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Performance evaluation of the βLACTA™ Test for rapid detection of ceftazidime resistance in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients

Pauline Ract[a,b], Redouane Dahoumane[d], Salah Gallah[c], Philippe Morand[e], Isabelle Podglajen[a,b], Fabrice Compain[a,b,f,g,*]

[a] Service de Microbiologie, Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France
[b] Université Paris Descartes, Sorbonne Paris Cité, Paris 75006, France
[c] Department of Microbiology, Saint-Antoine Hospital, Paris, France
[d] Department of Bacteriology, APHP Hôpitaux Universitaires Est Parisiens, Paris, France
[e] Department of Microbiology, Cochin Hospital, Paris, France
[f] INSERM UMRS 1138, Centre de Recherche des Cordeliers, Paris, France
[g] Sorbonne Universités, UPMC Univ Paris 06, Centre de Recherche des Cordeliers, Paris, France

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ABSTRACT

The chromogenic βLACTA™ test was evaluated to detect ceftazidime resistance in *P. aeruginosa* isolates from patients with cystic fibrosis. Best results were obtained after one hour of incubation with low sensitivity (64.1%), high specificity (98.3%), and negative and positive predictive values of 80.3% and 96.2%, respectively.

Pseudomonas aeruginosa is a Gram-negative nonfermenting bacillus, which commonly colonizes pulmonary airways of cystic fibrosis (CF) patients (Elborn, 2016). This pathogen is associated with chronic infections and acute pulmonary exacerbations, which result in high morbidity and mortality rates in this population. *P. aeruginosa* is intrinsically multidrug resistant (MDR) due to its low membrane permeability, multiple efflux pumps and the constitutive expression of a chromosomally-encoded β-lactamase (Lister et al., 2009). These resistance mechanisms are often deregulated in clinical isolates from CF patients, resulting in high-level resistance to most antipseudomonal β-lactams, including ceftazidime (Llanes et al., 2013; Tomás et al., 2010). Selection pressure with broad-spectrum antibiotics is known to promote the emergence of MDR isolates in CF patients (Giwercman et al., 1990; Mouton et al., 1993; Spencker et al., 2003). Conventional antibiotic susceptibility testing methods for Gram-negative bacilli provide results in 16–20 h. A rapid detection of ceftazidime-susceptible *P. aeruginosa* isolates on primary cultures could prove useful to spare broad-spectrum antibiotics, including new antipseudomonal treatments such as administration of the new cephalosporin ceftolozane or the ceftazidime-avibactam combination, which would otherwise be administered as empiric treatments (van Duin and Bonomo, 2016). This strategy could reduce the risk of emergence of higher drug resistance levels by guiding the use of ceftazidime alone as a first-line treatment (Carmeli et al., 2011; El Amari et al., 2001; Paramythiotou et al., 2004).

The βLACTA Test (BLT) (Biorad, Marnes-la-Coquette, France) is a rapid chromogenic assay, based on the hydrolysis of the third generation cephalosporin (3GC) HMRZ-86, which is structurally close to ceftazidime. It was initially developed for the rapid detection of resistance to 3GC in Enterobacteriaceae (Renvoisé et al., 2013), but was also reported to be reliable for the detection of resistance to ceftazidime in *P. aeruginosa* (including acquired-β-lactamase-producing strains and strains overproducing the AmpC chromosomal cephalosporinase) (Laurent et al., 2013). However, testing of *P. aeruginosa* by the βLACTA Test (BLT) remains an off-label use since it is only licensed to test isolates of Enterobacteriaceae. The aim of this study was to evaluate the performance of the BLT in detecting ceftazidime-resistance in *P. aeruginosa* clinical isolates from CF patients. A rapid detection of ceftazidime resistance would allow us to rapidly deescalate the empiric antibiotic treatment received by CF patients during acute pulmonary exacerbations.

A total of 97 non-duplicate *P. aeruginosa* isolates from pulmonary samples (including sputum samples, bronchial aspirates, and

* Corresponding author at: Microbiology, AP-HP, Hôpital Européen Georges Pompidou, Paris, France.
E-mail address: fabrice.compain@aphp.fr (F. Compain).

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bronchoalveolar lavage fluids) of 80 CF patients were included in this study. They were collected from four university hospitals in Paris, France, and were immediately stored at −80 °C before testing. Nineteen isolates originated from children and 78 from adult patients. Resistance to ceftazidime was assessed by the disk-diffusion method using 10-μg ceftazidime disks according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2018 recommendations (version 8.1) (http://www.eucast.org/clinical_breakpoints/). Mueller-Hinton agar plates containing cloxacillin (1000 μg/mL) were used for the detection of cephalosporinase overproduction as was previously described (Berrazeg et al., 2015). All ceftazidime-resistant isolates were screened for extended spectrum β-lactamase (ESBL) production by the double-disk synergy test (Laudy et al., 2017) using amoxicillin-clavulanate, ceftazidime, cefepime and aztreonam on Mueller-Hinton agar plates containing cloxacillin (1000 μg/mL). When resistance to ceftazidime was not related to the production of ESBL or to cephalosporinase overproduction, screening for the production of carbapenemases was performed using the Xpert® Carba-R molecular test (GeneXpert, Cepheid), which detects a large range of carbapenemase-encoding genes (including bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP-1}, and bla_{OXA-48}). An in-house PCR assay was additionally performed to detect all bla_{IMP} and bla_{VIM} carbapenemase genes (data not shown). Moreover, the β-CARBA Test, which has previously shown a good performance in the detection of carbapenemase activity in *P. aeruginosa* (Bernabeu et al., 2017), was performed on all the strains with unidentified resistance mechanisms.

All isolates were removed from storage at −80 °C, subcultured on Columbia agar plates supplemented with 5% horse blood (bioMérieux, La Balme-les-Grottes, France), and incubated for 16–24 h at 35 °C. The BLT was then performed on fresh colonies following the manufacturer's instructions. Briefly, colonies were harvested with a 1-μL loop and suspended in one drop of each of the R1 and R2 reagents of the BLT kit. Results were reported by two independent readers after 5 min, 15 min, 30 min, 1 h and 3 h of incubation at room temperature and interpreted as previously reported for *P. aeruginosa* (Laurent et al., 2013): yellow, negative; orange/red: positive. In case of discrepancy between the readings of the independent readers, or between the results from the BLT and the disk diffusion method, both experiments were repeated two more times to determine a majority result.

Disk diffusion showed ceftazidime resistance in 39 *P. aeruginosa* isolates, with most strains (34/39, 87%) showing high-level resistance (inhibition zone diameter = 6 mm). Resistance was due to overexpressed cephalosporinase (18/39, 46%, ceftazidime susceptibility was fully recovered using cloxacillin 1000 μg/mL), overexpressed cephalosporinase combined with possible deregulated efflux pumps (12/39, 31%, ceftazidime susceptibility was partially recovered using cloxacillin 1000 μg/mL), ESBL (1/39, 3%) and unidentified resistance mechanisms (8/39, 20%, ceftazidime susceptibility was not recovered using cloxacillin 1000 μg/mL, or no bacterial growth was observed using cloxacillin 1000 μg/mL). The full resistance phenotype to β-lactams of these last eight strains is detailed in Supplementary Table S1. No carbapenemase was detected in any of the strains using molecular methods coupled with the β-CARBA Test.

The distribution of BLT results compared to disk diffusion susceptibility testing is shown in Fig. 1. Detailed comprehensive results by each independent reader are shown in Supplementary Table S2. The BLT yielded positive results for up to 26/39 (66.7%) ceftazidime-resistant strains (Table 1). Results remained negative for 57/58 (98.3%) ceftazidime-susceptible strains after a 3-h incubation (the BLT turned orange after 30 min for the false positive result). Results of sensitivity and specificity according to durations of incubation are reported in Table 2. The performance of the test was the best after an incubation of one hour with sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of 64.1%, 98.3%, 80.3%, and 96.2%, respectively. This incubation is longer than stated in the manufacturer's instructions (15 min) and than previously used by Laurent et al. (30 min) (Laurent et al., 2013). There was an additional positive result for the ceftazidime-resistant strains after an incubation of 3 h (26 for a 3-h incubation versus 25 for 1 h, Table 1). However, the global reading of colorimetric changes was more difficult since after 3 h of incubation the red color began to fade back to orange for several tests (11/25, data not shown). Positive results obtained by the BLT were highly specific for ceftazidime resistance, suggesting that this test might be useful to exclude ceftazidime from empiric antibiotic therapy. However, this test should not be used to detect ceftazidime resistance in clinical isolates of *P. aeruginosa* since it generated an unacceptably high number of major errors by identifying 35.9% of the tested ceftazidime-resistant isolates as ceftazidime-susceptible.

No particular resistance profile was found to explain the lack of BLT sensitivity for 14/39 (35.9%) strains, nor could colorimetric changes be associated with any ceftazidime-resistance mechanism. Among these 14 strains, resistance to ceftazidime was associated with elevated AmpC production (8/14, 57%) and elevated AmpC production combined with probable deregulated efflux pumps (6/14, 43%). In comparison, ceftazidime-resistant strains yielding a positive BLT result showed overexpressed cephalosporinase (6/25, 24%) and overexpressed cephalosporinase combined with probable deregulated efflux pumps (10/25, 40%), while ESBL production was found in only one strain (causing the BLT to turn red in less than 15 min). We could not identify with certainty the resistance mechanisms in the eight (32%) remaining strains. No carbapenemase was found in the entire collection.

The BLT has previously shown high sensitivity and specificity in the detection of resistance to 3GC in Enterobacteriaceae (Renvoisé et al., 2013). However, its negative predictive value (NPV) decreased from 99% to 89% for the detection of a stable overproduction of AmpC in *Enterobacter* spp. and *Serratia* spp. due to a lower affinity of this β-lactamase for HMRZ-86 (Hanaki et al., 2007; Renvoisé et al., 2013). Moreover, the lack of sensitivity of the BLT for high-level active efflux strains was previously suggested for one ceftazidime-resistant *P. aeruginosa* isolate (Laurent et al., 2013). Ceftazidime resistance in *P. aeruginosa* isolates from CF patients is mainly due to deregulated efflux pumps and AmpC overproduction rather than the acquisition of extrinsic β-lactamases (Llanes et al., 2013; Tomás et al., 2010); such resistance mechanisms can also be found in collections of non-CF clinical isolates (Castanheira et al., 2014). This could explain why the performance of the BLT in our *P. aeruginosa* collection was poorer than that suggested by Laurent et al., who reported sensitivity, specificity, NPV and PPV of 95%, 87%, 99%, and 100%, respectively (Laurent et al., 2013). When only considering the 18 strains overexpressing cephalosporinase in our study (characterized by a full recovery of the susceptibility to ceftazidime by cloxacillin 1000 μg/mL), the sensitivity rate was 55.6% (versus 94.7% as reported by Laurent et al. (Laurent et al., 2013)).

In conclusion, the BLT colorimetric change (to orange or red) was highly specific for ceftazidime resistance after one hour of incubation at room temperature, for a collection of *P. aeruginosa* in which overexpression of cephalosporinase and efflux pumps was predominant. The high PPV of the BLT suggests that the test could be used to exclude ceftazidime from empiric antibiotic treatment when the result is positive. However, a negative result cannot be relied upon to provide an antibiotic treatment consisting of ceftazidime only to CF patients given

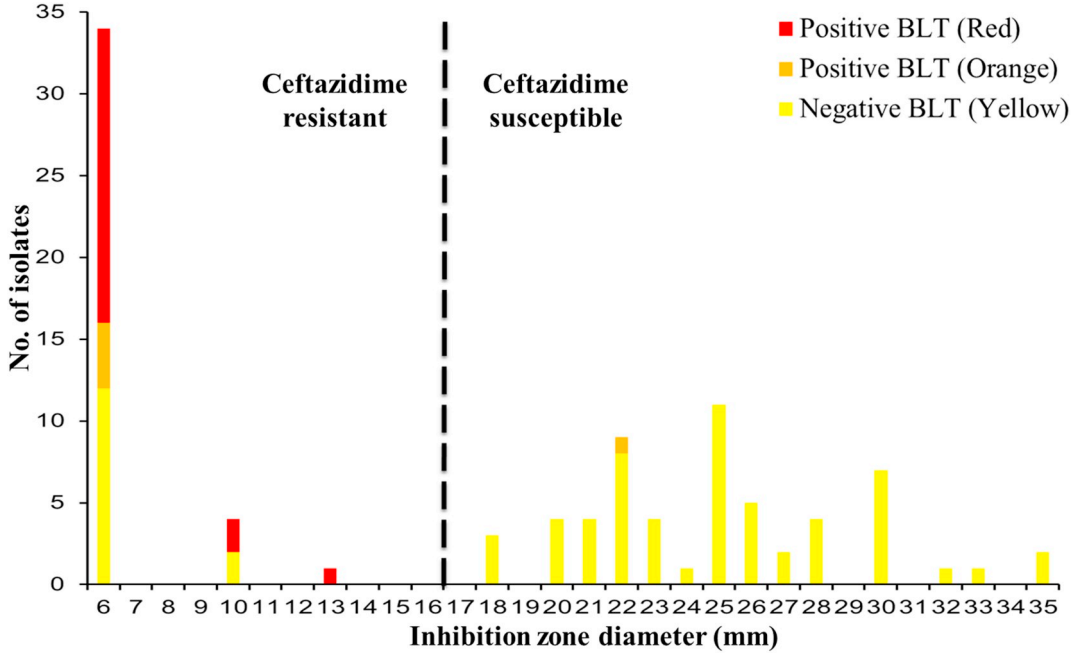

Fig. 1. β LACTA Test results and distribution of ceftazidime (10 μg) inhibition zone diameters for 97 *P. aeruginosa* clinical isolates collected from cystic fibrosis patients. Final colorimetric results (red, orange or yellow) were reported after 1-hour incubation at room temperature. The resistant and susceptible zones are defined following the EUCAST 2018 clinical breakpoints. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
β LACTA Test results for 97 *P. aeruginosa* clinical isolates collected from cystic fibrosis patients.

		β LACTA Test result at:				
		5 min	15 min	30 min	1 h	3 h
Ceftazidime-susceptible isolates (n = 58) [a]	Yellow	58	58	57	57	57
	Orange	0	0	1	1	1
	Red	0	0	0	0	0
Ceftazidime-resistant isolates (n = 39)	Yellow	30	21	16	14	13
	Orange	4	9	7	4	15
	Red	5	9	16	21	11

[a] Ceftazidime susceptibility was determined using the ceftazidime 10-μg disk diffusion method as described in the EUCAST 2018 guidelines.

Table 2
Performance of the β LACTA Test at various incubation periods compared to antibiotic susceptibility testing using the disk diffusion method.

	15 min	30 min	1 h	3 h
Sensitivity	46.2	59.0	64.1	66.7
Specificity	100.0	98.3	98.3	98.3
Negative predictive value	73.4	78.1	80.3	81.4
Positive predictive value	100.0	95.8	96.2	96.3

its low NPV in this context. This study has demonstrated that the BLT should not be used to determine resistance to ceftazidime in clinical isolates of *P. aeruginosa*.

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Conflicts of interest

None to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2019.01.019.

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