Direct Detection of Carbapenem-Resistant Organisms from Environmental Samples Using the GeneXpert Molecular Diagnostic System

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ABSTRACT In this pilot study, traditional culture and PCR methods were compared to the Cepheid GeneXpert IV molecular diagnostic system with the Xpert Carba-R assay (Carba-R assay) for detection of carbapenem resistance genes in primary environmental samples collected during a health care-related outbreak. Overall, traditional culture-dependent PCR and the Carba-R assay demonstrated 75% agreement. The Carba-R assay detected carbapenemase genes in five additional samples and in two samples that had additional genes when compared to culture-dependent PCR. The Carba-R assay could be useful for prioritizing further testing of environmental samples during health care-related outbreaks.

IMPORTANCE Use of the Carba-R assay for detection of carbapenem-resistant Gram-negative organisms (CROs) can provide data for implementation of a rapid infection control response to minimize the spread of CROs in the health care setting.

KEYWORDS Cepheid GeneXpert, PCR, carbapenem resistant, carbapenemase genes

Carbapenem-resistant Gram-negative organisms (CROs), such as carbapenem-resistant *Enterobacteriaceae* (CRE), are a global emerging antibiotic-resistant threat due to multidrug resistance and limited therapeutic treatment options. CRE alone have been estimated to cause 9,000 infections and 600 deaths per year in the United States (1, 2). Traditional methods such as differential plating and antimicrobial susceptibility testing are important in phenotypic detection of CROs, and real-time PCR is the gold standard for detecting specific carbapenemase genes from patient surveillance samples specimens (3, 4). Studies have shown that the Cepheid Xpert Carba-R assay (Carba-R assay) provides rapid detection of carbapenemase genes from rectal swabs to identify colonized persons (5–7). There is a need to extend this rapid testing capacity to environmental samples to identify potential reservoirs of antibiotic-resistant bacteria (8, 9). Culturing and detection of target antibiotic-resistant bacteria from the health care environment can be labor-intensive and time-consuming (10). Rapid culture-independent technology provides an opportunity to detect antibiotic resistance genes from environmental reservoirs quickly, and subsequently contribute to timely implementation of infection control measures to prevent transmission in health care facilities (11, 12).

In 2015, a tertiary care hospital (facility A) identified an outbreak of Verona integron-encoded metallo-β-lactamase (VIM)-producing CRE (13). This was a complex outbreak because the bla_{VIM} gene, identified by whole-genome sequencing as bla_{VIM-1}, was found in multiple species of the family *Enterobacteriaceae*, including *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*. As part of the outbreak investigation, environmental samples were collected for detection of carbapenemase genes in culturable CROs to determine whether a single point source existed. We used this opportunity to conduct a pilot study to compare culture-dependent PCR

methods to the culture-independent Carba-R assay for carbapenemase gene detection from environmental samples.

Primary environmental sample collection and culture. Thirty environmental samples were collected, including biofilm samples from 4 toilets, 8 sink drains, and 15 sink traps, 3 samples of high-frequency contact surfaces (e.g., environmental service [EVS] cart, diaper scale), and water from sink drains. A double-swab set (Copan, Murrieta, CA) was used to collect the biofilms, and a premoistened sponge wipe (3M Sponge-Stick; 3M, St. Paul, MN) was used for the high-frequency contact surfaces. Sponge wipe samples and one swab from each double-swab set were processed in phosphate-buffered saline containing 0.02% Tween 80 as previously described (10–14). The eluents were cultured on MacConkey II agar (Becton Dickinson, Sparks, MD) to select for Gram-negative organisms and on CHROMagar KPC medium (Becton Dickinson) to select for Gram-negative CROs.

Screening for CRO, PCR mechanism testing, and species identification. Representative suspect isolates from CHROMagar KPC medium and MacConkey II agar were subcultured to Trypticase II with 5% sheep blood agar plates (TSA II; Becton Dickinson) with a meropenem disc (10 μg; Becton Dickinson) between the first and second quadrants to screen for carbapenem resistance. The number of representative isolates picked for additional screening varied from sample to sample (range, 1 to 7 colonies). Isolates with inhibition zones of ≤22 mm were forwarded for lysate preparation (15) and mechanism testing by multiplex real-time PCR assays for the detection of bla_{KPC}, bla_{NDM}, $bla_{OXA-48-type}$, and bla_{VIM} genes with a cycle threshold value of ≤30 (15–17). Suspect CROs were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using a MALDI Biotyper system (Bruker Daltonics, Billerica, MA) using the Bruker and the CDC MicrobeNet (https://microbenet.cdc.gov/) databases; species-level identification is confident for scores of ≥2.0.

Primary environmental sample direct testing. The samples were tested on the Cepheid GeneXpert IV system with the Xpert Carba-R assay (Carba-R assay) (Cepheid, Sunnyvale, CA) according to the manufacturer's package insert (18). The second swab from each biofilm double-swab set was placed directly into the sample reagent vial and vortexed for approximately 10 s to mix. For samples with only liquid eluent available (i.e., water from sink drains, surface sponge wipes), 100 μl was directly inoculated into the sample reagent vial and vortexed. Approximately 1.7 ml of each was transferred into the sample chamber of the Carba-R assay cartridge and placed onto the instrument. If an invalid test resulted, a 1.5-ml aliquot from the original sample reagent vial was concentrated by centrifugation at 13,000 × g to pellet debris, the supernatant was added to a fresh sample reagent vial, and testing was repeated. If an invalid test resulted the second time, the sample was excluded. An invalid result indicates that the SPC (sample processing control) failed due to improperly processed samples, or because the PCR was inhibited, or the sample volume was inadequate. A Carba-R assay positive result was defined as detection of at least one carbapenemase gene (i.e., bla_{KPC}, bla_{NDM}, $bla_{OXA-48-type}$, bla_{IMP-1}, and bla_{VIM}) from a primary environmental sample.

Table 1 shows the comparison of traditional culture-dependent PCR methods and the culture-independent Carba-R assay. Of the 30 initial environmental samples, 16 were suspected to be culture positive for CROs (data not shown). From these 16, 37 representative isolates underwent confirmatory testing for carbapenemase genes by real-time PCR and identification by MALDI-TOF MS. Three of the sixteen samples were confirmed to have culture-positive CROs (19%): the room C sink drain from unit D, the housekeeping closet drain, and the EVS cart. Two isolates were identified as *Klebsiella pneumoniae* carbapenemase positive (KPC+) (13%): *Enterobacter gergoviae* from the housekeeping closet drain and *Klebsiella oxytoca* from the EVS cart. Two isolates were identified as VIM positive (VIM+) (13%); *Pseudomonas putida* from the EVS cart and *Citrobacter amalonaticus* from the room C sink drain. The 30 primary environmental samples were all tested using the Carba-R assay; seven samples were positive for at least one carbapenemase gene (23%). Six specimens were KPC+ (20%), three were

TABLE 1 Summary of the specimens tested and detection of carbapenemase genes by culture-dependent PCR and the Carba-R assay

| Ward | Location | Sample source | Culture-dependent results | | Carba-R assay result[c] |
			Identification[a]	PCR mechanism[b]	
Pediatric	Unit A	Bed 1 sink p-trap	NA	None	Negative
		Bed 1 sink p-trap	NA	None	NR
		Bed 1 sink p-trap	*Acinetobacter junii, Pseudomonas aeruginosa*	Negative	Negative
		Bed 2 sink p-trap	NA	None	Negative
		Bed 2 sink p-trap	NA	None	Negative
		Bed 2 sink p-trap	NA	None	NR
		Diaper scale surface	NA	None	Negative
	Unit B	Pod sink p-trap	NA	None	Negative
		Pod sink p-trap	*Citrobacter freundii, Pseudomonas aeruginosa, Stenotrophomonas maltophilia*	Negative	Negative
		Pod sink p-trap	*Citrobacter freundii, Pseudomonas aeruginosa*	Negative	NR
		Sink drain	*Escherichia coli*	Negative	Negative
		Diaper scale surface	NA	None	Negative
	Formula room	Handwashing sink drain	NA	None	Negative
		Formula sink drain	*Enterobacter asburiae, Klebsiella pneumoniae*	Negative	KPC+
Adult	Unit C	Room A sink p-trap	*Citrobacter freundii, Pseudomonas aeruginosa*	Negative	Negative
		Room A sink p-trap	*Achromobacter xylosoxidans, Citrobacter freundii, Stenotrophomonas maltophilia*	Negative	KPC+
		Room A sink p-trap	*Citrobacter freundii, Pseudomonas aeruginosa*	Negative	Negative
		Room A toilet swab	NA	None	KPC+
	Unit D	Room B toilet swab	NA	None	KPC+
		Room C sink drain	VIM+ *Citrobacter amalonaticus*	VIM+	Negative
		Room C sink drain	*Enterobacter cloacae, Klebsiella oxytoca*	Negative	Negative
		Room C toilet swab	NA	None	Negative
		Room D sink drain	NA	None	Negative
		Room D sink drain	*Achromobacter xylosoxidans, Citrobacter freundii, Pseudomonas monteilii, Klebsiella oxytoca, Klebsiella pneumoniae*	Negative	NR
		Room D toilet swab	NA	None	VIM+
		Hallway sink p-trap	*Pseudomonas aeruginosa*	Negative	Negative
		Hallway sink p-trap	*Klebsiella pneumoniae, Pseudomonas nitroreducens*	Negative	NR
		Hallway sink p-trap	*Citrobacter freundii, Klebsiella pneumoniae, Pseudomonas aeruginosa*	Negative	NR
	Housekeeping	Housekeeping closet drain	KPC+ *Enterobacter gergoviae*	KPC+[d]	KPC+, VIM+, OXA-48-like+
		EVS cart surface	KPC+ *Klebsiella oxytoca* VIM+ *Pseudomonas putida*	KPC+, VIM+	KPC+, VIM+, OXA-48-like+

[a]NA, not applicable (no zones of inhibition of ≤22 mm were found for bacteria in this sample, and therefore, no isolates were forwarded for PCR mechanism testing and identification).

[b]None, no zones of inhibition of ≤22 mm were found for bacteria in this sample, and therefore, no isolates were forwarded for PCR mechanism testing and identification; Negative, zone of inhibition of ≤22 mm was found by culture, but no carbapenemase genes were detected by PCR.

[c]NR, no result (no valid test result was obtained for these samples after two attempts).

[d]Two isolates tested positive for KPC for this sample.

VIM+ (10%), and two were OXA-48-like+ (7%). Water-related biofilms (i.e., sink or floor drains, in-room toilets) were the source of two of the three positive traditional PCR samples (67%) and five of the seven positive Carba-R assay samples (71%).

The Carba-R assay had seven invalid test results (23%) on the first attempt and six invalid test results (20%) on the second attempt, resulting in 24 valid tests for comparison to culture-dependent PCR. For the 24 valid sample results, samples that did not yield suspect isolates by traditional culture methods were considered negative along with those that tested negative by culture-dependent PCR. The overall agreement between the two methods was 75% (18/24) with a positive percent agreement of 67% (2/3) and a negative percent agreement of 76% (16/21) (Table 1).

Both methods identified at least one carbapenemase gene in 2 of 24 specimens (8.3%). Of note, for both these specimens, the Carba-R assay detected more carbapenemase genes than the culture-dependent PCR method. Sixteen out of 24 specimens tested negative by both methods (67%). Of the six (25%) specimens with total disagreement between the methods, culture-dependent PCR detected a carbapenemase

mechanism for only one specimen where the Carba-R assay did not. Conversely, the Carba-R assay detected carbapenemases for five specimens where culture-dependent PCR did not.

This pilot study represents a novel effort to compare performance of traditional culture-dependent methods to a rapid culture-independent technology for primary environmental samples from a health care-associated outbreak. There was 75% agreement between the Carba-R assay and the culture-dependent PCR methods for the primary environmental samples tested, and the Carba-R assay was more sensitive at detecting carbapenemase genes than the culture-dependent PCR approach. Limitations to this study are the small number of positive samples and the indescribable reasons for the differences in detection between the methods. More studies are needed to evaluate whether reduced cultivability of CRO was caused by disinfection residuals or other unknown inhibitors (19). In addition, only a single representative colony of similar phenotype for each suspect CRO from both agars was selected for further testing. Another potential weakness of the culture-based approach is the reduced ability of CHROMagar KPC to detect OXA-48-like and other CROs which may have low MICs to carbapenems (20). Finally, the Carba-R assay is designed to test whole rectal swabs, whereas culture-dependent methods test portions of the sample (19).

There are advantages and limitations to each approach when testing primary environmental samples. While the Carba-R assay requires less than 90 min for set-up and run time, which is far shorter than traditional culture-based methods (3 to 4 days), the cost of the Carba-R assay cartridges is more expensive. However, the Carba-R assay is extremely easy to use and requires little expertise, which reduces the need for technical knowledge and training. The Carba-R assay could quickly identify possible reservoirs and reduce the number of environmental sources requiring further culture-based investigation. However, the Carba-R assay alone cannot identify the organism harboring the carbapenemase gene, while traditional culture-based methods can. The culture method may have an advantage for phenotypic detection of carbapenem resistance not detected by the targeted PCR; supplemental tests (e.g., modified carbapenem inactivation method [mCIM]) may be performed to confirm carbapenemase production on detected CROs to evaluate other possible mechanisms of carbapenem resistance (21). Identification of target organisms is critical for determining the environmental source linked to a particular patient infection or transmission pathway. Currently, the Carba-R assay is validated only for diagnostic testing from pure isolates and rectal swabs. Additionally, our study samples had a 20% invalid test result rate when retested, which is markedly higher than previous studies performed on sputum specimens (22, 23). It is unknown what the effects of residual disinfectants, organic material, and surfactants, such as Tween 80, from the environmental samples have on this specific assay. Previous literature (24, 25) shows that, even at low concentrations, nonionic surfactants and other environmental inhibitors can affect PCR. Finally, the strategies for removing stool-derived inhibitors are likely different from environmental inhibitors.
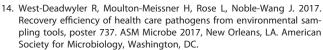
The results of this pilot study provide further evidence that health care indoor premise plumbing and water biofilms can act as a reservoir for CROs which informs interventions (26). The Carba-R assay could prove useful as a strategy for prioritizing environmental samples for further testing by rapidly identifying environmental sources of carbapenemase genes. Further validation with an array of environmental matrices is necessary before the Carba-R assay can be used reliably for this type of testing, but this approach could further our understanding of transmission dynamics and sources of infection. In addition, the rapid detection of CROs in health care environments allows for improved infection control and outbreak responses that are essential for prevention and containment of health care-associated antibiotic-resistant pathogens.

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