Multiple Broad-Spectrum Beta-Lactamase Targets for Comprehensive Surveillance

Kathy A. Mangold,^{a,b} Barbara L. Voss,^a Kamaljit Singh,^c Richard B. Thomson, Jr.,^{a,b} Donna M. Schora,^a Lance R. Peterson,^{a,b} Karen L. Kaul^{a,b}

Pathology, NorthShore University HealthSystem, Evanston, Illinois, USA^a; Pathology, Pritzker School of Medicine, University of Chicago, Chicago, Illinois, USA^b; Infectious Disease, Rush University Medical Center, Chicago, Illinois, USA^c

Real-time PCR testing for *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{CTX-M} was performed on rectal swabs obtained from residents of two long-term acute-care facilities. While *bla*_{KPC} was detected in 69/102 swabs (67.6%), testing for four other targets increased the positivity rate for a broad-spectrum β -lactamase to 73.5% (McNemar's $P = 0.03$).

Increased resistance to multiple antimicrobial agents is of growing concern worldwide and is now considered the greatest threat to human health (1). There is increasing concern about controlling spread in health care systems, where the majority of resistance emerges or disseminates. The control process is multifaceted and includes (i) screening and isolation, (ii) environmental disinfection, (iii) antibiotic stewardship, (iv) rapid diagnosis and detection of resistance, (v) hand hygiene, (vi) vaccination, (vii) education, (viii) research, (ix) communication and reporting of critical pathogens, and (x) outbreak tracking of emerging infections. Effectively dealing with these 10 key infection control functions involves the laboratory in at least six components, namely, i, iii, iv, viii, ix, and x. The use of molecular methods can rapidly identify patients harboring resistant pathogens as a critical first step in controlling the spread of multidrug-resistant organisms (MDROs), followed by proper patient isolation. Of particular concern is the frequent transfer of residents who are colonized with MDROs from long-term-care facilities to hospitals. Recently, we identified *Klebsiella pneumoniae* carbapenemase (KPC)-containing *Enterobacteriaceae*, currently referred to as carbapenem-resistant *Enterobacteriaceae* (CRE) (2), in two-thirds of residents from two Chicago-area long-term acute-care facilities, but there was also culture evidence of resistant bacteria that were not KPC positive (3). By using bacterial lysis that remained after initial testing for *bla*_{KPC}, real-time PCR testing for other β -lactamase genes was performed on these specimens to fully characterize the prevalence of MDROs in this select high-risk population.

Sites, patient selection, and collection of surveillance specimens. A single point prevalence study of *bla*_{KPC} rectal carriage among patients was conducted on separate days (one facility per day), 6 June and 22 June 2011, with no repeat sampling; details of the methods used were reported by Singh et al. (3). Rectal specimens were collected using premoistened double-headed rayon swabs (BBL CultureSwab; Becton, Dickinson, Sparks, MD) and transported in liquid Amies medium to the microbiology laboratory for processing. Samples were deidentified before the double-headed swabs were separated, with one sent to NorthShore University HealthSystem (NorthShore), Evanston, IL, for real-time PCR testing.

Specimen preparation. The swab was placed into a 5 ml tryptic soy broth (Remel, Lenexa, KS) containing one 30- μ g ceftazidime disk (BD Sensi-Disk; Becton, Dickinson) for overnight incubation at 35°C in ambient air. Following incubation, 250 μ l of the enrich-

ment broth was added to 200 μ l of lysis buffer (1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl [pH 8.0], and 1 mM EDTA), heated for 10 min at 99°C, and then microcentrifuged.

Real-time PCR. After the analysis for *bla*_{KPC} was completed (3), the remaining lysis supernatant from the original *bla*_{KPC} point prevalence specimens was tested for antibiotic resistance genes *bla*_{CTX-M}, *bla*_{NDM}, *bla*_{IMP}, and *bla*_{VIM} to determine if any of these were responsible for the observed non-KPC resistance (3). Two microliters of the original lysis supernatant per reaction was used for real-time PCR performed on a LightCycler 1.5 or 2.0 Instrument (Roche Diagnostics, Indianapolis, IN). The primers, probes, and PCR parameters are listed in Table 1. The assay for *bla*_{KPC} was previously published by our laboratory (4); all other assays for this study were designed and validated in our laboratory except for that for *bla*_{IMP} and *bla*_{VIM}, which was modified from the method of Bisiklis et al. to accommodate a SYBR green assay (5). Validation of each assay included the detection of isolates known to contain the antibiotic resistance gene in a blind analysis with negative specimens, the isolation of Gram-negative bacilli from enrichment broths that phenotypically matched the appropriate antibiotic resistance profile, and, in the case of *bla*_{CTX-M}, the sequencing of amplicons to determine the specificity of the CTX-M group 1 assay versus the CTX-M global assay.

Statistics. McNemar's test was used to compare paired proportions between testing for one target and testing for multiple targets.

Real-time PCR testing was negative for the genes *bla*_{NDM} and *bla*_{IMP}. As previously reported, *bla*_{KPC} was detected in 69/102 (67.6%) of the specimens (3). Testing for *bla*_{CTX-M} was positive in 31/102 (30.4%) of the specimens, and 22 of these 31 (70.1%) were further classified as *bla*_{CTX-M} group 1 in a separate real-time PCR assay; the most common member of this group found in North American patient populations is CTX-M-15 (6). All but five *bla*_{CTX-M}-positive specimens (16%) were also *bla*_{KPC} positive.

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Address correspondence to Kathy A. Mangold, KMangold@northshore.org

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TABLE 1 Real-time PCR assay parameters, including primer and probe sequences, used in this study

Assay	PCR parameters	Oligonucleotide name	Oligonucleotide sequence ^d
16S internal control for TaqMan assays ^a	Included with other targets; see below	16S792U22	GTA GTC CAC GCY GTA AAC GAT G
		16S1062L22	AAC CCA ACA TYT CAC RAC ACG A
		16STAQ2	Texas Red-CGA WGC AAC GCG AAG AAC CTT ACC T-BBQ
KPC TaqMan ^a	45 cycles of 10 s at 95°C, 30 s at 58°C, 20 s at 72°C	KPC294L20	ACC ACG GAA CCA GCG CAT TT
		KPC78U20	CAA CCT CGT CGC GGA ACC AT
		KPC220TAQ22	6FAM-TTT CTT GCT GCC GCT GTG CTG G-BBQ
NDM TaqMan ^b	45 cycles of 10 s at 95°C, 30 s at 58°C, 20 s at 72°C	NDM1-228R22	TGG TTC GAC AAC GCA TTG GCA T
		NDM1-96F20	ACC AGA CCG CCC AGA TCC TC
		NDM1TAQ	6FAM-TCG CAA TCC CCG CCG CAT-TMR
CTX-M group 1 TaqMan ^b	45 cycles of 10 s at 95°C, 30 s at 60°C, 20 s at 72°C	CTXM15-907F20	GAT AAA ACT GGC AGC GGT GG
		CTXM15-967PRB	6FAM-ATC GTG CGC CGC TGA TTC TGG TCA C-BBQ
		CTXM15-994R20	GGT TGA GGC TGG GTG AAG TA
CTX-M global TaqMan ^b	45 cycles of 10 s at 95°C, 30 s at 60°C, 20 s at 72°C	CTX-MgblFwd	CAA TGT GCA GYA CCA GTA ARG T
		CTX-MgblRev2a	GCG GRT CGC CYG GAA TG
		CTXMgrp1prb2R	6FAM-AGC TCA GCC AGT GAC ATC GTC CCA T-BBQ
		CTXMgrp2prb2	6FAM-AAA CAG AGC GAG AGC GAT AAG CAC CT-BBQ
		CTXMgrp9prb3	6FAM-CTG GAT CGC ACT GAA CCT ACG C-BBQ
IMP/VIM SYBR green ^c	40 cycles of 10 s at 95°C, 7 s at 48°C, 15 s at 72°C	IMP-as2	CAA ACC ACT ACG TTA TCT KGA GTG T
		IMP-fw	AAG TTA GTC AMT TGG TTT GTG GAG C
		VIM-fw	GTAC GCA TCA CCG TCG ACA C
		VIMspec1-re	TGA CGG GAC GTA TAC AAC CAG A
		VIMspec2-re	AGA CGG GAC GTA CAC AAC TAA G

^a Taken from Mangold et al. (4).^b Described in the present paper.^c Modified from the method of Bisiklis et al. (5).^d 6FAM, 6-carboxyfluorescein; TMR, TAMRA (tetramethylrhodamine) quencher; BBQ, Blackberry quencher.

Four specimens were positive for *bla*_{VIM}—one was only *bla*_{VIM} positive, two were *bla*_{VIM} and *bla*_{KPC} positive, and one was positive for *bla*_{VIM}, *bla*_{KPC}, and *bla*_{CTX-M}. Therefore, the number of specimens positive for at least one broad-spectrum β -lactamase increased significantly to 75/102 (73.5%; McNemar's $P = 0.03$), as indicated in Fig. 1. Since August 2009, we have done active surveillance testing at all four NorthShore intensive care units (ICUs) and continually detect the presence of MDROs in admitted patients. Figure 2 depicts the number of patients found since that time who were placed immediately under appropriate isolation precautions once an MDRO was found. We continually monitor for any organism transmission using contact tracing and pulsed-field gel electrophoresis; to date, only one instance of transmission to an existing patient has been detected during nearly 4 years of persistent transmission risk.

This study has some limitations, including small sample size. Additionally, ceftazidime was chosen as the antibiotic included in

the broth incubation to enrich for CRE, such as those harboring *bla*_{KPC}, the primary target of the point prevalence study. This antibiotic choice may limit the growth of bacteria with extended-spectrum β -lactamases (ESBLs) like *bla*_{CTX-M}, resulting in a possible underestimation of ESBL carriage in this study. Therefore, substituting ceftriaxone for ceftazidime could enhance the recovery of *bla*_{CTX-M} in future surveillance testing. Preliminary testing of this change in our current surveillance protocol yields more bacterial growth in the overnight enrichment broth, which in turn has resulted in increased background fluorescence observed in the SYBR green assay for *bla*_{IMP} and *bla*_{VIM}. To counteract this complication, we are now validating the probe-based assay as originally published by Bisiklis et al. (5).

Five separate assays were performed for each specimen in this study. By altering the fluorescent dyes attached to several probes, a multiplex real-time PCR assay could target *bla*_{KPC}, *bla*_{NDM}, and *bla*_{CTX-M}, as well as the 16S internal control in a single reaction (data not shown), reducing both the reagent costs and labor required to test for these targets. The assay for *bla*_{IMP} and *bla*_{VIM}, regardless of whether it is performed as a SYBR green assay or as a probe assay, cannot be multiplexed with the others due to distinctly different PCR parameters. Thus, the surveillance protocol as described is possible when using only two real-time PCR assays per sample.

The goal of surveillance testing is to identify patients with MDROs to facilitate early isolation. Our initial investigation found that real-time PCR is significantly more sensitive and faster than culture (3), suggesting that PCR currently is the optimal assay for MDRO surveillance. We have demonstrated that multiple ESBLs can be detected by using PCR as part of a comprehensive infection control program. In conclusion, we demonstrated

FIG 1 Venn diagram of the distribution of resistance genes identified during routine surveillance of 102 residents at two Chicago area long-term acute-care hospitals.

FIG 2 Time series plot of MDROs detected in four ICUs since the deployment of active surveillance testing in August 2009. Surveillance included culture for carbapenem-resistant *Pseudomonas aeruginosa* or *Acinetobacter baumannii* and PCR testing for critical β -lactamase gene targets. The y axis indicates the number of ICU patients identified with detectable MDROs.

that MDROs often harbor multiple resistance genes and when detection of multiple MDRO gene targets is indicated, specific molecular testing can be utilized as multiple individual PCR assays or as one or two multiplexed assays.

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