JCM
Journals.ASM.org

Rapid and Simultaneous Detection of Genes Encoding *Klebsiella pneumoniae* Carbapenemase (bla_{KPC}) and New Delhi Metallo-β-Lactamase (bla_{NDM}) in Gram-Negative Bacilli

Scott A. Cunningham,[a] Tabassum Noorie,[b] Daniele Meunier,[b] Neil Woodford,[b] Robin Patel[a,c]

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA[a]; Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, Health Protection Agency (HPA), London, United Kingdom[b]; Division of Infectious Diseases, Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota, USA[c]

We present a duplex, real-time PCR assay for detection of *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) and New Delhi metallo-β-lactamase (bla_{NDM}) genes. Accuracy was assessed with 158 Gram-negative bacillary isolates, including 134 carbapenemase producers. The assay had 100% sensitivity and specificity compared with reference methods and a turnaround time of 90 min.

Acquired carbapenem nonsusceptibility associated with carbapenemase production in Gram-negative bacilli is increasing, compromising treatment and raising concerns about nosocomial transmission (1, 2). Several genes encode carbapenemases, including serine carbapenemases bla_{KPC} and bla_{OXA-48} and metallo-β-lactamases bla_{IMP}, bla_{VIM}, and bla_{NDM} (3). In the United States, *Klebsiella pneumoniae* carbapenemase (KPC) is most common, followed by New Delhi metallo-β-lactamase (NDM). Plasmids carrying these genes have a range of host organisms and are spread efficiently (4, 5).

Carbapenemase detection using the modified Hodge test is neither sensitive nor specific and is subjective and requires follow-up molecular methods to characterize the underlying mechanism in positive isolates (6–12). Molecular methods can detect and characterize carbapenemases, including KPC- and NDM-mediated resistance (9). Confirmation of the PCR product by sequencing or target-specific probes in real-time assays has been described (13–21), as have microarray and loop-mediated isothermal amplification (LAMP) detection (22–25).

Here we describe a LightCycler (Roche Molecular Diagnostics, Indianapolis, IN) duplex real-time PCR assay employing fluorescence resonance energy transfer (FRET) hybridization probe-based detection of bla_{KPC} and bla_{NDM} which, when coupled with simple pre-PCR colony lysis, yields a "colony-to-result" time of 90 min, faster than any previously described assay. We validated our assay using a large number of carbapenemase-producing isolates, including *Enterobacteriaceae* and non-*Enterobacteriaceae*.

(This work was presented in part at the joint 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC]-46th Annual Meeting of the Infectious Diseases Society of America [IDSA], Washington, DC, 2008.)

Primers targeting bla_{KPC} (GenBank accession no. AF297554.1) and bla_{NDM} (GenBank accession no. FN396876.1) with exact sequence matches to all known genotypes of KPC (1 to 14) and NDM (1 to 7) were designed using LightCycler probe design software, version 2.0 (Roche Applied Science, Indianapolis, IN) (primers and probes are shown in Table 1). *K. pneumoniae* BAA-1705 (ATCC, Manassas, VA), containing bla_{KPC}, and *K. pneumoniae* NCTC 13443 (Health Protection Agency [HPA], London, United Kingdom), containing bla_{NDM-1}, were used as controls.

Each 15-μl master mix aliquot contained the following components: 9 μl molecular-grade water, 1.6 μl 10× DNA Master HybProbe mix (containing *Taq* DNA polymerase, reaction buffer, deoxyribonucleoside triphosphates with deoxyuridine triphosphate [dUTP] substituted for deoxyribosylthymine triphosphate [dTTP], and 1 mM $MgCl_2$) (Roche Applied Science), 2.4-μl volume of 25 μM $MgCl_2$ (supplemental), and 2.0 μl 10× primer-probe set 1716 (TIB MolBiol, Adelphia, NJ). Five microliters of colony lysate was added to 20 μl LightCycler reaction cuvettes containing the master mix. Cycling conditions were as follows: 95°C for 10 min; 45 cycles of 10 s at 95°C, 15 s at 55°C, and 15 s at 72°C; melting curve analysis for 0 s at 95°C, 20 s at 59°C, 20 s at 40°C (ramp rate of 0.2°C/s), and 0 s at 85°C (ramp rate of 0.2°C/s and continuous acquisition); and cooling for 30 s at 40°C. Analytical sensitivity for both targets was 10 CFU/μl. There was no cross-reactivity against a panel of 12 organisms (see Table S1 in the supplemental material).

Fifty-seven *Enterobacteriaceae* or nonfermenting Gram-negative bacillus isolates (46 of which were ertapenem nonsusceptible) were studied at Mayo Clinic (see Table S2 in the supplemental material). Isolation plate primary inoculation areas were gently swept using an inoculation loop and transferred to methicillin-resistant *Staphylococcus aureus* (MRSA) lysis tubes (Roche Molecular Diagnostics). Suspensions were heated and physically disrupted on a Thermomixer R (Eppendorf AG, Germany) for 6 min at 1,400 rpm, followed by centrifugation at 20,800 × *g* for 2 min. Thirty isolates were positive for bla_{KPC}, and three isolates were positive for bla_{NDM}. Concordant results were obtained with the Centers for Disease Control and Prevention's duplex real-time PCR assay (26).

To enrich for NDM-positive isolates, 101 isolates of *Enterobacteriaceae* or nonfermenting Gram-negative bacilli, which had been shown previously to produce an NDM-type carbapenemase by metallo-β-lactamase gene PCR, were additionally studied at the

Received 29 November 2012 Accepted 15 January 2013
Published ahead of print 23 January 2013
Address correspondence to Robin Patel, patel.robin@mayo.edu.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.03062-12.
Copyright © 2013, American Society for Microbiology. All Rights Reserved.
doi:10.1128/JCM.03062-12

TABLE 1 Primers and probes used in this study[a]

Primer or probe	Sequence
Primer	
KPC160F	5′ ATTGGCTAAAGGGAAACACGACC 3′
KPC160R	5′ GTAGACGGCCAACACAAT 3′
NDM1F	5′ ATTAGCCGCTGCATTGAT 3′
NDM1R	5′ GGCATGTCGAGATAGGAAGT 3′
Probe	
KPC160fl	5′ GAACCGCGGAGTGTATGGCACGG FITC 3′
KPC160iLC610	5′ AAATGACTATGCCGTCGTCTGGCCCACT Red610 3′
NDMfl	5′ CAACGGTTTGGCGATCTGGT FITC 3′
NDMiLC670	5′ Red670 TCCGCCAGCTCGCACCG TPO4 3′

[a] bla_{KPC} and bla_{NDM-1} 10× primer-probe set (number 1716; TIB MolBiol, Adelphia, NJ).

HPA (see Table S2 in the supplemental material). Colonies from overnight culture were suspended in 100 μl water, heated for 5 min at 95°C, and centrifuged at 8,000 rpm for 5 min; supernatant was tested by PCR. All were positive for bla_{NDM} (as expected) by the novel real-time PCR assay.

Recent publications have described molecular assays for carbapenemase genes. Spanu et al. evaluated 300 clinical isolates using the commercial NucliSENS EasyQ KPC (bioMérieux, Marcy l'Etoile, France) assay and found 100% sensitivity and specificity and an analytical sensitivity of 4 CFU/reaction (16). Manchanda et al. evaluated a laboratory-developed real-time PCR assay targeting bla_{NDM-1} by assaying 34 clinical isolates and compared results to a conventional PCR assay; concordant results and a sensitivity of 10 copies/reaction were reported (18). Diene et al. evaluated a collection of 44 clinical isolates using a real-time PCR assay targeting bla_{NDM-1}; results were concordant with conventional PCR, although only a single bla_{NDM-1}-positive isolate was studied (19). Ong et al. described a real-time PCR assay for bla_{NDM-1} using hydrolysis probes and assayed 47 isolates (12 of which were positive), reporting 100% sensitivity and specificity compared with conventional PCR and a limit of detection of 35 CFU/reaction (21). Qi et al. described a LAMP assay for detection of bla_{NDM-1} and tested it on 345 veterinary isolates also characterized by conventional PCR; their study included only a single bla_{NDM-1}-positive isolate (22). Finally, Monteiro et al. evaluated a multiplex real-time PCR assay, which detected six resistance genes, including bla_{KPC} and bla_{NDM-1}, using high-resolution melting-curve analysis. Fifty-eight isolates, which had been previously characterized by PCR and sequencing, were evaluated with 100% concordance (20). A commercial assay, hyplex SuperBug ID (Amplex, BioSystems GMBH, Geiβen, Germany), which detects bla_{KPC} and bla_{NDM-1} and other carbapenemase genes in 2.5 to 4 h, has been recently described (15). Although these assays performed equivalently to our assay, all except two used preparatory DNA extraction/purification and all had longer turnaround times than our 1.5-h estimate. The only reports that used a comparable lysis method either targeted only bla_{KPC} (16) or did not use real-time detection (15). Additionally, none of the reports detail the specificity of assay design for detection of NDM genotypes other than NDM-1; based on *in silico* analysis, NDM-1 through -7 would be detected by our assay.

Two evaluations utilizing microarrays that target multiple resistance genes reported similar results compared with conventional and real-time PCR methods (23, 24, 27, 28). While more information can be gained from this approach, the turnaround time is long and the instrumentation and expertise required are beyond the scope of most clinical laboratories.

In summary, this is the first description of a FRET hybridization probe-based real-time PCR assay that targets bla_{KPC} and bla_{NDM} in a single assay. It is simple to perform, as evidenced by its implementation in our laboratories in the United States and the United Kingdom. Including the rapid preparatory lysis procedure, results are obtained within 90 min, and the assay performs as well as a reference assay for use in the public health arena. It offers rapid detection and differentiation of bla_{KPC} and bla_{NDM} in multidrug-resistant Gram-negative isolates, resistance mechanisms that are important causes of carbapenem resistance worldwide, and provides information for patient care and for limiting the spread of resistant bacteria.

ACKNOWLEDGMENT

We thank the Minnesota Department of Health for providing two of the NDM-producing isolates studied.

REFERENCES

1. **Peirano G, Ahmed-Bently J, Woodford N, Pitout JD.** 2011. New Delhi metallo-beta-lactamase from traveler returning to Canada. Emerg. Infect. Dis. **17**:242–244.
2. **Paterson DL.** 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. Am. J. Infect. Control **34**(Suppl 1):S20–S28.
3. **Bush K, Fisher JF.** 2011. Epidemiological expansion, structural studies, and clinical challenges of new beta-lactamases from gram-negative bacteria. Annu. Rev. Microbiol. **65**:455–478.
4. **Carattoli A.** 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob. Agents Chemother. **53**:2227–2238.
5. **Potron A, Poirel L, Nordmann P.** 2011. Plasmid-mediated transfer of the bla_{NDM-1} gene in Gram-negative rods. FEMS Microbiol. Lett. **324**:111–116.
6. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement. CLSI document M100–S222012. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, Carey RB, Thompson A, Stocker S, Limbago B, Patel JB.** 2007. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in Enterobacteriaceae. J. Clin. Microbiol. **45**:2723–2725.
8. **Mochon AB, Garner OB, Hindler JA, Krogstad P, Ward KW, Lewinski MA, Rasheed JK, Anderson KF, Limbago BM, Humphries RM.** 2011. New Delhi metallo-beta-lactamase (NDM-1)-producing *Klebsiella pneumoniae*: case report and laboratory detection strategies. J. Clin. Microbiol. **49**:1667–1670.
9. **Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V.** 2012. Identification and screening of carbapenemase-producing Enterobacteriaceae. Clin. Microbiol. Infect. **18**:432–438.
10. **Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, Goto M.** 2000. Convenient test for screening metallo-beta-lactamase-producing gram-negative bacteria by using thiol compounds. J. Clin. Microbiol. **38**:40–43.
11. **Kim SY, Hong SG, Moland ES, Thompson KS.** 2007. Convenient test using a combination of chelating agents for detection of metallo-beta-lactamases in the clinical laboratory. J. Clin. Microbiol. **45**:2798–2801.
12. **Girlich D, Poirel L, Nordmann P.** 2012. Value of the modified Hodge test for detection of emerging carbapenemases in Enterobacteriaceae. J. Clin. Microbiol. **50**:477–479.
13. **Kruttgen A, Razavi S, Imhol M, Ritter K.** 2011. Real-time PCR assay and a synthetic positive control for the rapid and sensitive detection of the emerging resistance gene New Delhi Metallo-beta-lactamase-1 (bla_{NDM-1}). Med. Microbiol. Immunol. **200**:137–141.
14. **Richter SN, Frasson I, Biasolo MA, Bartolini A, Cavallaro A, Palu G.** 2012. Ultrarapid detection of $bla_{KPC1/2–12}$ from perirectal and nasal swabs by use of real-time PCR. J. Clin. Microbiol. **50**:1718–1720.
15. **Kaase M, Szabados F, Wassill L, Gatermann SG.** 2012. Detection of

carbapenemases in enterobacteriaceae by a commercial multiplex PCR. J. Clin. Microbiol. **50**:3115–3118.

16. **Spanu T, Fiori B, D'Inzeo T, Canu G, Campoli S, Giani T, Palucci I, Tumbarello M, Sanguinetti M, Rossolini GM.** 2012. Evaluation of the new NucliSENS EasyQ KPC test for rapid detection of *Klebsiella pneumoniae* carbapenemase genes (bla_{KPC}). J. Clin. Microbiol. **50**:2783–2785.
17. **Naas T, Ergani A, Carrer A, Nordmann P.** 2011. Real-time PCR for detection of NDM-1 carbapenemase genes from spiked stool samples. Antimicrob. Agents Chemother. **55**:4038–4043.
18. **Manchanda V, Rai S, Gupta S, Rautela RS, Chopra R, Rawat DS, Verma N, Singh NP, Kaur IR, Bhalla P.** 2011. Development of TaqMan real-time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. Indian J. Med. Microbiol. **29**:249–253.
19. **Diene SM, Bruder N, Raoult D, Rolain JM.** 2011. Real-time PCR assay allows detection of the New Delhi metallo-beta-lactamase (NDM-1)-encoding gene in France. Int. J. Antimicrob. Agents **37**:544–546.
20. **Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S.** 2012. Rapid detection of carbapenemase genes by multiplex real-time PCR. J. Antimicrob. Chemother. **67**:906–909.
21. **Ong DC, Koh TH, Syahidah N, Krishnan P, Tan TY.** 2011. Rapid detection of the bla_{NDM-1} gene by real-time PCR. J. Antimicrob. Chemother. **66**:1647–1649.
22. **Qi J, Du Y, Zhu X, Bai H, Luo Y, Liu Y.** 2012. A loop-mediated isothermal amplification method for rapid detection of NDM-1 gene. Microb. Drug Resist. **18**:359–363.
23. **Naas T, Cuzon G, Bogaerts P, Glupczynski Y, Nordmann P.** 2011. Evaluation of a DNA microarray (Check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. J. Clin. Microbiol. **49**:1608–1613.
24. **Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P.** 2012. Evaluation of a DNA microarray for the rapid detection of extended-spectrum beta-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.
25. **Fishbain JT, Sinyavskiy O, Riederer K, Hujer AM, Bonomo RA.** 2012. Detection of extended-spectrum beta-lactamase and *Klebsiella pneumoniae* carbapenemase genes directly from blood cultures by use of a nucleic acid microarray. J. Clin. Microbiol. **50**:2901–2904.
26. **Centers for Disease Control and Prevention.** 2011. Multiplex real-time PCR detection of *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-B-lactamase (NDM-1) genes. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/HAI/settings/lab/kpc-ndm1-lab-protocol.html.
27. **Woodford N, Warner M, Pike R, Zhang J.** 2011. Evaluation of a commercial microarray to detect carbapenemase-producing Enterobacteriaceae. J. Antimicrob. Chemother. **66**:2887–2888.
28. **Stuart JC, Voets G, Scharringa J, Fluit AC, Leverstein-Van Hall MA.** 2012. Detection of carbapenemase-producing Enterobacteriaceae with a commercial DNA microarray. J. Med. Microbiol. **61**:809–812.