AAC Journals.ASM.org

Strategy for Rapid Detection of Carbapenemase-Producing *Enterobacteriaceae*

Laurent Dortet,[a] Ludivine Bréchard,[a] Gaëlle Cuzon,[a] Laurent Poirel,[a,b] Patrice Nordmann[a,b]

INSERM U914, South-Paris Medical School, Le Kremlin-Bicêtre, France[a]; Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland[b]

A prospective survey was conducted on 862 *Enterobacteriaceae* isolates with reduced susceptibility to carbapenems. The Carba NP test, UV spectrophotometry, and a DNA microarray were used to detect carbapenemase producers, and the results were compared to those from PCR and sequencing. The 172 carbapenemase producers were detected using the Carba NP test and UV spectrophotometry, whereas the DNA microarray failed to detect IMI producers. The use of the Carba NP test as a first screening, followed by the use of molecular techniques, has been determined to be an efficient strategy for identifying carbapenemase-producing *Enterobacteriaceae*.

Carbapenemases have led to the ultimate evolution of resistance in *Enterobacteriaceae*, leaving virtually very few efficient antibiotics left for treating related infections (1, 2). The most clinically significant carbapenemases in *Enterobacteriaceae* are (i) Ambler class A enzymes, including KPC, IMI, and SME enzymes (1, 3, 4), (ii) metallo-β-lactamases (MBL) from the VIM, IMP, and NDM types (5, 6), and (iii) OXA-48-like enzymes (7). The detection of carbapenemase producers includes screening patients who are at risk for being carriers of carbapenemase producers, including patients who have been hospitalized abroad, as well as implementation of efficient isolation procedures for carriers; these are the main features for limiting the spread of this emerging resistance trait (8–10).

The biochemical Carba NP test, based on the detection of carbapenem hydrolysis, was recently developed (11). Molecular methods, such as simplex and multiplex PCRs, DNA hybridization, and sequencing are also used to identify carbapenemase genes.

The aim of this study was to evaluate prospectively an efficient and cost-effective strategy to detect and characterize carbapenemase-producing *Enterobacteriaceae*.

From June 2011 to July 2012, 862 nonduplicate clinical *Enterobacteriaceae* isolates of worldwide origins were tested in order to characterize the mechanisms leading to reduced susceptibility to carbapenems (Fig. 1). The isolates were identified using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Vitek MS, bioMérieux, La Balme-les-Grottes, France). Susceptibility testing was performed by determining MIC values using the Etest (bioMérieux) on Mueller-Hinton agar plates at 37°C, and the results were recorded according to U.S. guidelines (from the CLSI), as updated in 2013 (12). All tested isolates were nonsusceptible to at least one of the three carbapenem molecules, imipenem, meropenem, or ertapenem.

The detection of the bla_{KPC}, bla_{IMI}, bla_{SME}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{GIM}, and bla_{OXA-48} carbapenemase genes was performed by simplex PCR, followed by sequencing (13). The results of PCR and sequencing were used as standards to evaluate the other detection techniques. Molecular detection of the β-lactamase genes was also performed for all carbapenemase producers ($n = 173$) using a DNA hybridization array approach (Check-MDR CT103 array; Check-Points, Wageningen, The Netherlands) according to the manufacturer's instructions (14).

The detection of carbapenemase production was performed by UV spectrophotometry, as previously described (15). It was also performed by using the Carba NP test (11). An improved version (faster and easier) of this test was performed with isolates grown on Mueller-Hinton agar plates (Becton, Dickinson, Le Pont-de-Chaix, France) at 37°C for 18 to 22 h, as previously described (see supplemental material) (1, 16).

Statistical analyses were performed using a χ^2 test. P values of <0.05 were considered to be statistically significant.

Among the 862 enterobacterial isolates tested, the PCR-based techniques with subsequent sequencing identified 172 carbapenemase producers. Compared to the PCR-based detection method, the UV spectrophotometric method and the Carba NP test were found to be 100% sensitive and 100% specific for detecting carbapenemase producers (Table 1). Since the bla_{IMI} gene was not included in the panel of carbapenemase genes detected by the Check-MDR CT103 array, it failed to identify the two *Enterobacter* strains producing the IMI-1 carbapenemase, leading to 98.8% sensitivity and 100% specificity (Table 1). The positive predictive values (PPV) were 100% for all three techniques, and the negative predictive values (NPV) were 100% for UV spectrophotometry and the Carba NP test and 99.7% for the Check-MDR CT103 array (Table 1).

The DNA microarray was the only technique that identified additional noncarbapenemase β-lactamases. Indeed, 70% of the carbapenemase-producing *Enterobacteriaceae* additionally expressed at least one broad-spectrum β-lactamase, such as a plas-

Received 24 June 2013 Returned for modification 14 November 2013
Accepted 19 January 2014
Published ahead of print 27 January 2014
Address correspondence to Patrice Nordmann, patrice.nordmann@unifr.ch.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.01239-13.

doi:10.1128/AAC.01239-13

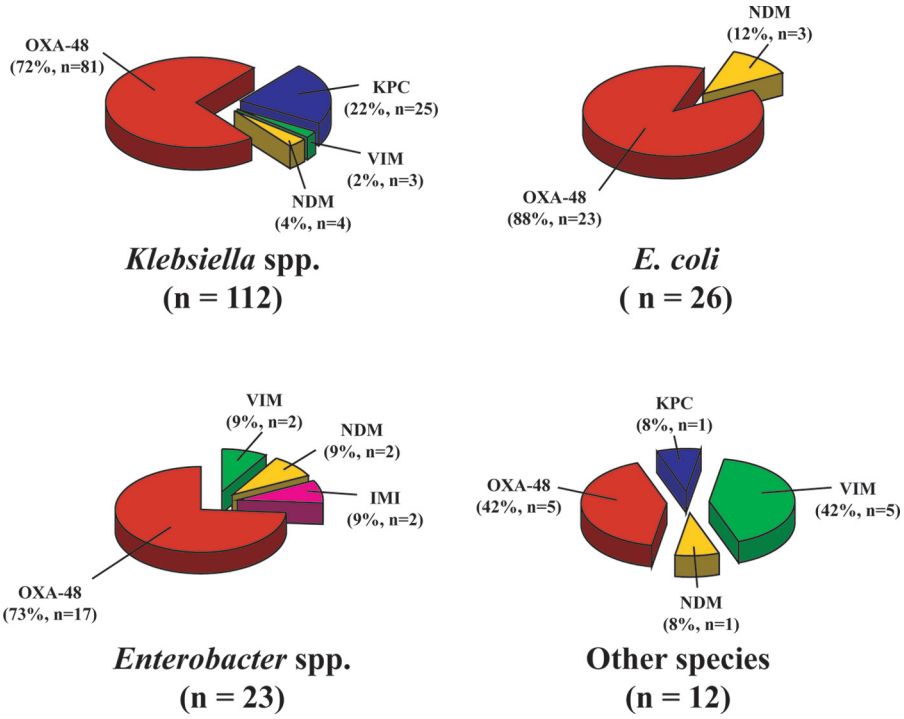

FIG 1 Distribution of the different carbapenemase types among carbapenemase-producing *Enterobacteriaceae*.

mid-mediated cephalosporinase and/or an extended-spectrum β-lactamase (Table 2).

Among the 172 carbapenemase producers, 65% were from *K. pneumoniae*, 15% were from *Escherichia coli*, 13% were *Enterobacter* spp., 5% were from *Citrobacter freundii*, 1% were from *Serratia marcescens*, and 1% were from *Salmonella enterica* (Table 2). The identified carbapenemases were of the OXA-48 (72%), KPC (15%), NDM (6%), VIM (6%), and IMI types (1%) (Table 2). The characterization of carbapenemase genes was done by sequencing, as listed in Table 2. Regardless of the enterobacterial species considered, OXA-48-like carbapenemases were predominant in our collection (Fig. 1). KPC producers were mostly identified in *K. pneumoniae* compared to the other enterobacterial species (96%, $P < 0.001$). Conversely, NDM producers were equally distributed ($P > 0.05$) among each type of enterobacterial species (Fig. 1).

TABLE 1 Main characteristics of Carba NP test, UV spectrophotometric method, and DNA microarray for the detection of carbapenemase-producing *Enterobacteriaceae*

Test parameters	Detection method characteristics			
	PCR + sequencing	Carba NP test	UV spectrophotometry	DNA microarray
Efficiency (%)[a]				
Sensitivity	100	100	100	98.8
Specificity	100	100	100	100
PPV	100	100	100	100
NPV	100	100	100	99.7
Other characteristics				
Rapidity (h)	24–48	<2	12–24	8–24
Cost[b]	$$	$	$	$$$
Expertise needs[c]	++	+	+++	++
Complete gene identification[d]	+	−	−	+/−

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] The number of $'s correlates with the effective (relative) price of the test.
[c] The number of +'s correlates with the expertise and training needed to perform and interpret the test.
[d] The + means that the technique is able to give a complete gene identification, the − means that the technique is not able to give a complete gene identification, and the +/− means that the technique is able to give a partial gene identification.

Overall, this study showed 100% specificity and sensitivity for the Carba NP test and UV spectrophotometry to detect the production of carbapenemases (Table 1) (11). Additionally, the PPV and NPV of both techniques were also 100%. The Carba NP test was as efficient as the UV spectrophotometric method to detect carbapenemase producers but with significant advantages, since the Carba NP test is more rapid (<2 h versus 24 h for UV spectrophotometry) and does not require any specific training to use. Its cost is <$1 per tested strain, whereas the UV spectrophotometric assay and PCR-based techniques require additional equipment (a UV spectrophotometer and sonicator for the UV spectrophotometric assay and consumables, reagents, and a thermocycler for the PCR assay). On the other hand, the Check-MDR CT103 array failed to detect two IMI-1 producers, leading to 100% specificity, 98.8% sensitivity, 100% PPV, and 99.7% NPV (Tables 1 and 2). Since the Check-MDR CT103 array is designed for clinical use, it may detect the most clinically relevant carbapenemase enzymes (KPC, VIM, IMP, and OXA-48-like carbapenemases). In addition, it cannot discriminate between the different variants of a given carbapenemase. Additionally, this technique requires several successive steps (DNA extraction, ligation, PCR amplification, hybridization, and detection) requiring 8 to 24 h total. It also requires additional equipment (DNA extraction kit, thermocycler, thermomixer, and Check-Points tube reader, including the software) that costs ~$16,000 (14). Additionally, the use of this array on a daily routine basis may be limited by its cost (~$100) compared to UV spectrophotometry ($2 to 3), the Carba NP test

TABLE 2 Molecular characterization of carbapenemase-producing *Enterobacteriaceae* using sequencing and DNA microarray

Carbapenemase type(s)	Carbapenemase variant(s)[a]	Species	*n*	DNA microarray type results			
				Acquired penicillinase(s)	ESBL(s)	Acquired cephalosporinase	Carbapenemase(s)
KPC	KPC-2	*K. pneumoniae*	7	TEM	None	None	KPC
			2	TEM	CTX-M-1	None	KPC
			1	TEM	CTX-M-9	None	KPC
			10	TEM	SHV	None	KPC
		C. freundii	1	TEM	None	None	KPC
	KPC-3	*K. pneumoniae*	4	TEM	None	None	KPC
			1	TEM	CTX-M-1	None	KPC
VIM	VIM-1	*K. pneumoniae*	2	TEM	SHV	None	VIM
		Enterobacter cloacae	1	TEM	None	None	VIM
			1	TEM	SHV	None	VIM
		C. freundii	1	TEM	None	None	VIM
	VIM-2	*C. freundii*	4	TEM	TEM	None	VIM
	VIM-4	*K. pneumoniae*	1	TEM	None	None	VIM
NDM	NDM-1	*E. coli*	1	None	CTX-M-1	None	NDM
			1	TEM	CTX-M-1	None	NDM
			1	TEM + SHV	CTX-M-1	None	NDM
		K. pneumoniae	1	None	None	CMY-2-like	NDM
			1	None	CTX-M-1	None	NDM
			1	TEM	CTX-M-1	None	NDM
		E. cloacae	1	TEM	CTX-M-1	None	NDM
			1	TEM	CTX-M-1 + SHV	None	NDM
		Salmonella spp.	1	TEM	None	DHA	NDM
IMI	IMI-1	*E. cloacae*	1	TEM	None	None	None
		Enterobacter asburiae	1	None	None	None	None
OXA-48-like	OXA-48	*E. coli*	4	None	None	None	OXA-48
			7	TEM	None	None	OXA-48
			1	TEM	None	CMY-2-like	OXA-48
			1	None	CTX-M-1	None	OXA-48
			7	TEM	CTX-M-1	None	OXA-48
			1	TEM + SHV	CTX-M-1	None	OXA-48
			1	TEM	CTX-M-9	None	OXA-48
		K. pneumoniae	5	None	None	None	OXA-48
			1	TEM	None	None	OXA-48
			1	None	None	DHA	OXA-48
			5	None	CTX-M-1	None	OXA-48
			64	TEM	CTX-M-1	None	OXA-48
			1	TEM	CTX-M-1	CMY-2-like	OXA-48
			1	None	CTX-M-9	None	OXA-48
		E. cloacae	1	None	None	None	OXA-48
			1	None	CTX-M-1	None	OXA-48
			10	TEM	CTX-M-1	None	OXA-48
			4	TEM + SHV	CTX-M-1	None	OXA-48
		Enterobacter hormaechei	1	TEM	CTX-M-1	None	OXA-48
		C. freundii	1	TEM	SHV	None	OXA-48
		C. freundii	1	SHV	CTX-M-1	None	OXA-48
		S. marcescens	1	None	None	None	OXA-48
			1	TEM	CTX-M-1	None	OXA-48
	OXA-162	*C. freundii*	1	None	SHV	None	OXA-48
	OXA-181	*E. coli*	1	None	CTX-M-1	None	OXA-48
		K. pneumoniae	2	TEM	CTX-M-1	None	OXA-48
NDM + OXA-48-like	NDM-1 + OXA-181	*K. pneumoniae*	1	TEM	CTX-M-1	None	NDM + OXA-48

[a] Carbapenemase variants were obtained after sequencing.

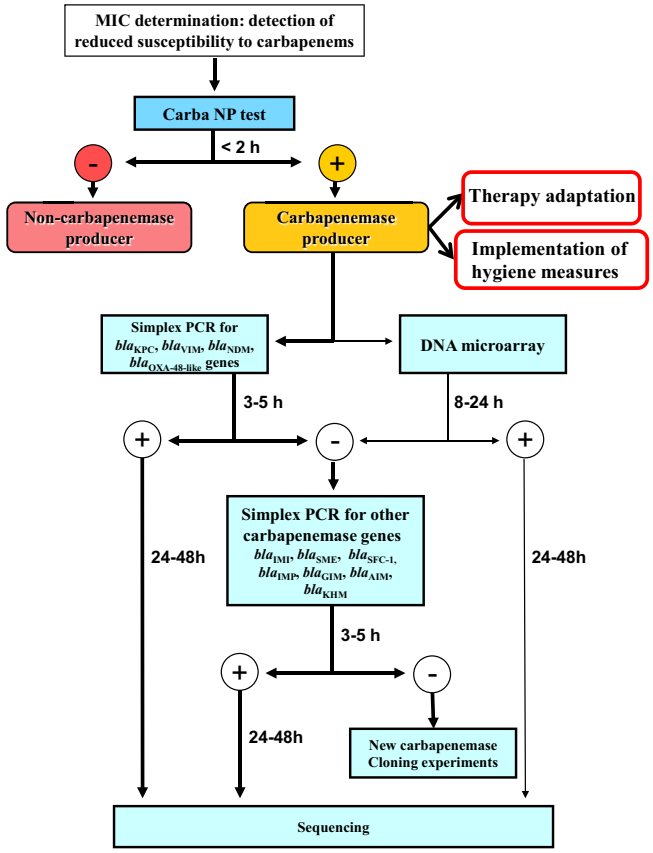

FIG 2 Flowchart for detection and characterization of carbapenemase producers among *Enterobacteriaceae*. The Carba NP test is used for rapid differentiation between carbapenemase and noncarbapenemase producers. The second step includes molecular techniques (PCRs or DNA microarray) for precise identification of carbapenemase genes. The bold arrows indicate the preferred way to identify carbapenemase genes. This second step may be followed only in university hospitals or large microbiology laboratories.

($1), and PCR-based testing ($30). However, the microarray technique may help to characterize the entire β-lactamase content of a single isolate by also detecting other broad-spectrum β-lactamase genes.

The diversity of the carbapenemases identified here mirrors the worldwide dissemination of the four main described enzymes (KPC, VIM, NDM, and OXA-48) (1). Additionally, our results further highlight the wide dissemination of the OXA-48 carbapenemase in Europe (particularly in France) accounting for 72% of the whole carbapenemases (Fig. 1) (7, 17), whereas KPC is the most widespread carbapenemase in the United States. Of note, the KPC carbapenemases are almost entirely restricted to the *K. pneumoniae* species (25/26). Conversely, OXA-48 and NDM were distributed among all enterobacterial species.

Since the management of patients requires the rapid identification of carbapenemase producers (regardless of its type) (18), a diagnostic strategy for the detection of carbapenemase producers in *Enterobacteriaceae* is proposed here (Fig. 2). This strategy is based on (i) the Carba NP test as the primary screening test for the detection of carbapenemase production, followed by (ii) a specific molecular characterization of the carbapenemase genes by simplex PCRs or the DNA microarray. The initial step (susceptibility testing and Carba NP test) may be developed in any laboratory worldwide. Molecular identification of the carbapenemase genes may be also performed locally, depending on the molecular techniques available; however, it is not required for antibiotic stewardship or infection control purposes.

In the case of a negative result obtained with the Carba NP test, the mechanism responsible for carbapenem-decreased susceptibility is not related to the production of a carbapenemase (e.g., reduced permeability of the outer membrane associated with overexpression of chromosomal or acquired AmpC and/or extended-spectrum β-lactamases [ESBL]); therefore, no additional test is required (17). In the case of a positive result with the Carba NP test, the use of a set of five simplex PCRs (bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMI}, and $bla_{OXA\text{-}48\text{-}like}$) may then identify all carbapenemase genes of our collection (Fig. 1 and Table 2). However, this screening may be adapted to local epidemiology, as was recently proposed for the detection of carbapenemase SME in the United States (4). The DNA microarray may be more useful for epidemiological purposes or for infection control studies, when high numbers of isolates have to be rapidly characterized (14). Additionally, this procedure may also detect potential new carbapenemases. Indeed, although molecular techniques are currently considered to be the gold standard for the detection of carbapenemase producers, they are only able to detect known carbapenemase genes. With the proposed strategy, a positive Carba NP test, followed by negative results using molecular techniques, may correspond to a novel carbapenemase that may be further characterized using cloning experiments (Fig. 2).

This is the first prospective study to evaluate at an international level the values of the different techniques for detecting carbapenemases. The strategy proposed for the detection of carbapenemase producers presents several advantages for treating infected patients and for the isolation of carriers. Indeed, it will lead to the rapid identification of carbapenemase producers (<2 h) using the Carba NP test, allowing for better antibiotic stewardship (18). This strategy may also have a significant impact on preventing the development of nosocomial outbreaks by acting rapidly on the management of carriers (through isolation and cohorting) as demonstrated for KPC outbreaks, at least in Israel (19). Finally, since the first step of this strategy, which includes susceptibility testing, and the Carba NP test are based on cheap techniques, it may be followed worldwide and therefore contribute to limiting the spread of what has been recently termed the new Red Plague (20).

ACKNOWLEDGMENTS

This work was partially funded by a grant from the INSERM (UMR914), by the Université of Fribourg, Switzerland, and by grants from the European Community (no. R-GNOSIS, FP7/HEALTH-F3-2011-282512, MAGIC-BULLET, and FP7/HEALTH-F3-2001-278232).

An international patent form for the Carba NP test has been filed on behalf of INSERM Transfert (Paris, France).

REFERENCES

1. **Nordmann P, Dortet L, Poirel L.** 2012. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! Trends Mol. Med. **18:**263–272. http://dx.doi.org/10.1016/j.molmed.2012.03.003.
2. **Schwaber MJ, Carmeli Y.** 2008. Carbapenem-resistant *Enterobacteriaceae*: a potential threat. JAMA **300:**2911–2913. http://dx.doi.org/10.1001/jama.2008.896.
3. **Nordmann P, Cuzon G, Naas T.** 2009. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect. Dis. **9:**228–236. http://dx.doi.org/10.1016/S1473-3099(09)70054-4.

4. **Bush K, Pannell M, Lock JL, Queenan AM, Jorgensen JH, Lee RM, Lewis JS, Jarrett D.** 2013. Detection systems for carbapenemase gene identification should include the SME serine carbapenemase. Int. J. Antimicrob. Agents **41:**1–4. http://dx.doi.org/10.1016/j.ijantimicag.2012.08.008.
5. **Nordmann P, Poirel L, Walsh TR, Livermore DM.** 2011. The emerging NDM carbapenemases. Trends Microbiol. **19:**588–595. http://dx.doi.org/10.1016/j.tim.2011.09.005.
6. **Walsh TR, Toleman MA, Poirel L, Nordmann P.** 2005. Metallo-β-lactamases: the quiet before the storm? Clin. Microbiol. Rev. **18:**306–325. http://dx.doi.org/10.1128/CMR.18.2.306-325.2005.
7. **Poirel L, Potron A, Nordmann P.** 2012. OXA-48-like carbapenemases: the phantom menace. J. Antimicrob. Chemother. **67:**1597–1606. http://dx.doi.org/10.1093/jac/dks121.
8. **Mitka M.** 2013. Indian public health leaders move to reduce antimicrobial resistance. JAMA **309:**531–532. http://dx.doi.org/10.1001/jama.2013.297.
9. **Infectious Diseases Society of America (IDSA), Spellberg B, Blaser M, Guidos RJ, Boucher HW, Bradley JS, Eisenstein BI, Gerding D, Lynfield R, Reller LB, Rex J, Schwartz D, Septimus E, Tenover FC, Gilbert DN.** 2011. Combating antimicrobial resistance: policy recommendations to save lives. Clin. Infect. Dis. **52**(Suppl 5)**:**S397–S428. http://dx.doi.org/10.1093/cid/cir153.
10. **Walsh TR, Toleman MA.** 2011. The emergence of pan-resistant Gram-negative pathogens merits a rapid global political response. J. Antimicrob. Chemother. **67:**1–3. http://dx.doi.org/10.1093/jac/dkr378.
11. **Nordmann P, Poirel L, Dortet L.** 2012. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg. Infect. Dis. **18:**1503–1507. http://dx.doi.org/10.3201/eid1809.120355.
12. **Clinical and Laboratory Standards Institute.** 2013. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. CLSI M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
13. **Poirel L, Dortet L, Bernabeu S, Nordmann P.** 2011. Genetic features of bla_{NDM-1}-positive *Enterobacteriaceae*. Antimicrob. Agents Chemother. **55:**5403–5407. http://dx.doi.org/10.1128/AAC.00585-11.
14. **Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P.** 2012. Evaluation of a DNA microarray for the rapid detection of extended-spectrum β-lactamases (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/MOX) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). J. Antimicrob. Chemother. **67:**1865–1869. http://dx.doi.org/10.1093/jac/dks156.
15. **Bernabeu S, Poirel L, Nordmann P.** 2012. Spectrophotometry-based detection of carbapenemase producers among *Enterobacteriaceae*. Diagn. Microbiol. Infect. Dis. **74:**88–90. http://dx.doi.org/10.1016/j.diagmicrobio.2012.05.021.
16. **Dortet L, Bréchard L, Poirel L, Nordmann P.** 2014. Comparison of diverse growing media for further detection of carbapenemase production using the Carba NP test. J. Med. Microbiol., in press.
17. **Dortet L, Cuzon G, Nordmann P.** 2013. Dissemination of carbapenemase-producing *Enterobacteriaceae* in France, 2012. J. Antimicrob. Chemother., in press. http://dx.doi.org/10.1093/jac/dkt433.
18. **Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, Polsky B, Adams-Haduch JM, Doi Y.** 2012. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. Antimicrob. Agents Chemother. **56:**2108-2113. http://dx.doi.org/10.1128/AAC.06268-11.
19. **Schwaber MJ, Lev B, Israeli A, Solter E, Smollan G, Rubinovitch B, Shalit I, Carmeli Y, Israel Carbapenem-Resistant *Enterobacteriaceae* Working Group.** 2011. Containment of a country-wide outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Israeli hospitals via a nationally implemented intervention. Clin. Infect. Dis. **52:**848–855. http://dx.doi.org/10.1093/cid/cir025.
20. **Looke DF, Gottlieb T, Jones CA, Paterson DL.** 2013. Gram-negative resistance: can we combat the coming of a new "Red Plague"? Med. J. Aust. **198:**243–244. http://dx.doi.org/10.5694/mja13.10190.