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Comparison of Five Media for Detection of Extended-Spectrum Beta-Lactamase by Use of the Wasp Instrument for Automated Specimen Processing

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Overall, 2,337 rectal screening samples (RSSs) were seeded by using the Wasp instrument for automated microbiological processing with five media for detection of extended-spectrum β-lactamase (ESBL): CHROMagar, ChromID, Brilliance, BD Drigalski, and HEGP media. Of 354 RSSs harboring ESBL-producing isolates, 89.3% were found to be positive on all media. Sensitivity and specificity ranged from 95.5 to 98.3% and from 57.9 to 72.3%, respectively. No medium was perfectly ESBL selective, and non-ESBL-producing strains were mainly *Enterobacteriaceae* overproducing AmpC β-lactamase and nonfermenting Gram-negative bacilli, mostly *Pseudomonas aeruginosa*.

In recent years, a dramatic increase in the prevalence of *Enterobacteriaceae* producing extended-spectrum β-lactamases (ESBLs) has been observed in Europe (1–3). In order to prevent nosocomial cross-transmission, systematic detection of carriage of ESBL-producing bacteria in the digestive tract of high-risk patients has been recommended by various academic societies (4, 5). In this context, hospital microbiology laboratories face several challenges. Systematic screening leads to an increased number of samples to be tested, while at the same time, budget restrictions entail reductions in human resources. Automation can be a relevant means of analyzing a large number of samples (6–9), but the delay in obtaining results still needs to be reduced. Moreover, if molecular tests for ESBL detection are expected to give a faster result than culture, the cost of these tests for large series is currently still too high relative to that of traditional culture. In this situation, agar manufacturers have improved their media for detection of ESBLs after only 24 h (10–16). Taking advantage of the Wasp instrument, an automated device for microbiological specimen processing, planting, and streaking (Copan, Brescia, Italy), we decided to compare the efficiencies of five selective media designed for ESBL detection.

The study was conducted at Hôpital Européen Georges-Pompidou (HEGP), an 830-bed acute-care teaching hospital with 23 wards and with 29,000 admissions in 2011. A rectal screening sample (RSS) was collected daily from each patient admitted to the intensive care units (ICUs) between 3 April and 3 July 2011, in compliance with French patient confidentiality regulations and ethical standards. The study was approved by an institutional review board (CCPPRB project no. ID-RCB 2011-A00259-32, University Paris XI, February 2011). Sampling was done by using the eSwab system (Copan). The swab sample was suspended in 1 ml of liquid Amies medium (Copan), and 10 μl was streaked by the automated system onto two sets of media, including (i) five media for the detection of ESBL, i.e., CHROMagar ESBL (CHROMagar, Paris, France), ChromID ESBL (bioMérieux, Lyon, France), Brilliance ESBL (Oxoid, Basingstoke, United Kingdom), BD Drigalski lactose agar with ceftazidime (Becton, Dickinson, Franklin Lakes, NJ), and in-house-made "HEGP medium" containing Drigalski agar (Oxoid, Basingstoke, Great Britain) and cefotaxime (2 μg/ml), taking into consideration the currently predominant producers of CTX-M family ESBLs in France (1, 2), and (ii) six additional media, some of which were used as controls, such as Drigalski agar without an ESBL selector (data not shown), and some of which were used to test the capability of the Wasp instrument to streak additional petri dishes including, e.g., biplates.

Of the 2,348 RSSs collected during the study period, only 11 RSSs were excluded from the study because they were improperly plated onto at least one medium by the Wasp instrument (mainly agar plowed). Since 11 media were seeded with each RSS by the Wasp instrument, a total of 25,828 petri dishes were plated by the end of the study period. Counting 1 min for plating of each medium manually (writing the patient name and sample number on the petri dish, opening the eSwab tube, depositing the sample onto agar, closing the eSwab tube, taking one loop, and streaking the sample), the time saved by using the Wasp automated instrument to plate 250 petri dishes daily was estimated to amount to two-thirds of the full working time of one technician. Plates were incubated at 37°C for 18 h. Growing colonies were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF-MS) using a Microflex Bruker Daltonics/BioTyper version 2.0 system (Bruker Daltonics, Bremen, Germany). To test if isolated colonies were ESBL producers as expected, antibiograms were performed with the disk diffusion method on Mueller-Hinton (MH) agar (Bio-Rad, Marne-la-Coquette, France), according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (17). Isolates were categorized as (i) "ESBL" (*Enterobacteriaceae* harboring ESBL) when a zone of synergy between third-generation cephalosporins (3GCs) and clavulanic acid was observed; (ii) "CASE" (*Enterobac-*

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TABLE 1 ESBL species isolated from ICU patients

ESBL species isolated from ICU patients	Result for no. of patients[a]								No. of isolates
	27	12	2	6	1	1	1	1	
E. coli	+	−	−	+	+	+	−	+	36
K. pneumoniae	−	+	−	+	−	−	+	+	20
Enterobacter cloacae	−	−	+	−	−	−	−	−	2
Klebsiella oxytoca	−	−	−	−	−	−	+	−	1
Morganella morganii	−	−	−	−	+	−	−	+	2
Enterobacter aerogenes	−	−	−	−	−	+	−	−	1
Total									62

[a] Total of 51 patients.

teriaceae overproducing AmpC cephalosporinase) when no synergy between any 3GC and clavulanic acid was observed and if there was at least a 5-mm increase in inhibition zone diameters around the 3GC disks, read on MH agar versus MH agar plus cloxacillin (250 μg/ml); and (iii) "others" for all isolates which did not belong to the ESBL or CASE category. SAS software version 9.2 (SAS Inc., Cary, NC) was used for statistical analysis. A gold standard (GS) value was defined for each RSS as follows: (i) positive GS (PGS) when at least one medium revealed ESBL production and (ii) negative GS (NGS) when no medium revealed ESBL production. The sensitivity of medium X was calculated as the number of RSSs containing an ESBL strain (ESBL-RSSs) detected by using medium X divided by the total number of PGSs. The specificity of medium X was calculated as the number of RSSs without any colony on medium X divided by the total number of NGSs. The positive predictive value (PPV) of medium X was calculated as the number of ESBL-RSSs detected by using medium X divided by the total number of RSSs yielding ESBL and non-ESBL colonies on this medium. The negative predictive value (NPV) of medium X was calculated as the number of ESBL-negative RSSs detected by using medium X divided by the total number of RSSs yielding no colonies on this medium. Finally, the reliability of medium X (only ESBL colonies on medium X when there is an ESBL in the RSS, or no colony on medium X when there is no ESBL in the RSS) was also calculated as the number of RSSs with only ESBL isolate colonies on medium X among PGSs or without any colony on medium X among NGSs divided by the total number of RSSs.

Overall, 2,337 RSSs were collected from 269 patients during the study period. After identification and interpretation of antimicrobial susceptibility patterns performed on the different colonies isolated from all selective media, it appeared that 354 RSSs collected from 51 patients contained ESBL isolates. In 36/51 (71%) cases, an ESBL strain was isolated on at least one medium for the first RSS collected, and in 25/51 (35%) cases, the ESBL-producing bacteria were isolated from all samples plated. The ESBLs observed were harbored mainly by *Escherichia coli* and *Klebsiella pneumoniae* isolates (Table 1).

As a primary analysis, only the first ESBL-RSS was examined. An ESBL-positive isolate was found on all five media in 38/51 (74.5%) ESBL-RSSs, while in 13/51 (25.5%) cases, an ESBL isolate was found on one to four media (Table 2). Thereby, the number of ESBL-RSSs ranged from 43 to 49 according to the medium. Using only the first ESBL-RSS, 84% of the 51 cases would have been detected by using BD medium, versus 96% with ChromID (Table 2).

When all ESBL-RSSs were taken into account, 316/354 (89.3%) were found to be positive on all five media, while 38/354 (10.7%) were positive on only one to four media, and the percentage of ESBL-RSSs varied from 94% to 97% depending upon the medium (Table 2). These discrepancies could be explained for 16/38 ESBL-RSSs by low inocula, since in these cases, fewer than 10 colonies were observed on the media on which they grew. Thus, aleatory repartition of bacteria in these ESBL-RSSs could lead to few colonies on one medium and no colonies on the other. In 6/38 ESBL-RSSs, high inocula of non-ESBL bacteria (mainly CASE and *Pseudomonas aeruginosa*) could have hidden growth of ESBL colonies.

When all RSSs were considered, depending upon the media, the number of ESBL-RSSs with only ESBL isolates varied from 283 to 312, and the number of negative RSSs (absence of any colony) varied from 1,154 to 1,454 (Table 3). For the detection of ESBL, no

TABLE 2 Results of detection of ESBL in RSSs

Medium	Result for total no. of ESBL-RSSs[a] (no. of 1st ESBL-RSSs[b])																No. (%) of ESBL-RSSs with ESBL isolate on medium plated	
	316 (38)	3	12 (4)	2 (2)	1 (1)	1 (1)	3	5 (1)	1	1 (1)	1	1	1	4 (3)	1	1	1st ESBL-RSS[a]	All ESBL-RSSs[b]
CHROMagar	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	46 (90)	342 (96)
ChromID	+	+	+	+	−	+	+	−	+	+	+	−	−	+	−	−	49 (96)	344 (97)
Brilliance ESBL	+	+	+	−	+	+	+	+	+	+	−	+	−	−	+	−	46 (90)	345 (97)
BD Drigalski	+	+	−	+	+	+	−	+	+	−	−	+	+	−	−	+	43 (84)	332 (94)
HEGP medium	+	−	+	+	+	+	−	−	−	+	+	−	+	−	−	−	47 (92)	335 (94)

[a] For each of the 51 patients with ESBL isolated, all ESBL-RSSs were taken into account (354 ESBL-RSSs).

[b] For each of the 51 patients with ESBL isolated, only the first ESBL-RSS was taken into account (51 ESBL-RSSs).

TABLE 3 Detection of ESBL by use of five media

Parameter[a]	Value for medium				
	CHROMagar	ChromID	Brilliance ESBL	BD Drigalski	HEGP medium
No. (%) of screening samples with result					
No colonies	1,439 (61.6)	1,454 (62.2)	1,154 (49.4)	1,278 (54.7)	1,191 (51.0)
ESBL only	312 (13.4)	303 (13.0)	289 (12.4)	296 (12.7)	283 (12.1)
ESBL + CASE	4 (0.2)	9 (0.4)	6 (0.3)	6 (0.3)	8 (0.3)
ESBL + others	26 (1.1)	32 (1.4)	50 (2.1)	30 (1.3)	44 (1.9)
ESBL + CASE + others	0	0	0	0	0
Total RSSs with ESBL	342 (14.6)	344 (14.8)	345 (14.8)	332 (14.2)	335 (14.3)
CASE only	187 (8.0)	152 (6.5)	173 (7.4)	286 (12.2)	236 (10.1)
Others only	318 (13.6)	321 (13.7)	567 (24.3)	392 (16.8)	498 (21.3)
CASE + others	51 (2.2)	66 (2.8)	98 (4.2)	49 (2.1)	77 (3.3)
Total RSSs without ESBL	556 (23.8)	539 (23.1)	838 (35.9)	727 (31.1)	811 (34.7)
Sensitivity (%) (95% CI)	98.3 (96.3, 99.4)	97.5 (95.2, 98.8)	98.6 (96.7, 99.5)	97.2 (94.9, 98.6)	95.5 (92.8, 97.4)
Specificity (%) (95% CI)	72.3 (70.2, 74.2)	72.9 (70.8, 74.8)	57.9 (55.7, 60.1)	63.9 (61.8, 66.1)	59.2 (57.1, 61.4)
PPV (%) (95% CI)	38.7 (35.5, 42.0)	39.1 (35.8, 42.4)	29.5 (26.9, 32.2)	32.5 (29.7, 35.4)	29.5 (26.9, 32.2)
NPV (%) (95% CI)	99.6 (99.1, 99.8)	99.4 (98.8, 99.7)	99.6 (99.0, 99.9)	99.2 (98.6, 99.6)	98.7 (97.8, 99.2)
Reliability [no. (%) of RSSs][b]	1,745 (74.7)	1,748 (74.8)	1,438 (61.5)	1,564 (66.9)	1,458 (62.4)

[a] ESBL, *Enterobacteriaceae* strain producing extended-spectrum beta-lactamase; CASE, *Enterobacteriaceae* strain overproducing cephalosporinase; others, *Enterobacteriaceae* without the ESBL and CASE phenotypes or other species (mainly nonfermenting Gram-negative bacilli, in particular *Pseudomonas aeruginosa*); CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[b] Reliability corresponds to the number of RSSs with only ESBL colonies on medium X when there is ESBL detected in the RSS, or no colonies on medium X when there is no ESBL detected in the RSS.

significant difference in sensitivity was observed for the five media (Table 3). This was not the case in other studies, where variations of ChromID sensitivity were 88%, 88.2%, 94.9%, and 100% compared to ESBL agar (AES) (14), CHROMagar (13), Brilliance (11), and MacConkey plus 1 μg/μl ceftazidime (12), respectively. Interestingly, high NPVs found for the five media (Table 3) attest that when these media remain sterile, the probability that an ESBL is present in the RSS is very low. In contrast, as shown in Table 3, strains harboring mechanisms of resistance other than ESBL production were selected on these "ESBL-specific media," and their specificities ranged from 57.9% to 72.3%, while the PPV for ESBL varied from 29.5 to 38.8%. These non-ESBL isolates were mainly CASE and nonfermenting Gram-negative bacilli. As shown by the PPV, these non-ESBL species were more often encountered on Brilliance, DB Drigalski, and HEGP media. *In fine*, the percentage of RSSs which yielded an unambiguous answer concerning the presence of ESBL (reliability; Table 3), i.e., which indicated the absence of ESBL when no ESBL strain was isolated from other media, or which yielded only an ESBL strain(s), varied from 61.5% to 74.8% depending upon the medium.

In conclusion, this study shows that (i) the Wasp instrument was very useful for seeding large series of samples, and all media were well adapted to this device; (ii) all media tested allow detection of ESBL strains; and (iii) no medium is fully ESBL selective, with CHROMagar and ChromID giving the lowest recovery of non-ESBL strains. Therefore, complementary tests are needed to ensure that the colonies growing on these "partially selective media" harbor ESBLs, explaining that a delay of 48 h between the seeding of the RSS and the availability of the definitive result remains necessary.

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