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Expression of the *adeB* gene and responsiveness to 1-(1-naphthylmethyl)-piperazine and phenylalanyl-arginyl-β-naphthylamide in clinical isolates of *Acinetobacter baumannii*

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Sir,

AdeABC, a resistance–nodulation–cell division (RND)-type efflux pump, is an attractive research target as a cause of multiple antibiotic resistance in *Acinetobacter baumannii*.[1] The overexpression of the *adeB* gene encoding the AdeB efflux protein has previously been associated with multidrug resistance in various *A. baumannii* strains.[1–3] These reports led us to perform a study in a group of clinical isolates of *A. baumannii*, to assess the relative expression of the *adeB* gene and responsiveness to the potential efflux pump inhibitors (EPIs), 1-(1-naphthylmethyl)-piperazine (NMP) and phenylalanyl-arginyl-β-naphthylamide (PAβN).[2–4] Fourteen multidrug-resistant *A. baumannii* isolates obtained from different patients in an intensive care unit and grouped previously into three clones were included in the study (Table 1).[5,6] *A. baumannii* ATCC 19606, *A. baumannii* SBMox2, *A. baumannii* U10247 and *Escherichia coli* ATCC 25922 were used as reference strains.[2,7] The MICs of ciprofloxacin, gentamicin, erythromycin, chloramphenicol, trimethoprim and tetracycline were determined by the broth microdilution test in the absence and presence of NMP and PAβN, at final concentrations of 100 mg/L.[2,8] For the detection of *adeB* transcripts, RT–PCR was performed (RNeasy Kit and Omniscript RT Kit from Qiagen, Hilden, Germany; and FastStart DNA Master SYBR Green I Kit from Roche, Mannheim, Germany) using LightCycler System 2 (Roche) with the primers previously described by Higgins *et al.*[7]

Eight isolates (MU-2, -5, -7, -9, -10, -12, -13 and -14) showed reductions in various antibiotic MICs after the addition of EPIs (Table 1). This demonstrates the ability of NMP and PAβN to partially reverse drug resistance in a significant portion of the isolates. We can conclude that the phenotypic changes in the drug susceptibilities of our EPI-responsive isolates might be associated with the inhibition of functional RND-type drug efflux.[2–4] In general, NMP was more active than PAβN. Such differences in the activities of the two compounds have been previously reported and associated with the different mechanisms of action of NMP and PAβN.[2] Significant effects of NMP were observed on ciprofloxacin, erythromycin and trimethoprim MICs (Table 1). Moreover, NMP switched the resistance phenotype to susceptible in two isolates (MU-7 and *A. baumannii* SBMox2) for trimethoprim and/or tetracycline.[2] This inhibitor may have affinity sites similar to those for these antibiotics inside the efflux pump.

The *adeB* expression of EPI-responsive isolates was as high as that found in *A. baumannii* SBMox2, which has been previously shown to be an *adeB* overexpresser (Table 1).[2] This supports our conclusion that the responsiveness of the isolates to EPIs might be due to the inhibition of functional RND-type drug efflux, particularly the AdeB pump. Earlier experiments have also highlighted the causal connection between *adeB* overexpression and responsiveness to EPIs in various *A. baumannii* strains.[2,3,7,9] Despite the fact that the aminoglycosides have been shown to be good substrates of the AdeB pump, the gentamicin MICs for most *adeB* overexpressers did not markedly change in the presence of EPIs.[1–3,7,9,10] This could be due to the presence of other resistance mechanisms, such as aminoglycoside modification enzymes and/or some recently recognized efflux pumps, which may have low affinity for the EPIs tested here.[10,11] Alternatively, the affinity constant for the efflux pump sites could be stronger for gentamicin in comparison with EPIs. In terms of changes in quinolone susceptibility, despite the fact that NMP decreased the ciprofloxacin MICs from 16–32 mg/L to 2–8 mg/L, the *adeB* overexpressers were still resistant to this antibiotic (Table 1). The persistence of ciprofloxacin resistance in these isolates might be associated with mutations in the *gyrA* gene.[2] Interestingly, the *adeB* expression of *A. baumannii* ATCC 19606 was high and similar to that of *A. baumannii* SBMox2.[12] Although this strain was initially found to be susceptible to ciprofloxacin and tetracycline, the addition of NMP caused reductions in their MICs (Table 1).[8] This supports earlier findings suggesting the efficiency of the AdeB pump alone was not sufficient to increase various antibiotic MICs to

Table 1. Expression of *adeB* and antibiotic MICs in the presence and absence of NMP and PAβN in *A. baumannii* isolates

Isolate	Clone	*adeB* expression[a]	EPI	MIC (mg/L)					
				CIP	GEN	ERY	CHL	TMP	TET
EPI-responsive isolates									
MU-2	A	0.81	none	16	16	32	128	16	32
			NMP	8	16	16	64	8	16
			PAβN	8	16	32	64	16	32
MU-9	A	1.06	none	32	2	16	64	16	16
			NMP	4	2	2	32	4	8
			PAβN	16	2	8	32	8	16
MU-5	B	0.93	none	16	256	4	32	32	512
			NMP	2	64	2	8	8	128
			PAβN	4	128	4	16	16	512
MU-10	B	0.75	none	32	128	32	64	32	16
			NMP	4	32	16	32	8	8
			PAβN	16	64	32	32	16	16
MU-12	B	0.81	none	16	256	32	16	32	256
			NMP	8	128	16	8	16	128
			PAβN	16	256	16	16	32	256
MU-13	B	1.5	none	32	2	32	64	32	256
			NMP	8	1	4	16	4	64
			PAβN	16	1	16	32	8	128
MU-14	B	1.1	none	32	4	32	256	32	128
			NMP	4	1	4	64	4	32
			PAβN	16	1	8	128	16	64
MU-7	C	1.08	none	16	512	16	512	8	16
			NMP	4	512	4	128	2	4
			PAβN	8	512	8	256	8	8
EPI-non-responsive isolates									
MU-3	B	0.04	none	8	128	32	32	8	64
			NMP	8	128	32	32	8	64
			PAβN	8	128	32	32	8	64
MU-11	B	0.04	none	8	128	32	32	16	32
			NMP	8	128	32	32	16	32
			PAβN	8	128	32	32	16	32
MU-1	C	0.57	none	16	512	16	128	16	32
			NMP	16	512	16	128	16	32
			PAβN	16	512	16	128	16	32
MU-4	C	0.66	none	16	512	16	8	16	32
			NMP	16	512	16	8	16	32
			PAβN	16	512	16	8	16	32
MU-6	C	0.54	none	32	512	16	32	32	512
			NMP	32	512	16	32	32	512
			PAβN	32	512	16	32	32	512
MU-8	C	0.1	none	0.25	0.25	16	16	64	16
			NMP	0.25	0.25	16	16	64	16
			PAβN	0.25	0.25	16	16	64	16
Reference strains									
SBMox2		0.9	none	64	>1024	128	128	16	32
			NMP	4	>1024	16	16	4	4
			PAβN	64	>1024	64	64	8	8
U10247		0.64	none	32	>1024	512	32	8	2
			NMP	8	>1024	128	2	4	<0.25
			PAβN	32	>1024	256	16	8	1

Continued

Table 1. *Continued*

Isolate	Clone	*adeB* expression[a]	EPI	MIC (mg/L)					
				CIP	GEN	ERY	CHL	TMP	TET
ATCC 19606		0.94	none	1	8	16	32	16	2
			NMP	<0.25	8	4	8	4	0.25
			PAβN	1	4	8	16	8	2
E. coli ATCC 25922		—	none	<0.25	2	—	16	<0.25	2

CIP, ciprofloxacin; GEN, gentamicin; ERY, erythromycin; CHL, chloramphenicol; TMP, trimethoprim; TET, tetracycline.
[a]Measured by RT–PCR relative to 16S rRNA expression.

levels above susceptibility breakpoints.[1,7] Regarding the clonality, the B clone was predominant among the *adeB* overexpressers. However, the presence of isolates expressing the *adeB* gene at low levels from the same clone suggests that the expression level of *adeB* is not a clone-specific characteristic. Hornsey *et al*.[6] have shown that antibiotic therapy may lead to an increase in the expression level of *adeB* in isolates belonging to the same clone.

In six isolates, no changes in antibiotic MICs were detected in the presence of EPIs (Table 1). The *adeB* expression of isolates MU-1, -4 and -6 in this group was similar to that of *A. baumannii* U10247 and high enough to display a response to EPIs. This may be explained by the presence of more effective antibiotic resistance mechanisms, which possibly masked the effect of EPIs on drug efflux in these isolates.[1,2,7]

In conclusion, this study demonstrated the prevalence of *adeB* expression among our isolates and supported the involvement of the AdeB pump at least partly in their multidrug resistance.

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Transparency declarations

None to declare.

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