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Detection of Patients Colonized with Carbapenemase-Producing Gram- Negative Bacilli Using the Xpert MDRO Assay

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Abstract

Detecting patients who are colonized with carbapenemase-producing bacteria can be difficult. This study compared the sensitivity and specificity of a PCR-based method (Xpert MDRO) for detecting *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ carbapenem resistance genes using GeneXpert cartridges to the results of culture with and without a broth enrichment step on 328 rectal, perirectal, or stool samples. The culture method included direct inoculation of a MacConkey agar plate on which a 10µg meropenem disk was placed, and plating on MacConkey agar after overnight enrichment of the sample in MacConkey broth containing 1µg/mL of meropenem. Forty-three (13.1%) samples were positive by PCR for *bla*$_{KPC}$ and 11 (3.4%) were positive for *bla*$_{VIM;}$ none were positive for *bla*$_{NDM.}$ The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the PCR assay for *bla*$_{KPC}$ were 100%, 99.0%, 93.0%, and 100%, respectively, compared to broth enrichment culture and sequencing of target genes. The sensitivity, specificity, PPV, and NPV of the assay for *bla*$_{VIM}$ were 100%, 99.4%, 81.8%, and 100%, respectively. Since none of the clinical samples contained organisms with *bla*$_{NDM,}$ 66 contrived stool samples were prepared at various dilutions using three *Klebsiella pneumoniae* isolates containing *bla*$_{NDM}$. The PCR assay showed 100% positivity at dilutions from 300-1800 CFU/ml and 93.3% at 150 CFU/ml. The Xpert MDRO PCR assay required 2 minutes of hands on time and 47 minutes to complete. Rapid identification of patients colonized with carbapenemase-producing organisms using multiplex PCR may help hospitals to improve infection control activities.

Introduction

The global spread of carbapenemase-producing *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter* species (i.e., multi-drug resistant organisms, or MDROs) is a critical medical and public health issue (1, 2). These bacteria are often resistant to all beta-lactam agents and frequently are co-resistant to multiple classes of other antimicrobial agents, leaving very few treatment options (3, 4). Tracing the epidemiologic spread of MDROs is complicated by the diversity of carbapenem-hydrolyzing enzymes that have emerged and the ability of the genes that encode them to spread among multiple bacterial species. Some of the resistance genes, such as those encoding the *Klebsiella pneumoniae* carbapenemases (KPC), which was first seen in *K. pneumoniae* in United States but has disseminated to many bacterial species worldwide, are often associated with successful clonal lineages of bacteria (e.g., *K. pneumoniae* ST258) (5), which have a selective advantage in those hospitals where antimicrobial use is high. Opportunities for transmission of organisms are often frequent, with further dissemination of the resistance genes via transmissible plasmids (6). *K. pneumoniae* strain ST258 has caused multiple outbreaks globally, especially in the United States (5) and Israel (7). Similarly, organisms containing the New Delhi metallo-beta-lactamase (NDM), first reported in *K. pneumoniae* and *Escherichia coli* isolates in 2010, have been introduced into Europe by individuals who, in many cases, have visited India or Pakistan (8) where multi-resistant organisms are more common (9). A third mechanism of carbapenem resistance, the Verona integron-mediated metallo-beta-lactamase (VIM), emerged in Italy in 2007 in *P. aeruginosa* (10) and has spread throughout Europe to enterobacterial species (11) and the rest of the world (3, 4, 6, 12). Additional metallo-

beta-lactamases, such as those in the IMP class from *Pseudomonas aeruginosa*, have been recognized for many years in organisms from Japan and other Asian countries, are present in the United States (13), and are spreading globally (3). Similarly, the Class D OXA-48 beta-lactamase, originally detected in 2001 in a *K. pneumoniae* isolate from Turkey, is now emerging in many parts of Europe (4, 14). This beta-lactamase often mediates low-level carbapenem resistance but not resistance to the extended-spectrum cephalosporins, which makes it harder to detect when selective media for extended-spectrum beta-lactamase-producing organisms are used for screening (15).

Currently, the standard method for detecting patients who are colonized with carbapenemase-producing organisms is to culture rectal or peri-anal swab samples on differential and selective agar plates, such as MacConkey agar, sometimes supplemented with a carbapenem disk, followed by antimicrobial susceptibility testing of lactose fermenting colonies (16). However, many carbapenemase-producing organisms are now present in multiple bacterial species, including non-fermenters, which would be ignored using this strategy (17). Selective agar media for carbapenemase-producing organisms have also been developed to facilitate screening studies (18-21). Selective media vary considerably in sensitivity and specificity depending on the formulation (6, 19). A rapid and accurate method for screening patients for colonization with carbapenemase-producing organisms could facilitate the ability of infection control programs to interrupt the spread of MDROs in hospitals and other healthcare venues (22, 23). Although molecular methods for detecting organisms in stool samples that contain the bla_{KPC} resistance gene have been described either for direct testing (24-26) or after broth

92 enrichment (22), clinical data regarding the sensitivity and specificity of those methods
93 are limited.

94 The goal of this study was to compare the sensitivity and specificity of a PCR-
95 based method for detecting *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ carbapenem resistance genes to the
96 results of culture with and without a broth enrichment step for detecting patients
97 colonized with carbapenemase-producing bacteria.

98

99 **Materials and Methods**

100 **Bacterial isolates.** Bacterial isolates containing a variety of beta-lactamases (Table 1)
101 were obtained from the American Type Culture Collection (ATCC, Rockville, MD), the
102 National Collection of Type Cultures (NCTC, Salisbury, United Kingdom), Northshore
103 HealthSystem (Evanston, IL, courtesy of Dr. Lance Peterson), Carlos G. Malbrán
104 Hospital (Buenos Aires, Argentina, courtesy of Dr. Marcelo Galas), Hôpital de Bicêtre,
105 (Kremlin-Bicêtre, France, courtesy of Dr. Patrice Nordmann), University of Medicine
106 and Dentistry of New Jersey (UNDMJ, Newark, NJ, courtesy of Dr. Barry Kreiswirth),
107 Stanford University Hospital (courtesy of Dr. Ellen Jo Baron), the Project ICARE
108 collection (27) (Emory University, courtesy of Dr. John McGowan), and the Cepheid
109 culture collection (Sunnyvale, CA).
110 **Clinical specimens.** Leftover swab samples were obtained from 2 hospitals in the United
111 States and 1 hospital in Spain. These included 121 single rectal swabs in Amies media,
112 74 single rectal swabs in Stuart's media, and 35 single perirectal swabs in Stuart's media.
113 In addition, 98 double swabs that were dipped in discarded stool specimens and placed in
114 Stuart's transport media were obtained from a third US hospital. All specimens were de-

identified prior to shipment to a central laboratory, removing all links to specific patients. None of the samples were more than 7 days old upon receipt in the reference laboratory. Institutional Review Board permission was received or waived at all testing sites for the study.

Reference culture method for swabs. For initial comparisons of ertapenem and meropenem disks, eighteen carbapenem non-susceptible isolates (including 9 containing *bla*$_{KPC}$, *bla*$_{NDM}$, or *bla*$_{VIM}$, and 6 containing *bla*$_{IMP}$ or *bla*$_{OXA48}$); 1 organism with *bla*$_{CMY-2}$ plus a porin mutation; and 2 additional carbapenem-resistant organisms of unknown mechanism (all negative for beta-lactamase resistance genes by microarray); and 22 carbapenem-susceptible organisms containing a variety of other beta-lactamase genes, including *bla*$_{CTXM}$, *bla*$_{SHV,}$ and *bla*$_{TEM}$ genes, were suspended in a 10% stool matrix and plated on MacConkey agar. The MacConkey agar plate was streaked for isolation and either a 10µg ertapenem disk or a 10µg meropenem disk (BD, Franklin Lakes, NJ) was placed between the first and second streak areas.

For studies with clinical specimens, swabs were inoculated on a MacConkey agar plate (Remel, Lenexa, KS), then the swab was vortexed in 10mls of MacConkey broth (General Laboratory Products, Yorkville, IL) to which a 10µg meropenem disk (BD, Franklin Lakes, NJ) was added. The MacConkey agar plate was streaked for isolation and a 10µg meropenem disk was placed between the first and second streak areas. The agar plates and broth cultures were incubated for 18-20 hours at 35ºC. After overnight incubation, a 100µl sample of the MacConkey broth was spread on a fresh MacConkey plate, a 10µg meropenem disk was placed in the center of the plate, and the plate was incubated for 18-20 hours at 35ºC. Based on the result of the initial studies, colonies on

the MacConkey agar plates (either direct inoculation or subcultures from enrichment broth) growing within a 27mm zone around the meropenem disk were investigated further for carbapenemase-producing organisms, as described by Lolans et al (16). Colonies were tested by disk diffusion and those classified as carbapenem non-susceptible [i.e., intermediate or resistant to ertapenem, imipenem, or meropenem using Clinical and Laboratory Standards Institute (CLSI) interpretive criteria in document M100-S23(28)] were tested by PCR for the presence of the *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ beta-lactamase genes and by Check-Points microarray (see below). Those containing any of the three target genes underwent bi-directional DNA sequence analysis (see below). A positive reference culture result for this study was defined as a carbapenem-non-susceptible organism that contained a *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ carbapenem resistance gene confirmed by DNA sequence analysis.

Organism and beta-lactamase gene characterization. Organisms growing on MacConkey agar within a 27mm zone around a carbapenem disk were identified to species level using the MicroScan WalkAway system (Siemens Healthcare Systems, Deerfield, IL) with Neg ID 2 panels. Antimicrobial susceptibility testing was performed using MicroScan WalkAway Neg MIC 38 panels [using revised CLSI breakpoints for carbapenems and cephalosporins in CLSI document M100-S23(28)] and disk diffusion as described in CLSI document M2-A10 (29) [also using revised carbapenem breakpoints (28)]. Quality control organisms included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853.

Check-Points microarray. The Check-Points CT103 beta-lactamase gene microarray, which contains probes for 57 beta-lactamase resistance genes, was performed as

161 described by the manufacturer (Check-Points, B.V., Wageningen, the Netherlands) on
162 pure cultures of organisms to confirm the presence of carbapenemase genes and other
163 beta-lactamase resistance genes. The Check-Points array system has been validated in
164 three clinical trials, including one multicenter evaluation (30-32).
165 **DNA sequence analysis.** M13 sequencing primers were added to each of the primer sets
166 used to amplify the *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ beta-lactamase genes. DNA was purified
167 from bacterial isolates using the Qiagen DNeasy Blood and Tissue Kit (Qiagen,
168 Germantown, MD) and amplified with the M13/MDRO sequencing primers. The
169 production of amplification products of appropriate size was confirmed on an Agilent
170 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) prior to initiating
171 bidirectional DNA sequence analysis. For discrepant analysis, when no carbapenem-
172 resistant colonies were obtained either from direct inoculation of MacConkey agar or
173 subculture of the MacConkey enrichment broth, DNA was extracted directly from 500µl
174 of the MacConkey enrichment broth, amplified using the M13/MDRO primers, and
175 sequenced (sequencing was performed by Sequetech, Mountain View, CA).
176 **Evaluation of the Xpert MDRO assay.** The Xpert MDRO assay, which detects the
177 *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ carbapenem resistance genes in a GeneXpert cartridge, was
178 run on the GeneXpert platform as per manufacturer's directions. All reagents for DNA
179 extraction, amplification, and detection are contained in the cartridge. The assay requires
180 47 minutes to complete. If two swabs were collected, the first swab was used for culture
181 and the second swab of each pair was vortexed in 3.0 mls of Xpert sample buffer and 2.0
182 mls of the sample buffer was transferred to an Xpert MDRO cartridge (Cepheid,
183 Sunnyvale, CA). If only a single swab was available for testing, the swab was vortexed

in 1.0 ml of phosphate buffered saline (PBS) to create a stool suspension and 500µl of the stool suspension was added to 3.0 mls of Xpert sample buffer. Then, 2.0 mls of the Xpert sample buffer was transferred to the Xpert MDRO cartridge. In addition, 50µl of the stool suspension was inoculated directly onto a MacConkey agar plate and streaked for isolation. A 10µg meropenem disk was placed between the first and second streak areas and the plate was incubated for 18-20 hours at 35ºC. The remainder of the stool suspension was inoculated into MacConkey enrichment broth, incubated overnight at 35ºC, and subcultured to MacConkey agar with a meropenem disk placed in the center of the plate, as described above.

Spiking experiments using NDM-containing strains. Simulated rectal swab samples were prepared by inoculating Copan Double Swabs (Copan, Murrieta, CA) with 75 µl of a stool matrix seeded with approximately 150, 300, 600, 1200, or 1800 colony forming units (CFU)/ml of a *bla*$_{NDM}$-positive *K. pneumoniae* isolate. Organisms included *K. pneumoniae* NCTC13443, *K. pneumoniae* ATCC BAA-2146, and *K. pneumoniae* UNDMJ 34262. The experiments were performed in replicates of five for the two lowest dilutions (150 and 300 CFU/ml) and replicates of four for the three higher dilutions (600, 1200, and 1800 CFU/ml).

Statistics. Ninety-five percent confidence intervals were calculated using Clopper-Pearson (Fisher's exact CI) (33) using Minitab version 16 (State College, PA).

Results

Comparison of MacConkey agar plates with ertapenem and meropenem disks for detection of carbapenem non-susceptible organisms. Organisms listed in Table 1 were inoculated onto MacConkey agar plates as described above. The diameters of the zones of inhibition around ertapenem and meropenem disks for carbapenem non-susceptible organisms (defined using the CLSI M100-S23 criteria) ranged from 6 to 21 mm for ertapenem and 6 to 25 mm for meropenem. The zone of inhibition around ertapenem disks for carbapenem-susceptible organisms, many of which contained beta-lactamases other than carbapenemases, ranged from 10-38 mm (average 25.7 mm), while the zones of inhibition ranged from 12 to 39 mm (average 28.3 mm) for meropenem disks. Using a zone diameter of ≤27mm around the carbapenem disk as the screening criteria [as described by Lolans et al. (16)], both ertapenem and meropenem disks showed 100% sensitivity for carbapenem non-susceptible organisms (defined using the CLSI M100-S23 criteria) but the specificity of the plates with ertapenem disks was only 50% (95% CI, 28.22-71.78) versus 73% (95% CI 49.78, 89.27) for meropenem. Ten organisms, including an imipenem- and meropenem-susceptible *P. aeruginosa*; and carbapenem-susceptible isolates of bla_{CTXM}, bla_{SHV}, and bla_{TEM} -producing strains of *Serratia marcescens*, *Citrobacter freundii*, *E. coli*, *K. pneumoniae*, *Salmonella* spp., and *E. cloacae* produced growth within 27mm around an ertapenem disk. All the organisms were confirmed to be imipenem- and meropenem-susceptible by disk diffusion testing and MicroScan testing. Six of the same 10 organisms grew on the MacConkey plate with meropenem disks, although the zone diameters were closer to the 27mm breakpoint.

Thus, a zone diameter of ≤27mm around a meropenem disk was selected as the screening test for further studies.

Evaluation of the Xpert MDRO assay. The control organisms listed in Table 1 were tested with the Xpert MDRO cartridge. Only the organisms containing the *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ beta-lactamase genes gave positive results. There were no false positive results. A total of 328 fecal samples were tested with the Xpert MDRO assay, of which 53 were positive for *bla*$_{VIM}$ or *bla*$_{KPC}$ or both genes. The Xpert MDRO results were available in 47 minutes after the sample buffer containing the sample eluate was added to the cartridge. Of the 53 positives, 10 were single rectal swab samples in Amies transport media that were positive for *bla*$_{VIM}$. The eleventh sample contained both a *P. aeruginosa* with *bla*$_{VIM}$ and a *K. pneumoniae* with *bla*$_{KPC}$. Eight of 10 samples each yielded 1 *bla*$_{VIM}$-positive organism, including 6 *K. pneumoniae*, 1 *P. putida*, and 1 *Raoultella ornithinolytica*. Nine isolates (including the *P. aeruginosa*) grew within 27mm of the meropenem disk on MacConkey agar and were positive for *bla*$_{VIM}$ when colonies were tested directly in the Xpert MDRO cartridge. All of the *bla*$_{VIM}$-positive isolates came from Spain, except the *P. putida*, which came from New York. All 9 colonies were positive on the microarray, and all yielded DNA sequence data consistent with *bla*$_{VIM}$. The10th Xpert positive sample did not grow on MacConkey agar, but the MacConkey enrichment broth contained amplifiable *bla*$_{VIM}$ sequences when DNA was extracted from 500µl of broth. No MDRO- positive isolate could be cultured from the broth, suggestive of the presence of a *bla*$_{VIM}$ gene in an organism with fastidious growth requirements. The remaining Xpert MDRO positive sample was negative by culture and no amplifiable DNA was obtained from sequence analysis of the enrichment broth. The

cycle threshold level for this sample was late (Ct=34.9) but within the assay cutoff (Ct ≤40). The sample that was only detected by sequencing the MacConkey broth was considered a false positive Xpert MDRO result. The sensitivity, specificity, PPV, and NPV of the assay for *bla*$_{VIM}$ are given in Table 2.

Forty-three single rectal swab samples in Stuart's media were positive by Xpert MDRO for *bla*$_{KPC}$; all came from New York. Of these, 40 yielded growth on MacConkey agar within a 27mm zone around the meropenem disk. All were positive for *bla*$_{KPC}$ when colonies were tested directly in the Xpert MDRO cartridge, all were positive by microarray testing, and all were positive for *bla*$_{KPC}$ by DNA sequence analysis. These included 34 *K. pneumoniae*, 3 *E. cloacae,* 1 *Escherichia coli,* 1 *Enterobacter aerogenes,* and 1 *Serratia marcescens*. Two Xpert MDRO-positive samples were negative by culture but DNA sequence analysis of the MacConkey enrichment broth was positive for *bla*$_{KPC}$. The final *bla*$_{KPC}$ positive sample (Ct= 30.1) was culture-negative on MacConkey agar, the enrichment broth was negative when tested with the Xpert MDRO assay, and no amplifiable DNA was recovered from the broth. The two samples that were only detected by sequencing the MacConkey broth were considered false positive Xpert MDRO results, since they did not yield colonies on MacConkey agar. The sensitivity, specificity, PPV, and NPV of the PCR assay for *bla*$_{KPC}$ are given in Table 2.

There were 275 samples that were Xpert MDRO-negative for all carbapenemase genes tested. Of these, 256 showed no growth of bacteria within 27mm of a meropenem disk either by direct inoculation on MacConkey agar or after broth enrichment in MacConkey broth. Nineteen samples yielded growth within the 27mm zone diameter. This included 7 isolates of *P. aeruginosa*, 3 *K. pneumoniae*, 4 *E. cloacae*, 2 *A. baumannii*,

1 *E. coli*, 1 *Stenotrophomonas maltophilia*, and 1 *P. putida* (Table 3). The *Enterobacteriaceae* were confirmed to be non-susceptible to at least one carbapenem by disk diffusion and MIC testing using the MicroScan WalkAway using the revised CLSI breakpoints for both methods. The *P. aeruginosa* and *A. baumannii* isolates were either resistant or intermediate to both imipenem and meropenem. The *S. maltophilia* is intrinsically resistant to all carbapenems. None of the organisms was positive when colonies were tested directly in the Xpert MDRO assay. Microarray analysis of the colonies demonstrated a variety of beta-lactamase genes among the isolates including the Class C (AmpC)-type beta-lactamase genes, and Class A beta-lactamase genes, including *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$; however, no *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ resistance genes were detected in the isolates. The isolates were not examined for porin changes and efflux-mediated resistance.

Nucleic acids were extracted from the 19 carbapenem-non-susceptible, Xpert MDRO-negative organisms and amplified using sequencing primers for all three target genes. DNA sequence analysis was negative for all colonies. A total of 100 samples from enrichment broth cultures that did not yield carbapenem-resistant colonies were also extracted and sequenced with negative results.

Spiked stool samples with NDM-containing organisms. Sixty-six stool swabs were inoculated with varying concentrations of one of three *bla*$_{NDM}$ –containing *K. pneumoniae* isolates. Sixty-five of the 66 samples were positive with the Xpert MDRO assay and grew on direct MacConkey agar plates and subcultures after broth enrichment within 27mm around a meropenem disk. The remaining sample, which contained approximately 150 CFU/ml, did not grow on the direct MacConkey agar plate but did

298 produce colonies with a diameter of 16mm around the meropenem disk on MacConkey
299 agar after overnight enrichment. Isolates recovered from all 66 mock rectal samples
300 plated on MacConkey agar were positive for *bla*$_{NDM}$ when tested directly in the Xpert
301 MDRO cartridge; six randomly selected isolates were positive for *bla*$_{NDM}$ on the
302 microarray and by DNA sequence analysis. The percent positive Xpert MDRO results
303 for the swabs inoculated with either 600, 1200, or 1800 CFU/ml of a *bla*$_{NDM}$-containing *K.*
304 *pneumoniae* isolate (n=12 for each dilution) was 100% (95% CI 77.9 to 100.00), it was
305 also 100% for swabs inoculated with 300 CFU/ml (95% CI 81.9 to 100.00) (n=15), and
306 93.3% (95% CI 68.1 to 99.8) for swabs inoculated with 150 CFU/ml (n=15).
307
308 **Discussion**
309 Screening patients for colonization with carbapenemase-producing organisms is
310 recommended by the Centers for Disease Control and Prevention whenever a
311 carbapenem-resistant strain of *Enterobacteriaceae* has been recognized in a hospitalized
312 patient (34). Many European countries also have published recommendations that
313 advocate screening patients for carbapenemase-producing bacteria on admission to the
314 hospital, especially if they previously have been hospitalized in a foreign country (17, 18).
315 Screening can be accomplished using either culture-based methods or molecular methods,
316 such as PCR. Culture-based methods have the advantage of detecting phenotypically
317 resistant organisms with carbapenemases, combinations of other beta-lactamases and
318 porin mutations, and organisms with efflux-mediated resistance. Many selective agar
319 formulations also include dyes that give presumptive species identifications for the
320 colonies that grow (19, 21). However, detecting resistant organisms by culture methods

can require several days to complete, which can delay infection control efforts to halt the transmission of multi-drug resistant organisms (18, 22, 34). In addition, many clinical laboratories do not have the capability to identify the mechanism of carbapenem resistance once a resistant organism has been isolated to confirm whether isolates from different patients have the same resistance mechanism and, thus, are potentially part of an outbreak. On the other hand, molecular methods offer rapid turn-around times, often <2 hours, and often have higher sensitivity than culture methods (35). Using a combination of culture and molecular methods to generate rapid results without missing novel resistance mechanisms that may emerge may be ideal but may be too costly for most laboratories to undertake.

This study used a combination of two culture methods, microarray analysis, and DNA sequence confirmation of the *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ genes to evaluate the PCR-based Xpert MDRO assay. We verified that the 27mm zone diameter cutoff used by Lolans et al.(16) for ertapenem disks also was appropriate when meropenem disks were substituted for ertapenem disks. However, using meropenem disks was critical because of the large number of stool samples that contained *P. aeruginosa* isolates, which are intrinsically resistant to ertapenem. The zone diameter selected in this study (i.e., 27mm) is larger than the susceptible meropenem breakpoints chosen by CLSI for *Enterobacteriaceae* (≥23mm), *P. aeruginosa* (≥19mm), and *A. baumannii* (≥16 mm), which increased the sensitivity of the reference culture method, potentially enabling organisms with low levels of carbapenem resistance to be detected. Interestingly, for several specimens, the zones of inhibition produced by growth around the meropenem disks were smaller on the direct MacConkey plate than on the MacConkey plate after

broth enrichment, especially for non-glucose fermenting organisms. This may reflect the interaction of several factors, including the concentration of the organisms present in the direct stool specimen, lower expression of resistance mechanisms after growth in broth, or partial inhibition of growth of organisms containing the target genes in MacConkey broth prior to subculture.

There were four instances where an Xpert MDRO-positive organism was only recovered after broth enrichment. This included one bla_{KPC}-producing *E. cloacae* and three bla_{VIM}-producing *K. pneumoniae* isolates. On the other hand, there was only one instance where the direct plate was positive and the enrichment broth was negative after subculture. This occurred with a *R. ornithinolytica* isolate harboring bla_{VIM}.

There were three instances in the study where no carbapenem non-susceptible organisms were recovered by culture, but DNA sequences consistent with the Xpert MDRO result (bla_{KPC} or bla_{VIM}) were detected by extraction of DNA directly from the enrichment broth. In each case, the DNA sequence of the nucleic acid amplified from the enrichment broth was consistent with the resistance gene detected by the PCR assay, suggesting the organisms harboring the genes were present but not growing. One possibility is that these patients may have been receiving antimicrobial agents, which, together with meropenem, inhibited the growth of the organisms. It is also possible that the three organisms had fastidious growth requirements or low meropenem MICs. Either may inhibit growth of organisms in or on MacConkey media. bla_{KPC} determinants have been detected in several non-fermenting organisms including *P. aeruginosa*, (36) *A. baumannii* (36), and *Pseudomonas putida* (37). Preliminary experiments with MacConkey broth in our laboratory demonstrated a 30-50% reduction in viable counts of

several *P. aeruginosa* isolates on MacConkey agar versus sheep blood agar after overnight incubation in MacConkey broth providing support for this hypothesis (data not shown). The bla_{VIM} determinants have also been described in a number of non-fermenting organisms in the United States, including *P. aeruginosa* and *A. baumanni* (38). To date, there have been no reports documenting the spread of bla_{KPC} or bla_{VIM} to anaerobic flora, but this remains a possible explanation of the results.

Among the carbapenem-non-susceptible organisms that grew on MacConkey agar but were negative with the Xpert MDRO assay, none contained any of the target carbapenemase resistance genes in the Xpert MDRO assay, but most of the non-*P. aeruginosa* organisms contained other beta-lactamase genes as shown by the microarray assay. It is probable that many of these organisms contained porin mutations in addition to beta-lactamases that contributed to their carbapenem resistance phenotype; however, we did not confirm the presence of porin mutations in these isolates. Among these organisms, several discrepancies between the categorical results of MIC testing and disk diffusion results were noted, but all but one were minor errors. Such discrepancies are commonplace when testing for carbapenem resistance and have been noted by other investigators (39, 40). In the case of the two samples where no DNA could be amplified from the enrichment broth, cross-reaction of the PCR primers with other non-target genes cannot be ruled out.

In an effort to determine whether samples that were negative by culture and Xpert MDRO contained fastidious organisms with low-level expression of the target resistance genes, nucleic acids were extracted from 100 broth samples from Xpert MDRO-negative/culture negative samples and amplified using DNA sequencing primers. No

390 *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ gene sequences were detected, suggesting that the presence of
391 fastidious organisms that may have been overlooked in our samples was negligible.
392 Several recent epidemiologic investigations that focused on controlling the spread
393 of carbapenem-resistant organisms in hospitals opted to use molecular assays either with,
394 or in lieu of, culture for detecting patients colonized with carbapenem-resistant organisms
395 (7, 22, 26, 35, 41). Speed of detecting colonized patients was noted as the key benefit
396 for using a molecular method. Diekema and Pfaller, in their recent review on the role of
397 molecular methods for epidemiologic studies of multidrug resistant organisms, noted that
398 the recent changes in the CLSI recommendations to drop ESBL testing and the Modified
399 Hodge test for carbapenemase activity have hindered infection control efforts by
400 eliminating data that epidemiologists found of considerable value (42). A molecular
401 method that identified carbapenemase-producing organisms rapidly, particularly if it
402 detected multiple resistance determinants, would be of potential value to hospital
403 epidemiologists who no longer had access to Modified Hodge test results. In this regard,
404 the Xpert MDRO assay described herein has the ability to differentiate among three beta-
405 lactamase gene families, i.e., *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM,}$ which includes a total of 53
406 genetic determinants. While still representing only a subset of carbapenem resistance
407 genes, the knowledge of which determinant is present could be of epidemiologic value
408 for tracing the spread of resistant organisms in hospitals.
409 The problems associated with using molecular assays to detect carbapenem
410 resistance genes, aside from the increased cost to the laboratory, include the failure to
411 detect novel carbapenemase resistance genes that may be introduced into a healthcare
412 setting (19), the lack of species identification of the organism that harbors the resistance

413 gene, and the lack of a pure culture on which to perform strain typing and additional
414 antimicrobial susceptibility testing. These have to be balanced against the ability of the
415 molecular method to generate rapid results that may lead to more effective infection
416 control interventions.

417 Our study has several limitations. The number of resistance genes surveyed in the
418 assay is limited to three families of beta-lactamases, i.e., *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM.}$
419 Other carbapenemases, including *bla*$_{OXA-48}$, *bla*$_{IMP}$, and *bla*$_{SPM}$ have emerged in various
420 countries around the world (3, 4), particularly outside of the United States, and such
421 isolates would not be detected in this assay. In addition, the number of organisms used to
422 establish the sensitivity and specificity of the Xpert MDRO assay did not cover the entire
423 range of the genes that could be detected, given that there are 15 *bla*$_{KPC}$, 10 *bla*$_{NDM}$, and
424 39 *bla*$_{VIM}$ determinants reported (http://www.lahey.org/Studies/other.asp#table1
425 accessed on 8/30/2013). However, in silico, there were no mismatches observed between
426 the primers and probes chosen for the assay and the mutations associated with alleles of
427 the three target gene families. In addition, the culture media used in this evaluation were
428 limited to MacConkey agar and did not include a broad range of agar plates that may
429 have supported growth of the more fastidious organisms for the three Xpert MDRO-
430 positive-culture negative samples. It is also possible that those organisms lost viability
431 during transport to the central testing laboratory.

432 In conclusion, these data suggest that the Xpert MDRO assay, which detects the
433 *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ beta-lactamase genes in 47 minutes directly from rectal swab
434 samples, can provide valuable information for infection control programs designed to
435 limit the spread of MDROs in healthcare settings.
436

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446 Table 1. Organisms used for evaluating the sensitivity and specificity of the reference

447 culture method and the Xpert MDRO assay.

Strain number[a]	Species	Source[b]	Carbapenem Non-susceptible	Resistance genes
KPC_6	*Citrobacter freundii*	NHS	Yes	*bla*$_{KPC}$; *bla*$_{AmpC}$; *bla*$_{SHV}$[c]
IC1698	*C. freundii*	Emory-ICARE	No	*bla*$_{TEM}$[c]
IC1008	*Enterobacter aerogenes*	Emory-ICARE	No	Carbapenem susceptible
KPC_11	*Enterobacter cloacae*	NHS	Yes	*bla*$_{KPC}$; *bla*$_{ACT/MIR}$[c]
IC260	*E. cloacae*	Emory-ICARE	No	*bla*$_{SHV}$; *bla*$_{TEM}$[c]
cor1	*E. cloacae*	Argentina	No	*bla*$_{CTX-M-2}$; *bla*$_{TEM}$
KPC_1	*Escherichia coli*	NHS	Yes	*bla*$_{KPC}$; *bla*$_{TEM}$[c]
148-2	*E. coli*	NHS	Yes	*bla*$_{KPC}$; *bla*$_{TEM}$[c]
C3015	*E. coli*	Hôpital de Bicêtre	Yes	*bla*$_{CMY-2}$; *bla*$_{TEM}$[c]
OM22	*E. coli*	Hôpital de Bicêtre	Yes	*bla*$_{OXA-48}$; *bla*$_{CTX-M-1}$; *bla*$_{CTX-M-15 like}$; *bla*$_{TEM}$[c]
NCTF 13476	*E. coli*	NCTC	Yes	*bla*$_{IMP}$; *bla*$_{TEM}$[c]
IC4566	*E. coli*	Emory-ICARE	No	ESBL, negative by microarray
5945	*E. coli*	Argentina	No	*bla*$_{CTX-M-2}$; *bla*$_{TEM}$
fbr22	*E. coli*	Argentina	No	*bla*$_{CTX-M-2}$; *bla*$_{TEM}$
NCTC 13441	*E. coli*	NCTC	No	*bla*$_{CTX-M-1}$; *bla*$_{CTX-M-15 like}$; *bla*$_{TEM}$[c]
ATCC 25922	*E. coli*	ATCC	No	Carbapenem susceptible
C421	*Klebsiella oxytoca*	NHS	Yes	Negative by microarray
NCTC 13438	*Klebsiella pneumoniae*	NCTC	Yes	*bla*$_{KPC}$; *bla*$_{SHV}$; *bla*$_{TEM}$[c]
NCTC 13443	*K. pneumoniae*	NCTC	Yes	*bla*$_{NDM}$; *bla*$_{CMY-2}$; *bla*$_{CTX-M-1}$; *bla*$_{CTX-M-15 like}$; *bla*$_{SHV}$; *bla*$_{TEM}$[c]
NCTF 13442	*K. pneumoniae*	NCTC	Yes	*bla*$_{OXA-48}$; *bla*$_{SHV}$; *bla*$_{TEM}$[c]
BAA-2146	*K. pneumoniae*	ATCC	Yes	*bla*$_{NDM}$; *bla*$_{CMY-2}$; *bla*$_{CTX-M-1}$; *bla*$_{CTX-M-15 like}$; *bla*$_{SHV}$; *bla*$_{TEM}$[c]
OM11	*K. pneumoniae*	Hôpital de Bicêtre	Yes	*bla*$_{OXA-48}$; *bla*$_{CTX-M-1}$; *bla*$_{CTX-M-15 like}$; *bla*$_{SHV}$;

				bla_{TEM}[c]
IMPBMI	*K. pneumoniae*	Hôpital de Bicêtre	Yes	bla_{IMP}[c]
NCTC 13465	*K. pneumoniae*	NCTC	No	bla_{SHV}; bla_{TEM}[c]
fle5	*K. pneumoniae*	Argentina	No	$bla_{CTX\text{-}M\text{-}2}$; bla_{TEM}
fan33	*K. pneumoniae*	Argentina	No	$bla_{CTX\text{-}M\text{-}2}$; bla_{TEM}
32443	*K. pneumoniae*	UMDNJ	No	$bla_{CTX\text{-}M\text{-}1}$; $bla_{CTX\text{-}M\text{-}15\ like}$; bla_{SHV}[c]
34262[d]	*K. pneumoniae*	UNDMJ	Yes	bla_{NDM}; $bla_{CMY\text{-}2}$; $bla_{CTX\text{-}M\text{-}1}$; $bla_{CTX\text{-}M\text{-}15\ like}$; bla_{SHV}; bla_{TEM}[c]
SH_1	*K. pneumoniae*	Stanford University Hospital	No	Carbapenem susceptible
fer29	*Morganella morganii*	Argentina	No	$bla_{CTX\text{-}M\text{-}2}$; bla_{TEM}
gut25	*Proteus mirabilis*	Argentina	No	$bla_{CTX\text{-}M\text{-}2}$; bla_{TEM}
IC472	*Providencia stuartii*	Emory-ICARE	No	Carbapenem susceptible
NCTC 13437	*Pseudomonas aeruginosa*	NCTC	Yes	bla_{VIM}[c]
168	*P. aeruginosa*	NHS	Yes	bla_{VIM}[c]
T158	*P. aeruginosa*	NHS	Yes	Negative by microarray
ATCC 27853	*P. aeruginosa*	ATCC	No	Carbapenem susceptible
3209	*Salmonella spp.*	Argentina	No	$bla_{CTX\text{-}M\text{-}2}$; bla_{TEM}
G029	*Salmonella spp.*	Hôpital de Bicêtre	Yes	bla_{IMP}[c]
gpp21	*S. marcescens*	Argentina	No	$bla_{CTX\text{-}M\text{-}2}$; bla_{TEM}
IC169	*S. marcescens*	Emory-ICARE	No	ESBL, negative by microarray
3331	*Shigella flexnerii*	Argentina	No	$bla_{CTX\text{-}M\text{-}2}$; bla_{TEM}

[a] NCTC, National Collection of Type Cultures, ATCC, American Type Culture Collection

[b] NHS, NorthShore University HealthSystem; UMDNJ, University of Medicine and Dentistry of New Jersey.

[c] confirmed using Checkpoints microarray assay

454 [d] This organism was used exclusively for the *bla*$_{NDM}$ spiking study experiments.

455 Table 2. Comparison of Xpert MDRO results to reference culture and DNA sequencing

456 (n=328)

	Reference culture and DNA sequencing results [a]							
Xpert result	Sensitivity (%, [TP/TP+FN])	95% CI	Specificity (%, [TN/TN+FP])	95% CI	PPV (%, [TP/TP+FP])	95% CI	NPV (%, [TN/TN+FN])	95% CI
bla$_{VIM}$ [b]	100 (9/9)	71.7 to 100	99.4 (315/317)	97.7 to 99.9	81.8 (9/11)	48.2 to 97.7	100 (317/317)	(99.1 to 100)
bla$_{KPC}$ [c]	100 (40/40)	92.8 to 100	99.0 (282/285)	97.0 to 99.8	93.0 (40/43)	80.9 to 98.5	100 (285/285)	(98.9 to 100)

457 a[b] CI, confidence interval using Clopper-Pearson (Fisher's Exact) CI; PPV, positive

458 predictive value; NPV, negative predictive value; TP, true positive; FP, false positive; TN,

459 true negative; FN, false negative.

460 [b] One specimen that was positive for *bla*$_{VIM}$ by Xpert MDRO and yielded *bla*$_{VIM}$

461 sequence data directly from the broth but was culture negative on agar was considered a

462 false positive result in this analysis.

463 [c] Two specimens that were positive for *bla*$_{KPC}$ by Xpert MDRO and yielded *bla*$_{KPC}$

464 sequence data directly from the broth but were culture negative on agar were considered

465 false positive results in this analysis.

466

467

468

469 Table 3. Characterization of carbapenem-non-susceptible organisms recovered on

470 MacConkey agar that were negative in the Xpert MDRO assay

Strain No.	Organism identification	Categorical interpretations of disk diffusion results (mm) for Ertapenem/ Imipenem/ Meropenem[a]	Categorical interpretations of Microscan MIC results (µg/ml) for Ertapenem/ Imipenem/ Meropenem[a]	Diameter of zone of inhibition around meropenem disk on MacConkey agar (direct)	Diameter of zone of inhibition around meropenem disk on MacConkey agar (broth enriched)	Checkpoints array results
C8812	*A. baumannii*	-/R/R	-/R/R	8	16	Negative
C8789	*A. baumannii*	-/R/R	-/R/R	26	20	bla_{TEM}
C8704	*E. cloacae*	R/S/R/	R/S/S	16	13	$bla_{AMPC,}$ $bla_{SHV,}$ bla_{TEM}
C8708	*E. cloacae*	R/S/S	I/S/S	24	25	Negative
C8719	*E. cloacae*	R/S/I	R/I/S	9	17	$bla_{AMPC,}$ $bla_{SHV,}$ bla_{TEM}
C8796	*E. cloacae*	I/S/S	S/S/S	>27	24	$bla_{TEM,}$ bla_{SHV}
C8633	*E. coli*	I/S/S	R/S/S	20	22	$bla_{AMPC,}$ $bla_{TEM,}$ $bla_{CTX-M-15}$
C8771	*K. pneumoniae*	R/S/R	R/S/R	20	16	$bla_{CTX-M-2,}$ $bla_{TEM,}$ bla_{SHV}
C8715	*K. pneumoniae*	R/I/R	R/I/R	12	12	$bla_{CTX-M-15,}$ $bla_{SHV,}$
C8717	*K. pneumoniae*	R/S/R	R/S/R	>27	25	$bla_{CTX-M-15,}$ bla_{SHV}
C8634	*P. aeruginosa*	-/I/R	-/R/R	12	6	Negative
C8698	*P. aeruginosa*	-/R/R	-/R/R	12	6	Negative

C8703	*P. aeruginosa*	-/R/R	-/R/R	6	6	Negative
C8710	*P. aeruginosa*	-/R/R	-/R/R	6	6	Negative
C8711	*P. aeruginosa*	-/I/R	-/R/I	>27	22	Negative
C8718	*P. aeruginosa*	-/R/R	-/R/R	>27	10	Negative
C8866	*P. aeruginosa*	-/R/R	-/R/R	8	16	Negative
C8700	*P. putida*	-/R/R	-/R/R	10	6	Negative
C8699	*S. maltophilia*	-/-/-	-/-/-	10	18	Negative

[a] ertapenem, imipenem, and meropenem; disk diffusion and MIC results are reported as: R, resistant; I, intermediate; S, susceptible; -, CLSI has not established breakpoints for ertapenem for *A. baumannii* or *P. aeruginosa*, and no carbapenem breakpoints have been established for *S. maltophilia,* since the organism is intrinsically resistant to these agents.

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