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In Vivo Selection of a Missense Mutation in *adeR* and Conversion of the Novel $bla_{OXA-164}$ Gene into bla_{OXA-58} in Carbapenem-Resistant *Acinetobacter baumannii* Isolates from a Hospitalized Patient▽

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The mechanism of stepwise acquired multidrug resistance in *Acinetobacter baumannii* isolates from a hospitalized patient was investigated. Thirteen consecutive multidrug-resistant isolates were recovered from the same patient over a 2-month period. The Vitek 2 system identified the isolates as meropenem-sensitive *Acinetobacter lwoffii*; however, molecular identification showed that the isolates were *A. baumannii*. Etest revealed that the isolates were meropenem resistant. The presence of oxacillinase (OXA)-type enzymes were investigated by sequencing. The clonal relatedness of isolates was assessed by pulsed-field gel electrophoresis (PFGE). Expression of the genes encoding the efflux pumps AdeB and AdeJ was performed by semiquantitative real-time reverse transcription-PCR (qRT-PCR). The *adeRS* two-component system was sequenced. All isolates had identical PFGE fingerprints, suggesting clonal identity. The first six isolates were positive for the novel $bla_{OXA-164}$ gene. The following seven isolates, recovered after treatment with a combination of meropenem, amikacin, ciprofloxacin, and co-trimoxazole showed an increase of >7-fold in *adeB* mRNA transcripts and a missense mutation in $bla_{OXA-164}$, converting it to bla_{OXA-58}. Sequencing revealed a novel mutation in *adeR*. These data illustrate how *A. baumannii* can adapt during antimicrobial therapy, leading to increased antimicrobial resistance.

Acinetobacter baumannii is a serious nosocomial pathogen. Infections are difficult to treat, as the organism is often multidrug resistant (4). The carbapenems have had good activity against *A. baumannii*, but resistance to this drug class is rising mainly through the action of intrinsic or acquired oxacillinases (OXAs) (18). OXAs exhibit weak activity against the carbapenems, but when they are overexpressed, they can confer resistance. This overexpression is associated with the presence of insertion elements, which are also a significant factor in *A. baumannii* multidrug resistance and genome plasticity (16, 19, 22). For example, the insertion element IS*Aba1* upregulates expression of the intrinsic bla_{OXA-51} gene and the efflux pump *adeABC* genes, but it can also mobilize antibiotic resistance genes (16, 21, 26). Efflux pumps such as AdeB and AdeJ also contribute to multidrug resistance (3, 14). Overexpression of these pumps has been selected *in vivo* where they also play a major role in the development of resistance to tigecycline (9, 21).

The aim of this study was to investigate the molecular epidemiology and the mechanism of increasing antimicrobial resistance observed in sequential *A. baumannii* isolates recovered from a patient during a prolonged hospitalization.

CASE REPORT

A 58-year-old patient was initially admitted to a community hospital for personality changes and confusion. After the diagnosis of an extremely large optic nerve sheath meningioma was established, the patient was transferred to the neurosurgery department of the Cologne University hospital in Cologne, Germany. The patient's condition rapidly deteriorated, and he required intubation and mechanical ventilation. The patient developed ventilator-associated pneumonia. Methicillin-resistant *Staphylococcus aureus* was isolated from a tracheal secretion, and the patient was started on vancomycin therapy. A follow-up sample obtained 11 days after admission to the hospital revealed a multiresistant *Acinetobacter* species that was identified by the Vitek 2 system (bioMérieux, Nürtingen, Germany) as *Acinetobacter lwoffii*, and therapy was switched to imipenem, although the isolate was tested intermediate to imipenem (MIC of 8 μg/ml). Since the patient did not improve, the antimicrobial therapy was changed several times during the patient's 96-day stay in the hospital (Fig. 1), including a short period of ciprofloxacin and co-trimoxazole for empirical coverage of *Stenotrophomonas maltophilia* (days 27 to 31). The *Acinetobacter* isolates after the ciprofloxacin–co-trimoxazole treatment course were increasingly resistant, however, and remained susceptible to colistin, which the patient received as intravenous (i.v.) therapy. Despite several attempts using combination therapy (Fig. 1), the multidrug-resistant organism could not be cleared from the respiratory tract and was later also isolated from blood cultures. Due to the poor prognosis of the underlying disease, an end-of-life decision was made, and the patient died 96 days after admission.

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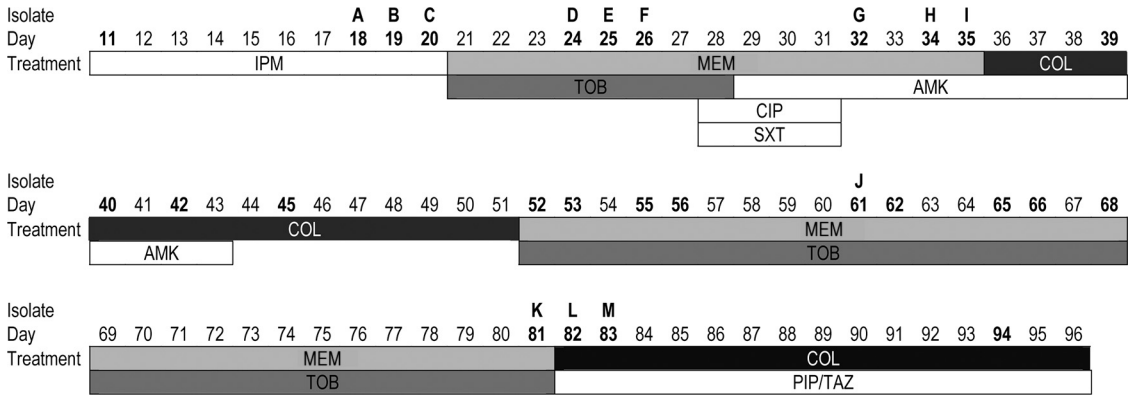

FIG. 1. Timeline of antimicrobial therapy and isolation of *A. baumannii.* The numbers in boldface type are the dates (number of days in the hospital) after the patient was admitted to the hospital when *A. baumannii* was isolated. Isolates A to M were retained for further investigation. Drug abbreviations: IPM, imipenem; MEM, meropenem; TOB, tobramycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; AMK, amikacin; COL, colistin; PIP/TAZ, piperacillin-tazobactam.

MATERIALS AND METHODS

Bacterial isolates. A total of 33 *Acinetobacter* isolates were recovered from the patient over 96 days (Fig. 1). The majority of isolates were recovered from tracheal secretions, but they were also recovered from tracheostoma (day 26), nose (day 32), bedsore (day 65), and bloodstream (days 81 and 82). Thirteen of these isolates were retained for further investigation (isolates A to M). Isolates were identified as *A. lwoffii* by the Vitek 2 system. Species identification was repeated by *gyrB* multiplex (7).

Susceptibility testing. Antimicrobial susceptibility of all isolates was initially tested by the Vitek 2 system using AST-N118 and AST-N110 cards. The susceptibilities of isolates A to M to imipenem, meropenem, tigecycline, levofloxacin, and tobramycin were tested by Etest (bioMérieux) following the manufacturer's instructions and retested by the Vitek 2 system. The breakpoint values used for interpretation were those proposed by the Clinical and Laboratory Standards Institute (CLSI) (2). Tigecycline MIC breakpoints are currently not available from CLSI for *A. baumannii*.

Molecular typing. Isolates were molecularly typed by repetitive-sequence-based PCR (rep-PCR) using the DiversiLab system (bioMérieux) and pulsed-field gel electrophoresis (PFGE) as previously described (5, 23).

Presence of carbapenemase genes. On the basis of the results of carbapenem susceptibility, the isolates were screened for known bla_{OXA} carbapenemase genes and sequenced (28). The presence of insertion elements was investigated as previously described (19, 26). Plasmids were isolated from the strains and used to transform electrocompetent *A. baumannii* ATCC 19606 and used as PCR template for the presence of bla_{OXA} carbapenemases to determine whether resistance to carbapenem was encoded in genes carried on a plasmid.

Cloning $bla_{OXA-164}$ and bla_{OXA-58}. The genes encoding oxacillinase 164 (OXA-164) and OXA-58 were amplified from isolates A and G using primers oxa-164/58_up and oxa-164/58_down (Table 1), which include 673 bp upstream of the start codon. PCR products were ligated into EcoRI/BamHI double digested pWH1266 which is an *Escherichia coli-Acinetobacter* shuttle plasmid (11) using the In-Fusion dry-down PCR cloning kit (Clontech, Saint-Germain-en-Laye, France) and transformed in *E. coli* NEB 5-alpha (New England BioLabs, Frankfurt, Germany). Plasmids were isolated from transformants and used to transform electrocompetent *A. baumannii* ATCC 19606 and *A. baumannii* ATCC 17978 selected on Mueller-Hinton agar containing 100 μg/ml ticarcillin. To confirm transfer of $bla_{OXA-164}$ and bla_{OXA-58}, plasmid inserts were sequenced. The susceptibility of the transformants to imipenem and meropenem was examined by Etest.

qRT-PCR. Expression of the efflux pump genes *adeB* and *adeJ*, the *adeABC* two-component system sensor (*adeS*) and regulator (*adeR*), the intrinsic oxacillinase bla_{OXA-51} gene, and the acquired bla_{OXA-58} and $bla_{OXA-164}$ genes (gene with a missense mutation in $bla_{OXA-164}$, converting it to bla_{OXA-58}) were investigated in five selected isolates by semiquantitative reverse transcription-PCR (qRT-PCR) as previously described with minor modifications (9). Bacterial isolates were grown in Luria-Bertani broth until mid-log phase. Aliquots were treated with RNA protect (Qiagen, Hilden, Germany), and RNA was isolated using the RNeasy minikit (Qiagen) following the manufacturer's instructions. cDNA was synthesized from 1 μg of total RNA using Quantitect reverse transcription kit (Qiagen), and real-time PCR was performed in a LightCycler with Quantifast SYBR green (Qiagen) following the manufacturer's instructions. Primer sequences are shown in Table 1. All qRT-PCR was done in triplicate using freshly prepared cDNA. qRT-PCR was repeated at least three times. *rpoB* was used as a control housekeeping gene and was quantified in tandem with genes under investigation (10).

Characterization of *adeB* upregulation. The genes encoding the two-component regulatory system AdeRS lie upstream of the *adeABC* operon. This area was investigated by sequencing. Primers adeS_F and adeR_R (Table 1) were designed using the published sequence of *A. baumannii* AB307-0294. The PCR product includes the upstream regions of both regulatory genes. PCR products from isolates A, F, G, and J were amplified using these primers and sequenced.

Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper have been submitted to the EMBL/GenBank nucleotide sequence database under accession numbers GU831575, HM440347, and HM440348.

RESULTS

Susceptibility testing. The results of susceptibility testing are summarized in Table 2. The Vitek 2 system recorded all isolates as resistant to cefepime, cefotaxime, cefoxitin, ceftazidime, ciprofloxacin, moxifloxacin, fosfomycin, gentamicin, piperacillin, piperacillin-tazobactam, and ampicillin-sulbactam and susceptible only to colistin (≤1 μg/ml) and tobramycin (≤1 μg/ml) (except for isolates J and K). Amikacin MICs were ≥32 μg/ml for all isolates except for isolates D and E, which had MICs of ≤2 μg/ml. Tigecycline MICs were 2 μg/ml for isolates A to F and ≥8 μg/ml for isolates G to M. According to Vitek 2, all isolates except isolate A were resistant to imipenem, and only isolates G and M were resistant to meropenem (MIC of ≥16 μg/ml). Etest recorded higher MICs than Vitek 2 did (Table 2). In particular, 12 isolates were resistant to meropenem (MIC range, 16 to ≥64 μg/ml), and one was intermediate. Isolates A to F exhibited heteroresistance to imipenem and meropenem. We define heteroresistance as a resistant subpopulation appearing within the Etest inhibition zone ellipse (Fig. 2 and 3). The patient received combination antimicrobial therapy on days 28 to 31, consisting of meropenem, ciprofloxacin, amikacin, and co-trimoxazole (Fig. 1). Posttherapy isolates G to M recorded higher MICs against co-trimoxazole, tigecycline, meropenem, and levofloxacin than those isolated before therapy (Table 2). This simultaneous rise

TABLE 1. Primer sequences used in this study

Primer	Primer sequence (5′–3′)	Application	Target gene(s)	Reference
rpob_rtF	GAGTCTAATGGCGGTGGTTC	qRT-PCR	*rpoB* housekeeping gene	10
rpob_rtR	ATTGCTTCATCTGCTGGTTG	qRT-PCR	*rpoB* housekeeping gene	10
adeB_rtF	GAATAAGGCACCGCAACAAT	qRT-PCR	*adeB*	This study
adeB_rtR	TTTCGCAATCAGTTGTTCCA	qRT-PCR	*adeB*	This study
adeJ_rtF	GCGAATGGACGTATGGTTCT	qRT-PCR	*adeJ*	This study
adeJ_rtR	CATTGCTTTCATGGCATCAC	qRT-PCR	*adeJ*	This study
adeR_rtF	TGGGTTAAAAGGCTTCACCA	qRT-PCR	*adeR*	This study
adeR_rtR	ACGCCAAAAAGCTCAGACTC	qRT-PCR	*adeR*	This study
adeS_rtF	GCATTTTTGACGGAAACCTC	qRT-PCR	*adeS*	This study
adeS_rtR	TTAGTCACGGCGACCTCTCT	qRT-PCR	*adeS*	This study
oxa51_rtF	GGAAGTGAAGCGTGTTGGTT	qRT-PCR	bla_{OXA-51}	This study
oxa51_rtR	CAAACTGTGCCTCTTGCTGA	qRT-PCR	bla_{OXA-51}	This study
oxa58_rtF	TCAAGAATTGGCACGTCGTA	qRT-PCR	bla_{OXA-58}	This study
oxa58_rtR	GCCCTTTCAACCAAAATTGA	qRT-PCR	bla_{OXA-58}	This study
oxa-164/58_up	TCGTCTTCAAGAATTCCCCTACGATCCGTGTTTTTG	Cloning $bla_{OXA-164}$ and bla_{OXA-58}	$bla_{OXA-164}$ and bla_{OXA-58} including the upstream promoter region	This study
oxa-164/58_down	CCGGCGTAGAGGATCCAATGACTTTGCTGAGGCTGAA	Cloning $bla_{OXA-164}$ and bla_{OXA-58}	$bla_{OXA-164}$ and bla_{OXA-58} including the upstream promoter region	This study
adeS_F	GAGGGAGTGCTCGAATTTGT	Sequencing	*adeS* and *adeR* including the promoter region of *adeS*	This study
adeR_R	GCTCAGCTTGAGCGACTTCT	Sequencing	*adeS* and *adeR* including the promoter region of *adeS*	This study

in MIC against four different drug classes was interpreted as enhanced efflux.

Species identification and presence of carbapenemase genes. The isolates failed to utilize D-glucose, D-mannose, and malonic acid, essential substrates for identification of *A. baumannii* in the Vitek 2 system, thus leading to their misidentification as *A. lwoffii*. Species identification using *gyrB* multiplex identified all isolates as *A. baumannii*. Sequencing of the intrinsic chromosomal bla_{OXA-51}-like gene revealed bla_{OXA-69}, which was not associated with IS*Aba1*. In addition, isolates A to F possessed the novel $bla_{OXA-164}$ gene, which is a bla_{OXA-58}-like variant. Isolates G to M all possessed bla_{OXA-58}, which differs from the latter by a single A-to-T mutation at position 342, resulting in the Leu114→Phe amino acid replacement. $bla_{OXA-164}$ and bla_{OXA-58} were flanked by IS*18* and IS*Aba*3 as previously described (29). The $bla_{OXA-164}$ and bla_{OXA-58} genes were not transferrable; however, plasmid preparations were positive by PCR for $bla_{OXA-164}$ or bla_{OXA-58} but not bla_{OXA-69}, suggesting a plasmid location.

Cloning $bla_{OXA-164}$ and bla_{OXA-58}. The results of cloning $bla_{OXA-164}$ and bla_{OXA-58} are summarized in Table 3. Clinical resistance to the carbapenems was achieved in *A. baumannii* transformants, but not *E. coli*. $bla_{OXA-164}$ and bla_{OXA-58} transformants recorded similar MICs. However, in both *E. coli* and *A. baumannii*, $bla_{OXA-164}$ transformants exhibited imipenem heteroresistance. The meropenem ellipse was reduced in *A. baumannii* bla_{OXA-58} transformants compared to that in $bla_{OXA-164}$ transformants (Fig. 4 to 7).

Molecular typing. All isolates had similar rep-PCR fingerprint and clustered with control isolates from European clonal complex I (WW1), which is in agreement with the presence of bla_{OXA-69}. All 13 isolates had an identical PFGE pattern, confirming their clonality (data not shown).

qRT-PCR and characterization of *adeB* upregulation. Expression of the *adeB* and *adeJ* efflux pump genes was investigated by qRT-PCR with isolates A, E, F, G, and J. We did not detect any difference in *adeJ* transcripts in isolates A, E, and J (Table 4). Isolates G and J expressed similar amounts of *adeB* mRNA, which were >7-fold greater than the amounts expressed by isolates A, E, and F obtained prior to hospital day 32. There was no difference in expression of bla_{OXA-69} transcripts. The bla_{OXA-58}-like gene was overexpressed in isolates J and G with levels 2- to 4-fold higher than those expressed by isolates A and F. We did not detect the presence of IS elements in the vicinity of the *adeABC* operon. The *adeR* and *adeS* genes of isolates A, F, G, and J were sequenced. Isolates G and J had a single missense mutation in *adeR* at nucleotide 58 (G→A) leading to an Asp20→Asn amino acid replacement. No other nucleotide changes were found. qRT-PCR with *adeR*

TABLE 2. MIC values of clinical isolates as determined by Vitek 2 and Etest

Isolate	bla_{OXA} gene	Day of isolation[a]	Source of isolate[b]	MIC (μg/ml) of the indicated drug[c] as determined by:										
				Vitek 2						Etest				
				IPM	MEM	TGC	TOB	AMK	SXT	IPM	MEM	TGC	TOB	LEV
A	$bla_{OXA-164}$	18	TS	8	2	2	≤1	≥64	40	32*	32*	4	2	8
B	$bla_{OXA-164}$	19	TS	≥16	4	2	≤1	≥64	40	32*	32*	4	4	8
C	$bla_{OXA-164}$	20	TS	≥16	8	2	≤1	32	40	32*	8	4	2	8
D	$bla_{OXA-164}$	24	TS	≥16	8	2	≤1	≤2	40	32*	32*	4	2	8
E	$bla_{OXA-164}$	25	TS	≥16	8	2	≤1	≤2	40	32*	32*	4	2	16
F	$bla_{OXA-164}$	26	TST	≥16	4	2	≤1	≥64	40	32*	16*	4	2	8
G	bla_{OXA-58}	32	TS	≥16	≥16	≥8	≤1	≥64	≥320	≥64	≥64	16	4	≥64
H	bla_{OXA-58}	34	TS	≥16	8	≥8	≤1	≥64	≥320	≥64	≥64	16	4	≥64
I	bla_{OXA-58}	35	TS	≥16	8	≥8	≤1	≥64	≥320	≥64	≥64	16	4	≥64
J	bla_{OXA-58}	61	BS	≥16	8	≥8	8	≥64	≥320	≥64	≥64	16	16	≥64
K	bla_{OXA-58}	81	BC	≥16	8	≥8	8	≥64	≥320	≥64	≥64	16	16	≥64
L	bla_{OXA-58}	82	TS	≥16	≥16	≥8	≤1	≥64	≥320	≥64	≥64	16	4	≥64
M	bla_{OXA-58}	83	TS	≥16	8	≥8	≤1	≥64	≥320	≥64	≥64	16	4	≥64

[a] The day of isolation is the date (number of days after the patient was admitted to the hospital) that the particular isolate was recovered from the patient.
[b] TS, tracheal secretion; TST, tracheostoma; BS, bedsore; BC, blood culture.
[c] Drug abbreviations: IPM, imipenem; MEM, meropenem; TGC, tigecycline; TOB, tobramycin; AMK, amikacin; SXT, trimethoprim-sulfamethoxazole; LEV, levofloxacin. The asterisks for IMP and MEM values indicate that the isolate exhibited heteroresistance.

and *adeS* primers showed no significant difference in expression of these genes in the four isolates.

DISCUSSION

The failure of the Vitek 2 system to correctly identify *A. baumannii* and to predict carbapenem susceptibility has been described previously (12, 13). More recently, almost half of the carbapenem-resistant *A. baumannii* isolates identified using routine microbiological methods were sensitive to carbapenems upon retesting by Etest (25). What makes the present study unusual is that Vitek 2 identified these isolates uniformly as *A. lwoffii*, a commensal species that is rarely involved or not at all involved in nosocomial infections (4). The species misidentification and false antimicrobial susceptibility subsequently may have compromised the clinical outcome. As these isolates cluster with WW1, one of the most frequently encountered carbapenem-resistant *A. baumannii* lineages (8), it opens the possibility that species misidentification may be more common than previously thought and warrants greater vigilance. It is of interest that isolates A to F were heteroresistant to the

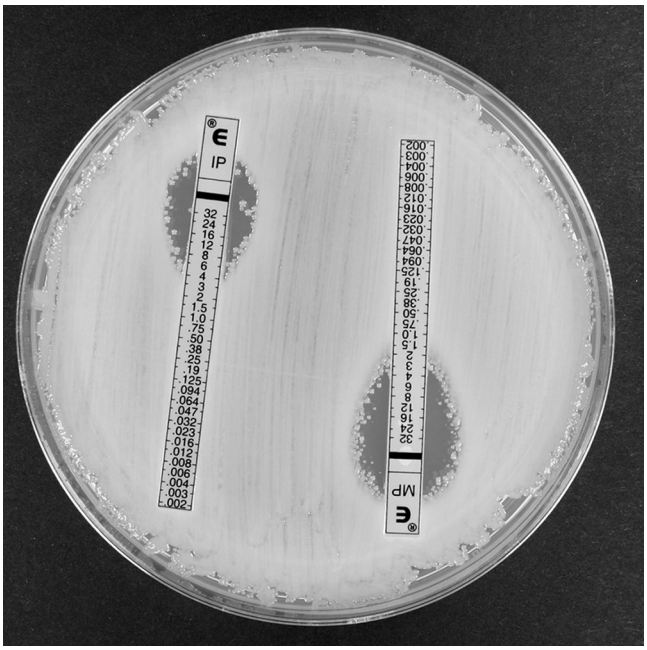

FIG. 2. Imipenem (IP) and meropenem (MP) Etests for *A. baumannii* clinical isolate A ($bla_{OXA-164}$) showing colonies within the imipenem and meropenem ellipse (heteroresistance).

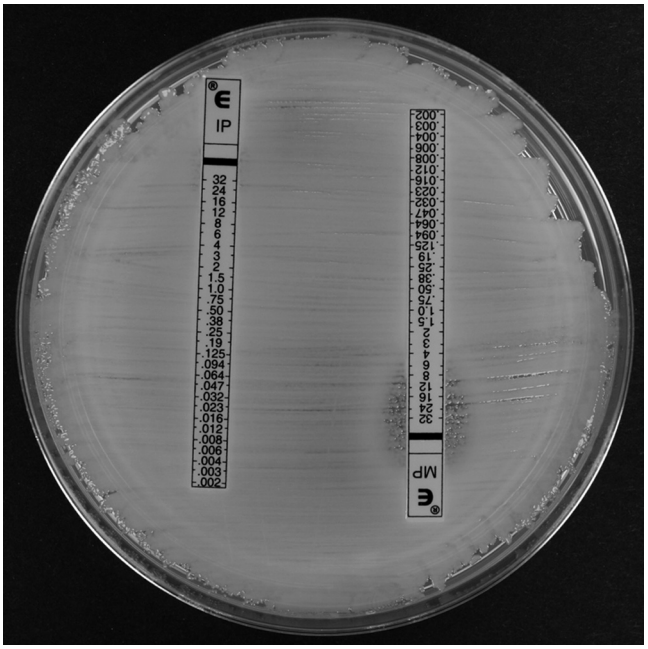

FIG. 3. Imipenem and meropenem Etests for *A. baumannii* clinical isolate G (bla_{OXA-58}) showing confluent growth up to the imipenem Etest strip and a reduced meropenem ellipse compared to that of the $bla_{OXA-164}$ isolate (Fig. 2).

TABLE 3. MICs determined by Etest for OXA-164- and OXA-58-producing *A. baumannii* ATCC 19606 and ATCC 17978 and *E. coli* NEB 5-alpha

Isolate	MIC (μg/ml)	
	Imipenem	Meropenem
E. coli NEB 5-alpha(pWH1266)	0.19	0.012
E. coli NEB 5-alpha(pWH1266::$bla_{OXA-164}$)	0.25	0.032
E. coli NEB 5-alpha(pWH1266::bla_{OXA-58})	0.5	0.032
A. baumannii ATCC 19606(pWH1266)	0.125	0.19
A. baumannii ATCC 19606(pWH1266::$bla_{OXA-164}$)	>32	32
A. baumannii ATCC 19606(pWH1266::bla_{OXA-58})	>32	>32
A. baumannii ATCC 17978(pWH1266)	0.25	0.25
A. baumannii ATCC 17978(pWH1266::$bla_{OXA-164}$)	>32	>32
A. baumannii ATCC 17978(pWH1266::bla_{OXA-58})	>32	>32

carbapenems. This may be due in part to slight differences between the biochemical properties of OXA-164 and OXA-58 as observed with the *E. coli* and *A. baumannii* transformants. However, we cannot discount the small increase in bla_{OXA-58} and *adeB* transcripts which may contribute to overall resistance levels (6, 9). Carbapenem-heteroresistant *A. baumannii* has been reported in the absence of an acquired carbapenemase (20); however, to our knowledge, this is the first description of heteroresistance with an acquired oxacillinase.

Overexpression of *adeB* has previously been attributed to mutations in the *adeS* and *adeR* genes and to insertion of IS*Aba1* in *adeS* (15, 21). We found a novel *adeR* mutation leading to an Asp20→Asn replacement in *adeB* overexpressors. AdeR is composed of two major domains, a signal receiver domain and an effector domain which is a DNA binding site (14). The signal receiver domain in AdeR is composed of a phosphorylation and dimerization site and receives the signal from its sensor partner (AdeS) (15, 17). The *A. baumannii* AdeR residue Asp20 corresponds to Asp10 in the *E. coli* PhoB

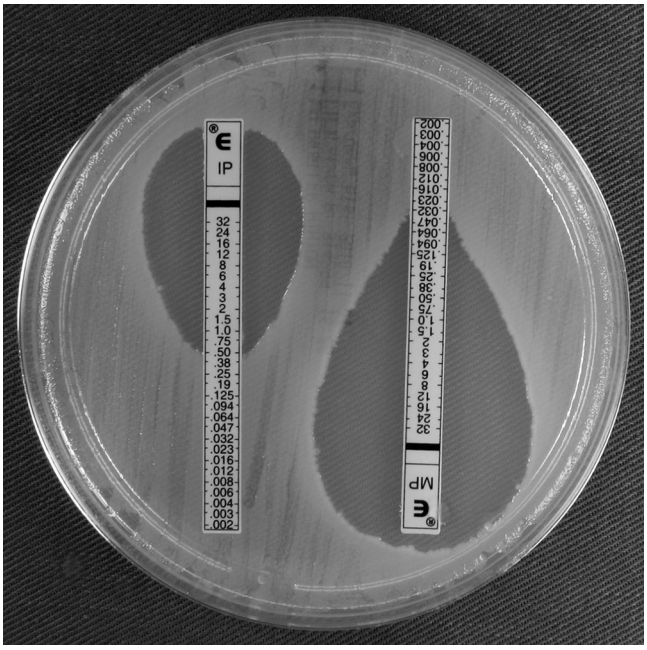

FIG. 5. Imipenem and meropenem Etests for *E. coli* NEB 5-alpha(pWH1266::bla_{OXA-58}).

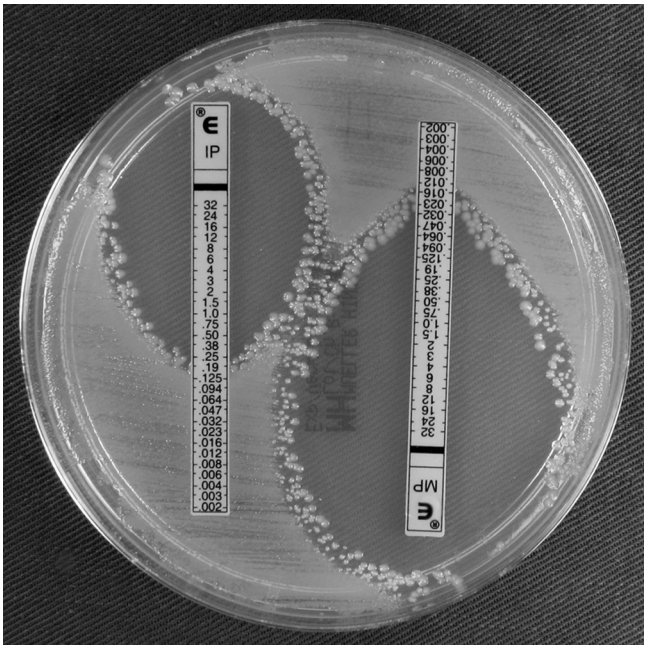

FIG. 4. Imipenem and meropenem Etests for *E. coli* NEB 5-alpha carrying pWH1266::$bla_{OXA-164}$ and showing imipenem and meropenem heteroresistance.

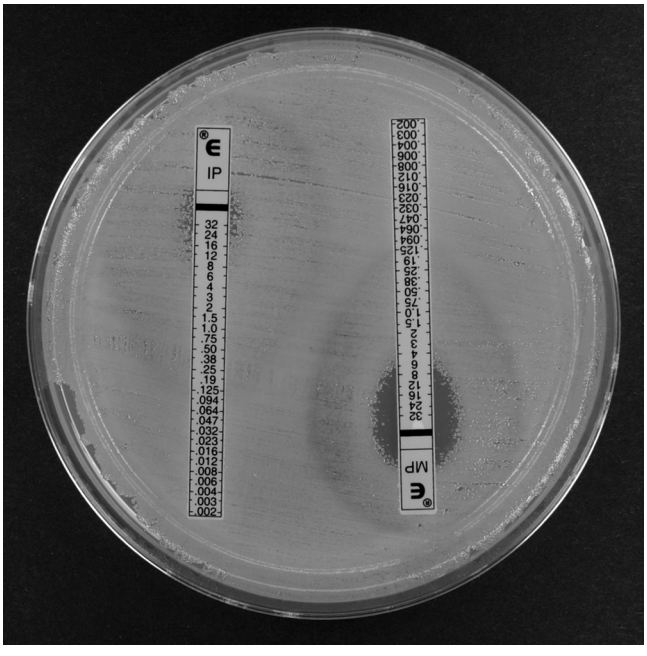

FIG. 6. Imipenem and meropenem Etests for *A. baumannii* 19606(pWH1266::$bla_{OXA-164}$) showing heteroresistance to imipenem and meropenem.

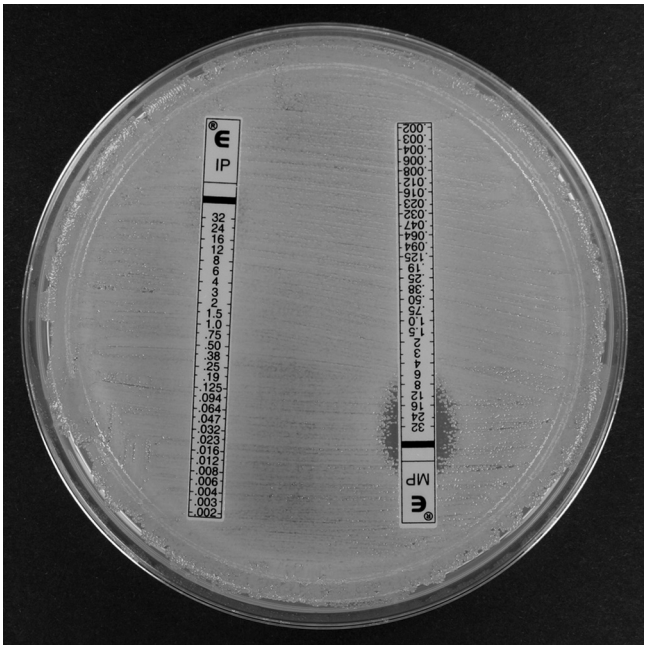

FIG. 7. Imipenem and meropenem Etests for *A. baumannii* 19606(pWH1266::bla_{OXA-58}) showing confluent growth up to the imipenem Etest strip and a reduction in meropenem heteroresistance compared to *A. baumannii* 19606(pWH1266::$bla_{OXA-164}$) (Fig. 6).

response regulator and is part of an acidic triad making up the active site for phosphorylation (1, 24). Thus, sustaining a mutation near this phosphorylation site may alter the interactions between the AdeS and AdeR subunit which likely results in the overexpression of the *adeABC* genes (27). However, the exact mechanistic details may need to be elucidated by site-directed mutagenesis.

The mechanism of tobramycin resistance in isolates J and K is not known. Gene disruption predicts tobramycin and amikacin to be substrates of AdeB (14). However, we found no significant differences in *adeB* or *adeJ* levels between tobramycin-sensitive and -resistant isolates. In addition, isolate E, which showed reduced amikacin susceptibility, expressed the same levels of *adeB* and *adeJ* mRNA as isolates A and F, suggesting that in this case, reduced amikacin susceptibly was not related to these efflux pumps.

We did not determine the agent(s) that drove the conversion of OXA-164 to OXA-58 and led to the *adeR* mutation. However, given that OXA-58 appears to confer greater resistance to both imipenem and meropenem, and meropenem was administered over the conversion period, it is not unreasonable to suggest that meropenem was the driving force. In addition, elevated meropenem MICs have also been associated with efflux overexpression (6, 9).

TABLE 4. Relative expression of *adeB*, *adeJ*, *adeR*, *adeS*, bla_{OXA-69}, and $bla_{OXA-164}$ and bla_{OXA-58} as determined by semiquantitative RT-PCR

Isolate	Relative expression[a]					
	adeB	*adeJ*	*adeR*	*adeS*	bla_{OXA-69}	$bla_{OXA-164/58}$
A	1	1	1	1	1	1
E	0.6 ± 0.2	1.3 ± 0.3	NT	NT	NT	NT
F	0.7 ± 0.1	NT	0.7 ± 0.03	0.9 ± 0.1	0.8 ± 0.1	1.1 ± 0.04
G	7.7 ± 1.5	NT	1.4 ± 0.2	1.8 ± 0.4	1.7 ± 0.2	4.2 ± 0.1
J	9.0 ± 1	1.3 ± 0.4	1 ± 0.1	1.5 ± 0.2	0.6 ± 0.1	2.8 ± 0.1

[a] The expression of the genes is normalized to *rpoB* expression and relative to the number of transcripts from isolate A. NT, not tested.

In summary, we have described the failure of the semiautomated system Vitek 2 to correctly identify carbapenem-resistant *A. baumannii*. The consequence was that meropenem was inappropriately administered for a prolonged period of time and may have contributed to the mutation in $bla_{OXA-164}$, leading to the selection and overexpression of bla_{OXA-58}. In addition, *adeB* overexpression was selected, leading to elevated tigecycline MICs without prior exposure to this drug. Our study demonstrates that *A. baumannii* has the propensity to rapidly acquire resistance by mutation during antimicrobial therapy. Of particular concern is the implication of the conversion of $bla_{OXA-164}$ to bla_{OXA-58}. These data serve to highlight the need for caution when interpreting the identification of a multidrug-resistant *Acinetobacter* isolate that is not *Acinetobacter baumannii* by Vitek 2.

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