Evaluation of the NucliSENS EasyQ KPC Assay for Detection of *Klebsiella pneumoniae* Carbapenemase-Producing *Enterobacteriaceae*

A. S. McEwan,[a] A. Derome,[b] D. Meunier,[c] P. J. Burns,[a] N. Woodford,[c] A. R. Dodgson[a,d]

Microbiology, Manchester Royal Infirmary, Manchester, United Kingdom[a]; bioMérieux, Grenoble, France[b]; Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, HPA Colindale, London, United Kingdom[c]; University of Manchester, Manchester, United Kingdom[d]

The NucliSENS EasyQ KPC assay (bioMérieux SA, Marcy l'Etoile, France) was compared with a routinely used phenotypic method for detection of *Enterobacteriaceae* producing *Klebsiella pneumoniae* carbapenemase (KPC)-type carbapenemases, using 806 stool samples and rectal swabs. Compared with the phenotypic method, the EasyQ KPC assay had a sensitivity and specificity of 93.3% and 99.0%, respectively, in this setting, with diverse KPC producers not limited to ST258 *Klebsiella pneumoniae*.

The *Klebsiella pneumoniae* carbapenemases (KPC) are among the most frequently encountered carbapenemases in *Enterobacteriaceae*. Isolates that produce KPC enzymes are often also resistant to other classes of antibiotics, and thus the infections they cause are difficult to treat. Infection with KPC-producing organisms can result in delayed effective antimicrobial treatment, and affected patients often have comorbidities. Severe infections have been associated with a mortality of up to 70% (1, 2), but the overall mortality rate of infection is around 40% (3).

KPC carbapenemases have become disseminated globally and have attained endemic status in the United States, Greece, and Israel, with hospital outbreaks having been reported in several other European countries, including the United Kingdom, France, and Germany (4, 5). *Enterobacteriaceae* with KPC enzymes may be carried asymptomatically in the gastrointestinal tract and can act as a reservoir for potential hospital transmission. Hospital outbreak prevention and control require targeted infection control interventions, which must include active surveillance, screening, and timely detection of KPC-producing organisms.

The screening method recommended by the Centers for Disease Control and Prevention (CDC) for detection of KPC producers in surveillance specimens uses carbapenem-supplemented broth enrichment followed by culture on MacConkey agar (6). Lolans et al. compared this method with bla_{KPC} PCR testing and demonstrated that the CDC method had a sensitivity of only 65.6% (7). However, screening with an ertapenem disk on MacConkey agar, using a cutoff of ≤27 mm, had a sensitivity of 97.0% (7).

Once carbapenem-resistant bacteria have been isolated, carbapenemase production must be confirmed. This can be done phenotypically using meropenem discs supplemented with different β-lactamase inhibitors. Synergy between meropenem and boronic acid but not between meropenem and cloxacillin indicates a likely KPC-producing organism (8). Carbapenemase production can also be confirmed with the modified Hodge test (MHT) (9).

Phenotypic methods of KPC detection can be subjective, labor-intensive, and time-consuming, requiring up to 72 h to obtain a definitive result. Sensitive and specific molecular methods, detecting bla_{KPC} using real-time nucleic acid amplification (10) or DNA microarrays (11, 12), offer the potential to overcome these problems and may be able to provide same-day results, allowing prompt infection control intervention.

We undertook a prospective study to evaluate the performance of the NucliSENS EasyQ KPC assay (bioMérieux SA, Marcy

l'Etoile, France) for detection of KPC-producing organisms within stool and rectal swab surveillance specimens in a hospital setting. The EasyQ KPC assay is a real-time nucleic acid sequence-based amplification (NASBA) assay for qualitative detection of bla_{KPC} (13). The assay required minimal interpretation, and results for a full run of 48 samples were obtained within 5 h.

The EasyQ KPC assay was compared with the microbiology laboratory's routinely used, culture-based method. A total of 806 consecutive surveillance specimens (619 rectal swabs and 187 stools) were tested. The only inclusion criterion for use of a specimen in the study was that the specimen had been routinely taken for surveillance of KPC-producing organisms. The choice of when and from whom specimens were taken was dependent on implementation of screening policies.

All specimens were obtained over a 2-month period from a large tertiary-care center in the northwest of England, in which KPC has been the dominant carbapenemase for over 2 years, in diverse producers that are not limited to *K. pneumoniae* of the international ST258 lineage. Specimens were tested by the routine method, and following this, specimens were anonymized and tested the next day by the EasyQ KPC assay. The investigator performing the EasyQ KPC assay was blind to the routine test results, and the routine laboratory was blind to the EasyQ KPC assay test results. Routine results and EasyQ KPC assay results were saved and unlinked from patient and clinical identifiers. EasyQ KPC assay results could not therefore be used to alter standard clinical practice, and the local ethics committee confirmed that formal ethical review was not required.

For the routine method, the specimen was plated on ChromID ESBL agar (bioMérieux SA) with a 10-μg ertapenem disk and incubated overnight at 37°C. *Enterobacteriaceae* growth with a zone of inhibition around the ertapenem disc of ≤27 mm, or colonies growing within 28 mm of the ertapenem disc, were deemed potential carbapenemase producers and were tested by

TABLE 1 Comparison of the bioMérieux EasyQ KPC with a routine, phenotypic method of KPC detection

EasyQ KPC[a]	No. of specimens with routine method result			% (95 CI)[b]			
	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV
All specimens				93.3 (77.9–99.2)	99.0 (98.0–99.6)	77.8 (60.9–89.9)	99.7 (99.1–100)
Positive	28	8	36				
Negative	2	768	770				
Total	30	776	806				
Stools				90.0 (55.5–99.8)	98.3 (95.1–99.7)	75.0 (42.8–94.5)	99.4 (96.9–100.0)
Positive	9	3	12				
Negative	1	174	175				
Total	10	177	187				
Swabs				95.0 (75.1–99.9)	99.2 (98.1–99.7)	79.2 (57.9–92.9)	99.8 (99.1–100.0)
Positive	19	5	24				
Negative	1	594	595				
Total	20	599	619				

[a] Final EasyQ result, after retests to resolve invalid results.
[b] CI, confidence interval.

MHT; isolates with a zone of inhibition of >28 mm were not processed further. MHT-positive isolates were subjected to antimicrobial susceptibility testing (AST) and interpretation of resistance mechanisms using the Vitek 2 system. A positive carbapenemase-producing isolate was defined as (i) one for which the Vitek Advanced Expert System inferred KPC or MBL (metallo-β-lactamase) or (ii) one with a meropenem MIC of >4 mg/liter or an ertapenem MIC of >1 mg/liter, in conjunction with a positive MHT result. Isolates which were positive for carbapenemase production based upon MHT and Vitek AST were sent to the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI) for confirmatory testing using bla_{KPC} PCR ([14]). All colonies that grew within 28 mm of the ertapenem disk on a ChromID ESBL agar plate and all *Enterobacteriaceae* with a zone of inhibition around the ertapenem disc of ≤27 mm were collected and anonymized by the routine laboratory and given to the study investigator to be tested by the EasyQ KPC assay. Before testing, isolates were subcultured on to tryptic soy agar and incubated overnight at 37°C.

Nucleic acid was extracted from clinical specimens on the NucliSENS easyMAG system (bioMérieux SA) with onboard lysis and with positive and negative extraction controls in each run. The easyMAG run time was approximately 50 min. However, when bacterial isolates were tested, DNA was extracted by heating a 0.5 McFarland suspension of the organism at 95°C for 5 min.

The KPC-positive *K. pneumoniae* control strain was ATCC BAA-1705, and the KPC-negative *K. pneumoniae* strain was ATCC BAA-1706. The EasyQ KPC assay was performed according to the manufacturer's instructions. Each EasyQ KPC assay NASBA reaction included an internal control. Invalid tests were defined as reactions in which there was inhibition of amplification of the internal control. Invalid tests were repeated by retesting both untreated extract and also a DNase-treated extract.

Using the routine method, KPC-positive *Enterobacteriaceae* were detected in 30/806 (3.7%) specimens (20 rectal swabs and 10 stool samples). There were 36 positive specimens with the EasyQ KPC assay, 28 of which were also positive using the routine method. Two specimens that tested positive by the routine method, and yielded isolates positive by bla_{KPC} PCR, tested negative by the EasyQ KPC assay (Table 1). The sensitivity and specificity of the EasyQ KPC assay compared with the routine method were 93.3% and 99.0%, respectively (Table 1). There was no significant difference in the performance characteristics of the assay when it was used on stool samples or rectal swabs (Table 1). Using the two-tailed McNemar test for paired samples, the performance of each test was not statistically different ($P = 0.114$).

Sixty-five bacterial isolates grew within 28 mm of the ertapenem disk on ChromID ESBL agar. Of these, 34 isolates were identified phenotypically as carbapenemase producers by the routine method, and these were also all positive by both bla_{KPC} PCR and the EasyQ KPC assay. The 34 isolates comprised *K. pneumoniae* (26 isolates), *Escherichia coli* (four isolates), *Enterobacter cloacae* (three isolates), and *Citrobacter braakii* (one isolate). The 31 isolates that were phenotypically negative for carbapenemase production were also negative by the EasyQ KPC assay. A study ([13]) recently reported the EasyQ KPC assay to be 100% sensitive and specific when bacterial isolates were tested directly, with the assay correctly detecting all 111 bla_{KPC}-positive isolates from a panel of 300 isolates of *Enterobacteriaceae*.

In our study, 41 specimens (5.1%) gave an invalid EasyQ result on first testing and thus required repeat testing. The invalid rate for stool specimens was 9.6% (18 specimens), more than twice that of rectal swabs at 3.7% (23 specimens). Direct retesting of the original nucleic acid extract by the EasyQ KPC assay resolved 51.2% of invalid results. However, DNase treatment of extracts resolved all invalid tests that had failed a direct repeat. That a high number of specimens required repeat testing poses the problem of delayed reporting of results, which compromises the advantage of speed gained by use of the EasyQ KPC assay.

The positivity rates of stool specimens by the routine method and EasyQ KPC were 5.3% and 6.4%, respectively. This was higher than the positivity rates of rectal swabs of 3.2% and 3.9%, respectively. This may be due to variability in the amount and quality of specimen obtained with rectal swabs. While stools or rectal swabs are recommended for surveillance, rectal swabs are often the specimen of

TABLE 2 Summary of discrepant results between the routine method and the EasyQ KPC

Result by:		No. of discrepant results	
Routine method	EasyQ KPC	Initially	After EasyQ retesting[a]
Positive	Negative	2	1
Negative	Positive	8	4

[a] A discrepancy was considered resolved when the repeat EasyQ result was in agreement with the result of the routine method.

choice in surveillance studies (7), due to the ease and convenience with which they may be obtained in comparison to fecal samples.

Ten specimens gave discrepant results between the EasyQ KPC assay and the routine method, comprising two apparent false-negative and eight false-positive EasyQ KPC results (Table 2). The repeat testing of discrepant specimens by EasyQ KPC resolved five of the discrepancies, in favor of the routine method. Four culture-negative specimens with EasyQ KPC-positive results remained positive on retesting by EasyQ KPC and may represent genuine positive specimens missed by the routine method (Table 2). For the four false-positive EasyQ KPC results that were negative on repeat testing, it is possible the positive results were due to contamination, or alternatively, the repeat negative results may have been due to the specimens' containing an amount of RNA at the lower limit of detection which failed to be detected upon retesting. It is possible that the bla_{KPC} gene may have been carried by other components of the bowel flora and that these were not cultured by the routine method.

The EasyQ KPC had a high negative predictive value of 99.7%, important for an effective screening tool. However, the positive predictive value was poorer at just 77.8%. This may be due to the possibility that the EasyQ KPC assay's sensitivity was greater than that of the routine method, as suggested by the apparent false-positive results. The lower PPV is also a consequence of the low number of positive samples found in the study.

A limitation of the study was that discrepant results were not investigated further, which would have been beneficial, particularly for investigation of the apparent EasyQ KPC assay false-positive results. However, since there were no isolates, this was not possible.

In conclusion, this study found the EasyQ KPC assay to be a sensitive and specific method for screening surveillance specimens for KPC-positive *Enterobacteriaceae*. The high negative predictive value of the assay would allow rapid and accurate identification of patients who do not have gastrointestinal carriage of KPC-producing organisms. This may be of use in prioritizing the use of infection control interventions, such as patient isolation, to prevent or contain hospital outbreaks of KPC-producing organisms.

It is noteworthy that the assay can only detect bla_{KPC} and does not identify the species harboring the gene. The assay may therefore not be appropriate as a standalone tool for routine surveillance, given the increasingly diverse range of implicated bacterial species and the numerous types of clinically important carbapenemase enzymes encountered in the health care setting. However, the assay may be useful in the setting of an outbreak of KPC-producing *Enterobacteriaceae* or in institutions where such organisms are prevalent.

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REFERENCES

1. Endimiani A, Depasquale JM, Forero S, Perez F, Hujer AM, Roberts-Pollack D, Fiorella PD, Pickens N, Kitchel B, Casiano-Colón AE, Tenover FC, Bonomo RA. 2009. Emergence of blaKPC-containing *Klebsiella pneumoniae* in a long-term acute care hospital: a new challenge to our healthcare system. J. Antimicrob. Chemother. **64**:1102–1110.
2. Borer A, Saidel-Odes L, Riesenberg K, Eskira S, Peled N, Nativ R, Schlaeffer F, Sherf M. 2009. Attributable mortality rate for carbapenem-resistant *Klebsiella pneumoniae* bacteremia. Infect. Control. Hosp. Epidemiol. **30**:972–976.
3. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, Spanu T, Ambretti S, Ginocchio F, Cristini F, Losito AR, Tedeschi S, Cauda R, Bassetti M. 2012. Predictors of mortality in bloodstream infections caused by Klebsiella pneumoniae carbapenemase-producing *K. pneumoniae*: importance of combination therapy. Clin. Infect. Dis. **55**:943–950.
4. Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, Brolund A, Giske CG. 2009. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. Antimicrob. Agents Chemother. **53**:3365–3370.
5. Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos J, Vatopoulos A, Gniadkowski M, Toth A, Pfeifer Y, Jarlier V, Carmeli Y, CNSE Working Group. 2010. Carbapenem-non-susceptible *Enterobacteriaceae* in Europe: conclusions from a meeting of national experts. Euro Surveill. **15**:19711.
6. Centers for Disease Control and Prevention. 2009. Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing, *Klebsiella* spp. and *E. coli* from rectal swabs. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/HAI/pdfs/labSettings/Klebsiella_or_Ecoli.pdf.
7. Lolans K, Calvert K, Won S, Clark J, Hayden MK. 2010. Direct ertapenem disk screening method for identification of KPC-producing *Klebsiella pneumoniae* and *Escherichia coli* in surveillance swab specimens. J. Clin. Microbiol. **48**:836–841.
8. Giske CG, Gezelius L, Samuelsen Ø, Warner M, Sundsfjord A, Woodford N. 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. 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.
10. Hindiyeh M, Smollen G, Grossman Z, Ram D, Davidson Y, Mileguir F, Vax M, Ben David D, Tal I, Rahav G, Shamiss A, Mendelson E, Keller N. 2008. Rapid detection of blaKPC carbapenemase genes by real-time PCR. J. Clin. Microbiol. **46**:2879–2883.
11. Endimiani A, Hujer AM, Hujer KM, Gatta JA, Schriver AC, Jacobs MR, Rice LB, Bonomo RA. 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.
12. 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.
13. 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 (blaKPC). J. Clin. Microbiol. **50**:2783–2785.
14. Woodford N, Zhang J, Warner M, Kaufmann ME, Matos J, Macdonald A, Brudney D, Sompolinsky D, Navon-Venezia S, Livermore DM. 2008. Arrival of *Klebsiella pneumoniae* producing KPC carbapenemase in the United Kingdom. J. Antimicrob. Chemother. **62**:1261–1264.