Clinical Chemistry 62:2
353–359 (2016)

Analytical Performance of Multiplexed Screening Test for 10 Antibiotic Resistance Genes from Perianal Swab Samples

G. Terrance Walker,[1*] Tony J. Rockweiler,[1] Rossio K. Kersey,[1] Kelly L. Frye,[1] Susan R. Mitchner,[1] Douglas R. Toal,[1] and Julia Quan[1]

BACKGROUND: Multiantibiotic-resistant bacteria pose a threat to patients and place an economic burden on health care systems. Carbapenem-resistant bacilli and extended-spectrum β-lactamase (ESBL) producers drive the need to screen infected and colonized patients for patient management and infection control.

METHODS: We describe a multiplex microfluidic PCR test for perianal swab samples (Acuitas® MDRO Gene Test, OpGen) that detects the vancomycin-resistance gene *vanA* plus hundreds of gene subtypes from the carbapenemase and ESBL families *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona integron-mediated metallo-β-lactamase (VIM), imipenemase metallo-β-lactamase (IMP), OXA-23, OXA-48, OXA-51, CTX-M-1, and CTX-M-2, regardless of the bacterial species harboring the antibiotic resistance.

RESULTS: Analytical test sensitivity per perianal swab is 11–250 CFU of bacteria harboring the antibiotic resistance genes. Test throughput is 182 samples per test run (1820 antibiotic resistance gene family results). We demonstrate reproducible test performance and 100% gene specificity for 265 clinical bacterial organisms harboring a variety of antibiotic resistance genes.

CONCLUSIONS: The Acuitas MDRO Gene Test is a sensitive, specific, and high-throughput test to screen colonized patients and diagnose infections for several antibiotic resistance genes directly from perianal swab samples, regardless of the bacterial species harboring the resistance genes.

Multidrug resistant organisms (MDROs)[2] are a growing concern globally. Every year, >2 million Americans are infected with antibiotic-resistant bacteria, resulting in 23 000 deaths because of a lack of therapeutic options to combat these bacteria *(1)*. Associated annual direct health care costs exceed $20 billion in the US *(1)*. Gram-negative MDROs are especially worrisome, according to the Centers for Disease Control and Prevention (CDC), which reports that carbapenemase-resistant *Enterobacteriaceae* (CRE) have been found in medical facilities in 47 of the 50 states, with major outbreaks occurring in the Northeast, Chicago, and Denver *(2)*.

Because clinical cultures usually identify only a fraction of all patients with CRE, the CDC recommends a range of CRE screening measures to identify carriers and prevent the spread of infection, including (*a*) point prevalence surveys of patients and health care providers in hospitals and long-term care facilities; (*b*) active surveillance testing of prespecified high-risk patients (e.g., intensive care and long-term care); and (*c*) epidemiological linkage of colonized carriers and infected patients *(3)*. Asymptomatic individuals and patients colonized with MDROs are reservoirs for transmission *(4)*. During an outbreak at the NIH Clinical Center, for example, a carbapenem-resistant *Klebsiella pneumoniae* (KPC) strain that affected 18 patients, 11 of whom died, resided undetected in the facility for 3 weeks between patient transmissions *(5)*.

β-Lactamases are the primary threat of antibiotic resistance among gram-negative MDROs, as categorized by penicillinases, extended-spectrum β-lactamases (ESBLs), and carbapenemases, on the basis of degradation of penicillins, cephalosporins, monobactams, and carbapenems. Genes for KPC, New Delhi metallo-β-lactamase (NDM), Verona integron-mediated metallo-β-lactamase (VIM), and imipenemase metallo-β-lactamase (IMP) encode serine and metallo-β-lactamases with carbapenemase and broad-spectrum inhibition of antibiotics. Increased prevalence of carbapenemases has

[1] OpGen, Gaithersburg, MD.
* Address correspondence to this author at: OpGen, 708 Quince Orchard Road, Gaithersburg, MD 20878. E-mail twalker@opgen.com.
Received July 20, 2015; accepted October 19, 2015.
Previously published online at DOI: 10.1373/clinchem.2015.246371

[2] Nonstandard abbreviations: MDRO, multidrug resistant organism; CDC, Centers for Disease Control and Prevention; CRE, carbapenemase-resistant *Enterobacteriaceae*; ESBL, extended-spectrum β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; VIM, Verona integron-mediated metallo-β-lactamase; IMP, imipenemase metallo-β-lactamase.

been identified in gram-negative bacilli, including *Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Serratia marcescens*, *Salmonella enterica*, *Raoultella* spp., and *Proteus mirabilis*. KPC is associated with mortality rates of >50% in the US *(6)*, whereas NDM, VIM, and IMP are associated with mortality rates of 18%–67% *(5)*. The OXA gene family codes a diverse set of β-lactamases against penicillins, cephalosporins, and carbapenems. The CTX-M gene family represents the most common ESBLs among *Enterobacteriaceae* worldwide, with the US recently experiencing a rapid emergence and spread of *K. pneumoniae* and *E. coli* expressing CTX-M *(7)*.

The *vanA* operon encodes Enterococci resistance to vancomycin and teicoplanin in *Enterococcus faecalis*, *E. faecium*, *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. raffinosus*, and *E. avium (8)*. Identifying asymptomatic carriers is essential to controlling vancomycin-resistant Enterococci in hospitals and long-term care facilities.

We describe the Acuitas® MDRO Gene Test (OpGen) for detection of antibiotic resistance gene families (KPC, NDM, VIM, IMP, OXA-23, OXA-48, OXA-51, CTX-M-1, CTX-M-2, and VanA) associated with MDROs including CREs, carbapenem-resistant gram-negative bacilli, and ESBLs, plus vancomycin-resistant Enterococci. The multiplex microfluidic PCR test provides sensitive, specific, and high-throughput analysis of perianal swab samples from infected or colonized patients as an aid to health care, patient management, and infection control in diagnostic or surveillance settings where identification of infected and colonized patients can minimize transmission and reduce outbreaks.

Materials and Methods

SAMPLE EXTRACTION

Perianal swabs were collected with the BD Liquid Amies Elution Swab (ESwab) Collection and Transport System (#480C), a sample collection device that is designed to release bacterial organisms from the swab into 1 mL transport media during sample transport. We extracted total nucleic acids from 500 μL transport media per ESwab with the MagNA Pure 96 DNA and Viral NA Large Volume Kit (P/N 06374891001, Roche) on the MagNA Pure 96 System, which produces 100 μL extracted nucleic acids from each perianal swab sample.

PCR

Nested PCR was performed with outside primers for the first PCR (see Supplemental Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol62/issue2) followed by inside primers and a fluorescent reporter probe for the second PCR (see online Supplemental Table 2). PCR primers and fluorescent reporter probes were designed against homologous sequences within gene families and contained degenerate nucleotides to cover several subtypes per gene family. Fluorescent reporter probes were Applied Biosystems Custom TaqMan® MGB™ Probes with 5′-FAM™ and a 3′ nonfluorescent quencher. All PCRs used dUTP instead of TTP along with uracil-DNA glycosylase before the first multiplex PCR to guard against accidental amplicon contamination. We prepared an internal amplification control (gBlocks Gene Fragment from Integrated DNA Technologies) in 1 μg/mL calf thymus DNA in Tris-EDTA, pH 8 (Fisher BP2473–1), that was added to all samples before PCR to monitor potential PCR inhibition.

We performed the first step of nested PCR as 3 separate multiplex PCRs per perianal swab sample (see online Supplemental Table 1) with the following gene targets per multiplex PCR: (*a*) KPC, NDM, VIM, and the internal amplification control; (*b*) OXA-51, OXA-23, OXA-48, VanA, and IMP; and (*c*) CTX-M-1 and CTX-M-2. Each multiplex PCR (50 μL) contained 10 μL extracted target DNA, 61 nmol/L of each outside PCR primer (see online Supplemental Table 1), and 1× Express qPCR Supermix Universal master mix (11785–01K, Life Technologies). We added the internal amplification control to all samples at 100 molecules per PCR. PCR was performed with the following cycling program: 2 min at 50 °C, 10 min at 95 °C, and 25 cycles of 15 s at 95 °C, 1 min at 61 °C, followed by incubation at 4 °C.

We combined 5-μL aliquots from each of the 3 completed multiplex PCRs per perianal swab sample, along with 6 μL of 4 U/μL *E. coli* exonuclease I in 1× buffer (M0293S, New England Biolabs) for a final volume of 21 μL per perianal swab sample. Exonuclease digestion occurred at 37 °C for 30 min with subsequent exonuclease inactivation at 80 °C for 15 min. Each exonuclease-treated sample was diluted to 75 μL with 10 mmol/L Tris and 1 mmol/L EDTA, pH 7.8. Aliquots (2.6 nL) from each resulting sample served as targets for the second PCR detection step.

The second PCR detection step (see online Supplemental Table 2) was performed with the BioMark HD System and 192.24 Dynamic Array Integrated Fluidic Circuit (Fluidigm), which is a microfluidic array capable of analyzing 192 samples with 24 separate PCR assays. Each PCR on the circuit contained 2.6 nL target from the first PCR step, plus 900 nmol/L of each inside PCR primer, 200–500 nmol/L fluorescent reporter probe (see online Supplemental Table 2), and 1× FastStart Universal Probe Master Mix (Rox) (P/N 04914139001, Roche) with $MgCl_2$ supplemented to an additional 3 mmol/L. PCR was performed with the following cycling program: 10 min at 95 °C and 30 cycles of 15 s at 95 °C, 1 min at 60 °C, followed by incubation at 4 °C.

SENSITIVITY TESTING

We demonstrated clinical analytical sensitivity for each gene family assay by spiking a pool of negative perianal swab samples from human volunteers with mixtures of clinical bacterial organisms (IHMA and ATCC). Perianal swab samples were collected under approval by OpGen's Internal Review Board. We estimated the number of spiked bacterial cells per perianal swab sample by parallel counting of colony forming units (CFU) on culture plates (BD Trypticase™ Soy Agar with 5% Sheep Blood, TSA II™) as follows. We created 0.5 McFarland standards from individual bacterial cultures for each organism and then prepared 10 serial 10-fold dilutions in 1× PBS (BP2438–20, Fisher) from each 0.5 McFarland standard. We used 100 μL from each serial dilution to inoculate blood agar plates with overnight incubation at 37 °C. The number of bacterial colonies per agar plate was used to calculate the number of CFU per mL for each serial dilution, which in turn was used to calculate spiked CFU per perianal swab sample. Only agar plates with 10–300 colonies were used to estimate CFU levels. Each spiked perianal swab sample containing a mixture of bacterial organisms at estimated CFU levels underwent DNA extraction in duplicate and subsequent testing in triplicate. Limits of detection were defined as the lowest CFU level per perianal swab for each bacterial organism where all 6 test replicates were positive for the appropriate gene family assay.

GENE SPECIFICITY TESTING

We tested gene specificity for each gene family assay by use of 265 clinical bacterial organisms (IHMA and ATCC) with a variety of antibiotic-resistance genotypes. We prepared individual 0.5 McFarland standards from individual bacterial cultures per organism and then prepared 3 serial 10-fold dilutions in 1× PBS from each 0.5 McFarland standard. We tested 500 μL from the third serial dilution for each clinical organism, which represented approximately 10^6 CFU per sample. Each spiked sample was extracted and tested in duplicate. Acuitas MDRO Gene Test results were compared with reported genotypes from IHMA and reference PCR results *(9–11)*. Reference PCR results for gene families CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 were also obtained (Beta-Lactamase Detection, Comprehensive Gram-negative Bacteria Panel, Test Code 91334, Quest).

REPRODUCIBILITY TESTING

Analytical intra- and interday reproducibility was demonstrated by use of negative perianal swab samples spiked with mixtures of clinical bacterial organisms harboring β-lactamase genes as described above for sensitivity testing. Each spiked clinical organism was present at approximately 100–10 000 CFU per perianal swab sample as indicated. Each spiked swab sample was prepared in triplicate and tested in duplicate (6 replicates) on each of 3 days, each day by 1 of 2 blinded laboratory technicians.

Table 1. Gene subtypes per gene family assay.

Assay	Gene subtypes
KPC	KPC-1 through -6, -8 through -19, -21, -22, -24
NDM	NDM-1 through -9, -11, -13 through -16
VIM	VIM-1 through -6, -8 through -12, -14 through -19, -23 through -29, -31 through -38
IMP	IMP-1 through -4, -6, -8, -10, -14, -16, -18 through -20, -24 through -26, -30, -32 through -34, -38, -40, -42
OXA-51	OXA-51, -64 through -70, -76 through -78, -80, -82 through -84, -86 through -95, -98 through -100, -106 through -111, -113, -115, -120, -128, -130, -132, -138, -144, -148 through -150, -174 through -180, -194 through -197, -201, -202, -206, -208, -216, -217, -219, -223, -242, -248, -249, -254
OXA-23	OXA-23, -49, -73, -146, -165, -167 through -171, -225, -239
OXA-48	OXA-48, -162, -163, -181, -199, -204, -232, -244, -245, -247, -370
CTX-M-1	CTX-M-1, -3, -11, -12, -15, -22, -28, -32, -33, -36, -42, -52, -54, -55, -57, -58, -60, -61, -64, -69, -71, -72, -79, -82, -88, -96, -101, -103, -107, -108, -109, -114, -117, -123, -132, -136
CTX-M-2	CTX-M-2, -5, -20, -31, -43, -44, -56, -59, -76, -77, -92, -95, -97, -131
VanA	*vanA*

Results

The Acuitas MDRO Gene Test was designed to detect the vancomycin-resistance gene *vanA* plus hundreds of gene subtypes from the carbapenemase and ESBL families KPC, NDM, VIM, IMP, OXA-23, OXA-48, OXA-51, CTX-M-1, and CTX-M-2, regardless of the bacterial species harboring the antibiotic resistance. Table 1 indicates the antibiotic resistance gene subtypes for which each gene family assay was designed. PCR primers and probes are perfect matches for most reported subtypes for the gene families KPC, NDM, VIM, IMP, OXA-23, OXA-48, OXA-51, CTX-M-1, and CTX-M-2 as described in Table 1. The test was not designed to distinguish subtypes within the gene families KPC, NDM, VIM, IMP, OXA-23, OXA-48, OXA-51, CTX-M-1, and CTX-M-2.

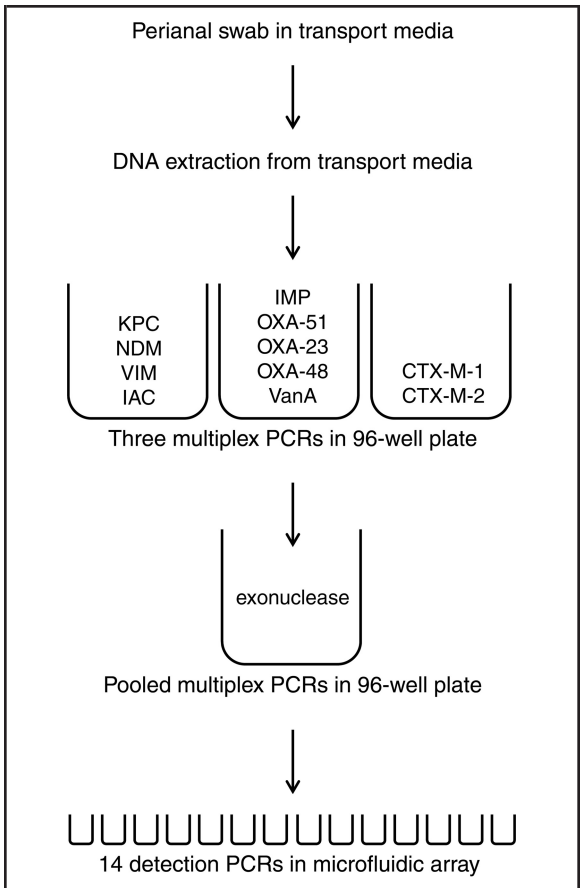

Fig. 1. Test workflow per sample.

DNA is extracted from bacterial cells in transport media of a perianal swab sample. Three separate multiplex PCRs for the indicated gene families are performed per extracted DNA sample in 3 wells of a 96-well PCR plate. Multiplex PCRs are pooled and treated with exonuclease followed by 14 detection PCRs per exonuclease-treated sample in the microfluidic array. IAC, internal amplification control.

The test uses nested PCR with real-time PCR detection in a microfluidic array. The first PCR step was performed as 3 separate multiplex PCRs per perianal swab sample (Fig. 1) with the following gene targets per multiplex PCR: (*a*) KPC, NDM, VIM, and the internal amplification control; (*b*) OXA-51, OXA-23, OXA-48, VanA, and IMP; and (*c*) CTX-M-1 and CTX-M-2. The 3 completed PCR samples were pooled and treated with exonuclease to remove primers, with the resulting sample serving as target for a series of real-time detection PCRs on the 192.24 Dynamic Array Integrated Fluidic Circuit, which is a microfluidic array capable of analyzing 192 samples with 24 separate PCR assays. The test was designed for operation in a central testing laboratory receiving hundreds of samples per day, analyzing as many as 182 patient samples in a single laboratory shift.

Table 2. Analytical sensitivity.

Assay	Spiked clinical bacterial organism (gene subtype)	Limit-of-detection (CFU per perianal swab specimen)
KPC	*E. cloacae* (KPC-3)	84
NDM	*K. pneumoniae* (NDM-1)	93
VIM	*S. marcescens* (VIM-4)	154
	P. aeruginosa (VIM-2)	37
	E. cloacae (VIM-5)	154
IMP	*K. pneumoniae* (IMP-1)	13
	K. pneumoniae (IMP-8)	66
OXA-51	*A. baumannii* (OXA-51)	11
OXA-23	*A. baumannii* (OXA-23)	109
OXA-48	*K. pneumoniae* (OXA-48)	79
CTX-M-1	*K. pneumoniae* (CTX-M-28)	79
CTX-M-2	*K. pneumoniae* (CTX-M-2)	151
VanA	*E. faecium* (*vanA*)	250

TEST SENSITIVITY

We determined clinical analytical sensitivity of the test in our CLIA-certified laboratory by collecting negative perianal swab samples from human volunteers and quantitatively spiking the samples with clinical bacterial organisms harboring β-lactamase genes (e.g., *E. cloacae* with gene subtype KPC-3) as shown in Table 2. Spiked levels of the clinical organisms were determined by parallel counting of bacterial CFU on agar culture plates. The test exhibited limits of detection of 11–250 CFU per perianal swab sample across the gene family assays (Table 2). Because only 5% of the anal swab sample was analyzed in the test, the corresponding PCR sensitivity was 0.6–13 CFU per PCR, where detection of <1 CFU reflects detection of dead bacteria plus target antibiotic resistance genes carried on multiple plasmid copies per bacterial cell. The test exhibited analytical sensitivity of approximately 10 target molecules per PCR when evaluated by use of synthetic PCR targets (gBlocks Gene Fragment, Integrated DNA Technologies) (data not shown).

GENE SPECIFICITY

We demonstrated 100% gene specificity for the test with 265 clinical bacterial organisms with a variety of antibiotic-resistance genes, including various subtypes for gene families KPC, NDM, VIM, IMP, OXA-23, OXA-48, OXA-51, CTX-M-1, and CTX-M-2. The specificity panel included common clinical enteric species (*E. coli*, *K. pneumoniae*, *K. oxytoca*, *C. freundii*, *E. cloacae*, *S. marcescens*, *P. aeruginosa*, *A. baumannii*, *E. faecium*, *E. avium*, *E. canintestini*, and *E. raffinosus*). The test correctly detected all gene subtypes for which it was designed (Table 1) and did not yield false positives for

Table 3. Analytical reproducibility.

	Range of positive cycle threshold values across 6 replicates								
	Day 1			Day 2			Day 3		
Assay	10,000 CFU per swab	1000 CFU per swab	100 CFU per swab	10,000 CFU per swab	1000 CFU per swab	100 CFU per swab	10,000 CFU per swab	1000 CFU per swab	100 CFU per swab
KPC	5–6	9	12–13	5–6	8–9	12–13	5–6	8–9	11–13
NDM	6–7	9–12	12–14	5–6	7–9	11–12	6–7	9–10	11–12
VIM-1	7–8	11–12	14–15	7	10–11	13–14	7	10–11	14–15
VIM-2	6–8	11–12	13–15	6–7	10–11	13–14	7	10–11	14–15
VIM-5	4–5	7–9	9–11	4–5	6–7	9–10	4	6	8–9
IMP-1	5–7	9–10	12–14	5–6	8–10	12–14	6–7	9–10	12–13
IMP-2	8–10	12–14	15–16	8–9	11–12	14–16	8–9	11–12	15–16
OXA-51	6–7	10–11	13–14	6–7	8–10	12–13	5–6	8–10	12–13
OXA-23	4–5	6–9	9–11	5–6	6–8	10–11	4–5	6–7	9–10
OXA-48	6–8	10	13–14	5–6	9–10	12–14	6–7	9–10	12–13
CTX-M-1	6–8	10	13–14	6–7	9–10	13–14	6–7	10	13–14
CTX-M-2	5–6	8–9	11–12	5–6	8–9	11–12	5–6	8–9	12
VanA	10–12	14–16	17–20[a]	9–10	12–14	14–19[a]	10–11	13–15	17–19[a]

[a] One of 6 replicates was negative

isolates lacking target genes or harboring additional antibiotic-resistance genes for which the test was not designed (e.g., OXA-24, OXA-72, CTX-M-8, CTX-M-9, CTX-M-14, CTX-M-27, CTX-M-65, SHV, TEM, ACT, MIR, CMY, DHA, ACT, and VEB). The test was negative for 99 clinical organisms lacking reported antibiotic-resistance genes. The presence and absence of the *vanA* gene in 33 vancomycin-resistant and 24 vancomycin-sensitive Enterococci were also correctly detected. Gene specificity results are shown in online Supplemental Table 3 for a representative set of 265 clinical organisms with a variety of antibiotic-resistance genes.

TEST REPRODUCIBILITY

We demonstrated intra- and intertest reproducibility over 3 days by use of negative perianal swab samples quantitatively spiked with mixtures of clinical bacterial organisms harboring target resistance genes. Swab samples were spiked with mixtures of bacterial organisms from Table 2 at approximately 100, 1000, or 10 000 CFU per spiked sample for each organism. Each spiked swab sample was prepared in triplicate and tested in duplicate (6 replicates) on each of 3 days. The range of PCR cycle threshold values across the 6 replicates is shown in Table 3 for each gene family assay over 3 days of testing. The test exhibited reproducible and quantitative results for all 10 antibiotic resistance genes across a range of target levels, although the test was not designed to be quantitative in clinical practice, because of the nonquantitative nature of sample collection with perianal swabs.

The VanA assay exhibited poorer sensitivity and reproducibility compared with the other assays (Tables 2 and 4). For instance, negative VanA results were obtained for 1 of 6 replicates on each day of reproducibility testing at the lowest spiked level of approximately 100 CFU per swab sample (Table 3), which is below the assay's limit of detection of 250 CFU per sample reported in Table 1. The VanA assay was evaluated by use of perianal swab samples spiked with the gram-positive organism *E. faecium*, whereas the other assays were evaluated via spiking with gram-negative organisms (Table 2). The VanA assay was found to be as sensitive and reproducible as the other assays when evaluated by use of synthetic PCR targets (gBlocks Gene Fragments, Integrated DNA Technologies) (data not shown). Assay performance would be expected to be influenced by the efficiency of sample extraction for gram-positive vs -negative and/or target gene copy numbers. *VanA* tends to be a single-copy chromosomal gene, whereas β-lactamase genes are typically carried on plasmids at approximately 2–10 copies per bacterial cell.

Discussion

We have described a sensitive and high-throughput multiplex molecular diagnostic test that is provided through

OpGen's CLIA testing laboratory as a tool for screening colonized patients and diagnosing infections for several antibiotic resistance genes directly from perianal swab samples, regardless of the bacterial species harboring the resistance gene. The test was found to provide exquisite sensitivity for asymptomatic colonized individuals and broad gene coverage for carbapenem-resistant gram-negative bacilli, cephalosporin-resistant (third- or fourth-generation) gram-negative bacilli, and vancomycin-resistant Enterococci that commonly colonize the enteric tract. The sensitivity of the PCR test matched or exceeded microbiology methods exemplified by the benchmark agar culture plate protocol described in this study. Practical clinical sensitivity will be limited by sample collection that uses perianal swabs.

The test detected the most prevalent and resistant carbapenemases in *Enterobacteriaceae* and *Acinetobacter baumannii* (KPC, NDM, VIM, IMP, OXA-48, and OXA-23) *(11, 12)* plus the 2 most common gene families of ESBLs (CTX-M-1 and CTX-M-2) *(13)*, along with the vancomycin resistance gene *vanA*, which is prevalent across clinical strains of *E. faecalis* and *E. faecium*. The test also detected the vast majority of subtypes for the gene families KPC, NDM, VIM, IMP, OXA-23, OXA-48, OXA-51, CTX-M-1, and CTX-M-2 as curated by databases such as the Lahey Clinic (http://www.lahey.org/studies/other.asp#Table1). The test thereby provides broad multiplex coverage for the most common and critical carbapenemase and ESBL genes from clinical gram-negative bacilli across the world while maintaining essential detection sensitivity. Future versions of the test may include additional albeit less prevalent ESBL genes (e.g., CTX-M-9) plus cephalosporinase and AmpC β-lactamase genes for broader coverage of antibiotic-resistant gram-negative bacilli.

The test was designed for high-throughput screening in a central testing laboratory receiving hundreds of samples per day. Results from 182 patient samples (1820 reported gene tests results) were obtained with high sensitivity in a single laboratory shift to meet the clinical demands of molecular diagnostic screening for bacterial colonization of perianal swab samples.

MDROs in hospitals and long-term care facilities pose a serious threat to patient health and a challenge to patient management. According to the CDC, antibiotic-resistant bacteria, especially CRE, are among the most serious health threats to the US population, leading to death in ≤50% of patients who become infected *(1)*. Recent emergence of dangerous carbapenem-resistant bacilli and ESBL producers with transmissible plasmids has accelerated the need for the development of new methods to diagnose infected patients, screen high-risk individuals, and track infection outbreaks.

A report from the Agency for Healthcare Research and Quality suggests that active screening programs can effectively control MDRO prevalence when they identify colonized patients and place them into contact isolation precautions *(14)*. Further, traditional infection-control strategies that only target monitoring of clinical isolates as a trigger for initiating control interventions have not proved effective for KPC control, and are only addressing the tip of the iceberg, since there are about 100 colonized patients for every infected patient *(14)*.

Clinical evidence suggests that asymptomatic individuals and patients colonized with MDROs are especially problematic. One study found that healthy patients who tested positive for carbapenem-resistant *A. baumannii* cultures had 8.4 times the risk of developing a subsequent infection than patients who remained negative on surveillance cultures *(15)*. In another study, 32 of 433 patients (7.4%) with carbapenem-resistant *K. pneumoniae* developed an infection within 2–40 days from colonization to infection *(16)*.

The CDC recommends that providers use a range of CRE screening measures to identify carriers and prevent the spread of infection *(1)*. Current clinical methods, such as microbe detection on the basis of growing cells in a laboratory culture, a process that can take up to 4 days, may identify only a fraction of all patients with CREs. Investigators in a study of 36 patients with CRE bloodstream infections reported that time to treatment had a significant effect on the course of infection, with rectal screening leading to earlier recognition and prompt empirical treatment *(17)*.

The Acuitas MDRO Gene Test (OpGen) represents a sensitive and high-throughput tool to assist patient management in health care settings through surveillance testing or diagnosis of actively infected patients, with excellent sensitivity and gene coverage for the most common carbapenemase and ESBL genes.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: G.T. Walker, OpGen, Inc.
Consultant or Advisory Role: D.R. Toal, OpGen, Inc.
Stock Ownership: G.T. Walker, OpGen, Inc.; D.R. Toal, OpGen, Inc.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: G.T. Walker, 61/952795; T.J. Rockweiler, OPGN-014/001US.

Role of Sponsor: No sponsor was declared.

References

1. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013. http://www.cdc.gov/drugresistance/threat-report-2013/index.html. (Accessed November 2014).
2. Singh K, Mangold KA, Wyant K, Schora DM, Voss B, Kaul KL et al. Rectal screening for Klebsiella pneumoniae carbapenemases: comparison of real-time PCR and culture using two selective screening agar plates. J Clin Microbiol 2012;50:2596–600.
3. Centers for Disease Control and Prevention. 2012 CRE Toolkit–Guidance for control of carbapenem-resistant Enterobacteriaceae (CRE). http://www.cdc.gov/hai/organisms/cre/cre-toolkit/index.html. (Accessed November 2014).
4. Calfee D, Jenkins SG. Use of active surveillance cultures to detect asymptomatic colonization with carbapenem-resistant Klebsiella pneumoniae in intensive care unit patients. Infect Control Hosp Epidemiol 2008;29:966–8.
5. Snitkin ES, Zilazny AM, Thomas PJ, Stock F, NISC Comparative Sequencing Program, Henderson DK et al. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. Sci Transl Med 2012;4:148ra116.
6. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2011;17:1791–8.
7. Wang G, Huang T, Surendraiah PK, Wang K, Komal R, Zhuge J et al. CTX-M beta-lactamase-producing *Klebsiella pneumoniae* in suburban New York City, New York, USA. Emerg Infect Dis 2013;19:1803–10.
8. Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob Agents Chemother 1993;37:2311–7.
9. Mostachio A.K., van der Heidjen I, Rossi F, Levin AS, Costa SF. Multiplex PCR for rapid detection of genes encoding oxacillinases and metallo-beta-lactamases in carbapenem-resistant Acinetobacter spp. J Med Microbiol 2009;58:1522–4.
10. van der Zee, A, Roorda L, Bosman G, Fluit AC, Hermans M, Smits PHM et al. Multi-centre evaluation of real-time multiplex PCR for detection of carbapenemase genes OXA-48, VIM, IMP, NDM and KPC. BMC Infect Dis 2009;14:27.
11. Böckelmann U, Dörries HH, Ayuso-Gabella MN, Salgot de Marçay M, Tandoi V, Levantesi C, Masciopinto C et al. Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. Appl Environ Microbiol 2009;75:154–63.
12. Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP. Epidemiology of carbapenemase-producing *Enterobacteriaceae* and *Acinetobacter baumannii* in Mediterranean countries. Biomed Res Int 2014;2014:305784.
13. Woerther P-L, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev 2013;26:744–58.
14. Parker VA, Logan CK, Currie B (Boston University School of Public Health and Montefiore Medical Center). Carbapenem-resistant *Enterobacteriaceae* (CRE) control and prevention toolkit. Rockville (MD): Agency for Healthcare Research and Quality (AHRQ); 2014 Apr. Contract No. 290-2006-0012-I. AHRQ Publication No. 14-0028. Available from: http://www.ahrq.gov/professionals/quality-patient-safety/patient-safety-resources/resources/cretoolkit/index.html
15. Latibeaudiere R, Rosa R, Laowansiri P, Arheart K, Namias N, Munoz-Price LS. Surveillance cultures growing carbapenem-resistant *Acinetobacter baumannii* predict the development of clinical infections: a cohort study. Clin Infect Dis 2015;60:415–22.
16. Borer A, Eskira S, Nativ R, Saidel-Odes L, Riesenberg K, Livshiz-Riven I et al. A multifaceted intervention strategy for eradication of a hospital-wide outbreak caused by carbapenem-resistant *Klebsiella pneumoniae* in Southern Israel. Infect Control Hosp Epidemiol 2011;32:1158–65.
17. Balkan II, Aygün G, Aydın S, Mutcal SI, Kara Z, Kuşkucu M et al. Blood stream infections due to OXA-48-like carbapenemase-producing *Enterobacteriaceae*: treatment and survival. Int J Infect Dis 2014;26:51–6.