JCM
Journals.ASM.org

Evaluation of the New NucliSENS EasyQ KPC Test for Rapid Detection of *Klebsiella pneumoniae* Carbapenemase Genes (bla_{KPC})

Teresa Spanu,[a] Barbara Fiori,[a] Tiziana D'Inzeo,[a] Giulia Canu,[a] Serena Campoli,[a] Tommaso Giani,[c] Ivana Palucci,[a] Mario Tumbarello,[b] Maurizio Sanguinetti,[a] and Gian Maria Rossolini[c,d]

Institutes of Microbiology[a] and Infectious Diseases,[b] Agostino Gemelli Hospital, Catholic University of the Sacred Heart, Rome, Italy, and Section of Microbiology, Department of Biotechnologies, University of Siena,[c] and Clinical Microbiology and Virology Unit, Siena University Hospital,[d] Siena, Italy

KPC-type carbapenemases are emerging in *Klebsiella pneumoniae* and other Gram-negative pathogens worldwide. Rapid and sensitive detection of these resistance determinants has become relevant to clinical management and infection control. We evaluated the bioMérieux EasyQ real-time PCR assay for bla_{KPC} detection with 300 members of the *Enterobacteriaceae*, including 29 control strains producing known carbapenemases and 271 nonreplicate clinical isolates. The EasyQ assay correctly detected all of the 111 isolates harboring bla_{KPC} genes, with no false positives, and results were available within 2 h.

Klebsiella pneumoniae carbapenemases (KPCs) can confer resistance to virtually all β-lactam antimicrobials, not just carbapenems. For this reason, they are among the most challenging antibiotic resistance determinants that have emerged in the last decade (14, 17, 24). KPC-producing strains often exhibit multidrug resistance phenotypes and the ability to spread rapidly within hospital settings (13, 14, 25). These enzymes are most commonly produced by *K. pneumoniae*, although they have also been detected in other species of the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter* species (3, 7, 25, 27).

Rapid, reliable detection of KPC-producing members of the *Enterobacteriaceae* is essential both for clinical management of infected patients and for interventions aimed at limiting their spread (11, 19, 21). Phenotypic testing can putatively detect KPC-mediated resistance, but it requires at least an overnight incubation and cannot definitively confirm the nature of the carbapenemase gene (8, 10, 11, 22, 23, 26). PCR and sequencing are currently considered the gold standard to identify isolates harboring bla_{KPC} genes, but this approach is also time-consuming and requires considerable technical expertise. Many of these shortcomings can reportedly be overcome with new molecular methodologies. They offer considerably shorter turnaround times, and they may also prove to be accurate (2,4–6,15).

The EasyQ KPC test (bioMérieux, Marcy l'Etoile, France) is a novel real-time PCR assay that has recently been developed for bla_{KPC} detection. It is designed for use with the bioMérieux NucliSENS platform. Amplification, real-time detection, and automated interpretation of the test results are done on a NucliSens EasyQ analyzer. In this work we evaluated the performance of this new system in the identification of clinical isolates of bla_{KPC}-positive members of the *Enterobacteriaceae*.

Study design. The study was conducted with a total of 300 bacterial isolates: *K. pneumoniae* ($n = 157$) and *Escherichia coli* ($n = 143$). These included 29 strains (28 *K. pneumoniae* strains and 1 *E. coli* strain) producing known carbapenemases belonging to β-lactamase class A (KPC-2 and -3 types), class B (VIM-1 and NDM-1), and class D (OXA-48), isolated from various clinical sources (Table 1). The remaining 271 isolates (129 *K. pneumoniae* and 142 *E. coli* isolates) were selected from a consecutive series of nonreplicate, multidrug-resistant *K. pneumoniae* and *E. coli* isolates recovered from blood cultures between January 2009 and December 2011 in the clinical microbiology laboratory of the Catholic University of the Sacred Heart Medical Center and had not been characterized for resistance determinants at the time of the study. Identification and antibiotic susceptibility profiling of these 271 isolates had initially been performed with ID-GN and NO89 cards and the Vitek 2 system (bioMérieux). Isolates were subsequently reidentified with the Bruker Daltonics MALDI Biotyper system (Bruker Daltonik GmbH, Leipzig, Germany). Vitek 2 antibiogram results for oxyiminocephalosporins and carbapenems were confirmed by Etest (bioMérieux) determination of MICs, and results were interpreted according to EUCAST breakpoints (document version 2.0, 2 January 2012; http://www.eucast.org/clinical_breakpoints). Multidrug resistance was defined as nonsusceptibility to at least one agent in three or more of the following antimicrobial categories: oxyiminocephalosporins, carbapenems, aminoglycosides (amikacin and/or gentamicin), and quinolones (ciprofloxacin and levofloxacin) (12).

NucliSENS EasyQ KPC testing was performed according to the protocol suggested by the manufacturer. Briefly, colonies from plated cultures (on tryptic soy agar [TSA] agar plates; bioMérieux) were suspended in saline at a density of 0.5 McFarland standard. Suspensions were heated for 10 min at 95°C, and aliquots of 2.5 μl (each) were used for real-time PCR. Data were analyzed with the NucliSENS EasyQ Director software.

The comparison method for assessment of the NucliSENS EasyQ KPC test consisted of PCR amplification and sequencing, as described elsewhere, to identify genes for class A carbapenemases (KPC and GES enzymes), class B metallo-β-lactamases (VIM, IMP, and NDM enzymes), class D carbapenemases (OXA-23, -24/40, -48, -51, -55, -58, and -143), extended-spectrum β-lactamases (ESBLs) (TEM type, SHV type, CTX-M type, and OXA-2 and -10

Received 31 January 2012 Returned for modification 12 March 2012
Accepted 18 May 2012
Published ahead of print 23 May 2012
Address correspondence to Teresa Spanu, tspanu@rm.unicatt.it.
T.S. and B.F. contributed equally to this work.

doi:10.1128/JCM.00284-12

TABLE 1 Performance of EasyQ KPC for detection of bla_{KPC} genes in 111 KPC-producing and 189 non-KPC-producing *Klebsiella pneumoniae and Escherichia coli* isolates[a]

Microorganism (n[b])	No. of KPC-positive isolates identified by EasyQ as:		No. of KPC-negative isolates identified by EasyQ as:		% sensitivity	% specificity
	KPC positive	KPC negative	KPC positive	KPC negative		
Reference isolates	18	0	0	11	100	100
Escherichia. coli[c] (1)	0	0	0	1	ND[e]	ND
Klebsiella pneumoniae[d] (28)	18	0	0	10	100	100
Bloodstream isolates	93	0	0	178		
Escherichia coli[f] (142)	2	0	0	140	100	100
Klebsiella pneumoniae[g] (129)	91	0	0	38	100	100
Total (300)	111	0	0	189	100	100

[a] Isolates were classified as KPC positive when bla_{KPC} was identified by standard DNA sequence analysis.
[b] n, no. of isolates.
[c] *E. coli* with NDM-1 carbapenemase.
[d] Eighteen *K. pneumoniae* isolates were KPC producers: 14 (9 with KPC-2 and 5 with KPC-3) belonged to sequence type (ST) 258, 3 with KPC-3 belonged to ST 512, and one with KPC-2 belonged to ST 101, respectively. Ten KPC-negative isolates produced the VIM-1 ($n = 9$) or OXA-48 ($n = 1$) carbapenemase, respectively.
[e] ND, not determined. Sensitivity was not calculated when <5 isolates were found.
[f] *E. coli* isolates with KPC-3 ($n = 2$), CTX-M-1 ($n = 1$), CTX-M-1/SHV-12 ($n = 4$), CTX-M-3 ($n = 1$), CTX-M-10 ($n = 2$), CTX-M-15 ($n = 98$), CTX-M-15/SHV-12 ($n = 12$), SHV-2 ($n = 1$), and SHV-12 ($n = 21$).
[g] *K. pneumoniae* isolates with KPC-3 ($n = 91$), CTX-M-15 ($n = 12$), SHV-5 ($n = 4$), and SHV-12 ($n = 22$).

ESBLs), and plasmid-mediated AmpC β-lactamases (CMY-2 group, DHA group, ACC-1, MOX-1, FOX-1, MIR-1, and ACT-1 group) (1, 9, 16, 18, 20, 28). This analysis was carried out with all 271 bloodstream isolates.

To define the analytical sensitivity of the NucliSENS EasyQ assay, we tested (in triplicate) 3 isolates harboring bla_{KPC-2} or bla_{KPC-3} genes. The detection limit assays were carried out by preparing saline suspension of each isolate to a density equivalent to a 0.5 McFarland turbidity standard. Suspensions were diluted in a 10-fold dilution series, and aliquots of 2.5 μl of each 10-fold dilution were then processed by the real-time bla_{KPC} assay. The numbers of CFU per suspension (CFU/ml) were evaluated by standard plating procedures.

Reproducibility was assessed by testing in triplicate 12 reference strains, including 6 with KPC-type, 4 with VIM-1, 1 with OXA-48, and 1 with NDM-1 carbapenemase genes. The turnaround time for the PCR assay was recorded.

All isolates were stored in Cryobank vials (Mast Group Ltd., Bootle, Merseyside, United Kingdom) at −70°C.

Performance of NucliSENS EasyQ assay with strains carrying known carbapenemase genes. In preliminary testing, carried out with the 29 strains carrying known carbapenemase genes, the EasyQ KPC assay correctly detected the presence of the bla_{KPC} gene in all 18 strains producing KPC carbapenemases (Table 1). False-positive results were not observed. The detection limit of the real-time bla_{KPC} assay was 4 CFU per reaction (mean). The reproducibility was excellent, with no false negatives and no false positives in the replicate test with the 12 tested strains.

Performance of NucliSENS EasyQ assay with clinical isolates. The results of the EasyQ KPC assay were concordant with those of the molecular comparison method for all the 271 bloodstream isolates tested (Table 1). The mean turnaround time for the PCR assay was 1.49 h. The cost per test for PCR assay was EUR 22.50, and the mean technician time was 8 min.

The 93 bla_{KPC}–positive isolates (91 *K. pneumoniae* and 2 *E. coli*) expressed high-level ertapenem resistance (MICs of >32 μg/ml), while imipenem and meropenem MICs ranged from 2 to >32 μg/ml and from 4 to >32 μg/ml, respectively. The remaining 178 isolates (38 *K. pneumoniae* and 140 *E. coli*) were susceptible to carbapenems while showing various levels of resistance to the oxyiminocephalosporins, cefepime, cefotaxime, and ceftazidime (MIC range, 0.5 to >64 μg/ml). These isolates were found to carry genes encoding various types of ESBLs (including SHV-2, SHV-5, SHV-12, and CTX-M-1, CTX-M-3, CTX-M-10, and CTX-M-15).

Concluding remarks. To our knowledge, this is the first study evaluating the performance of the EasyQ KPC assay with a large series of reference and clinical isolates of KPC-producing bacteria. In our series of 300 well-characterized members of the *Enterobacteriaceae*, this test showed a sensitivity of 100% and a specificity of 100% compared with reference molecular tests.

The real-time PCR assay has several advantages. First, results can be obtained within 2 h (from the initiation of the procedure), which may help clinicians to provide more adequate therapy during the early period of clinical illness and facilitates rapid decisions regarding isolation of patients to prevent dissemination. Second, it is simple and easy to perform and requires minimal training. Third, its theoretical processing capacity (up to 48 samples in 24 h) makes it suitable for testing even large collections of isolates (as may be required with proactive surveillance programs in outbreak situations). On the whole, compared to standard DNA sequence analysis, the EasyQ KPC test should be of benefit since it appears to allow fast, accurate, and cost-effective detection of KPC-producing members of the *Enterobacteriaceae* with considerable savings in terms of time and work.

One limitation of this study is that bla_{KPC} allelic variants other than bla_{KPC-2} and bla_{KPC-3} were not investigated. However, this appears to be a minor limitation, since bla_{KPC-2} and bla_{KPC-3} are by far the most common allelic variants encountered in clinical settings (4, 7, 19, 24), while the sequence variability among known bla_{KPC} variants is very limited (GenBank database, accessed on 12 May 2012). Nonetheless, further investigation may be needed to

confirm the reliability of the EasyQ KPC assay for detecting other bla_{KPC} variants.

ACKNOWLEDGMENT

This work was supported by Università Cattolica del Sacro Cuore (Fondi Ateneo, Linea D1-2010).

REFERENCES

1. **Al Naiemi N, Schipper K, Duim B, Bart A.** 2006. Application of minimal sequence quality values prevents misidentification of the bla_{SHV} type in single bacterial isolates carrying different SHV extended-spectrum β-lactamase genes. J. Clin. Microbiol. **44**:1896–1898.
2. **Bogaerts P, et al.** 2011. Multicenter evaluation of a new DNA microarray for rapid detection of clinically relevant *bla* genes from beta-lactam-resistant gram-negative bacteria. Antimicrob. Agents Chemother. **55**: 4457–4460.
3. **Bratu S, Landman D, Alam M, Tolentino E, Quale J.** 2005. Detection of KPC carbapenem-hydrolyzing enzymes in *Enterobacter* spp. from Brooklyn, New York. Antimicrob. Agents Chemother. **49**:776–778.
4. **Chen L, et al.** 2011. Multiplex real-time PCR assay for detection and classification of *Klebsiella pneumoniae* carbapenemase gene (bla KPC) variants. J. Clin. Microbiol. **49**:579–585.
5. **Cole JM, Schuetz AN, Hill CE, Nolte FS.** 2009. Development and evaluation of a real-time PCR assay for detection of *Klebsiella pneumoniae* carbapenemase genes. J. Clin. Microbiol. **47**:322–326.
6. **Endimiani A, et al.** 2010. Evaluation of a commercial microarray system for detection of SHV-, TEM-, CTX-M-, and KPC-type beta-lactamase genes in Gram-negative isolates. J. Clin. Microbiol. **48**:2618–2622.
7. **Giani T, et al.** 2009. Emergence in Italy of *Klebsiella pneumoniae* sequence type 258 producing KPC-3 carbapenemase. J. Clin. Microbiol. **47**:3793–3794.
8. **Giske CG, et al.** 2011. A sensitive and specific phenotypic assay for detection of metallo-β-lactamases and KPC in *Klebsiella pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clin. Microbiol. Infect. **17**:552–556.
9. **Gootz TD, et al.** 2009. Genetic organization of transposase regions surrounding bla_{KPC} carbapenemase genes on plasmids from *Klebsiella* strains isolated in a New York City hospital. Antimicrob. Agents Chemother. **53**:1998–2004.
10. **Grundmann H, et al.** 2010. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro Surveill. 15:pii=19711. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19711.
11. **Hirsch EB, Tam VH.** 2010. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J. Antimicrob. Chemother. **65**:1119–1125.
12. **Magiorakos AP, et al.** 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. **18**:268–281.
13. **Marchaim D, et al.** 2011. Outbreak of colistin.-resistant, carbapenem-resistant *Klebsiella pneumoniae* in metropolitan Detroit, Michigan. Antimicrob. Agents Chemother. **55**:593–599.
14. **Miriagou V, et al.** 2010. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin. Microbiol. Infect. **16**:112–122.
15. **Naas T, Cuzon G, Truong H, Bernabeu S, Nordmann P.** 2010. Evaluation of a DNA microarray, the check-points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and KPC carbapenemases. Antimicrob. Agents Chemother. **54**:3086–3092.
16. **Pagani L, et al.** 2003. Multiple CTX-M-type extended-spectrum beta-lactamases in nosocomial isolates of *Enterobacteriaceae* from a hospital in northern Italy. J. Clin. Microbiol. **41**:4264–4269.
17. **Poirel L, Pitout JD, Nordmann P.** 2007. Carbapenemases: molecular diversity and clinical consequences. Future Microbiol. **2**:501–512.
18. **Pérez-Pérez FJ, Hanson ND.** 2002. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol. **40**:2153–2162.
19. **Qureshi ZA, et al.** 2012. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. Antimicrob. Agents Chemother. **56**:2108–2113.
20. **Rasheed JK, Cockerill F, Tenover FC.** 2007. Detection and characterization of antimicrobial resistance genes in pathogenic bacteria, p 1248–1267. *In* Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (ed), Manual of clinical microbiology, 9th ed, vol 1. American Society for Microbiology, Washington, DC.
21. **Schwaber MJ, et al.** 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.
22. **Tsakris A, et al.** 2010. A simple phenotypic method for the differentiation of metallo-β-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. J. Antimicrob. Chemother. **65**:1664–1671.
23. **Tenover FC, et al.** 2006. Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. Emerg. Infect. Dis. **12**: 1209–1213.
24. **Walsh TR.** 2008. Clinically significant carbapenemases: an update. Curr. Opin. Infect. Dis. **21**:367–371.
25. **Woodford N, et al.** 2004. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A β-lactamase, KPC-3, in a New York medical center. Antimicrob. Agents Chemother. **48**:4793–4799.
26. **Woodford N, et al.** 2010. Comparison of BD Phoenix, Vitek 2, and MicroScan automated systems for detection and inference of mechanisms responsible for carbapenem resistance in Enterobacteriaceae. J. Clin. Microbiol. **48**:2999–3002.
27. **Yigit H, et al.** 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45**:1151–1161.
28. **Yong D, et al.** 2009. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob. Agents Chemother. **53**:5046–5054.