Molecular β -Lactamase Characterization of Aerobic Gram-Negative Pathogens Recovered from Patients Enrolled in the Ceftazidime-Avibactam Phase 3 Trials for Complicated Intra-abdominal Infections, with Efficacies Analyzed against Susceptible and Resistant Subsets

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ABSTRACT The correlation of the clinical efficacy of ceftazidime-avibactam (plus metronidazole) with that of meropenem was evaluated in subjects infected with Gram-negative isolates having characterized β -lactam resistance mechanisms from the complicated intra-abdominal infection (cIAI) phase 3 clinical trials. *Enterobacteriaceae* isolates displaying ceftriaxone and/or ceftazidime MIC values of ≥ 2 $\mu\text{g/ml}$ and *Pseudomonas aeruginosa* isolates with ceftazidime MIC values of ≥ 16 $\mu\text{g/ml}$ were characterized for extended-spectrum- β -lactamase (ESBL) content. *Enterobacteriaceae* and *P. aeruginosa* isolates with imipenem and meropenem MIC values of ≥ 2 and ≥ 8 $\mu\text{g/ml}$, respectively, were tested for carbapenemase genes. The primary efficacy endpoint was clinical cure at test of cure (TOC) among the members of the microbiologically modified intention-to-treat (mMITT) population. A total of 14.5% (56/387) and 18.8% (74/394) of patients in the ceftazidime-avibactam and meropenem arms had isolates that met the MIC screening criteria at the baseline visit, respectively. CTX-M variants alone (29.7%; 41/138) or in combination with OXA-1/30 (35.5%; 49/138), most commonly, *bla*_{CTX-M} group 1 variants (79/90; 87.8%), represented the β -lactamases most frequently observed among *Enterobacteriaceae* isolates. Among the patients infected with pathogens that did not meet the screening criteria, 82.2% showed clinical cure in the ceftazidime-avibactam group versus 85.9% in the meropenem group. Among patients infected with any pathogens that met the MIC screening criteria, clinical cure rates at TOC were 87.5% and 86.5% for the ceftazidime-avibactam and meropenem groups, respectively. Ceftazidime-avibactam had clinical cure rates of 92.5% to 90.5% among patients infected with ESBL- and/or carbapenemase-producing *Enterobacteriaceae* strains at the baseline visit, while meropenem showed rates of 84.9% to 85.4%. The ceftazidime-avibactam and meropenem groups had cure rates of 75.0% and 86.7%, respectively, among patients having any pathogens producing AmpC enzymes. The efficacy of ceftazidime-avibactam was similar to that of meropenem for treatment of cIAI caused by ESBL-producing organisms. (This study has been registered at ClinicalTrials.gov under registration no. NCT01499290 and NCT01500239.)

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isolates of *Enterobacteriaceae* are common causes of community-acquired infections and health care-associated infections (HAI), and the latter are particularly evident among intensive care unit (ICU) patients (1). In a worldwide multicenter study (75 countries; 1,265 ICUs), Gram-negative pathogens were estimated to cause 62% of HAI in ICU patients (2). An earlier study reported that Gram-negative bacilli were associated with 22% of HAI in U.S. hospitals (3), whereas a more recent investigation reported a prevalence of 39% of HAI caused by Gram-negative pathogens (or 27% caused by *Enterobacteriaceae*) among acute-care U.S. hospitals (4). A study performed in a hospital in Florence, Italy, reported a prevalence of 65% of Gram-negative organisms causing HAI in ICU patients (5).

In addition to the high prevalence rates, reports of antimicrobial resistance in Gram-negative pathogens causing community-acquired infections and HAI have increased (6, 7). Carbapenem-resistant *Enterobacteriaceae* (CRE) strains and ceftriaxone-resistant *Enterobacteriaceae* strains have been reported to account for 35,000 HAI each year in U.S. hospitals (6), and the percentage of CRE among U.S. medical centers increased from 1.2% in 2001 to 4.2% in 2011 (8). A combined prevalence of 2% carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* isolates from U.S., European, and Latin American centers was reported (9), and a prevalence of 6% among *K. pneumoniae* isolates in the United States alone was reported (10). Moreover, a more recent study has documented that 2% of CRE *E. coli* isolates from the United States carried the *mcr-1* plasmid-borne polymyxin resistance determinant (11).

The scenario described above, the lack of new antimicrobial agents approved in the last decades, the clinical challenges faced during management of infections caused by multidrug-resistant (MDR) organisms, and the limited therapeutic options have recently prompted several agencies to promote the development of new antimicrobial agents (12, 13). The use of ceftazidime-avibactam for the treatment of complicated intra-abdominal infections (cIAs), among other indications, was approved by the U.S. Food and Drug Administration (FDA) in 2015 (14) and by the European Medicines Agency in 2016 (15). The present investigation characterized the β -lactamase content of baseline pathogens recovered from patients with cIA enrolled in two phase 3 clinical trials of ceftazidime-avibactam and correlated the clinical efficacy of ceftazidime-avibactam and that of the comparator meropenem (16).

RESULTS

Study population. Randomized patients from 136 centers in 30 countries participated in the phase 3 clinical trial for intraabdominal infections. Overall, 58.9% of patients were recruited in the Eastern Europe region, 16.6% in North America and Western Europe, and 24.5% in other regions. Countries that recruited >5% of the total comprised the Czech Republic (22.8%), Romania (13.5%), India (11.7%), Ukraine (9.3%), the United States (8.0%), and Bulgaria (6.2%). A total of 1,149 patients were enrolled, 1,066 were randomized, and 1,058 (529 in each treatment group) received one or more doses of study therapy (16). Among the members of the microbiologically modified intention-to-treat (mMITT) population, 413 and 410 patients (823 total) were from the ceftazidime-avibactam plus metronidazole group and the meropenem group, respectively.

A total of 56 patients (54 with *Enterobacteriaceae* infections and 2 with *Pseudomonas aeruginosa* infections) in the ceftazidime-avibactam arm had baseline isolates that met the MIC screening criteria warranting molecular characterization for β -lactamases. Three subjects were infected with two *Enterobacteriaceae* species (that met the MIC screening criteria) at the baseline visit. In the comparator arm, 74 subjects (64 infected with *Enterobacteriaceae* and 4 infected with *P. aeruginosa*) were infected with baseline isolates that met the MIC screening criteria and 5 subjects had multiple isolates. Patients from either arm that had additional baseline isolates that did not meet the MIC screening criteria were not characterized.

Antimicrobial susceptibility profiles. The vast majority of *Enterobacteriaceae* isolates (85.9%; 806/938) did not meet the β -lactamase (MIC screening) criteria (Table 1).

TABLE 1 Summary of MIC results obtained against baseline pathogens (mMITT population)^a

Pathogen	No. of isolates tested	Phenotype/genotype ^b	Agent ^c	MIC (μg/ml) ^d			
				50%	90%	Min	Max
<i>Enterobacteriaceae</i>	806	MIC screen negative	CAZ	0.12	0.25	≤0.03	2
			CAZ-AVI	0.06/4	0.12/4	≤0.008/4	1/4
			MER	0.015	0.03	0.008	0.5
	132	MIC screen positive	CAZ	16	>64	0.12	>64
			CAZ-AVI	0.25/4	2/4	0.015/4	>256/4
			MER	0.03	0.25	0.015	>8
	5	Carbapenemase	CAZ	>64		>64	>64
			CAZ-AVI	>256/4		1/4	>256/4
			MER	>8		8	>8
	99	ESBL	CAZ	16	>64	0.12	>64
			CAZ-AVI	0.12/4	1/4	0.015/4	4/4
			MER	0.015	0.12	0.015	1
	28	AmpC	CAZ	64	>64	0.25	>64
			CAZ-AVI	0.5/4	4/4	0.015/4	4/4
			MER	0.03	0.12	0.015	0.5
<i>P. aeruginosa</i>	66	MIC screen negative	CAZ	2	4	0.5	8
			CAZ-AVI	2/4	4/4	0.5/4	4/4
			MER	0.12	0.5	0.03	8
	6	MIC screen positive	CAZ	>64		32	>64
			CAZ-AVI	16/4		8/4	32/4
			MER	2		2	>8

^aNote that with respect to the table data, a patient can have more than 1 pathogen in any given category.

^bFor positive phenotypic MIC screening, *Enterobacteriaceae* with ceftioxone and/or ceftazidime MIC results of ≥2 μg/ml and *P. aeruginosa* with ceftazidime MIC results of ≥16 μg/ml were selected for molecular characterization of β-lactamase content. *Enterobacteriaceae* and *P. aeruginosa* isolates exhibiting imipenem/meropenem MIC results of ≥2 and ≥8 μg/ml, respectively, were tested for the presence of carbapenemase-encoding genes. Carbapenemase-producing *Enterobacteriaceae* consisted of 5 isolates (see Table 2). The AmpC group is represented by isolates with any plasmid AmpC and/or overexpression of the intrinsic AmpC gene with or without other β-lactamases, while the remaining isolates were classified as ESBL.

^cCAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; MER, meropenem.

^dMax, maximum; Min, minimum; MIC₅₀, MIC required to inhibit the growth of 50% of organisms; MIC₉₀, MIC required to inhibit the growth of 90% of organisms.

These isolates had ceftazidime-avibactam MIC₅₀ and MIC₉₀ values of 0.06 and 0.12 μg/ml, respectively. Those *Enterobacteriaceae* isolates (14.1%; 132/938) that met the MIC screening criteria showed ceftazidime-avibactam MIC₅₀ and MIC₉₀ results of 0.25 and 2 μg/ml, respectively (Table 1). Furthermore, all *Enterobacteriaceae* isolates were inhibited by ceftazidime-avibactam at ≤4 μg/ml, except for four NDM-producing isolates (ceftazidime-avibactam MIC, >256/4 μg/ml). The comparator agent meropenem had MIC₅₀ and MIC₉₀ values of 0.015 and 0.03 μg/ml, respectively, against MIC screen-negative *Enterobacteriaceae* isolates, whereas MIC₅₀ and MIC₉₀ values of 0.03 and 0.25 μg/ml were obtained against MIC screen-positive isolates, respectively. Most *P. aeruginosa* isolates were MIC screen negative (66/72; 91.7%). Among those *P. aeruginosa* isolates that did not meet the MIC screening criteria, similar MIC values were observed for ceftazidime and ceftazidime-avibactam (MIC_{50/90} of 2/4 μg/ml for both), whereas the MIC screen-positive isolates (8.3%; 6/72) had MIC₅₀ levels of >64 and 16/4 μg/ml for ceftazidime and ceftazidime-avibactam, respectively (Table 1).

β-Lactamase profiles. CTX-M variants alone (29.7%; 41/138) or in combination with OXA-1/30 (35.5%; 49/138) represented the most common β-lactamases observed among *Enterobacteriaceae* isolates that met the MIC screening criteria (Table 2). Among the MIC screen-positive *E. coli* isolates, most pathogens (82.7%; 67/81) carried *bla*_{CTX-M} alone or with additional genes (*bla*_{OXA-1/30} and/or *bla*_{CMY}). Other *E. coli* isolates had SHV-12 (2.5%; 2/81), TEM-40 (2.5%; 2/81), or CMY (7.4%; 6/81), while resistance mechanisms were not detected in two isolates (Table 2). A total of 26/28 (92.9%) *K. pneumoniae* isolates had group-1 CTX-M variants (i.e., 1 *bla*_{CTX-M-3} and 25 *bla*_{CTX-M-15}), including one strain each that also had *bla*_{OXA-48}, *bla*_{NDM-1}, and *bla*_{NDM-4} (Table 2). These carbapenemase-producing *K. pneumoniae* isolates were detected as follows: one NDM-1 from Romania (ceftazidime-avibactam MIC, >256/4 μg/ml); one NDM-4 from India (ceftazidime-avibactam MIC, >256/4 μg/ml); and one OXA-48 from Romania (ceftazidime-avibactam MIC, 1/4 μg/ml; Tables 1 and 2). Moreover, one *K. pneumoniae*

TABLE 2 Summary of β -lactamase-encoding genes detected among baseline pathogens recovered from patients enrolled in the cIAI phase 3 trials for ceftazidime-avibactam that met the MIC screening criteria^a

Pathogen (no.; % of total)	Result ^b (no. of isolates)
<i>E. coli</i> (81; 58.7)	CTX-M-15 (10) CTX-M-14 (2) CTX-M-1 (2) CTX-M-2 (1) CTX-M-27 (8) CTX-M-3 (3) CTX-M-55 (1) CTX-M-55 + OXA-1/30 (1) CTX-M-1 + CTX-M-3 (1) CTX-M-15 + CMY (2) CTX-M-27 + CMY (1) CTX-M-15 + OXA-1/30 (31) CTX-M-142 + OXA-1/30 (1) CTX-M-15 + OXA-1/30 + CMY (2) CTX-M-15 + CTX-M-14 + OXA-1/30 + CMY-2 (1) SVH-12 (2) TEM-40 (2) CMY + OXA-1/30 (2) CMY (4) cAmpC (2) Unknown (2)
<i>K. pneumoniae</i> (28; 20.3)	NDM-1/-4 + CTX-M-15 (2) OXA-48 + CTX-M-15 + OXA-1/30 (1) CTX-M-15 (8) CTX-M-3 (1) CTX-M-15 + OXA-1/30 (13) CTX-M-15 + OXA-1/30 + SHV-27 (1) DHA-1 + OXA-1/30 + TEM-209 (1) Unknown (1)
<i>Enterobacter</i> spp. (12; 8.7) ^c	NDM-1 + CTX-M-15 + cAmpC (1) NDM-1 + OXA-1/30 (1) CTX-M-15 + OXA-1/30 (1) CTX-M-15 + OXA-1/30 + cAmpC (4) CTX-M-15 + OXA-1/30 + SHV-12 (1) CTX-M-3 (1) SVH-12 + cAmpC (1) cAmpC (2)
<i>P. mirabilis</i> (5; 3.6)	ACC-4 (4) CMY-16 (1)
<i>C. freundii</i> (3; 2.2)	cAmpC (1) CTX-M-15 + OXA-1/30 (1) CTX-M-3 (1)
<i>S. marcescens</i> (3; 2.2)	CTX-M-15 + OXA-1/30 (1) CTX-M-3 (2)
<i>P. aeruginosa</i> (6; 4.3)	cAmpC (1) cAmpC + OXA-10 (1) cAmpC + PER-1 (4)

^aNote that with respect to the table data, a patient can have more than 1 pathogen in any given category.

^bcAmpC represents overexpression of the intrinsic *ampC* gene. Genes encoding non-ESBL enzymes, such as TEM-1, TEM-79, SHV-1, SHV-11, SHV-33, and SHV-76, were detected but not included within the profiles described. TEM-209 enzyme possesses an unknown spectrum and was listed.

^cData include 11 *E. cloacae* and 1 *E. aerogenes* isolates.

showed *bla*_{DHA-1} and *bla*_{OXA-1/30}, whereas a second isolate did not have any of the screened resistance mechanisms.

CTX-M enzymes (58.3%; 7/12) prevailed among *Enterobacter* spp. (10 *E. cloacae* and 2 *E. aerogenes* isolates) and were most frequently (85.7%; 6/7) associated with OXA-1/30

TABLE 3 Clinical response at TOC (mMITT population)

Patient subgroup	Clinical cure rate ^a				% difference between groups ^b
	Ceftazidime-avibactam (n = 413)		Meropenem (n = 410)		
	n	m (%)	n	m (%)	
Renal status					
Normal function	476	407 (85.5)	478	410 (85.8)	−0.3
Moderate impairment	41	20 (48.8)	43	32 (74.4)	−25.6
MIC screen negative	331	272 (82.2)	320	275 (85.9)	−3.8
MIC screen positive	56	49 (87.5)	74	64 (86.5)	1.0
Enterobacteriaceae	54	47 (87.0)	70	60 (85.7)	1.3
ESBL/carbapenemase	42 ^c	38 (90.5)	55 ^d	47 (85.4)	4.6
ESBL	40 ^e	37 (92.5)	53 ^f	45 (84.9)	7.6
AmpC	12 ^g	9 (75.0)	15 ^h	13 (86.7)	−11.7
<i>P. aeruginosa</i>					
MIC screen negative	31	27 (87.1)	31	29 (93.5)	−6.5
MIC screen positive	2	2 (100.0)	4 ⁱ	4 (100.0)	0.0
Gene/infection status ^j					
Single gene	26	21 (80.8)	30	27 (90.0)	−9.2
Multiple genes/polymicrobial	30	27 (90.0)	42	36 (85.7)	4.3
Total	413	337 (81.6)	410	349 (85.1)	−3.5

^aPercentages are calculated as *m/n*, where *m* was defined as the number of patients with a clinical response at the test-of-cure visit and *n* was defined as the combined number of patients with clinical cure, clinical failure, and indeterminate outcome. Efficacy results for all patients from the ceftazidime-avibactam and meropenem arm were adapted from Mazuski et al. 2016 (16). Renal function was based on CrCl reported by the site using the Cockcroft-Gault method (16) and based on local laboratory data (normal renal function, CrCl = >50 ml/min; moderate renal impairment, CrCl = >30 to ≤50 ml/min). Patients with a baseline CrCl of <31 ml/min were recorded as having a missing renal status and were not included in the analysis. The clinical cure rate in patients in the mMITT population with missing renal status receiving ceftazidime-avibactam or meropenem was 2 of 3 patients (66.6%) or 2 of 2 (100%), respectively.

^bDifferences were calculated as the value for the ceftazidime-avibactam plus metronidazole treatment group minus the value for the meropenem treatment group.

^cData include patients with isolates carrying multiple non-ESBL- and/or ESBL genes, including one isolate with *bla*_{OXA-48} (cure at TOC) and two *bla*_{NDM-1/4} (failure). One patient had a polymicrobial infection.

^dData include patients with isolates carrying multiple non-ESBL and/or ESBL genes, including two subjects with NDM-1-producing isolates (clinical cure). Two patients had polymicrobial infections.

^eData include patients with isolates carrying multiple non-ESBL and/or ESBL genes.

^fData include patients with isolates carrying multiple non-ESBL and/or ESBL genes. Data include one patient infected with two *E. coli* strains each harboring *bla*_{SHV-12} and *bla*_{CTX-M-15} plus *bla*_{OXA-1/30}.

^gData include patients with isolates with documented overexpression of the intrinsic AmpC gene and/or carrying plasmid AmpC and/or with multiple non-ESBL and/or ESBL genes. One patient had a polymicrobial infection (failure).

^hData include isolates with documented overexpression of the intrinsic AmpC gene or carrying plasmid AmpC alone or with multiple non-ESBL and/or ESBL genes.

ⁱTwo patients had concomitant ESBL-producing *Enterobacteriaceae* isolates at the baseline visit.

^jData represent patients infected with pathogens where a single ESBL-encoding gene was detected versus patients that had polymicrobial infections or where pathogens had multiple ESBL genes.

(Table 2). In addition, two *E. cloacae* isolates from India carried *bla*_{NDM-1} (ceftazidime-avibactam MIC, >256/4 µg/ml; Tables 1 and 2). All *Proteus mirabilis* isolates that met the MIC screening criteria carried plasmid-encoded AmpC enzymes (ACC-4 and CMY-16; Table 2), whereas *Serratia marcescens* and *Citrobacter freundii* isolates produced CTX-M-3 or CTX-M-15 (plus OXA-1/30) and one *C. freundii* isolate had elevated expression of AmpC. All *P. aeruginosa* isolates demonstrated overexpression of the intrinsic AmpC gene alone or associated with *bla*_{OXA-10} or *bla*_{PER-1} (Table 2).

Efficacy analysis of ceftazidime-avibactam and carbapenems (mMITT population). Overall, the clinical cure rates at test of cure (TOC) were high (>80%) and similar for the two treatment groups of the mMITT population (Table 3). There was a response trend in favor of meropenem in the mMITT population with moderate renal impairment, as reported previously (16). Among the patients infected with pathogens that did not meet the screening criteria, 82.2% showed clinical cure in the ceftazidime-avibactam group versus 85.9% in the meropenem group. Among the patients infected with any pathogens that met the phenotypic MIC screening criteria, clinical cure rates at TOC were 87.5% and 86.5% for the ceftazidime-avibactam and meropenem groups, respectively. Data for patients having baseline isolates that met the MIC screening

TABLE 4 Summary of patients in the CAZ-AVI arm with baseline isolates that met the MIC screening criteria who had clinical failure or had an indeterminate status at TOC^a

Pathogen(s)	CAZ-AVI MIC ($\mu\text{g/ml}$)	Molecular characterization
<i>E. coli</i>	0.12/4 2/4 0.12/4	CMY-2 CMY-42 CTX-M-27
<i>E. coli</i> and <i>K. pneumoniae</i>	0.25/4 and 0.12/4	CMY-59 and CTX-M-15/OXA-30
<i>C. freundii</i>	0.12/4	CTX-M-3
<i>K. pneumoniae</i>	0.5/4	CTX-M-15/OXA-30
<i>E. cloacae</i> and <i>K. pneumoniae</i>	>256/4 and >256/4	NDM-1/OXA-30 and NDM-4/CTX-M-15

^aCAZ-AVI, ceftazidime-avibactam. Patients in the comparator arm that failed treatment or had an indeterminate status were infected with *Enterobacteriaceae* isolates (meropenem MIC, 0.015 to 1 $\mu\text{g/ml}$) producing CTX-M with or without OXA-1/30, except for one isolate that also had TEM-40. One patient had a polymicrobial infection.

criteria and presented clinical failure or had an indeterminate status at TOC in both arms are summarized in Table 4.

Clinical cure rates of 90.5% to 92.5% and 85.4% to 85.7% were noted for ceftazidime-avibactam and meropenem, respectively, in patients infected with *Enterobacteriaceae* isolates with any extended-spectrum- β -lactamase (ESBL)- and/or carbapenemase-encoding genes detected at baseline. Clinical cure rates of 75.0% in the ceftazidime-avibactam group and 86.7% in the meropenem group were observed in patients harboring any *Enterobacteriaceae* pathogens producing plasmid AmpC and/or overproducing the intrinsic AmpC enzyme, with or without the presence of additional β -lactamase enzymes (Table 3).

All patients infected by *P. aeruginosa* isolates that met the MIC screening criteria reached clinical cure at TOC, regardless of the additional concomitant presence of *Enterobacteriaceae* isolates or genotype (Table 3). Moreover, the presence of a polymicrobial infection caused by at least two pathogens that met the MIC screening criteria or of an infection caused by a pathogen having multiple ESBL enzymes did not alter the clinical cure rates for the two arms in comparison to those cIAI caused by isolates with a single ESBL enzyme (Table 3).

DISCUSSION

A total of 14.1% of baseline *Enterobacteriaceae* isolates collected from the mMITT population met the screening criteria for β -lactamase-encoding genes. This prevalence is lower than that obtained in the cIAI phase 2 trial for ceftazidime-avibactam (33.8%) (17) but higher than that reported in a more recent trial for ceftolozane-tazobactam (7.2%) (18). These differences in the prevalences of ESBL-producing pathogens can probably be attributed to geographical differences and probably reflect the epidemiology of participating hospitals. However, these studies do share the high proportions of CTX-M-producing isolates causing cIAI, frequently associated with OXA-1/30 (17, 18). The study results presented here corroborate those of previous investigations reporting the overwhelming dominance of *Enterobacteriaceae* isolates producing such enzymes (i.e., CTX-M), which has been very well documented in both nosocomial and community settings during the last several years (17, 19).

The overall clinical cure rates at TOC were similar for the arms (81.6% and 85.1% for ceftazidime-avibactam and meropenem, respectively). This trend was also observed among patients with MIC screen-negative pathogens (82.2% and 85.9% for ceftazidime-avibactam and meropenem, respectively) and MIC screen-positive pathogens (87.5% and 86.5% for ceftazidime-avibactam and meropenem, respectively) (16). Among the patients in the ceftazidime-avibactam arm infected with MIC screen-positive *Enterobacteriaceae* isolates, four patients showed clinical failure, whereas three had an indeterminate status at TOC due to having been lost to follow-up or to death to which

cIAI was noncontributory. Among the indeterminate cases, one patient was infected with two NDM-producing *Enterobacteriaceae* isolates (ceftazidime-avibactam MIC, >256/4 $\mu\text{g/ml}$), and the metallo- β -lactamase (MBL) group of enzyme is known to be refractory to inhibition by avibactam (20). Interestingly, the accumulation of ESBL genes did not affect the clinical outcomes obtained for ceftazidime-avibactam or meropenem, which also suggests that characteristics other than strain background and phenotype/genotype (except for the presence of MBL) play more important roles.

All subjects infected with *P. aeruginosa* at the baseline visit on both arms had clinical cure at TOC. These isolates exhibited ceftazidime-avibactam and meropenem MIC values of 8 and 0.03 to 2 $\mu\text{g/ml}$, respectively. However, the number of *P. aeruginosa* isolates that met the screening criteria was low and should be considered a study limitation. The low number of *P. aeruginosa* isolates could be attributed to the screening criteria applied (ceftazidime and imipenem/meropenem MIC results of $\geq 16 \mu\text{g/ml}$ and $\geq 8 \mu\text{g/ml}$, respectively). In addition, these *P. aeruginosa* isolates were screened only for resistance mechanisms mediated by β -lactamase enzymes, while it is well known that decreased permeability plays an important role in causing resistance to ceftazidime-avibactam and other antimicrobial agents (21).

Clinical cure rates for meropenem were numerically higher than those for ceftazidime-avibactam among patients with moderate renal function (Table 3). This finding was reported previously, and subsequent pharmacokinetic/pharmacodynamic modeling was utilized to modify dose adjustments for moderate (and severe) renal impairment at baseline to optimize ceftazidime-avibactam plasma levels to ensure the highest possible target attainments are achieved (14, 16). In summary, this report provides new insights for the treatment of cIAI caused by multidrug-resistant (MDR) pathogens by analyzing the rates of clinical cure according to β -lactamase production and expands on the results obtained from the phase 2 clinical trial study (16). Ceftazidime-avibactam became an addition to the antimicrobial armamentarium for treatment of infections caused by MDR isolates, and this report emphasizes its potential clinical value as a carbapenem-sparing agent since 14.1% mMITT patients had ESBL pathogens. Moreover, a similar molecular characterization was performed in isolates from additional phase 3 trials for urinary tract infections and nosocomial pneumonia (22, 23), which will enable further data analysis to complement the findings documented here.

MATERIALS AND METHODS

Patients, clinical isolates, study treatment, and endpoints. Two identical, prospective, randomized, multicenter, and double-blind comparative studies (ClinicalTrials registration no. NCT01499290 and NCT01500239) were conducted between March 2012 and April 2014 (16). In each trial, eligible patients were randomized to one of two treatment groups in a 1:1 ratio according to a central randomization schedule to receive either ceftazidime-avibactam (2 g of ceftazidime combined with 0.5 g of avibactam as a 2-hour intravenous infusion every 8 h, followed by 0.5 g metronidazole as a 60-min intravenous infusion every 8 h) or meropenem (1 g as a 30-min intravenous infusion every 8 h) for 5 to 14 days. After at least 5 full days of intravenous study therapy, treatment could be discontinued if the patient showed clinical improvement. If *Enterococcus* species or methicillin-resistant *Staphylococcus aureus* were suspected or isolated, vancomycin, linezolid, or daptomycin could be added to either of the study regimens at the discretion of the investigator. Antifungal therapy was not permitted (16).

Study protocols were designed to minimize the potential for prior use of other antibiotics that might confound the assessment of the treatment effect. For patients with moderate renal impairment at baseline (estimated Cockcroft-Gault-calculated creatinine clearance [CrCL] of >30 to $\leq 50 \text{ ml/min}$, based on local laboratory serum creatinine levels at screening), adjustments were made to the ceftazidime-avibactam dose (1 g of ceftazidime combined with 0.25 g of avibactam as a 2-h intravenous infusion every 12 h) or to the meropenem dose (1 g as a 30-min infusion every 12 h). No adjustment was made for metronidazole. Cultures were obtained for all patients either from blood samples taken at the baseline visit or (intraoperatively) from the intra-abdominal site of infection. Patients with bacteria not expected to respond to either study drug (e.g., *Stenotrophomonas* spp. and *Acinetobacter* spp.) were considered to have ineligible baseline pathogens and were excluded from this analysis.

Clinical cure was determined by the local investigators at the TOC visit, which occurred 28 to 35 days after randomization. Clinical cure was defined as resolution or as improvement in signs and symptoms of cIAI such that no further treatment was required. Other defined primary endpoints were failure or indeterminate status (see Mazuski et al. for additional information) (16).

Susceptibility testing, selection criteria, and screening of β -lactamases. All baseline clinical isolates were centrally tested for susceptibility by broth microdilution (Clinical and Laboratory Standards Institute [CLSI]; M07-A10; 2015) (24). *Enterobacteriaceae* isolates displaying ceftriaxone and/or ceftazidime MIC results of ≥ 2 $\mu\text{g/ml}$ (25) and *P. aeruginosa* isolates with ceftazidime MIC results of ≥ 16 $\mu\text{g/ml}$ were selected for further characterization of β -lactamase content, including ESBL genes. *Enterobacteriaceae* and *P. aeruginosa* isolates exhibiting imipenem/meropenem MIC results of ≥ 2 and ≥ 8 $\mu\text{g/ml}$, respectively, were also tested for the presence of carbapenemase-encoding genes, as previously described (17).

Isolates that met the MIC screening criteria described above were subjected to testing with a microarray-based assay Check-MDR CT101 kit according to the manufacturer's instructions (Check-Points, Wageningen, Netherlands). This kit has the capability to detect CTX-M groups 1, 2, 8 + 25, and 9, non-ESBL and ESBL variants of TEM and SHV, plasmid AmpC (ACC, ACT/MIR, CMY, DHA, and FOX), and KPC- and NDM-encoding genes (17). Supplemental multiplex PCR assays were utilized to detect additional ESBL-encoding genes (*bla*_{GES}, *bla*_{VEB}, *bla*_{PER}, and oxacillinase enzyme-encoding genes [the *bla*_{OXA-2}, *bla*_{OXA-10}, and *bla*_{OXA-13} groups, *bla*_{OXA-18}, and *bla*_{OXA-45}]) and carbapenemase-encoding genes (*bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM-1}, *bla*_{OXA-48}, *bla*_{GES}, *bla*_{NMC-A}, *bla*_{SME}, and *bla*_{IMI}) (17). All results obtained by Check-Points and by supplemental PCR assays were confirmed by single PCRs and subsequent determination of gene alleles by sequencing analysis of both strands (Sanger method); nucleotide and amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Amino acid sequences were compared with those available through the internet using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>).

The transcription levels of chromosomally encoded AmpC were determined in *P. aeruginosa*, *E. coli*, and *Enterobacter cloacae* isolates by the quantification of the target gene mRNA using a normalized expression analysis method and comparisons to susceptible control strains (17). A given isolate for which the level of *ampC* transcripts was found to be at least 10-fold greater than that seen with a species-specific wild-type reference control strain was defined as exhibiting overexpression of the *ampC* gene.

Statistical analyses. The clinical responses obtained for ceftazidime-avibactam and meropenem at TOC were summarized. Percentages of clinical responses were calculated as m/n , where m was defined by the number of patients with a clinical response at TOC visit and n was represented by the combined number of patients with clinical cure, clinical failure, and indeterminate outcome. Differences in clinical responses between the ceftazidime-avibactam and meropenem groups are also described. Analyses of results of comparisons between arms stratified by group of organisms/species, phenotype, and β -lactamase resistance mechanisms of baseline isolates were performed. All statistical analyses were performed using SAS software Version 9.1 or higher (SAS Institute, Inc., Cary, North Carolina).

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