Comparison of Two Phenotypic Algorithms To Detect Carbapenemase-Producing *Enterobacteriaceae*

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ABSTRACT A novel algorithm designed for the screening of carbapenemase-producing *Enterobacteriaceae* (CPE), based on faropenem and temocillin disks, was compared to that of the Committee of the Antibiogram of the French Society of Microbiology (CA-SFM), which is based on ticarcillin-clavulanate, imipenem, and temocillin disks. The two algorithms presented comparable negative predictive values (98.6% versus 97.5%) for CPE screening among carbapenem-nonsusceptible *Enterobacteriaceae*. However, since 46.2% ($n = 49$) of the CPE were correctly identified as OXA-48-like producers by the faropenem/temocillin-based algorithm, it significantly decreased the number of complementary tests needed (42.2% versus 62.6% with the CA-SFM algorithm).

KEYWORDS OXA-48, KPC, NDM, VIM, IMP disk diffusion susceptibility testing, bacterial susceptibility testing, carbapenemase, disk diffusion

Carbapenem resistance caused by carbapenemase production has been increasingly reported worldwide in *Enterobacteriaceae* (1, 2). In most of the cases, these carbapenemases belong to Ambler class A (mostly KPC and GES types) (3), Ambler class B or metallo- β -lactamases (MBLs) of VIM, IMP (1, 2), and NDM types (4), or carbapenem-hydrolyzing Ambler class D β -lactamases (CHDLs) (mostly OXA-48-like) (5). The spread of carbapenemase-producing *Enterobacteriaceae* (CPE) represents a serious threat for public health and requires their rapid identification to implement proper infection control measures to prevent further spread in hospitals and initiate proper treatments. Although very few novel antibiotics are, or will be, available for the treatment of CPE, the most promising therapies involve combinations of a broad-spectrum β -lactam and novel β -lactamase inhibitors (e.g., ceftazidime-avibactam, imipenem-relebactam) active on class A carbapenemases and on class D (for avibactam only) but not on MBLs (6, 7). During the last 3 years, several methods have been developed for the detection of CPE, including (i) tests able to detect carbapenem-hydrolyzing activity (Carba NP test and derivatives [8–10], matrix-assisted laser desorption/ionization–time of flight [MALDI-TOF] protocols [11, 12], Bogaerts-Yunus-Glupczynski [BYG] test [13], carbapenem inactivation method [CIM] test [14, 15], and β Carba [16]), (ii) immuno-chromatographic tests for the rapid detection of KPC, OXA-48-type, and NDM carbapenemases (17–19), (iii) combination disk diffusion assays (20), and (iv) molecular-based techniques that aim to detect the most widespread carbapenemase-encoding genes (21–23).

The ability to infer from routine antibiogram the presence of a carbapenemase is often complicated and requires complementary tests, leading to additional delay and cost for clinical laboratories. Accordingly, it is of importance to develop solutions (e.g., algorithms) with high sensitivities and negative predictive values (NPVs) to discriminate

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non-CPE from those that require complementary testing (20, 24). Recently, an algorithm proposed by the Committee of the Antibiogram of the French Society of Microbiology (CA-SFM), based on the inhibition zone diameters of three antibiotics (ticarcillin-clavulanate, imipenem, and temocillin), could correctly identify 50% of non-CPE producers (Table 1), thus avoiding additional testing for CPE in one-third of the enterobacterial isolates with decreased susceptibility to carbapenems (24). However, ticarcillin-clavulanate was discontinued in many countries by the manufacturer in November 2014, and this molecule has been reserved for *Stenotrophomonas maltophilia* infections only. Accordingly, ticarcillin-clavulanate susceptibility testing is not relevant anymore for *Enterobacteriaceae*.

Here, we have evaluated a novel phenotypic algorithm based on the interpretation of two disks: a faropenem-containing disk (CAT-ID, product D71C; Mast Diagnostic, Amiens, France) and a temocillin 30- μ g disk (TEM 30C; MAST Diagnostic). This algorithm has been compared to that proposed by the CA-SFM for the detection of CPE.

In July 2016, 211 nonduplicate clinical enterobacterial isolates were received at the French National Reference Center for CPE for expert evaluation. The isolates were reidentified at the species level using the Bruker Biotyper MALDI-TOF mass spectrometry (MS) system (Bruker Daltonique S.A., Wissembourg, France), and susceptibility testing performed by the disk diffusion method on Mueller-Hinton agar plates (Bio-Rad, Marnes-La-Coquette, France) according to EUCAST guidelines (as updated in 2016; <http://www.eucast.org/>) revealed that they were nonsusceptible to at least one of the three carbapenems tested as inferred by inhibition diameters of <25 mm, <23 mm, and <25 mm for meropenem, imipenem, and ertapenem, respectively.

The mechanisms involved in the decreased carbapenem susceptibility were characterized as previously described (24). These isolates included 105 non-CPE, 3 KPC-2 producers, 17 NDM producers (13 NDM-1 and 4 NDM-5), 2 VIM-1 producers, 1 IMP-4 producer, 79 CHDL producers (73 OXA-48 and 6 OXA-181), and 4 multiple carbapenemase producers (3 NDM-1 + OXA-48 and 1 NDM-1 + OXA-232) (Fig. 1).

Inhibition zone diameters of a faropenem-containing disk and a temocillin 30- μ g disk were measured and subjected to the algorithm described in Fig. 1A. Inhibition zone diameters of ticarcillin-clavulanate (75/10 μ g), imipenem (10 μ g) (Oxoid, Dardilly, France), and temocillin (30 μ g) were measured and subjected to the CA-SFM algorithm as previously described (Fig. 1B) (24).

Applied to our prospective collection of 211 *Enterobacteriaceae* with decreased susceptibility to carbapenems, the CA-SFM and the faropenem/temocillin-based algorithms had negative predictive values (NPVs) of 97.5% (95% confidence interval [95% CI], 90.3% to 99.6%) and 98.6% (95% CI, 91.2% to 99.9%) and sensitivities of 98.1% (95% CI, 92.6% to 99.7%) and 99.1% (95% CI, 94.1% to 99.9%) for the detection of CPE, respectively (Table 1). The faropenem/temocillin-based algorithm gave one false-negative result (0.9% of the isolates) for an OXA-48-producing *K. pneumoniae* isolate, and the CA-SFM algorithm gave two false-negative results (1.9% of the isolates) including the same OXA-48-producing *K. pneumoniae* isolate and one KPC-2-producing *K. pneumoniae* isolate (Fig. 1). In addition, and unlike the CA-SFM algorithm, the faropenem/temocillin-based algorithm correctly identified 49 isolates (23.3% [49/211] of the total isolates) as OXA-48-like producers with a positive predictive value (PPV) and a specificity of 92.5% (95% CI, 80.9% to 97.6%) and 96.9% (95% CI, 91.7% to 99.0%), respectively (Table 1). Finally, the number of complementary tests that needed to be performed using the faropenem/temocillin-based algorithm was significantly lower (chi-square, $P < 0.0001$; 42.2% [$n = 89$]) than that of the CA-SFM algorithm (62.6% [$n = 132$]) (Table 1).

Recently, an algorithm developed by the CA-SFM and based on the results of inhibition zone diameters of three molecules (ticarcillin-clavulanate, imipenem, and temocillin) could directly categorize 32.9% of the isolates as non-CPE without any further testing (24). However, ticarcillin-clavulanate was discontinued by the manufacturer in November 2014 and must be reserved for *Stenotrophomonas maltophilia* infections only. Accordingly, ticarcillin-clavulanate susceptibility testing seems to be irrelevant for *Enterobacteriaceae*, thus rendering impossible the further use of the

TABLE 1 Performances of the faropenem/temocillin-based and CA-SFM algorithms

Study or reference	Study type	No. of isolates tested	Algorithm used	% (ratio) accurately detected isolates for:		% (ratio) false negatives (detected as non-CPE instead of CPE)	% (ratio) false positives (detected as OXA-48 producer instead of non-CPE)	% (ratio) complementary tests required	Performance for CPE detection (%) ^a				Performance for OXA-48-like producer detection (%) ^a			
				Non-CPE	OXA-48-like producers				Se	Sp	PPV	NPV	Se	Sp	PPV	NPV
24 This study	Prospective	621	CA-SFM	50.0 (204/408)	0 (0/183)	0 (0/213)	ND ^b	67.1 (417/621)	100	46	51	100	ND	ND	ND	ND
	Prospective	211	CA-SFM	73.3 (77/105)	0 (0/79)	1.9 (2/106)	ND	62.6 (132/211)	98.1	73.3	78.8	97.5	ND	ND	ND	ND
			Faropenem/temocillin	65.7 (69/105)	59.0 (49/83)	0.9 (1/106)	3.8 (4/106)	42.2 (89/211)	99.1	65.7	74.5	98.6	59.0	96.9	92.5	78.5

^aSe, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

^bND, could not be determined.

FIG 1 Prospective comparison of the faropenem/temocillin-based algorithm (A) and the CA-SFM algorithm (B) on 211 consecutive enterobacterial isolates with reduced susceptibility to carbapenems.

CA-SFM algorithm. Accordingly, we proposed and validated a novel algorithm based on two disks (faropenem and temocillin). Our results showed that this algorithm was as efficient as that of the CA-SFM for the screening of CPE among *Enterobacteriaceae* with decreased susceptibility to carbapenems (99.1% [95% CI, 94.1% to 99.9%] sensitivity and 98.6% [95% CI, 91.2% to 99.9%] NPV for the faropenem/temocillin-based algorithm versus 98.1% [95% CI, 92.6% to 99.7%] sensitivity and 97.5% [95% CI, 90.3% to 99.6%] NPV for the CA-SFM algorithm) (Table 1). In addition, due to its ability to directly detect OXA-48-like producers with an excellent specificity (96.9% [95% CI, 91.7% to 99.0%]) and a good PPV (92.5% [95% CI, 80.9% to 97.6%]), this novel algorithm led to significantly decrease the number of complementary tests needed (Table 1) and thus to reduced associated costs. In addition, this efficient early detection of OXA-48-like producers is also of clinical importance with the commercialization of ceftazidime-avibactam combinations, which may be a relevant alternative to treat OXA-48-like-producing enterobacterial infections.

As previously observed (24), using the CA-SFM algorithm, we identified only one isolate with ticarcillin-clavulanate of ≥15 mm and imipenem of <22 mm that required complementary tests (Fig. 1B), and this isolate was a non-CPE. Accordingly, we confirmed that the CA-SFM algorithm may be simplified just by considering isolates with inhibition zones of ≥15 mm to ticarcillin-clavulanate as non-CPE without any consequence on the NPV (24).

Finally, we demonstrated that the faropenem/temocillin-based algorithm may accurately replace that of the CA-SFM, resulting in a reduced cost and in a gain of time for clinical microbiology laboratories. However, this algorithm suffers the same disadvantages as those previously mentioned for the CA-SFM algorithm (24). (i) The suscep-

tibility testing has to be performed using disk diffusion methods, which is not the case for many laboratories that usually use automated liquid methods for susceptibility testing. (ii) It cannot be directly applied on colonies grown on CPE-selective medium as opposed to most CPE detection kits (e.g., Carba NP test, MALDI-TOF, immunochromatography, molecular biology, etc.).

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We declare no conflicts of interest.

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