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Evaluation of the Check-Points Check MDR CT103 and CT103 XL Microarray Kits by Use of Preparatory Rapid Cell Lysis

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Using a rapid bacterial lysis method, the Check MDR CT103 and CT103 XL microarrays demonstrated accuracies of 98.1% and 94.2%, respectively, for detection of known resistance genes in 108 multidrug-resistant Gram-negative bacilli. In 45 isolates, 49 previously unrecognized extended-spectrum β-lactamase or plasmid AmpC targets were detected and confirmed by conventional PCR.

Multidrug-resistant *Enterobacteriaceae* (MDRE), especially those producing carbapenemases, are of concern to physicians and laboratorians worldwide. MDRE have acquired resistance through plasmid transfer, with or without chromosomal mutation (1). The ability of clinical laboratories to rapidly detect and characterize MDRE is a sentinel defense against these threatening pathogens. Current laboratory tools directed at MDRE and their associated resistances are largely focused on phenotypic methods, such as selective indicator media (e.g., commercial chromogenic media), antimicrobial susceptibility testing (AST) systems to assess MICs, and confirmatory tests for enzyme expression (e.g., modified Hodge test, Carba NP test) (2–5).

A multitude of PCR assays have been described for detecting resistance genes in isolated bacteria (6–10). Sensitivity and specificity are generally excellent; however, such assays typically target a relatively small number of β-lactamase genes based largely on prevalence. MDRE may carry one or more plasmids and may simultaneously harbor multiple antimicrobial resistance genes and resistance mechanisms that affect the same antimicrobial agents (11). Given this, a comprehensive approach to detect resistance genes may be appropriate for MDRE. Studies employing microarray-based molecular tests that target a large number of resistance genes suggest that such platforms may serve this role (5, 12–15). Cuzon et al. evaluated the Check-Points Check MDR CT103 (Check-Points Health B.V., Wageningen, The Netherlands) microarray kit by using 187 clinical isolates and reported excellent specificity (16). This kit can detect 6 AmpC genes/gene groups (CMY-I/MOX, ACC, DHA, ACT/MIR, CMY-II, FOX), 5 carbapenemase genes/gene groups (KPC, NDM, VIM, IMP, OXA-48-like), and genes encoding CTX-M-1, -2, -8, -9, and -25 groups and can detect and distinguish wild-type bla_{SHV} and bla_{TEM} alleles from those with point mutations conferring extended-spectrum β-lactamase (ESBL) production (16).

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We evaluated the Check MDR CT103 kit and the expanded CT103 XL kit (Check-Points Health B.V.) following the manufacturer's suggested protocol but substituting a previously described time- and reagent-saving rapid bacterial lysis step (6) for DNA preparation. The CT103 XL kit contains additional targets beyond those included in the CT103 kit, including carbapenemases most commonly found in nonfermenting Gram-negative bacilli rather than *Enterobacteriaceae*, specifically GES, GIM, SPM, OXA-23-like, OXA-24/40-like, and OXA-58-like, as well as the infrequently encountered ESBLs VEB, PER, BEL, and GES-type.

As the evaluation panel, 108 isolates, including 107 isolates of *Enterobacteriaceae* and 1 *Pseudomonas aeruginosa* isolate, were studied. Isolate resistance mechanisms were previously determined with a variety of phenotypic and genotypic methods at Mayo Clinic (Rochester, MN) ($n = 21$), University of Minnesota (Minneapolis, MN) ($n = 75$), Rush University Medical Center (Chicago, IL) ($n = 11$), or Loyola University Medical Center (Maywood, IL) ($n = 1$) (17–24). Genes included in the collection consisted of 35 $bla_{CTX\text{-}M\text{-}1}$ group genes, 16 $bla_{CTX\text{-}M\text{-}9}$ group genes, 21 bla_{SHV}, 30 bla_{TEM}, 9 $bla_{CMY\text{-}II}$, 1 bla_{FOX}, 1 bla_{VEB}, 6 bla_{KPC}, 1 bla_{VIM}, and 1 bla_{NDM}, with 17 isolates possessing more than one resistance gene. Two isolates that had originally been shown to harbor bla_{SHV} or bla_{KPC} presumably lost plasmids during cryopreservation, as they were PCR negative for these genes; they were included as negative controls. Discordant results were resolved with additional conventional PCR testing (25, 26). The CT103 or CT103 XL was repeated at least once if falsely negative results were obtained (i.e., discordant with the known genotype of the isolate).

Overall, compared to the known genotypes, the CT103 and CT103 XL kits detected 97.1% (102/105) and 94.2% (97/103) of the genes that they were designed to detect (Table 1). The denominator for assessing the CT103 kit was 105, as this excluded the two negative controls and bla_{VEB}, which is not included on the panel. The denominator for assessing the CT103 XL kit was 103, as this excluded the negative controls and three isolates that failed to

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TABLE 1 Distribution of resistance targets identified by CT103 and CT103 XL according to species

Species (no. of isolates)[a]	Resistance gene target	No. of resistance gene variants						
		Total no. of targets[b]	Detected by CT103	Additionally detected by CT103[c]	Missed by CT103 (false negative)	Detected by CT103 XL	Additionally detected by CT103 XL[c]	Missed by CT103 XL (false negative)
Escherichia coli (93)	CTX-M-1 group[d]	36	36	2[e]	0	36	2[e]	0
	CTX-M-9 group[d]	19	17	3	2[e]	18	3	1[e]
	SHV[f]	19	18	5	1[g]	18	5	1[g]
	TEM[h]	58	57	29	1[i]	53	29	5[i]
	Plasmid-borne AmpC[j]	13	13	3	0	12[k]	3	0[k]
	VEB	1	NA[l]	NA	NA	1	0	0
	NDM	1	1	0	0	1	0	0
	KPC	1	1	0	0	1	0	0
Klebsiella pneumoniae complex (8)	TEM[m]	3	3	1	0	3	1	0
	SHV[n]	8	8	1	0	6[o]	1	0[o]
	CTX-M-1 group[p]	1	1	0	0	1	0	0
Citrobacter koseri (1)	TEM[q]	1	1	1	0	1	1	0
	KPC	1	1	0	0	1	0	0
Enterobacter aerogenes (1)	KPC	1	1	0	0	1	0	0
	SHV[g]	1	0	0	1	1	1	0
	TEM[q]	1	0	0	1	1	1	0
Enterobacter cloacae complex (1)	KPC	1	1	0	0	1	0	0
Proteus mirabilis (1)	KPC	1	1	0	0	1	0	0
Providencia stuartii (1)	KPC	1	1	0	0	1	0	0
Serratia marcescens (1)	TEM[q]	1	1	1	0	1	1	0
	KPC	1	1	0	0	1	0	0
Pseudomonas aeruginosa (1)	VIM	1	1	0	0	1	0	0

[a] Includes negative controls comprising 2 *E. coli* isolates, which lost plasmids carrying bla_{SHV} or bla_{KPC} during cryopreservation. These two isolates were found, based on the studies performed herein, to harbor $bla_{CTX\text{-}M\text{-}9}$/$bla_{TEM}$ and $bla_{CTX\text{-}M\text{-}9}$.
[b] As previously characterized, as part of reference collections and also if detected as present by the CT103 or CT103 XL assay, and confirmed by conventional PCR.
[c] Resistance target was not previously known to be present but was identified by the CT103 or CT103 XL assays and confirmed by conventional PCR.
[d] The CTX-M-1 group comprises (no. of isolates) CTX-M-1 (2), CTX-M-1 like (1), CTX-M-3 like (4), CTX-M-15 (26), CTX-M-22 (1), CTX-M-27 (1), and CTX-M-28 (1); The CTX-M-9 group comprises (no. of isolates) CTX-M-9 (1), CTX-M-14 (14), CTX-M-27(1), and CTX-M-9 (unspecified) (3).
[e] CT103 and CT103 XL detected a CTX-M-1 target in a reference CTX-M-14 isolate, which is part of the CTX-M-9 group, but did not detect CTX-M-9. Conventional PCR confirmed the presence of CTX-M-1 and CTX-M-9. In addition, CT103 missed a CTX-M-27 (CTX-M-9 group).
[f] The SHV group comprises (no. of isolates) SHV-1 (1), SHV-12 (4), and SHV (unspecified) (14).
[g] SHV (unspecified).
[h] The TEM group comprises (no. of isolates) TEM-1 (4), TEM-1a (7), TEM-1c (2), TEM-1d (1), TEM-6 (1), TEM-10 (1), TEM-12 (1), TEM-26b (2), TEM-43 (1), TEM-104 (1), and TEM (unspecified) (37).
[i] One TEM (unspecified) was missed by CT103, and two TEM-1a, one TEM-1d, one TEM-6, and one TEM (unspecified) was missed by CT103 XL.
[j] Plasmid-borne AmpC comprises (no. of isolates) CMY-2 (11), CMY-8 (1), and FOX-5 (1).
[k] One nonviable isolate was not tested.
[l] NA, not available.
[m] The TEM group comprises (no. of isolates) TEM-1 (1), TEM-1/TEM-10 cocarried (1), and TEM (unspecified) (1).
[n] The SHV group comprises (no. of isolates) SHV-5/55 (4), SHV-12 (1), and SHV (unspecified) (3).
[o] Two nonviable isolates were not tested.
[p] One CTX-M-12 isolate.
[q] TEM (unspecified).

grow on subculture. Regarding the false-negative results (compared to the known genotypes), the CT103 microarray failed to detect one isolate each with bla_{SHV}, $bla_{CTX\text{-}M\text{-}14}$, and $bla_{CTX\text{-}M\text{-}27}$ (the last two representing the $bla_{CTX\text{-}M\text{-}1}$ group), whereas the CT103 XL microarray failed to detect two $bla_{TEM\text{-}1a}$, one $bla_{TEM\text{-}1d}$, one $bla_{TEM\text{-}6}$, one bla_{SHV}, and one $bla_{CTX\text{-}M\text{-}14}$ targets. All missed targets were shown to be present by conventional PCR. Also, CT103 and CT103 XL identified the $bla_{CTX\text{-}M\text{-}1}$ group in a $bla_{CTX\text{-}M\text{-}14}$ ($bla_{CTX\text{-}M\text{-}9}$ group)-containing isolate; conventional PCR confirmed the presence of both $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}9}$ group targets. The observed false-negative results for bla_{SHV} and bla_{TEM} were similar to those described by Las-

cols et al., who found the Check-KPC ESBL microarray to exhibit incomplete sensitivity for these targets (13).

Compared to the historical characterization of the study isolates, the new testing identified an additional 49 resistance genes (34 bla_{TEM}, 7 bla_{SHV}, 3 bla_{CMY-II}, 3 $bla_{CTX-M-9}$ group genes, and 2 $bla_{CTX-M-1}$ group genes) in 45 isolates (46 and 48 targets detected by CT103 and CT103 XL, respectively), all of which were confirmed with conventional PCR, highlighting the added utility of microarray testing (Table 1). This included the unexpected detection of the $bla_{CTX-M-9}$ group/bla_{TEM} and the $bla_{CTX-M-9}$ group in the two isolates that were included as negative controls. Many of the newly detected genes were found in isolates known to contain genes encoding broad-spectrum enzymes, such as bla_{CTX-M} variants, AmpC-encoding genes, bla_{KPC}, and bla_{NDM}.

Limitations in our study were that a majority of the isolates studied were *Escherichia coli* isolates (93 of 108, including negative controls) and that we studied only nine carbapenemase-producing isolates, 13 AmpC-producing isolates, and no rare ESBL- or carbapenemase-producing types.

Microarray platforms may provide valuable data for epidemiologic analyses; however, they are expensive, somewhat laborious, and not rapid. The current emphasis on cost containment and test turnaround time in many health care institutions may impact the use of the described assays in direct patient care. Besides microarray preparation, the mini-column DNA preparation represents a substantial proportion of the hands-on time associated with assay performance. Our approach to DNA preparation improves turnaround time (7 min for rapid bacterial lysis) in the analytical process compared to that of the magnetic bead or mini-column DNA preparation recommended by the manufacturer (30 min to ~1 h depending on method used and number of samples processed concurrently). We were able to test up to 16 isolates during an 8-h shift, which suggests that results can be provided within a working day. Previous studies evaluating this platform, prior versions of it, and similar microarray platforms have not addressed analytical turnaround (12, 14–16, 27).

While not the direct focus of our current evaluation, a previous version of the Check-Points microarray (CT102) has been applied with success to direct genotypic characterization of resistance in Gram-negative bacilli from positive blood cultures (28). Rapid and robust direct-from-sample detection of resistance genotypes in patients with bacteremia may help direct care by allowing the most appropriate antimicrobials to be selected. This may be increasingly important with the introduction of newer antimicrobials, such as the avibactam-based combinations, where different combinations have various activities against isolates harboring the various carbapenemases (29).

By design, microarrays detect only what is included in each platform. As such, manufacturers must stay abreast of the rapidly changing molecular epidemiology of MDRE. Without continuous updating, these platforms will likely become antiquated. Further, in the near future, they may be surpassed by emerging technologies, such as whole-genome sequencing (30).

Overall, our results demonstrate the high accuracy of a microarray-based approach for resistance mechanism characterization in MDRE. Despite the high cost compared to that of real-time or conventional PCR and phenotypic methods, the additional information provided by such arrays may be helpful for infection prevention and control, and potentially, antimicrobial stewardship and patient care (28, 30).

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