>64	8	16	>8	≤0.25	0.5	1
C23	pus	medicine	Israel	<i>K. pneumoniae</i>	KPC	1024	>64	>64	>64	>128	>32	32	>32	>64	4	32	>8	>2	0.5	0.5

TMC, temocillin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin/tazobactam; ETP, ertapenem; IPM, imipenem; MEM, meropenem; ATM, aztreonam; GEN, gentamicin; AMK, amikacin; TOB, tobramycin; CIP, ciprofloxacin; TGC, tigecycline; CST, colistin.

^aMIC determined by an Etest method for temocillin.

within the susceptible category. All CPE isolates, apart from one KPC-positive *Klebsiella oxytoca* strain, were resistant to temocillin. Remarkably, all OXA-48 producers exhibited high-level resistance to temocillin (MIC ≥ 512 mg/L), while KPC- and NDM-positive isolates showed more variable temocillin MIC levels (16–1024 mg/L). Only one NDM-positive *K. pneumoniae* was resistant to colistin and two OXA-48-positive *K. pneumoniae* showed intermediate resistance to tigecycline.

Our study has some limitations. First, ~20% of the CNSE isolates detected locally were not referred to the reference laboratory, suggesting that the CPE prevalence of 0.28% should be considered as a minimal estimate. Also, the carriage rate of CPE is probably underestimated because most isolates were collected from clinical specimens and not from CPE screening samples, which are still rarely taken in Belgian hospitals. Finally, only the subset of CNSE isolates were screened for CPE, thus not excluding the possible occurrence of poorly expressed carbapenemases among carbapenem-susceptible isolates. Previous studies have suggested that the susceptibility breakpoints of carbapenems should be modified for optimizing the detection of CPE.^{16,17} We believe indeed that additional evaluations are necessary to further improve the accuracy of laboratory detection strategies of CPE, including adjustment of the currently recommended screening cut-offs for carbapenems and/or the consideration of other antimicrobial markers, such as associated high-level resistance to temocillin and piperacillin/tazobactam.

In conclusion, the prevalence of CNSE is low in Belgian hospitals, although CPE strains were detected in one-third of the participating centres. Overall, carbapenemases were found mostly in *K. pneumoniae*, emphasizing that detection efforts should be concentrated on this species. Further studies are warranted to monitor trends over time and to evaluate the prevalence of CPE carriage in hospitals as well as in other medical-care sectors, such as nursing homes.

Acknowledgements

We acknowledge the participation of all members in the multicentre study group (see below).

Members of the multicentre study group

J. Caddrobi (CSE, Namur); I. Leroux and G. Claeys (UZ Gent); E. Oris and G. Coppens (ZOL Genk); A. Dediste and O. Vandenberg (CHU St-Pierre, Brussels); Y. Degheldre (CHIREC, Brussels); C. Nonhoff and O. Denis (Hôpital Erasme, Brussels); A. Smismans and J. Frans (Bonheiden); M.-G. Garrino (CHR Namur); J.-S. Goffinet (Arlon); T.-D. Huang and Y. Glupczynski (Yvoir); M. Ieven (UZ Antwerpen); B. Lissioir (Charleroi); K. Magerman (Hasselt); M. Dodemont and P. Melin (CHU Liege); Y. Miendje (CHU Brugmann, Brussels); E. Nulens (AZ St-Jan, Brugge); A. Schallier and D. Pierard (UZ Brussel); A. Pernet and C. Potvlieghe (La Louvière); H. Rodriguez-Villalobos and A. Simon (UCL St-Luc, Brussels); M. Carpentier and J.-M. Senterre (CHR Citadelle, Liege); K. Van Vaerenbergh and A. Boel (OLV Aalst); A.-M. Vandenabeele (AZ St-Lucas, Gent); V. Verbelen (Ottignies); and V. Saegeman and Jan Verhaegen (UZ Leuven).

Funding

This work was funded in part by a grant from the Belgium National Health Insurance Institute INAMI-RIZIV.

Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

References

- 1 Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2011; **17**: 1791–8.
- 2 Nordmann P, Cornaglia G. Carbapenemase-producing Enterobacteriaceae: a call for action! *Clin Microbiol Infect* 2012; **18**: 411–2.
- 3 European Centre for Disease Prevention and Control. *Antimicrobial Resistance Surveillance in Europe 2011. Interactive Database of the European Antimicrobial Resistance Surveillance Network (EARS-Net): Tables of Antimicrobial Resistance*. <http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/database/Pages/database.aspx> (1 November 2012, date last accessed).
- 4 Canton R, Akova M, Carmeli Y *et al.* Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012; **18**: 413–31.
- 5 Huang TD, Bogaerts P, Berhin C *et al.* Rapid emergence of carbapenemase-producing Enterobacteriaceae isolates in Belgium. *Euro Surveill* 2011; **16**: pii=19900.
- 6 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement M100-S22*. CLSI, Wayne, PA, USA, 2012.
- 7 Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2012; **18**: 1503–7.
- 8 Bogaerts P, Rezende de Castro R, de Mendonça R *et al.* Validation of carbapenemase and extended-spectrum β -lactamase multiplex endpoint PCR assays according to ISO 15189. *J Antimicrob Chemother* 2013; **68**: 1576–82.
- 9 European Society of Clinical Microbiology and Infectious Diseases/EUCAST. *Clinical Breakpoints*. <http://www.eucast.org/> (1 November 2012, date last accessed).
- 10 Fuchs PC, Barry AL, Thornsberry C *et al.* Interpretive criteria for temocillin disk diffusion susceptibility testing. *Eur J Clin Microbiol* 1985; **4**: 30–3.
- 11 Yagi T, Wachino J, Kurokawa H *et al.* Practical methods using boronic acid compounds for identification of class C β -lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli*. *J Clin Microbiol* 2005; **43**: 2551–8.
- 12 Grundmann H, Livermore DM, Giske CG *et al.* Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. *Euro Surveill* 2010; **15**: pii=19711.
- 13 Woodford N, Dallow JW, Hill RL *et al.* Ertapenem resistance among *Klebsiella* and *Enterobacter* submitted in the UK to a reference laboratory. *Int J Antimicrob Agents* 2007; **29**: 456–9.
- 14 Miró E, Agüero J, Larrosa MN *et al.* Prevalence and molecular epidemiology of acquired AmpC β -lactamases and carbapenemases in

Enterobacteriaceae isolates from 35 hospitals in Spain. *Eur J Clin Microbiol Infect Dis* 2012; **32**: 253–9.

- 15** Giani T, Pini B, Arena F *et al.* The increasing spread of KPC-producing *Klebsiella pneumoniae* in Europe: results from an Italian nationwide survey. In: *Abstracts of the Fifty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2012*. Abstract C2-1211. American Society for Microbiology, Washington, DC, USA.
- 16** Pasteran F, Mendez T, Guerriero L *et al.* Sensitive screening tests for suspected class A carbapenemase production in species of Enterobacteriaceae. *J Clin Microbiol* 2009; **47**: 1631–9.
- 17** Vading M, Samuelsen Ø, Haldorsen B *et al.* Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing *Klebsiella pneumoniae* with the EUCAST and CLSI breakpoint systems. *Clin Microbiol Infect* 2011; **17**: 668–74.