JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2011, p. 2985–2987 Vol. 49, No. 8
0095-1137/11/$12.00 doi:10.1128/JCM.02087-10

New Diagnostic Microarray (Check-KPC ESBL) for Detection and Identification of Extended-Spectrum Beta-Lactamases in Highly Resistant *Enterobacteriaceae*▽†

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Received 15 October 2010/Returned for modification 22 March 2011/Accepted 31 May 2011

The performance of a microarray for the detection of extended-spectrum beta-lactamases was determined on a collection of 638 highly resistant members of the family *Enterobacteriaceae* collected from patients in 18 hospitals in The Netherlands. The microarray had a significantly higher specificity than the phenotypic assays. It also detects carbapenemases and characterizes the resistance genes, providing epidemiological insight.

The worldwide prevalence of extended-spectrum beta-lactamase-producing members of *Enterobacteriaceae* (ESBL-E) is increasing rapidly (1). Controlling ESBL-E is difficult, as the resistance genes are located on plasmids and may be transferred between species and even different genera of the *Enterobacteriaceae* family (10). The rapid laboratory detection of this resistance trait is important to guide antimicrobial therapy and to take appropriate infection control measures.

We evaluated a ligation-mediated amplification in combination with a microarray to detect and characterize ESBL-E in a contemporary collection of *Enterobacteriaceae* from a representative sample of hospitals in The Netherlands.

Multicenter prospective surveillance was performed in 18 Dutch hospitals during 6 months in 2007. All newly identified patients with highly resistant members of the family *Enterobacteriaceae* were included. The criteria for highly resistant *Enterobacteriaceae* are defined in the Dutch national guideline for the control of highly resistant microorganisms (6).

Susceptibility tests were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2). ESBL production was determined as previously described (9). All *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Salmonella* spp., and *Shigella* spp. (group I) were tested with ceftazidime and cefotaxime with and without clavulanic acid. All *Enterobacter* spp., *Serratia* spp., *Providencia* spp., *Citrobacter freundii*, *Morganella morganii*, and *Hafnia alvei* (group II) were tested with cefepime with and without clavulanic acid (12). When Etest results were not conclusive, a disk diffusion test (Roscodiagnostica, Taastrup, Denmark) was performed with the double-disk methodology, using a similar algorithm.

DNA isolation was performed with the Easymaq system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The Check-KPC ESBL array (Check-Points, Wageningen, The Netherlands) was used as previously described (12). bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ genes were amplified by PCR (3). Sequence analysis and alignments were performed with the Bionumerics 6.01 software program (Applied Maths, Sint-Martens-Latem, Belgium), BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and information from the www.lahey.org/studies website.

All isolates with concordant phenotypic and genotypic ESBL detection results were considered identified correctly. For discordant findings, the results of sequencing were considered the gold standard.

A total of 638 highly resistant members of the family *Enterobacteriaceae* were included (Table 1). On the basis of the phenotypic test results, 355 (55.6%) isolates were considered ESBL producers. The microarray detected one or more ESBL genes in 345 of the 638 (54.1%) isolates.

The overall phenotypic and genotypic results for 590 out of 638 (92.5%) isolates were concordant. In group I, the majority (24/30) of discordant results had a positive phenotypic test and a negative microarray result. In group II, the majority (12/18) had a negative phenotypic test and a positive microarray result.

Table 2 shows the performance of the phenotypic and microarray tests when the results of sequencing are incorporated into the gold standard. For group I, the sensitivities were comparable, but the microarray was more specific. For group II, the microarray was more sensitive, and the specificities were comparable.

Seven of the false-positive phenotypic tests were negative upon retesting, and six of the false-negative test results were positive. Also, both false-positive microarray results were negative upon retesting and five false-negative microarray results were positive on retesting. In Table S1 in the supplemental material, all discordant findings are shown.

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† Supplemental material for this article may be found at http://jcm.asm.org/.

▽ Published ahead of print on 8 June 2011.

TABLE 1. Distribution of bacterial species

Group and species	No. of isolates (%)
Group I (n = 501)	
Escherichia coli	332 (66.3)
Klebsiella pneumoniae	93 (18.6)
Proteus mirabilis	41 (8.2)
Klebsiella oxytoca	29 (5.8)
Salmonella spp.	3 (0.6)
Shigella spp.	2 (0.4)
Pantoea spp.	1 (0.2)
Group II (n = 137)	
Enterobacter cloacae	87 (63.5)
Citrobacter freundii	24 (17.5)
Morganella morganii	9 (6.6)
Serratia marcescens	6 (4.4)
Citrobacter spp.	6 (4.4)
Enterobacter aerogenes	3 (2.2)
Providencia spp.	2 (1.5)

The 345 isolates that produced ESBLs according to the microarray had various types of ESBL genes (Table 3). More than half of the group I isolates from the *Enterobacteriaceae* had $bla_{\text{CTX-M-1}}$ family ESBL genes, whereas in group II, the $bla_{\text{CTX-M-1}}$ family of ESBL genes constituted a minority of the genes. The most prevalent ESBL type in group II was the $bla_{\text{CTX-M-9}}$ family (61.6%). Some isolates contained more than one ESBL gene. bla_{TEM} coexisted once with a $bla_{\text{CTX-M-1}}$ family ESBL gene and once with a $bla_{\text{CTX-M-9}}$ family gene both in group I organisms. bla_{SHV} was found six times in combination with $bla_{\text{CTX-M-1}}$ family in group I organisms and nine times in combination with the $bla_{\text{CTX-M-9}}$ family in group I (n = 7) and group II (n = 2) organisms. One *K. oxytoca* isolate contained three different genes (bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M-9}}$ family).

TABLE 2. Results of the phenotypic tests and microarray testing after resolution of discordant results[a]

Test, group, and isolates	No. of results		Total no. of results
	ESBL-negative	ESBL-positive	
Phenotypic tests			
Group I			
ESBL negative	205	5	210
ESBL positive	16	275	291
Total	221	280	501
Group II			
ESBL negative	61	11	72
ESBL positive	5	59	64
Inconclusive	0	1	1
Total	66	71	137
Microarray			
Group I			
ESBL negative	220	8	228
ESBL positive	1	272	273
Total	221	280	501
Group II			
ESBL negative	65	0	65
ESBL positive	1	71	72
Total	66	71	137

[a] The performance of the phenotypic and microarray test when the results of sequencing are incorporated into the gold standard. For phenotypic tests on group I, the sensitivity was 98.2%, and the specificity was 92.8%. For phenotypic tests on group II, the sensitivity was 83.1%, and the specificity was 92.4%. For the microarray on group I, the sensitivity was 97.2%, and the specificity was 99.5%. In group I, the specificity of the microarray was statistically significantly higher than that of the phenotypic test ($P < 0.001$). For the microarray on group II, the sensitivity was 100%, and the specificity was 99.5%. In group II, the sensitivity of the microarray was statistically significantly higher than that of the phenotypic test ($P < 0.001$).

TABLE 3. Identification of extended-spectrum beta-lactamase genes of group I and group II isolates in *Enterobacteriaceae* by microarray

ESBL gene type	No. of genes (%)		Total no. of genes (%)
	Group I	Group II	
TEM	27 (9.5)	3 (3.8)	30 (8.2)
SHV	47 (16.5)	20 (25.3)	67 (18.4)
CTX-M-1	153 (53.7)[a]	6 (7.6)[a]	159 (43.7)
CTX-M-2	0 (0)	4 (5.1)	4 (1.1)
CTX-M-9	58 (20.4)[a]	45 (57.0)[a]	103 (28.3)
CTX-M-8/M-25	0 (0)	1 (1.3)	1 (0.3)
Total	285 (100)	79 (100)	364 (100)

[a] The differences between the values for group I and group II are statistically significant for CTX-M-1 and CTX-M-9.

In addition to ESBL genes, the microarray detects carbapenemase genes: these genes were detected in two *Klebsiella pneumoniae* isolates, both from the same patient. This patient who had been hospitalized in Greece after a traffic accident was subsequently transferred to a general hospital in The Netherlands and from there to a university hospital. Both hospitals in The Netherlands participated in our survey. Resistance to carbapenems had not been detected in the diagnostic laboratory with the standard laboratory procedures but was confirmed upon retesting. The Etest MIC for ertapenem was 24.0 mg/ml.

This evaluation showed that the diagnostic microarray was more accurate than the current phenotypic methods to detect ESBL genes in a representative sample of clinical isolates. This microarray has recently been evaluated on three collections of selected isolates containing the majority of known ESBL and KPC genes (3, 5, 7). In the first evaluation (3), it detected 95% of the isolates that contained an ESBL gene and did not produce false-positive results. The second evaluation (7) included a well-defined collection of ESBL and KPC producers and confirmed the ability of the array to detect most resistance genes. In one case, the array failed to detect a KPC gene, but the authors concluded that plasmid instability was the most likely explanation for this negative result. The third study tested the array on 106 Gram-negative strains (5). The following sensitivities and specificities, respectively, were recorded: 98.8% and 100% for bla_{SHV}, 100% and 96.4% for bla_{TEM}, and 100% and 100% for $bla_{\text{CTX-M}}$ and bla_{KPC}. These promising results from the analytic evaluations were confirmed in our study. The false-positive phenotypic tests observed in group I were often negative upon retesting. This reflects the subjectivity involved with the interpretation of the results. In group II, chromosomal AmpC production is a known pitfall for the phenotypic methods, resulting in a substantially reduced specificity (11). As we did not include all known ESBL genes in the sequencing reactions, there is a possibility that some of the

false-positive phenotypic test results are in fact true-positive results that were not detected by the molecular tests. However, the ESBL genes that were not included are rare. In conclusion, there were only a few failures of the array to detect ESBL genes, and the specificity of the microarray was superior to the phenotypic tests, which makes this commercially available microarray a highly reliable tool to detect and identify ESBL genes in the clinical setting. In addition, it also detects the presence of carbapenemase genes, which are nowadays considered to be the most important threats of antimicrobial resistance (8). Finally, the array identifies the type of ESBL that is present, as shown in Table 3. The epidemiology of ESBL-E is rapidly changing, and it is poorly understood how the resistance genes are spreading and which reservoirs are involved (4). This new tool will likely improve insight into the epidemiology of resistance genes, which may be an aid in the further control of resistance.

Check-Points BV, Wageningen, The Netherlands, supplied the materials for the microarray.

There was no external funding for this project. There are no potential conflicts of interest.

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