Journal of Clinical Microbiology

Current Trends in Culture-Based and Molecular Detection of Extended-Spectrum-β-Lactamase-Harboring and Carbapenem-Resistant Enterobacteriaceae

Muriel Gazin, Fabienne Paasch, Herman Goossens and Surbhi Malhotra-Kumar
J. Clin. Microbiol. **2012, 50(4):1140. DOI: 10.1128/JCM.06852-11.**
Published Ahead of Print 18 January 2012.

JCM
Journals.ASM.org

Current Trends in Culture-Based and Molecular Detection of Extended-Spectrum-β-Lactamase-Harboring and Carbapenem-Resistant *Enterobacteriaceae*

Muriel Gazin, Fabienne Paasch, Herman Goossens, and Surbhi Malhotra-Kumar on behalf of the MOSAR WP2 and SATURN WP1 Study Teams

Laboratory of Medical Microbiology, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium

The ever-expanding role of extended-spectrum-β-lactamase (ESBL)-harboring and carbapenem-resistant *Enterobacteriaceae* in causing serious infections poses a grave public health threat because these organisms also tend to be multidrug resistant, for which very few (if any) antibiotic options remain available. Rapid detection of such panresistant organisms offers one of the best solutions to improve patient screening and hospital infection control practices as well as curb inappropriate antibiotic use. This review discusses and compares primarily the current state-of-the-art culture-based and molecular methods that are commercially available for detection or screening of ESBL-harboring and carbapenem-resistant *Enterobacteriaceae*.

The alarming spread of β-lactam-resistant *Enterobacteriaceae* poses a serious public health threat and has increasingly captured the attention of scientists, politicians, and the general public (26). Resistance to β-lactams in *Enterobacteriaceae* is due primarily to β-lactamase-mediated antibiotic hydrolysis, while an altered expression of efflux pumps and/or porins play only a minor role. Based on substrate specificities, the β-lactamase family is divided into 4 functional groups: penicillinases, extended-spectrum-β-lactamases (ESBLs), carbapenemases, and AmpC-type cephalosporinases (reviewed in reference 4). Of these, ESBLs, which can hydrolyze virtually all penicillins and cephalosporins, including the extended-spectrum cephalosporins, like cefotaxime or ceftazidime, comprise the largest and most prevalent group of enzymes (http://www.lahey.org). A high prevalence of ESBL-harboring *Enterobacteriaceae* has resulted in the increased use of carbapenems that exhibit potent activity against many ESBL-harboring organisms. This in turn has led to an emergence and increase in resistance to carbapenems among the *Enterobacteriaceae*, also mediated primarily by hydrolyzing enzymes (carbapenemases) that are functionally divided into serine-dependent (e.g., KPC, OXA-48) and metallo-β-lactamase groups, the latter including some of the most "famous" β-lactamases, VIM, IMP, and NDM. Of major concern is the coexistence of multiple ESBL and carbapenemase genes as well as that of other antibiotic resistance determinants on mobile elements that, along with the genetic plasticity of the *Enterobacteriaceae*, has led to rampant intra- and interspecies transfer of these elements and emergence of organisms with resistance to virtually all antibiotics (4). With the pharmaceutical pipeline running almost dry, rapid detection of such panresistant organisms offers one of the best solutions to improve patient screening and hospital infection control practices as well as curb inappropriate antibiotic use, thus prolonging the efficacy of the currently available antibiotics. This review discusses and compares primarily the current state-of-the-art culture-based and molecular methods that are commercially available for detection or screening of ESBL-harboring and carbapenem-resistant *Enterobacteriaceae*.

CULTURE-BASED DETECTION OF ESBL-HARBORING *ENTEROBACTERIACEAE*

The simplest method for direct detection of ESBL producers in patient screening samples is the use of selective culture media (e.g., MacConkey and Drigalski agar) supplemented with cefotaxime and/or ceftazidime at different concentrations. Commercially, such media are available as biplates, such as BLSE agar (AES Chemunex, Bruz, France) and EbSA ESBL agar (AlphaOmega B.V., The Netherlands), with the latter also able to inhibit AmpC producers and Gram-positive bacteria due to additional supplementation with cloxacillin and vancomycin, respectively. While these commercial media allow direct and rapid detection of ESBL producers from screening samples, confirmatory testing of the ESBL phenotype (using manufacturers' recommendations) and organism identification are necessary. Currently employed confirmatory tests, such as combination disk testing, double-disk synergy testing, and Etest, are generally based on the principle that an ESBL producer in the presence of a β-lactamase inhibitor (clavulanate) exhibits enhanced susceptibility to a cephalosporin or a monobactam. These tests are undoubtedly useful, although the presence of KPCs or hyperproducing K1 penicillinases and high-level AmpC production can result in false-positive and -negative results, respectively (25). Minor adaptations, such as decreasing the interdisk distance or adding an AmpC inhibitor, significantly improve test performance. Also, automated tests such as Vitek 2 (bioMérieux, Craponne, France) and Phoenix (BD Diagnostics, San Diego, CA) are commercially available for ESBL detection and confirmation from pure cultures.

Chromogenic media are the next generation of media and can be considered truly "rapid" culture-based methods as they com-

Published ahead of print 18 January 2012
Address correspondence to Surbhi Malhotra-Kumar, surbhi.malhotra@ua.ac.be.
M.G. and F.P. contributed equally to this article.
Supplemental material for this article may be found at http://jcm.asm.org/.

doi:10.1128/JCM.06852-11

bine presumptive ESBL detection with organism identification. These incorporate a chromogenic substrate that builds up as a colored dye in the bacterial colony upon hydrolysis by the targeted bacterial enzyme, thus resulting in easy differentiation of targeted (potential) pathogens from the adjunct flora. The recent incorporation of selective antibiotics into chromogenic media has been a breakthrough for the direct detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens. Development of similar chromogenic media for ESBL detection, however, has been rather challenging due to the need for (i) color-based differentiation of several genera of *Enterobacteriaceae* or Gram-negative bacteria (GNB) harboring ESBLs, (ii) maximal suppression of growth of the highly complex and abundant interfering resident flora present in fecal samples (as the gastrointestinal tract is the primary screening site for ESBL producers), and (iii) an indicator β-lactam(s) that offers maximal coverage of the ESBL spectrum, as these enzymes vary widely in resistance profiles that are further complicated by the presence of multiple β-lactamases in the same organism. Currently available commercial chromogenic media for detection of ESBL-producers include chromID ESBL (bioMérieux), Brilliance ESBL (Oxoid Ltd., Basingstoke, United Kingdom), and CHROMagar ESBL (CHROMagar, Paris, France). In contrast to the other ready-to-use media, CHROMagar ESBL needs to be prepared by adding a proprietary selective mix to the CHROMagar Orientation agar base. For specific detection of CTX-M producers, the CHROMagar CTX supplement is added to the CHROMagar ECC base. The performance and characteristics of some of these media have been assessed in analytical and clinical studies that are discussed in the following sections.

Antibiotics used as selective agents. Antibiotic combinations and their concentrations are of primary importance in determining media selectivity, with the main aims here being to suppress growth of Gram-positive organisms and yeasts and to select for ESBL producers. While the antibiotic combinations utilized in the chromogenic media are not entirely disclosed, cefpodoxime is currently the cephalosporin of choice for selection of ESBL producers and is utilized in chromID ESBL (4 μg/ml) and Brilliance ESBL (personal communications from the manufacturers). Cefpodoxime was already known as a reliable, selective substrate for most TEM- and SHV-derived ESBLs more than a decade ago, and it is also preferred because it can be utilized as a single substrate, in contrast to ceftazidime, which is combined with cefotaxime, in order to reliably detect both CTX-M producers and those with ceftazidimase-type TEM variants. The superior recovery of CTX-M-type ESBLs on chromogenic media containing cefpodoxime (chromID ESBL and Brilliance ESBL) compared to that on MacConkey agar supplemented with ceftazidime (2 μg/ml) alone or with a ceftazidime disk (30 μg) has been demonstrated in several studies (9, 10). Nonetheless, a reported lack of specificity due to growth of AmpC and K1 hyperproducers while using cefpodoxime disks (5 or 10 μg) indicates that cefpodoxime concentrations incorporated in media need to be chosen carefully, as MICs to cefpodoxime in the range of 2 to 4 μg/ml for *Escherichia coli* might be due to changes in porin or AmpC overexpression rather than ESBL production (15). Finally, the additional antibiotics incorporated in chromID ESBL also allow a better inhibition of the adjunct flora in comparison to BLSE agar (22).

Efficacy of chromogen combinations in differentiating GNB genera/species. chromID ESBL and Brilliance ESBL contain two chromogens that can differentiate *E. coli* from strains belonging to the *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter* (KESC) group. One of the chromogens in chromID ESBL is metabolized by the β-glucuronidase enzyme that is highly specific to *E. coli* and results in pink to burgundy colonies, whereas on Brilliance ESBL agar, *E. coli* cells expressing β-galactosidase and β-glucuronidase appear as blue colonies (pink if β-galactosidase negative). The KESC group grow as green and green/blue to brownish-green colonies on Brilliance and chromID ESBL due to β-galactosidase and β-glucosidase expression, respectively. Tryptophan is added to both media to detect members of the *Proteus*, *Morganella*, and *Providencia* (PMP) group that appear tan-colored with a brown halo as a result of tryptophan deaminase production.

Colorless *E. coli* colonies on chromID ESBL, especially after 24 h of incubation, have been observed (10, 22) and might be due to these strains being deficient in β-glucuronidase expression. However, these *E. coli* colonies were easily detectable on Brilliance ESBL (10), probably because of a dual enzyme targeting by the chromogens in this medium. On the other hand, *E. coli* colonies can display variable coloration (turquoise instead of blue-violet) on Brilliance ESBL, compared to a homogenous burgundy on chromID ESBL, causing difficulties in differentiating *E. coli* from KESC group strains (10). Regarding the KESC group, a major difference between the two media was observed for ESBL-producing *Citrobacter freundii*, wherein all isolates exhibited an expected green colony color on Brilliance ESBL in contrast to colorless colonies on chromID ESBL (10), probably due to lack of the β-glucosidase enzyme in these strains. A direct oxidase test on colorless colonies can increase the sensitivity of chromID ESBL for detection of enterobacterial ESBL producers (22, 23). Also, *Klebsiella oxytoca* can be rapidly differentiated on chromID ESBL from other KESC group members by direct detection of indole production on green/blue-colored colonies (22), while on Brilliance ESBL, *K. oxytoca* colonies appear as turquoise, slightly different from the green-colored KESC group (10), and might be differentiated simply based on colony color. However, differentiation between the metallic-blue colonies of *Klebsiella* spp. and turquoise colonies of *Enterococcus* spp., which are not inhibited on CHROMagar ESBL, was found to be rather difficult (S. Malhotra-Kumar, unpublished data). Concerning nonfermenters, *Pseudomonas aeruginosa* colonies are usually colorless on chromID ESBL, although colonies can exhibit the typical pyocyanin-related green-brown pigmentation on both chromID ESBL and Brilliance ESBL (9, 22, 23). A direct oxidase test can differentiate these from the similarly brown-colored *Proteus* spp. colonies.

Effect of incubation time. The impact of incubation time on the performance of ESBL chromogenic media is not yet well defined. One study showed an increase in sensitivity (88% to 94%) and a decrease in specificity (94% to 91%) of chromID ESBL with prolonged incubation (48 h), while another did not observe an increase in sensitivity at 48 h, although the recovery of ESBL producers was complicated due to growth of associated flora on this medium (9, 22). On the other hand, another study reported no difference in growth with prolonged incubation of samples on chromID ESBL (18).

Analytical sensitivity and specificity. Evaluation studies of ESBL chromogenic media using well-characterized strains are summarized in Table 1. Most studies comparing the performance of chromID ESBL to selective media, automated tests, or other chromogenic media have shown almost uniformly high sensitivities (>95%) of this medium (8, 10, 16, 21, 22). The majority of

TABLE 1 Overview of analytical studies evaluating available chromogenic media for detection of ESBL producers[a]

Chromogenic medium	Organism(s) tested (*n*)	Proportion of ESBL producers in total tested strains (%)	Incubation time (h)	Comparator(s)	Sensitivity (%)	Specificity (%)	Reference
chromID ESBL	*E. coli* (334) *Klebsiella* spp. (124) *Proteus* spp. (42) *Salmonella* spp. (3) *Shigella* spp. (2)	291/505 (57.6)	18–24	EbSA	97.3	93.9	16
	Enterobacter spp. (90) *Citrobacter* spp. (30) *Morganella morganii* (9) *Serratia marcescens* (6) *Providencia* spp. (2)	65/137 (47.4)	18–24	EbSA	98.5	44.3	16
	Enterobacteriaceae (156) Gram-negative nonfermenters (44)	98/200 (49)	24	Brilliance ESBL	ND	ND	10
	Enterobacteriaceae and *Pseudomonas* spp. (150)	101/150 (67.3)	ND	CHROMagar CTX, CHROMagar ECC + CTX[c] or CAZ[c]	98	25.5	21
	E. coli (72) *Klebsiella pneumoniae* (21) *K. oxytoca* (4) *Enterobacter cloacae* (11) *Enterobacter aerogenes* (1) *S. marcescens* (1) *Citrobacter koseri* (1) *Proteus mirabilis* (1) *Proteus vulgaris* (1) *M. morganii* (1)	99/114 (86.8)	18–24 and 48	Vitek 2, Phoenix	95.8	10.5	8
Brilliance ESBL	*Enterobacteriaceae* (156) Gram-negative nonfermenters (44)	98/200 (49)	24	chromID ESBL	ND	ND	10
CHROMagar CTX	*Enterobacteriaceae* and *Pseudomonas* spp. (150)	70[b]/150 (46.6)	ND	chromID ESBL, CHROMagar ECC + CTX[c] or CAZ[c]	100	64.2	21

[a] CTX, cefotaxime; CAZ, ceftazidime; ND, not described.
[b] CTX-M producers.
[c] Cefotaxime or ceftazidime was incorporated at concentrations of 1, 2, 4, and 8 μg/ml.

these utilized strains expressing commonly occurring TEM-, SHV-, and CTX-M-type ESBLs, although successful recovery of enterobacterial strains harboring ESBLs isolated less frequently (VEB, GES, BEL, PER, TLA-1, TLA-2, BES, and OXA-18) has also been demonstrated on chromID ESBL (22). However, the specificities observed for this medium have been very low (11% to 44%) due to growth of AmpC-overproducing organisms (8, 10, 16, 21). A drastic decrease in specificity (44%) of chromID ESBL was observed when strains with chromosomal AmpC β-lactamases were tested compared to the high specificity (94%) of this medium while evaluating non-AmpC-producing *Enterobacteriaceae* (Table 1) (16). Similar problems with AmpC-overproducing isolates were also reported with Brilliance ESBL (10). In contrast, EbSA demonstrated significant suppression of AmpC producers (due to incorporation of cloxacillin) and higher specificities compared to chromID ESBL (78% versus 44%) (16). Besides AmpC, specificities of chromID ESBL and Brilliance ESBL were also compromised due to growth of non-ESBL K1-OXY penicillinase overproducing *K. oxytoca* and few OXA-30 penicillinase-producing *E. coli* (10). Brilliance ESBL and chromID ESBL showed similar performance with an enterobacterial strain collection, wherein every growing colony was considered positive regardless of color (10). Nonfermenters *P. aeruginosa* and *Acinetobacter baumannii* grew on both media, whereas *Stenotrophomonas maltophilia*, which exhibits intrinsic β-lactam resistance even to the carbapenems and might be present in respiratory and urinary specimens, was markedly inhibited on Brilliance ESBL (10).

CHROMagar CTX, which has been specifically designed to target the ubiquitous and globally disseminated CTX-M-type ESBLs, demonstrated excellent sensitivity (100%), although specificity (64%) was compromised due to recovery of non-CTX-M-type ESBLs (21). Also noteworthy was its marked inhibition of AmpC producers; only 14% grew on CHROMagar CTX compared to 76% on chromID ESBL (21).

Clinical sensitivity and specificity. Performance studies using clinical samples have compared primarily chromID ESBL, Brilliance ESBL, and CHROMagar ESBL, either to selective culture media or to each other, and are summarized in Table 2. Five studies evaluated chromID ESBL using mainly fecal, respiratory, and urine samples and observed high sensitivities (88% to 100%) and

TABLE 2 Overview of clinical studies evaluating chromogenic media for detection of ESBL producers and CRE[a]

Chromogenic medium/use	Sample(s) (*n*)	Proportion of samples harboring ESBL producers/carbapenem resistant strains in total tested samples (%)	Incubation time (h)	Comparator(s)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Reference
ESBL GNB detection									
chromID ESBL	Fecal (344) Respiratory tract (134) Wound, urine, vaginal, blood (50)	59/528 (11)	24	Brilliance ESBL, MAC + CAZ (30 μg)	94.9	95.5	70.8	98.2	10
	Fecal (500)	41/500 (8.2)	24	MAC + CAZ (1 μg/ml), MAC + CTX (1 μg/ml)	100	94.8	63	100	18
	Stool (186) Urine (48) Sputum (12) Wound (10)	17/256 (6.6)	24	CHROMagar ESBL	88.2	92.9	46.9	99.1	23
	Rectal swab (468)	32/765 (4.2)	24	BLSE	88	94.4	38.7	99.6	22
	Urine (255) Pulmonary aspiration (42)		48		94	90.5	28.4	99.9	
	Fecal (561) Lower respiratory tract (63) Wound, ear/nose/throat (20)	37/644 (5.7)	18–24	MAC + CAZ (2 μg/ml)	97.7	90.4	ND	ND	9
CHROMagar ESBL	Stool (186) Urine (48) Sputum (12) Wound (10)	17/256 (6.6)	24	chromID ESBL	100	93.3	51.5	100	23
Brilliance ESBL	Fecal (344) Respiratory tract (134) Wound, urine, vaginal, blood (50)	59/528 (11)	24	chromID ESBL, MAC + CAZ (30 μg)	94.9	95.7	73.7	99.3	10
CRE detection									
CHROMagar KPC/Colorex KPC									
	Rectal swab (139)	33/139 (24)	24	MAC + IPM (1 μg/ml), MAC + IPM, MEM and ERT disks (10 μg)	84.9	88.7	70	95	1
	Rectal swab (126)	46/126 (36.5)	24 and 48	MAC+ IPM (1 μg/ml)	97.8	100	100	98.8	17
	Stool (200)	37/200 (18.5)	20	ID Carba	97	96	71.9	25.8	19
	Rectal swab (122)	41/122 (33.6)	24	MAC + IPM, MEM and ERT disks (10 μg)	100	98.4	ND	ND	24
ID Carba	Stool (200)	37/200 (18.5)	20	Colorex KPC	100	93	78.8	52.9	19

[a] CRE, carbapenem-resistant *Enterobacteriaceae*; PPV, positive predictive value; NPV, negative predictive value; MAC, MacConkey agar; CAZ, ceftazidime; CTX, cefotaxime; IPM, imipenem; MEM, meropenem; ERT, ertapenem; ND, not described.

specificities (90% to 96%) for this medium (Table 2). NPVs were uniformly high (>98%), while positive predictive values (PPVs) varied between studies (39% to 74%), depending on the prevalence of ESBL producers at study sites that ranged from 4% to 11% (9, 10, 18, 22, 23). Higher sensitivities and PPVs were reported for CHROMagar ESBL (100% and 52%) than chromID ESBL (88% and 46%), as two ESBL-harboring *E. coli* strains appeared colorless on the latter medium (23). It is noteworthy, though, that the criteria employed by most studies to identify an isolate as truly positive on chromogenic media are either not clearly specified or

vary widely between studies, making direct comparisons difficult. This was well illustrated by Huang and colleagues, who found high sensitivities for both chromID ESBL and Brilliance ESBL (95%) when total growth (including colorless colonies) was considered; however, when only oxidase-negative and colored colonies were considered, sensitivity of chromID ESBL decreased (86%), whereas that of Brilliance ESBL remained the same (10). Similar to the observations in the analytical studies, false-positive results on all three chromogenic media were attributed mainly to strains expressing plasmidic AmpC or hyperproducing chromosomal AmpC (mostly *Enterobacter* spp. and *Citrobacter* spp.) and penicillinases (*K. oxytoca*), and, to a minor extent, also narrow-spectrum TEM and SHV and OXA penicillinases (9, 10, 18, 22, 23). Importantly, frequency of false-positive results after 24 h of incubation was 3-fold higher on BLSE agar compared to that on chromID ESBL agar (22). Nonfermenters and a few enterococci were also detected on chromID ESBL, although the former appear as colorless, nonspreading colonies and are not likely to interfere with detection of ESBL producers (9, 10, 22).

CULTURE-BASED DETECTION OF CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE*

Detection of carbapenem-resistant *Enterobacteriaceae* (CRE) is highly challenging owing to the wide heterogeneity in resistance levels to carbapenems that depend on the enzyme and the host organism, which also makes it impossible to set up uniform screening and confirmatory tests for detection of carbapenemase producers. Recently, utilization of the carbapenemase inhibitors phenylboronic acid and EDTA to detect and differentiate metallo-β-lactamases and KPCs directly from rectal swabs was shown to be a highly sensitive (90%) and specific (100%) method (28). The modified Hodge test, which allows growth of a susceptible indicator strain toward a carbapenem disk while being adjacent to a carbapenemase producer, has been used for confirmation (25). However, it has low specificity and lacks sensitivity for metallo-β-lactamase detection (25).

Several commercial chromogenic media for screening of CRE have also been recently introduced and include CHROMagar KPC (also available as a ready-to-use medium called Colorex KPC), Brilliance CRE (Oxoid), and Hardy CHROM carbapenemase (Hardy Diagnostics, Santa Maria, CA). Recovery of high-level resistant CRE harboring KPC, IMP, or VIM is achieved easily on CHROMagar KPC, with detection limits up to 14 CFU/ml, although when CRE with carbapenem MICs of <4 μg/ml are included, detection limits are higher (1×10^1 to 2×10^5 CFU/ml) (5). In comparison, the same set of strains was easily detected on chromID ESBL, due to the hydrolysis of cephalosporins by carbapenemases, and showed lower limits of detection (10 to 80 CFU/ml) than on CHROMagar KPC. Similarly, NDM-1 harboring CRE (meropenem MIC 1.5 to 32 μg/ml) that also confer resistance to extended-spectrum cephalosporins were also detected at lower concentrations on chromID ESBL (8 to 500 CFU/ml) than on CHROMagar KPC (1×10^1 to 5×10^5 CFU/ml) (14). However, OXA-48 producers that hydrolyze penicillins and carbapenems, but not extended-spectrum cephalosporins, have proven particularly difficult to detect with phenotypic detection methods. Recovery of OXA-48-producers on media like chromID ESBL would require ESBL coexpression, and these strains also show variable growth patterns on CHROMagar KPC due to wide variations in carbapenem MICs (5). Of note, noncarbapenemase-producing CRE exhibiting cell wall impermeability and AmpC hyperproduction are also recovered on CHROMagar KPC (11). Nonetheless, despite the problems noted on analytical studies, chromogenic media for detection of CRE have generally performed well in the field (17, 24), although discrepancies in performance have been noted between studies (Table 2). For instance, two surveillance studies carried out in Israel using rectal swabs showed variable sensitivity (100% and 85%) and specificity (98% and 89%) of CHROMagar KPC (1, 24). Importantly, the CRE prevalent in Israel belong primarily to a KPC-harboring *K. pneumoniae* clone (carbapenem MIC $\geq$ 16 μg/ml) (1) and are easily detected on CHROMagar KPC. However, the use of a less-sensitive comparator (carbapenem disks on MacConkey agar) by one study might also have (artificially) enhanced the performance of CHROMagar KPC (24). Another study utilized stool sample suspensions to compare Colorex KPC and ID Carba (bioMérieux), a prototype medium, and found a significantly higher strain recovery on ID Carba than on Colorex KPC, primarily because the latter medium could sustain growth of CRE only with meropenem MICs $\geq$ 4 μg/ml (19). Nonetheless, despite this limitation, only one additional patient was identified by ID Carba due to the coexistence of other carbapenemase producers in the stool, resulting in a sensitivity of 100% versus 97% for ID Carba and Colorex KPC, respectively.

MOLECULAR DETECTION OF ESBL-HARBORING AND CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE*

Recent advancements in molecular assays for ESBL/carbapenemase detection have resulted in commercial assays that are highly flexible and can easily incorporate new, emerging β-lactam resistance genes as well as resistance targets to other antibiotic classes to give a comprehensive picture of multidrug resistant GNB. In addition to this distinct advantage over culture-based tests, molecular detection of carbapenemase producers is also rather straightforward and does not have the inherent drawback of detecting all CRE, including those that owe their phenotypes to a combination of ESBL or AmpC enzymes and porin changes. Finally, detection of resistance genes expressed at low levels is also easily achieved with molecular assays. A classic example that demonstrates the utility of the molecular approach for detection of carbapenemase producers is the recent outbreak in a Dutch hospital caused by an OXA-48-harboring *K. pneumoniae* strain that exhibited low-level resistance to carbapenems and could be efficiently detected by PCR-based tests and appropriate containment measures instituted (http://www.rivm.nl/en/).

Currently available molecular methods for detection of ESBLs and carbapenemases are broadly based on DNA amplification followed by amplicon detection either on a tube microarray (Check-Points assays, Check-Points Health, Wageningen, The Netherlands; and Identibac AMR-ve assays, Alere GmbH, Cologne, Germany) or by enzyme-linked immunosorbent assay (ELISA) (Hyplex assays, Amplex Diagnostics, Gars, Germany). The microarray-based systems have especially undergone strong advancements, resulting in newer and improved versions over the last few years.

The Check-Points assays are based on ligation-mediated amplification, wherein each target-specific probe consists of two oligo arms that ligate and produce amplification products only when the target sequence is a perfect match. Every target-specific probe is equipped with the same consensus primer pair for ampli-

fication, as well as a unique "ZIP code" region required for a specific hybridization on the tube array (for more details, refer to references 6 and 7). Hybridization and detection on the microarray requires 1 to 2 h of processing, depending on the number of samples (up to 3 amplicons can be analyzed in parallel on one microarray) (7), and, along with the amplification step, gives a turnaround time of approximately 6 h (manufacturer's specifications). These assays are available in several combinations for ESBL and carbapenemase gene detection and collectively cover 95% (84/88), 77% (27/35), and 100% of the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes described in the Lahey database, respectively, while minor EBSLs, such as bla_{VEB}, bla_{PER}, and bla_{GES}, are not included (see Table S1 in the supplemental material). The Check-ESBL assay detects and differentiates the narrow-spectrum bla_{TEM} and bla_{SHV} β-lactamases from the ESBL variants, which has important therapeutic implications. In addition, bla_{CTX-M} can also be classified into groups 1, 2, 9, and 8/25. The assay was evaluated using ESBL-positive and -negative enterobacterial isolates (β-lactamase gene contents of all isolates tested in molecular studies are detailed in the Table S1 in the supplemental material) and, in comparison to PCR and sequencing, showed 95% sensitivity and 100% specificity for ESBL detection. The assay failed to detect bla_{TEM-5}, bla_{TEM-7}, and bla_{TEM-75}, one $bla_{CTX-M-39}$ (group 8/25), and one bla_{SHV-57}, the last because the array does not include a bla_{SHV-57} probe. Importantly, K1 hyperproducers misclassified as ESBL positive by phenotypic tests could be resolved (6). The Check-KPC ESBL assay has a detection profile similar to that of the Check-ESBL and additionally detects bla_{KPC}. The assay evaluation showed excellent ESBL and bla_{KPC} detection sensitivities and specificities ranging from 95% to 100% and 99% to 100%, respectively (see Table S1) (7, 12, 20). Failure to detect ESBLs was primarily because some gene probes were not included on the array (e.g., bla_{PER} and bla_{GES}), although a few bla_{TEM} and bla_{SHV} ESBLs were also missed (7, 12). Sensitivity for narrow-spectrum bla_{TEM} detection was 100%; however, up to 17% bla_{SHV} were missed (7, 13). A few problems with identification of bla_{CTX-M} group 1 genes could be attributed to either a relative insensitivity of the bla_{CTX-M} group 1 probe or detection limits of the identification software (20). The Check-MDR CT101 assay targets the bla_{TEM}, bla_{SHV}, bla_{CTX-M} ESBLs, bla_{KPC}, and bla_{NDM-1}, as well as 6 groups of plasmid-mediated bla_{AmpC} but not narrow-spectrum β-lactamases. The assay performance was evaluated using members of the *Enterobacteriaceae* possessing different *bla* genes, which yielded 100% sensitivities and specificities for detection of bla_{AmpC}, bla_{KPC}, and bla_{NDM}; however, 56% of the chromosomal bla_{AmpC} tested also hybridized to the plasmidic bla_{AmpC} array probes (3). The Check-MDR CT102 assay (previously the Check-Carba ESBL assay) is an updated version of the Check-KPC ESBL assay that additionally detects bla_{OXA-48}, bla_{VIM}, bla_{IMP}, and bla_{NDM-1}. The assay showed overall ESBL and carbapenemase detection sensitivities and specificities of 96% to 100%, although a few narrow-spectrum bla_{SHV} and bla_{TEM} genes and two bla_{KPC} genes were not detected, probably owing to plasmid instability (12, 27).

The Identibac AMR-ve assay is based on a linear multiplex-PCR amplification wherein biotin-labeled DNA amplicons are hybridized on the array and visualized colorimetrically (horseradish peroxidase-streptavidin conjugation with seramun green staining), giving a turnaround time of approximately 8 h (M. Gazin, unpublished data). Detection of 70 genes encoding resistance to commonly used antibiotic groups, including up to 25 resistance determinants to β-lactams (including bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{CMY}, and bla_{ACC} as well as minor ESBLs such as bla_{PER}, bla_{LEN}, etc.), is possible. Similar to the Check-Points array, bla_{CTX-M} ESBLs can also be classified into groups 1, 2, 8, 9, and 25. A drawback of the assay is its inability to differentiate narrow- and extended-spectrum β-lactamase genes. We recently evaluated this assay using multiresistant *Enterobacteriaceae* and observed 93% sensitivity for ESBL detection in comparison to PCR and sequencing (M. Gazin, unpublished data) (see Table S1 in the supplemental material). ESBL genotypes could be correctly ascribed to ESBL variants of bla_{TEM} (96%), bla_{CTX-M} (61%), and bla_{SHV} (29%); 100% sensitivities were observed for bla_{OXA} and bla_{CMY}. The majority (91%) of the bla_{CTX-M} could be correctly classified to their groups.

The Hyplex assays involve multiplex PCR amplification followed by amplicon hybridization to reverse probes that are immobilized on ELISA-microwell plates, and hybridization complexes are visualized through peroxidase-conjugated antibodies. Befitting a rapid test, these assays can be utilized directly on patient samples; however, the ELISA-hybridization "modules" for consensus and specific detection of *bla* genes tend to be rather laborious and time consuming. The Hyplex MBL ID multiplex PCR-ELISA detects metallo-β-lactamases bla_{VIM} and bla_{IMP} and was tested with clinical samples (urine, pus swabs, respiratory samples, and positive blood cultures) for bla_{VIM} detection and showed sensitivity and specificity of 98% and 99%, respectively (2).

CONCLUSIONS

With the ever-expanding role of ESBL-harboring and carbapenem-resistant *Enterobacteriaceae* in causing serious infections, a rapid assay that can detect carriage of such organisms in patients at the time of hospital admission features high on the infectious disease specialist/clinician's wish list. Our critical assessment of the current state-of-the-art methods identified some promising assays; however, none could as yet be defined as a truly rapid diagnostic approach. The use of chromogenic agars for detection of ESBL-harboring and carbapenem-resistant *Enterobacteriaceae* is associated with an inherent diagnostic delay of at least 18 h. The currently available PCR-microarray-based molecular assays are also subject to delays, as these require bacterial DNA as template and, hence, despite being genotypic identification systems, are heavily reliant on conventional culture techniques. Nonetheless, the PCR-microarray approach seems to hold great potential for further development as a rapid diagnostic test that could be utilized on clinical samples to enable direct detection and differentiation of the vast numbers of ESBL and carbapenemase genes. Attempts to further this approach as sample-in-answer-out miniaturized systems with a turnaround time of a few hours are currently ongoing as part of several European projects (InTopSens [http://www.ee.kth.se/intopsens/], RAPP-ID [http://www.rapp-id.eu], RGNOSIS [http://www.r-gnosis.eu/]), which, if successful, would arm us with truly rapid diagnostic assays to combat the global pandemic of antibiotic resistance.

ACKNOWLEDGMENTS

We thank Olympia Zarkotou (Tzaneio General Hospital, Piraeus, Greece) for critical review of the manuscript.

M.G. is supported by funding from the European Community (InTopSens network contract FP7-ICT-2007-223932). F.P. received a doctoral scholarship from the Flemish Interuniversity Council (Vlaamse

Interuniversitaire Raad/VLIR). We declare that we have no conflict of interest.

REFERENCES

1. **Adler A, et al.** 2011. Laboratory and clinical evaluation of screening agar plates for detection of carbapenem-resistant *Enterobacteriaceae* from surveillance rectal swabs. J. Clin. Microbiol. **49**:2239–2242.
2. **Avlami A, et al.** 2010. Detection of metallo-beta-lactamase genes in clinical specimens by a commercial multiplex PCR system. J. Microbiol. Methods **83**:185–187.
3. **Bogaerts P, et al.** 2011. Multicenter evaluation of a new DNA microarray for rapid detection of clinically relevant *bla* genes from beta-lactam-resistant gram-negative bacteria. Antimicrob. Agents Chemother. **55**: 4457–4460.
4. **Bush K.** 2010. Alarming beta-lactamase-mediated resistance in multidrug-resistant *Enterobacteriaceae*. Curr. Opin. Microbiol. **13**:558–564.
5. **Carrër A, Fortineau N, Nordmann P.** 2010. Use of chromID extended-spectrum beta-lactamase medium for detecting carbapenemase-producing *Enterobacteriaceae*. J. Clin. Microbiol. **48**:1913–1914.
6. **Cohen Stuart J, et al.** 2010. Rapid detection of TEM, SHV and CTX-M extended-spectrum beta-lactamases in *Enterobacteriaceae* using ligation-mediated amplification with microarray analysis. J. Antimicrob. Chemother. **65**:1377–1381.
7. **Endimiani A, et al.** 2010. Evaluation of a commercial microarray system for detection of SHV-, TEM-, CTX-M-, and KPC-type beta-lactamase genes in Gram-negative isolates. J. Clin. Microbiol. **48**:2618–2622.
8. **Färber J, et al.** 2008. Extended-spectrum beta-lactamase detection with different panels for automated susceptibility testing and with a chromogenic medium. J. Clin. Microbiol. **46**:3721–3727.
9. **Glupczynski Y, Berhin C, Bauraing C, Bogaerts P.** 2007. Evaluation of a new selective chromogenic agar medium for detection of extended-spectrum beta-lactamase-producing *Enterobacteriaceae*. J. Clin. Microbiol. **45**:501–505.
10. **Huang TD, Bogaerts P, Berhin C, Guisset A, Glupczynski Y.** 2010. Evaluation of Brilliance ESBL agar, a novel chromogenic medium for detection of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae*. J. Clin. Microbiol. **48**:2091–2096.
11. **Moran Gilad J, Carmeli Y, Schwartz D, Navon-Venezia S.** 2011. Laboratory evaluation of the CHROMagar KPC medium for identification of carbapenem-nonsusceptible *Enterobacteriaceae*. Diagn. Microbiol. Infect. Dis. **70**:565–567.
12. **Naas T, Cuzon G, Bogaerts P, Glupczynski Y, Nordmann P.** 2011. Evaluation of a DNA microarray (Check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. J. Clin. Microbiol. **49**:1608–1613.
13. **Naas T, Cuzon G, Truong H, Bernabeu S, Nordmann P.** 2010. Evaluation of a DNA microarray, the check-points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and KPC carbapenemases. Antimicrob. Agents Chemother. **54**:3086–3092.
14. **Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR.** 2011. How to detect NDM-1 producers. J. Clin. Microbiol. **49**:718–721.
15. **Oliver A, et al.** 2002. Mechanisms of decreased susceptibility to cefpodoxime in *Escherichia coli*. Antimicrob. Agents Chemother. **46**:3829–3836.
16. **Overdevest IT, Willemsen I, Elberts S, Verhulst C, Kluytmans JA.** 2011. Laboratory detection of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae*: evaluation of two screening agar plates and two confirmation techniques. J. Clin. Microbiol. **49**:519–522.
17. **Panagea T, et al.** 2011. Evaluation of CHROMagar KPC for the detection of carbapenemase-producing *Enterobacteriaceae* in rectal surveillance cultures. Int. J. Antimicrob. Agents **37**:124–128.
18. **Paniagua R, Valverde A, Coque TM, Baquero F, Canton R.** 2010. Assessment of prevalence and changing epidemiology of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* fecal carriers using a chromogenic medium. Diagn. Microbiol. Infect. Dis. **67**:376–379.
19. **Perry JD, et al.** 2011. Prevalence of faecal carriage of *Enterobacteriaceae* with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. J. Antimicrob. Chemother. **66**:2288–2294.
20. **Platteel TN, et al.** 2011. Evaluation of a commercial microarray as a confirmation test for the presence of extended-spectrum beta-lactamases in isolates from the routine clinical setting. Clin. Microbiol. Infect. **17**: 1435–1438.
21. **Randall LP, et al.** 2009. Evaluation of CHROMagar CTX, a novel medium for isolating CTX-M-ESBL-positive *Enterobacteriaceae* while inhibiting AmpC-producing strains. J. Antimicrob. Chemother. **63**:302–308.
22. **Réglier-Poupet H, et al.** 2008. Performance of chromID ESBL, a chromogenic medium for detection of *Enterobacteriaceae* producing extended-spectrum beta-lactamases. J. Med. Microbiol. **57**:310–315.
23. **Saito R, et al.** 2010. Evaluation of a chromogenic agar medium for the detection of extended-spectrum β-lactamase-producing *Enterobacteriaceae*. Lett. Appl. Microbiol. **51**:704–706.
24. **Samra Z, et al.** 2008. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant *Enterobacteriaceae*. J. Clin. Microbiol. **46**:3110–3111.
25. **Thomson KS.** 2010. Extended-spectrum-beta-lactamase, AmpC, and carbapenemase issues. J. Clin. Microbiol. **48**:1019–1025.
26. **Walsh TR, Toleman MA.** 2012. The emergence of pan-resistant Gram-negative pathogens merits a rapid global political response. J. Antimicrob. Chemother. **67**:1–3.
27. **Woodford N, Warner M, Pike R, Zhang J.** 2011. Evaluation of a commercial microarray to detect carbapenemase-producing *Enterobacteriaceae*. J. Antimicrob. Chemother. **66**:2887–2888.
28. **Zarkotou O, et al.** 2011. A simple phenotypic algorithm for direct and specific detection of KPC and MBL carbapenemase-producing Enterobacteriaceae in rectal screening. *In* Abstracts of 21st European Congress of Clinical Microbiology and Infectious Diseases in Milan, Italy.

Surbhi Malhotra-Kumar is an Associate Professor of Medical Microbiology at the University of Antwerp, Belgium. She has a double master's in Medical Microbiology and Molecular Biology with a Ph.D. in Medical Microbiology and has been a fellow of Research Foundation Flanders and a recipient of the 2011 ESCMID Young Investigator Award. Her research interests include studying the molecular epidemiology and genetics of beta-lactam resistance in the *Enterobacteriaceae* and developing rapid diagnostic assays for pathogens causing community-acquired and nosocomial infections.