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 asymptptomatically 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*<sub>KPC</sub> 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 cloxacinil 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*<sub>KPC</sub> 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*<sub>KPC</sub> (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
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 Anti-microbial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI) for confirmatory testing using \( \text{bla}_{KPC} \) PCR (13). All colonies that grew within 28 mm of the ertapenem disk on ChromID ESBL agar 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 \( \text{bla}_{KPC} \) 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).

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 \( \text{bla}_{KPC} \) 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 \( \text{bla}_{KPC} \)-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.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>No. of specimens with routine method result</th>
<th>% (95 CI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All specimens</td>
<td>28</td>
<td>8</td>
<td>36</td>
<td>93.3 (77.9–99.2)</td>
<td>99.0 (98.0–99.6)</td>
<td>77.8 (60.9–89.9)</td>
</tr>
<tr>
<td>Stools</td>
<td>9</td>
<td>3</td>
<td>12</td>
<td>90.0 (55.5–99.8)</td>
<td>98.3 (95.1–99.7)</td>
<td>75.0 (42.8–94.5)</td>
</tr>
<tr>
<td>Swabs</td>
<td>19</td>
<td>5</td>
<td>24</td>
<td>95.0 (75.1–99.9)</td>
<td>99.2 (98.1–99.7)</td>
<td>79.2 (57.9–92.9)</td>
</tr>
</tbody>
</table>

* a Final EasyQ result, after retests to resolve invalid results.
* b CI, confidence interval.
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 discrepencies, 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 blaKPC 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-negative 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 carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro Surveill. 15:19711.


REFERENCES


