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SUSCEPTIBILITY

Aztreonam plus Clavulanate, Tazobactam, or Avibactam for Treatment of Infections Caused by Metallo-β-Lactamase-Producing Gram-Negative Bacteria

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ABSTRACT Metallo-β-lactamase (MBL)-producing Gram-negative bacteria are often extremely resistant, leading to a real therapeutic dead end. Here, we evaluated the *in vitro* and *in vivo* efficacy of aztreonam in combination with ceftazidime-avibactam, ceftolozane-tazobactam, or amoxicillin-clavulanate for the treatment of infections caused by MBL-producing *Enterobacteriaceae*, MBL-producing *Pseudomonas aeruginosa*, and extremely drug-resistant *Stenotrophomonas maltophilia*. First, we report two clinical cases, namely, a urinary tract infection caused by an NDM-5-producing *Escherichia coli* isolate and a pulmonary infection caused by a *S. maltophilia* isolate efficiently treated with the association of aztreonam-ceftazidime-avibactam and aztreonam-amoxicillin-clavulanate, respectively. Then, a total of 50 MBL-producing *Enterobacteriaceae* isolates, 3 MBL-producing *P. aeruginosa* isolates, and 5 extremely drug-resistant *S. maltophilia* isolates were used to test aztreonam susceptibility in combination with ceftolozane-tazobactam, ceftazidime-avibactam, or amoxicillin-clavulanate. The Etest strip superposition method was used to determine the MICs of the aztreonam/inhibitor combinations. According to CLSI breakpoints, aztreonam susceptibility was fully restored for 86%, 20%, and 50% of the MBL-producing *Enterobacteriaceae* isolates when combined with ceftazidime-avibactam, ceftolozane-tazobactam, and amoxicillin-clavulanate, respectively. In *P. aeruginosa*, the aztreonam-ceftazidime-avibactam combination was the most potent, even though the reduction in MICs was at most 2-fold. With the 5 *S. maltophilia* isolates, aztreonam-ceftazidime-avibactam and aztreonam-amoxicillin-clavulanate were found to be equal (100% susceptibility). Overall, aztreonam-ceftazidime-avibactam was the most potent combination to treat infections caused by MBL producers compared with aztreonam-amoxicillin-clavulanate and aztreonam-ceftolozane-tazobactam. However, in many cases aztreonam-amoxicillin-clavulanate was found to be as efficient as aztreonam-ceftazidime-avibactam, offering the main advantage to be markedly cheaper. We also confirmed the validity of Etest superpositions as a very simple method to determine MICs of aztreonam combinations.

KEYWORDS IMP, NDM, VIM, carbapenemase, case report, monobactam, therapeutic impasse

Carbapenem-resistant Gram-negative bacilli are increasingly reported worldwide (1). In *Enterobacteriaceae*, carbapenem resistance is mainly mediated by the production of carbapenemases belonging to (i) Ambler class A carbapenemases

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(mostly KPC types) (2), (ii) Ambler class B carbapenemases or metallo-β-lactamases (MBLs) (mostly NDM, VIM, or IMP types) (3), and (iii) Ambler class D carbapenem-hydrolyzing β-lactamases of OXA-48-like types (4). In *Pseudomonas aeruginosa*, carbapenem resistance might be caused by a membrane permeability defect, overexpression of the chromosome-encoded cephalosporinase, overexpression of efflux pumps, or carbapenemase production (5, 6). In this species, the most prevalent carbapenemases are MBLs (mainly VIM and IMP types) (5, 6). All these carbapenemase-producing bacteria are a major threat to public health, as carbapenem resistance is often coupled with additional resistance mechanisms, such as extended-spectrum-β-lactamases (ESBLs), aminoglycoside-modifying enzymes, and mutations involved in fluoroquinolone resistance, precluding the clinical use of most antibiotics. Recently, new molecules (β-lactam/β-lactamase-inhibitor combinations) have been developed, including (i) ceftazidime-avibactam (7, 8) and (ii) ceftolozane-tazobactam (9). Despite that ceftazidime-avibactam and ceftolozane-tazobactam offer therapeutic alternatives for the treatment of infections caused by ESBL producers and class A and D carbapenemase producers, there is still no commercially available treatment for MBL producers. *Stenotrophomonas maltophilia*, which produces an MBL (L1) and an ESBL (L2), is also naturally resistant to many antimicrobials (e.g., aminoglycosides) and remains susceptible to very few molecules, such as ticarcillin-clavulanate, sulfamethoxazole-trimethoprim, and colistin. However, resistance to these antimicrobials may occur, and the production of ticarcillin-clavulanate has been stopped worldwide, fearing cases of pandrug-resistant *S. maltophilia* infections.

To fill this gap in the antibacterial armamentarium, the combination of two antibiotics, including aztreonam, which is still active on MBLs alone, with an antimicrobial association containing a β-lactamase inhibitor (avibactam, tazobactam, or clavulanic acid), inhibiting ESBLs if present, might be useful to treat infections with MBL producers. Recently, the association of aztreonam and ceftazidime-avibactam was proposed to treat infections caused MBL-producing *Klebsiella pneumoniae* and NDM-1-producing *P. aeruginosa* (10–13). The association aztreonam-avibactam that is still in clinical phase 2 development was also suggested to be a promising alternative to treat *S. maltophilia*-related infections (14).

Here, we report two clinical cases, namely (i) a urinary tract infection (UTI) caused by an NDM-5-producing *Escherichia coli* isolate and (ii) a pulmonary infection caused by *S. maltophilia* efficiently treated with an association of aztreonam-ceftazidime-avibactam and aztreonam-amoxicillin-clavulanate, respectively. In addition, we have evaluated the susceptibility of MBL-producing *Enterobacteriaceae*, MBL-producing *P. aeruginosa*, and extremely drug resistant *S. maltophilia* isolates to aztreonam in association with (i) ceftazidime-avibactam, (ii) ceftolozane-tazobactam, or (iii) amoxicillin-clavulanate.

RESULTS

Aztreonam-ceftazidime-avibactam combination to treat a pyelonephritis caused by NDM-5-producing *E. coli*. A 70-year-old man without a particular medical history, except a trip to India 1 month earlier, was admitted at our hospital for acute pyelonephritis. At admission, the temperature was of 40°C, the pulse at 120 beats per minute, and the blood pressure at 100/80 mm Hg. The white blood cell (WBC) count was 20,000 per mm^3, the C-reactive protein (CRP) at 338 mg/liter, the procalcitonin at 36.73 μg/liter, and the creatinine level at 173 μmol/liter (glomerular filtration rate [GFR] at 34). Cytobacteriological examination of the urine revealed 127,900 red blood cells/ml, 125,000 WBC/ml, and $>10^7$/ml *E. coli* which was found to be resistant to all tested antimicrobial agents except colistin, gentamicin, and fosfomycin, with MICs at 0.25 mg/liter, 0.38 mg/liter, and 0.75 mg/liter, respectively. This extremely drug resistant *E. coli* isolate belonged to sequence type 167 (ST-167) and produced an NDM-5 carbapenemase associated with an OXA-1 penicillinase and a CTX-M-15 ESBL. This strain was also isolated from three consecutive blood cultures. Treatment comprising colistin, fosfomycin, and gentamicin was started. Despite the strict surveillance and dosages of

TABLE 1 MIC breakpoints for antimicrobials according to CLSI guidelines

Antimicrobial(s)	MIC (mg/liter) breakpoints for:			
	Enterobacteriaceae spp.		*Pseudomonas* spp.[a]	
	S[b]	R[b]	S	R
Aztreonam	≤4	≥16	≤8	≥32
Amoxicillin-clavulanate	≤8	≥32	—[c]	—
Ceftolozane-tazobactam	≤2	≥8	≤4	≥16
Ceftazidime-avibactam	≤8	≥16	≤8	≥16

[a]Due to the absence of breakpoints, *Pseudomonas* spp. breakpoints were used to categorize *S. maltophilia*.
[b]S, susceptible; R, resistant.
[c]—, No breakpoint (natural resistance).

gentamicin and colistin, the renal function deteriorated rapidly (creatinine at 350 μmol/liter and GFR at 15). Gentamicin was stopped at day 7 and colistin and fosfomycin at day 10. Despite 10 days of treatment with antibiotics active *in vitro*, the patient still had fever with elevated inflammatory markers. Thus, MICs to aztreonam-ceftazidime-avibactam and aztreonam-amoxicillin-clavulanate were determined at 0.094 mg/liter and 0.38 mg/liter, respectively. Antimicrobial treatment was switched to aztreonam (2 g three times a day) plus ceftazidime-avibactam (2 g to 0.5 g three times a day) for 7 days. The patient became apyretic 48 h later with improvement of the renal function (creatinine at 172 μmoles/liter and GFR at 34) and resolution of the inflammatory syndrome. At day 60, microbiological and clinical cure was confirmed with complete restoration of renal function (creatinine 130 μmoles/liter and GFR at 47).

Aztreonam-amoxicillin-clavulanate combination to treat a pulmonary infection caused by highly resistant *Stenotrophomonas maltophilia*. A 60-year-old woman was hospitalized in the pneumology intensive care unit for decompensated chronic obstructive pulmonary disease, associated with hypercapnic acidosis. Prior to hospitalization, the patient received amoxicillin-clavulanate empirical treatment for 1 week for purulent sputum. At admission, the temperature was 38.5°C, the WBC count was 18,900 per mm^3, and the CRP was 189 mg/liter. A chest X-ray revealed left-sided basal pneumonia that was microbiologically confirmed to be due to a *S. maltophilia* isolate resistant to all β-lactams, including ticarcillin-clavulanate (MIC, >256 mg/liter), fluoroquinolones (levofloxacine MIC at 16 mg/liter), and sulfametoxazole-trimetoprim (MIC at 128 mg/liter). It remained susceptible only to colistin (MIC at 0.25 mg/liter). An evaluation of aztreonam-ceftazidime-avibactam and aztreonam-amoxicillin-clavulanate associations revealed an MIC at 6 mg/liter for both. Using CLSI breakpoints available for *Pseudomonas* spp. this strain was considered susceptible (Table 1). Aztreonam (1 g three times a day) plus amoxicillin-clavulanate (1 g three times a day) for 15 days was started. The patient's evolution was rapidly favorable with resolution of the fever and improvement of the inflammatory syndrome (CRP from 189 to 88 mg/liter in less than 48 h and 21 mg/liter after 5 days), and microbiological and clinical cure was confirmed at day 60.

***In vitro* evaluation of aztreonam-β-lactamase inhibitor combinations on collection isolates.** To assess the potential efficacy of the aztreonam-inhibitor combination, we have tested these combinations *in vitro* on a large collection of MBL-producing isolates. All tested isolates were resistant or intermediate to aztreonam (MIC range, 6 to >256 mg/liter), to ceftazidime-avibactam (MIC range, 16 to >256 mg/liter), to ceftolozane-tazobactam (MIC range, 6 to >256 mg/liter), and to amoxicillin-clavulanate (MIC range, 12 to >256 mg/liter) (Table 2). According to CLSI breakpoints (Table 1), susceptibility rates (≤4 mg/liter) of aztreonam-ceftazidime-avibactam, aztreonam-ceftolozane-tazobactam, and aztreonam-amoxicillin-clavulanate were 86% ($n = 43/50$), 20% ($n = 10/50$), and 50% ($n = 25/50$) for *Enterobacteriaceae*, respectively (Table 2). No difference was observed depending on the type of MBL produced by the enterobacterial isolate. MICs of aztreonam-inhibitor combinations were higher with NDM-producing *E. coli* than that of NDM-producing *K. pneumoniae*. However, whole-genome

TABLE 2 MICs and categorization according to CLSI breakpoints for antimicrobials on MBL-producing *Enterobacteriaceae*, MBL-producing *P. aeruginosa*, and *S. maltophilia*

		MICs (mg/liter) by treatment[a]						
Enterobacteriaceae sp.	β-Lactamases	ATM	CZA	C/T	AMC	ATM+ CZA	ATM+ C/T	ATM+ AMC
E. coli	NDM-1 + OXA-1 + OXA-10 + CMY-16 + TEM-1	32	>256	>256	16	0.125	24	8
E. coli	NDM-1 + CTX-M-15 + TEM-1	>256	>256	>256	12	1	>256	2
E. coli	NDM-1 + OXA-1 + OXA-2 + CTX-M-15 + TEM-1	>256	>256	>256	24	2	>256	8
E. coli	NDM-1 + CTX-M-15 + TEM-1	>256	>256	>256	32	6	>256	8
E. coli	NDM-4 + CTX-M-15 + OXA-1	>256	>256	>256	96	6	>256	4
E. coli	NDM-4 + CTX-M-15 + CMY-6	>256	>256	>256	>256	6	>256	24
E. coli	NDM-5 + TEM-1 + CTX-M-15	>256	>256	>256	96	8	>256	64
E. coli	NDM-6 + CTX-M-15 + OXA-1	>256	>256	>256	16	1	>256	2
E. coli	NDM-7 + ESBL	>256	>256	>256	96	4	>256	32
K. pneumoniae	NDM-1 + CTX-M-15 + SHV-11 + OXA-1	>256	>256	>256	12	0.125	24	0.38
K. pneumoniae	NDM-1 + CTX-M-15 + CMY-4 + OXA-1	>256	>256	>256	32	0.75	>256	16
K. pneumoniae	NDM-1 + CTX-M-15 + OXA-1 + OXA-9 + TEM-1 + SHV-28 + SHV-11	>256	>256	>256	32	0.25	>256	3
K. pneumoniae	NDM-1 + OXA-1 + SHV-11	>256	>256	>256	12	0.047	0.094	0.094
K. pneumoniae	NDM-1 + OXA-1 + CTX-M-15 + TEM-1 + SHV-28 + OXA-9 + CMY-6	>256	>256	>256	16	0.047	3	0.25
K. pneumoniae	NDM-1 + TEM-1 + CTX-M-15 + SHV-12 + OXA-9	>256	>256	>256	12	0.125	96	1
K. pneumoniae	NDM-1 + TEM-1 + CTX-M-15 + SHV-12 + OXA-9	>256	>256	>256	12	0.125	96	0.5
K. pneumoniae	NDM-1 + TEM-1 + CTX-M-15 + SHV-11 + OXA-1	>256	>256	>256	12	0.064	8	0.38
Salmonella enterica	NDM-1 + CTX-M-15 + TEM-1 + OXA-1 + OXA-9 + OXA-10	>256	>256	>256	16	0.125	16	0.5
E. coli	VIM-1 + CTX-M-3	>256	>256	>256	16	0.125	24	0.5
E. coli	VIM-4 + ESBL	16	>256	>256	24	1.5	24	16
K. pneumoniae	VIM-1 + SHV-5	>256	>256	>256	>256	0.25	192	1.5
K. pneumoniae	VIM-1 + SHV-12	>256	>256	>256	16	0.125	4	0.25
K. pneumoniae	VIM-1 + ESBL	>256	>256	>256	>256	12	16	12
K. pneumoniae	VIM-1 + SHV-5	16	>256	>256	>256	6	12	32
K. pneumoniae	VIM-1 + TEM-1 + SHV-5	96	>256	>256	>256	96	64	48
K. pneumoniae	VIM-1 + SHV-5	>256	>256	>256	24	0.25	8	0.75
K. pneumoniae	VIM-1 + SHV-5	>256	>256	>256	12	0.125	2	0.38
K. pneumoniae	VIM-19 + CTX-M-3 + TEM-1 + SHV-1	6	32	>256	16	0.047	2	1.5
Enterobacter cloacae	VIM-1 + SHV-70	256	128	>256	48	0.094	0.25	0.19
E. cloacae	VIM-4 + CTX-M-15 + TEM-1 + SHV-31	64	>256	>256	64	1	64	32
Citrobacter freundii	VIM-2 + TEM-1 + ESBL	16	16	>256	32	0.25	2	24
C. freundii	VIM-2 + TEM-1 + OXA-9 + OXA-10	32	24	>256	32	1.5	16	24
E. coli	IMP-8 + SHV-12	128	>256	>256	24	0.19	2	0.38
K. pneumoniae	IMP-8 + SHV-12	>256	48	>256	12	0.094	32	0.25
E. cloacae	IMP-8 + SHV-12	12	>256	>256	24	0.032	0.064	0.094
E. cloacae	GIM-1 + ESBL	12	>256	48	24	0.5	8	16
Enterobacter hormaechei	TMB-1 + overexpressed Case[b]	64	64	32	32	0.5	12	12
C. freundii	TMB-1 + overexpressed Case	64	96	32	12	0.125	12	12
K. pneumoniae	NDM-1 + OXA-181 + SHV-11 + TEM-1 + CTX-M-15 + OXA-1	64	>256	>256	48	0.094	8	2
K. pneumoniae	NDM-1 + OXA-181 + SHV-27 + CTX-M-15 + TEM-1 + OXA-1	128	>256	>256	96	0.25	16	3
K. pneumoniae	NDM-1 + OXA-181 + SHV-11 + CTX-M-15 + OXA-1	256	>256	>256	>256	0.19	32	3
K. pneumoniae	NDM-1 + OXA-181 + SHV-11 + TEM-1 + CTX-M-15 + OXA-9	>256	>256	>256	>256	0.19	>256	12
K. pneumoniae	NDM-1 + OXA-181 + SHV-2 + CTX-M-15 + OXA-1	>256	>256	>256	32	0.125	32	1.5
C. freundii	NDM-1 + OXA-181 + OXA-1 + OXA-9 + OXA-10 + CTX-M-15 + TEM-1	>256	>256	>256	64	0.75	>256	12
E. coli	NDM-1 + OXA-48 + ESBL	32	>256	>256	48	0.094	12	8
E. coli	NDM-1 + OXA-48 + ESBL	>256	>256	>256	>256	0.75	>256	4
E. coli	NDM-1 + OXA-48 + ESBL	>256	>256	>256	>256	1	>256	4
K. pneumoniae	NDM-1 + OXA-232 + ESBL	64	>256	>256	>256	0.094	24	3
E. coli	NDM-1 + OXA-232 + ESBL	>256	>256	>256	>256	1	>256	8
E. coli	NDM-5 + OXA-232 + ESBL	>256	>256	>256	96	1	>256	64
S. maltophilia		>256	>256	>256	32	2	128	2
S. maltophilia		>256	>256	6	96	1.5	6	2
S. maltophilia		>256	>256	>256	>256	4	>256	4
S. maltophilia		>256	16	72	16	1	8	2
S. maltophilia		>256	>256	>256	>256	0.75	24	0.75
P. aeruginosa	VIM-2 + overexpressed cephalosporinase	16	24	>256	>256	8	12	16
P. aeruginosa	IMP-2 + overexpressed cephalosporinase	12	>256	>256	>256	6	12	24
P. aeruginosa	IMP-1 + overexpressed cephalosporinase	128	>256	>256	>256	96	48	64

[a]Black, gray, and white colored MICs correspond to resistant, intermediate, and susceptible categorization, respectively, according to CLSI breakpoints, as updated in 2018. *Pseudomonas* sp. breakpoints were used for *Stenotrophomonas maltophilia*. ATM, aztreonam; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam; AMC, amoxicillin-clavulanate.

[b]Case, chromosome-encoded cephalosporinase.

sequence (WGS) analyses did not reveal any correlation between β-lactamase contents and these higher MICs. Two of the three *P. aeruginosa* isolates were categorized as susceptible to the aztreonam-ceftazidime-avibactam combination, which remained the most potent association, even though the reduction in MICs was at most 2-fold. Aztreonam susceptibility was restored for all *S. maltophilia* isolates when combined with ceftazidime-avibactam or amoxicillin-clavulanate, whereas two isolates remained resistant to aztreonam-ceftolozane-tazobactam (Table 2).

DISCUSSION

We demonstrated that the associations of aztreonam with ceftazidime-avibactam or amoxicillin-clavulanate are useful therapeutic options to treat infections caused by MBL-producing Gram-negative bacteria. Our results confirm the efficacy of the aztreonam-ceftazidime-avibactam combination for the treatment of most MBL-producing *Enterobacteriaceae* bacteria (86%), as previously observed (10–13). Although, we showed that this combination is the most powerful, aztreonam-amoxicillin-clavulanate was found to be as efficient as aztreonam-ceftazidime-avibactam in many cases (Table 2). It suggests that the aztreonam-amoxicillin-clavulanate association should be tested systematically. Indeed, in case of equal or close MICs, the aztreonam-amoxicillin-clavulanate association offers the main advantage to be markedly cheaper than aztreonam-ceftazidime-avibactam. Accordingly, we described the successful treatment of a pneumonia caused by an extremely drug resistant *S. maltophilia* using the aztreonam-amoxicillin-clavulanate combination. However, it could be noticed that the efficiency of aztreonam/inhibitor combinations for *P. aeruginosa* should be tested on a larger collection. Indeed, the observed 2-fold MIC reduction was obtained on strains with low aztreonam MICs. It could be interesting to test these combinations on strains with additional resistance mechanisms, such as ESBL.

It is assumed that the β-lactamase inhibitor (avibactam, clavulanate, or tazobactam) is responsible for the restoration of aztreonam susceptibility. Accordingly, we have demonstrated that Etest superpositions and aztreonam Etest performed on clavulanate- (2 mg/liter) or tazobactam- (4 mg/liter) supplemented Mueller Hinton (MH) gave the same results (see Fig. S1 in the supplemental material). It confirmed the validity of Etest superpositions as a very simple method to determine MICs of aztreonam combinations in any clinical microbiology laboratory. In addition, the reproducibility of the Etest superposition method was tested on 10 isolates (3 times). The same MICs were systematically obtained. We assume that other combinations, such as aztreonam-ampicillin-sulbactam should be tested and might be successful for the treatment of MBL producers. Finally, our results confirm the potential activity of the aztreonam-avibactam association (in phase 2 development) for the treatment of MBL producers, as it has been previously reported on a few Gram-negative collections (14–17).

MATERIALS AND METHODS

Bacterial isolates. A total of 50 MBL-producing *Enterobacteriaceae* isolates, 3 MBL-producing *P. aeruginosa* isolates, and 5 *S. maltophilia* isolates were used to test the aztreonam/inhibitor combinations (Table 2). All selected strains were also resistant to aztreonam due to the production of an ESBL and/or a cephalosporinase. The MBL-producing *Enterobacteriaceae* group included 30 NDM producers with 12 isolates coproducing an OXA-48-like carbapenemase, 14 VIM producers, 3 IMP producers, 1 GIM-1 producer, and 2 TMB-1 producers. The MBL-producing *P. aeruginosa* bacteria were VIM-2, IMP-1, and IMP-2 producers. The five *S. maltophilia* isolates were resistant to all β-lactams, including ticarcillin-clavulanate, and to all other antimicrobials, including fluoroquinolones, colistin, and sulfamethoxazole-trimethoprim.

MIC determination. MICs to aztreonam, ceftazidime-avibactam, ceftolozane-tazobactam, and amoxicillin-clavulanate antimicrobials were determined by Etest (bioMérieux, La Balme-les-Grottes, France). The Etest strip superposition method was used to determine the MICs of the aztreonam-inhibitor combinations. MICs were determined after 16 h of incubation at 37°C and interpreted according to CLSI guidelines, as updated in 2018 (18). Since no recommendation exists regarding these molecules for *S. maltophilia*, interpretation criteria of *Pseudomonas* spp. were used (Table 1).

Etest strip superposition method. To determine the MICs of the aztreonam/inhibitor combinations, the Etest strips of ceftolozane-tazobactam, ceftazidime-avibactam, or amoxicillin-clavulanate were ap-

plied on MH agar plates (inoculated with the bacteria to test) for 10 minutes and then removed, and the aztreonam strip was subsequently deposited on the exact same place. The ceftolozane-tazobactam, ceftazidime-avibactam, and amoxicillin-clavulanate Etest strips contain a fixed concentration of tazobactam (4 mg/liter), avibactam (4 mg/liter), and clavulanate (2 mg/liter).

The validity of the Etest strip superposition method was validated by performing aztreonam Etest on Mueller-Hinton agar supplemented with tazobactam (4 mg/liter) (Sigma-Aldrich) or clavulanate (2 mg/liter) (Sigma-Aldrich) (Fig. S1).

Whole-genome sequencing. The DNA libraries were prepared using the NexteraXT v3 kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions, and then run on the HiSeq system (Illumina) for generating paired-end 150-bp reads. *De novo* assembly of Illumina reads was performed using CLC genomic workbench 10.0 according the manufacturer's recommendations (Qiagen). The genome was annotated using the Rapid Annotations using Subsystem Technology (RAST) tool (http://rast.nmpdr.org/rast.cgi) (19).

ResFinder online software (https://cge.cbs.dtu.dk/services/ResFinder/) was used for the detection of resistance determinants other than β-lactamase (20). Sequence type determination was performed using the multilocus sequence type (MLST) 2.0 software available online at https://cge.cbs.dtu.dk/services/MLST/ (21).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00010-19.

SUPPLEMENTAL FILE 1, PDF file, 1.6 MB.

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We have no conflicts of interest to declare.

L.D. and C.E. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

L. Dortet contributed to study concept and design; all authors contributed to the acquisition, analysis, or interpretation of data; C. Emeraud, L. Dortet, L. Escaut, R.A. Bonnin, and T. Naas drafted the manuscript; and L. Dortet, R.A. Bonnin, and T. Naas performed a critical revision of the manuscript for important intellectual content.

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