

Multi-drug resistance profiles and the genetic features of *Acinetobacter baumannii* isolates from Bolivia

Bruno S. Lopes¹, Lucía Gallego², Sebastian G. B. Amyes¹

¹Medical Microbiology, The University of Edinburgh, Edinburgh, United Kingdom

²Departamento Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco, Campus de Bizkaia, Leioa, Spain

Abstract

Introduction: *Acinetobacter baumannii* is opportunistic in debilitated hospitalised patients. Because information from some South American countries was previously lacking, this study examined the emergence of multi-resistant *A. baumannii* in three hospitals in Cochabamba, Bolivia, from 2008 to 2009.

Methodology: Multiplex PCR was used to identify the main resistance genes in 15 multi-resistant *A. baumannii* isolates. RT-PCR was used to measure gene expression. The genetic environment of these genes was also analysed by PCR amplification and sequencing. Minimum inhibitory concentrations were determined for key antibiotics and some were determined in the presence of an efflux pump inhibitor, 1-(1-naphthylmethyl) piperazine.

Results: Fourteen strains were found to be multi-resistant. Each strain was found to have the *bla*_{OXA-58} gene with the *ISAba3*-like element upstream, responsible for over-expression of the latter and subsequent carbapenem resistance. Similarly, *ISAba1*, upstream of the *bla*_{ADC} gene caused over-expression of the latter and cephalosporin resistance; mutations in the *gyrA* (Ser83 to Leu) and *parC* (Ser-80 to Phe) genes were commensurate with fluoroquinolone resistance. In addition, the *adeA*, *adeB* efflux genes were over-expressed. All 15 isolates were positive for at least two aminoglycoside resistance genes.

Conclusion: This is one of the first reports analyzing the multi-drug resistance profile of *A. baumannii* strains isolated in Bolivia and shows that the over-expression of the *bla*_{OXA-58}, *bla*_{ADC} and efflux genes together with aminoglycoside modifying enzymes and mutations in DNA topoisomerases are responsible for the multi-resistance of the bacteria and the subsequent difficulty in treating infections caused by them.

Key words: *Acinetobacter baumannii*; insertion sequences; beta-lactamases; carbapenems; gene; environment

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Introduction

Acinetobacter baumannii is a pathogenic bacterium responsible for a wide range of infections such as septicaemia, meningitis, pneumonia and urinary tract infections and is one of the most important Gram-negative pathogens causing infections in immuno-compromised patients [1]. *Acinetobacter baumannii* has been considered the paradigm of multi-resistant bacteria because of emerging multi-drug resistance to various antimicrobial agents [2], notably mutations in *gyrA* and *parC* genes that confer fluoroquinolone resistance and activation of the aminoglycoside inactivating enzymes [2,3,4]. Beta-lactam antibiotics (mainly carbapenems) are now the first drug of choice to treat these microorganisms; however, in the last decade, resistance to carbapenems has appeared in hospitals worldwide owing to the production of beta-lactamases, changes in

permeability, increase in efflux, and modification of the affinity of penicillin-binding proteins (PBPs) in these bacteria [2,3].

Transposable elements also play a major role in gene expression. They are generally tightly regulated and exercise their role in a strategic manner [5]. Insertion sequences carrying promoters are often responsible for driving the expression of the downstream antibiotic resistance gene, often leading to the over-expression of the gene, and making the bacterium resistant to various antibiotics [6].

A. baumannii is considered an emerging pathogen but very few reports are available from developing countries in Latin America on the mechanisms responsible for carbapenem resistance. In this study we analysed the factors involved in antibiotic resistance in various clinical strains isolated from three major hospitals in Cochabamba, Bolivia. We also

investigated the genetic environments responsible for carbapenem and ceftazidime resistance as well as the mutations that confer resistance to fluoroquinolones.

Methodology

Fifteen isolates thought to have a multi-resistant profile were selected for this study. The isolates were obtained from three different hospitals in Cochabamba in 2008-2009 [Table]. The isolates were identified as *A. baumannii* by PCR amplification and sequencing of the *bla*_{OXA-51-like} [7] and *rpoB* genes [8].

Antimicrobial susceptibility testing

All the isolates were tested for their susceptibility to imipenem (IPM), meropenem (MEM), ceftazidime (CAZ), ciprofloxacin (CIP), and gentamicin (CN). Minimum inhibitory concentrations (MICs) were determined by the agar double dilution method according to the British Society for Antimicrobial Chemotherapy (BSAC) methodology [9]. The MICs of ciprofloxacin and gentamicin were also determined in the presence of an efflux pump inhibitor 1-(1-naphthylmethyl), piperazine (NMP) (100mg/L). The results were interpreted according to BSAC guidelines [9]. The reference strains used for MIC testing were *Escherichia coli* NCTC 10418, *P. aeruginosa* ATCC 10662, and *S. aureus* NCTC 6571.

Screening for antimicrobial resistance genes of the *bla*_{OXA} family

All the isolates were screened for the presence of genes of the *bla*_{OXA-23-like}, *bla*_{OXA-40-like}, *bla*_{OXA-51-like}, *bla*_{OXA-58-like} and *bla*_{OXA-143-like} families by multiplex PCR described by Higgins and colleagues [10]. The primers described by Héritier *et al.* [11] were used for the amplification and sequencing of the *bla*_{OXA-58-like} gene. The primer SM2, described by Poirel and Nordmann [12], was used in combination with walk-58-R [13] for amplification and sequencing of the region upstream of the *bla*_{OXA-58-like} gene.

Screening for the disruption of the *carO* gene

PCR amplification for the insertions causing disruption of the *carO* gene (29kDa OMP) was also completed for all the isolates using primers as described by Mussi *et al.* [14].

Screening for the presence of aminoglycoside resistance genes

A multiplex PCR was performed for the screening of aminoglycoside resistance genes as described earlier by Noppe-Leclercq *et al.* [4]. PCR was also

performed to check for the presence of the *armA* gene and other rRNA methylases such as *rmtA*, *rmtB* and *rmtC* using primers described by Yamane *et al.* [15]. The *rmtD* gene screening was completed using primers described by Doi *et al.* [16].

Screening for the presence of other antimicrobial determinants

PCR arrays were performed for the identification of *bla*_{TEM}, *bla*_{SHV}, *bla*_{PER-2} and *bla*_{CTX-M-2} enzymes using primers described by Celenza *et al.* [17]. Amplification of the *bla*_{VEB} and *bla*_{GES} genes was performed as described by Moubareck *et al.* [18].

Analysis of the genetic environment of the *bla*_{ADC} gene

Primers FU (5'-GCGCCGTGAATTCTTAAGTG-3') and RU (5'-AGCCATACCTGGCACATCAT-3') were used for amplification of the intergenic region upstream of the *bla*_{ADC} gene. Primers FD (5'-CAGCTTATGCTGTGCTGGAT-3') and RD (5'-GAGCTGCCATATTGGGAAGA-3') were used to amplify the intergenic region downstream of the *bla*_{ADC} gene.

Analysis of the quinolone resistance-determining region (QRDR)

PCR assays as described by Valentine and colleagues [3] were used for the amplification of the QRDRs of the *gyrA* and *parC* genes. The gene fragments were sequenced and checked for specific amino acid changes.

Analysis of the *adeABC* and *adeRS* genes involved in efflux

PCR was performed to check for the presence of *adeABC* and *adeRS* genes. The intergenic regions of *adeRS* were amplified using the primers *adeRF* (5'-GCA TTA CGC ATA GGT GCA GA-3') and *adeSR* (5'-GAG GTC GCC GTG ACT AAT TT-3'). The primers *adeRA* (5'-TCA CGG GAG TCT GAG CTT TT-3') and *adeAB* (5'-AAT AGG CGC TCG AAC TGT TG-3') were used to check for any insertions between the *adeRA* genes. The primers *adeAF* (5'-CGC AAG TCG GAG GTA TCA TT-3') and *adeAR* (5'-TAT ACC TGA GGC TCG CCA CT-3') were used for the amplification of *adeA*, while the primers *adeBF* (5'-CCCTAATCAAGGACGTATGC-3') and *adeBR* (5'-TAG AGT GCA GCC AAG ACA AG-3') were used for the amplification of the *adeB* gene. The primers *adeCF* (5'-AGCCTGCAATTACATCTCAT-3') and *adeCR* (5'-TGGCACTTCACTATCAATAC-

3') were used to check for the presence of the *adeC* outer membrane gene.

Analysis of gene expression

Expression of the *bla*_{ADC}, *bla*_{OXA-58-like}, *adeA*, *adeB* and *adeC* genes was studied by RT-PCR. The primers for the *bla*_{ADC} gene were those defined by Ruiz *et al.* [19] and those used for *bla*_{OXA-58-like} gene expression were the multiplex primers described by Woodford *et al.* [20]. The *adeAF* and *adeAR*, *adeBF* and *adeBR*, and *adeCF* and *adeCR* primers were used to determine *adeA*, *adeB*, and *adeC* expression. Total RNA was extracted from isolates in the exponential growth phase using the RiboPure Bacteria kit (Ambion, Paisley, United Kingdom) and treated with the DNase I provided. cDNA was synthesised from 100ng of RNA using the Access quick RT-PCR system kit (Promega, Southampton, United Kingdom). The PCR products were quantified using the Bio-Rad Quantity One Software 4.6.1 (Bio-Rad, Hemel Hempstead, United Kingdom). 16S-rRNA gene primers as described by Lin *et al.* [21] were used for normalization. The results were confirmed three times and were based on the average of the mean increase or decrease of the individual strains.

Results

Isolates were confirmed as *A. baumannii* by *bla*_{OXA-51-like} (Table) and *rpoB* gene PCR. All strains except isolate 2 were resistant to one or more antibiotics tested. They had previously been confirmed to be clonally related according to their PFGE profiles [22].

The table shows the MIC values of IPM, MEM, CAZ, CIP, CIP+NMP, CN and CN+NMP in each individual isolate. It shows that all the strains except two were multi-resistant. The MIC of gentamicin did not change upon the addition of NMP; however, NMP did give a two- to four-fold decrease in isolates resistant to CIP, suggesting that efflux pumps partially contribute to resistance in these isolates.

The table identifies the specific *bla*_{OXA-51-like} gene in each isolate; none of them had insertion sequences upstream of the *bla*_{OXA-51-like} gene. All isolates except number 2 were positive for the *bla*_{OXA-58-like} gene determined by multiplex PCR. Sequencing of the *bla*_{OXA-58-like} gene revealed no nucleotide changes. The primers SM-2 and walk-58-R detected the insertion of an IS*Aba3*-like structure 17bp upstream of the *bla*_{OXA-58} gene. Putative promoters with -35 (TTTATC) and -10 (TTTCTT) motifs were detected in the IS*Aba3*-like element. The promoters had previously been identified

by Poirel and Nordman [11] and correlate with the high MICs of carbapenems resistance in these isolates (Table). The IS*Aba3*-like element had a single codon change at position 25 (GAT to AAT) which led to an aspartate to asparagine amino acid change; however, this but did not affect its activity. The role of the insertion sequence is supported by the observation that the RT-PCR for the *bla*_{OXA-58-like} gene expression also recorded high levels of expression in the isolates positive for the *bla*_{OXA-58-like} gene (Table). The *bla*_{OXA-58-like} gene was found to be present on the 40kb plasmid in all strains resistant to carbapenems except isolate 2 (which was carbapenem sensitive).

The *carO* gene fragment was not disrupted by any insertion element (as indicated earlier by Mussi *et al.* [14]) and thus did not contribute to reduced carbapenem susceptibility.

Multiplex PCR revealed that all isolates except isolate 2 were positive for two aminoglycoside resistance enzymes, the *aac(6')-Ib* and *aph(3')-VI*. As reviewed by Shaw *et al.* [23] the *aac(6')-Ib* class of aminoglycoside inactivating enzymes has been shown to confer resistance to tobramycin, dibekacin, amikacin, 5-episisomicin, netilmicin, 2'-N-ethylnetilmicin, and sisomicin, whereas the *aph(3')-VI* group of enzymes is characterized by resistance to kanamycin, neomycin, paromomycin, ribostamycin, butirosin, amikacin, isepamicin and gentamicin. Isolate 2, on the other hand, was positive for three enzymes: *aph(3')-Ia*, which confers resistance to various aminoglycosides including gentamicin; *aac(3)-Ia*, responsible for resistance to gentamicin and fortimicin; and *ant(2'')-Ia*, which hydrolyzes tobramycin, dibekacin, sisomicin, kanamycin and gentamicin. In each strain these combinations of enzymes contribute to the high MIC of gentamicin. The MICs of gentamicin did not decrease after the addition of NMP, indicating that resistance to gentamicin may not be efflux mediated. Aminoglycoside resistance is common in *Acinetobacter* spp and often derives from inactivation of the antibiotic by the specific modifying enzymes described above [24]. The PCR assays for the rRNA methylase genes such as *armA*, *rmtA*, *rmtB*, *rmtC*, and *rmtD* were all negative.

PCR performed for the identification of *bla*_{SHV}, *bla*_{PER-2}, *bla*_{CTX-M-2}, *bla*_{VEB} and *bla*_{GES} genes were negative. All isolates had the *bla*_{TEM-1} gene which was amplified and sequenced.

IS*Aba1* was found to be present upstream of the *bla*_{ADC} gene in all the strains except isolate 2; the latter had a low MIC of ceftazidime. There was no insertion

Table. *In vitro* activities of antibiotics, levels of gene expression and the mutations in QRDR's in the *A. baumannii* clinical isolates

No	Hospital	MIC (mg/L)							The level of gene expression*						<i>gyrA</i> ser83	<i>parC</i> ser80
		IPM	MEM	CN	CN+NMP	CIP	CIP+NMP	CAZ	<i>bla</i> _{OXA-}		<i>bla</i> _{ADC}	<i>adeA</i>	<i>adeB</i>	<i>adeC</i>		
									<i>51-like</i>	<i>58</i>						
1	GBJ	8	2	128	128	256	64	128	65	14	15	28	29	15	Leu	Phe
2	V	0.25	0.5	256	256	1	1	16	66	-	5	20	18	14	Ser	Ser
3	V	8	2	>256	256	256	64	128	65	14	14	31	31	14	Leu	Phe
4	V	8	2	256	256	256	64	128	65	14	15	25	29	14	Leu	Phe
5	V	8	2	64	64	128	32	128	65	15	15	27	29	13	Leu	Phe
6	GBJ	16	4	64	64	128	32	128	65	14	16	26	28	13	Leu	Phe
7	V	8	2	128	128	256	64	128	65	15	15	27	30	11	Leu	Phe
8	V	8	2	64	64	256	64	128	65	15	15	26	29	14	Leu	Phe
9	CO	8	2	>256	256	>256	64	128	65	14	14	31	36	15	Leu	Phe
10	V	8	2	64	64	128	32	128	65	14	15	26	28	15	Leu	Phe
11	CO	8	2	128	128	256	64	128	65	15	14	27	29	14	Leu	Phe
12	V	16	2	128	128	256	64	128	65	14	15	27	27	13	Leu	Phe
13	V	8	2	256	256	128	64	128	65	15	14	28	27	15	Leu	Phe
14	V	8	2	256	256	128	64	128	65	15	14	27	28	17	Leu	Phe
15	V	8	2	256	256	128	64	128	65	14	15	28	28	15	Leu	Phe

GBJ = Gastroenterológico Boliviano-Japonés, V = Viedma, CO = Clínica Olivos

* = Relative gene expression was determined by RT PCR and quantified by Bio-Rad quantity I software.

+ = present

- = absent

IPM = imipenem, CN = gentamicin, CIP = ciprofloxacin, CAZ = ceftazidime, NMP = 1-(1-naphthylmethyl) piperazine

detected downstream of the *bla*_{ADC} gene in any of the isolates. Expression of the *bla*_{ADC} gene in the strains was identified by the high MIC values of ceftazidime and confirmed by RT-PCR.

The intergenic regions of *adeRS* were amplified using the primers *adeRF* (5'-GCA TTA CGC ATA GGT GCA GA-3') and *adeSR* (5'-GAG GTC GCC GTG ACT AAT TT-3'); these amplified a 790bp product, indicating that there was no insertion. The primers *adeRA* (5'-TCA CGG GAG TCT GAG CTT TT-3') and *adeAB* (5'- AAT AGG CGC TCG AAC TGT TG-3') amplified a product of 929bp, indicating there was no insertion between these two genes. Ruzin *et al.* [25] previously reported that *ISAbal* may contribute to the expression of the efflux genes. The primers *adeCF* (5'-AGC CTG CCA TTA CAT CTC AT-3') and *adeCR* (5'-TGG CAC TTC ACT ATC AAT AC-3') detected the presence of the *adeC* gene. Because the strains had a very high MIC of ciprofloxacin, which decreased after the addition of NMP (Table), we hypothesized that the efflux pump genes would be highly expressed. RT-PCR for analysis of the *adeA* (encoding for the membrane fusion protein) and the *adeB* (encoding the RND-transporter) genes revealed that both the genes were highly expressed as they are co-transcribed (Table). The *adeC* (encoding the outer membrane protein) was not as highly expressed as *adeA* and *adeB*; the reason may be that its expression is not necessary to confer resistance [26] as alternative outer membrane proteins can be used by bacteria to export substrates for effluxing any poisons or antibiotics. We checked further for mutations in *gyrA* and *parC* as well as the antibiotic determinants responsible for ciprofloxacin resistance in these isolates. Sequencing of the *gyrA* and *parC* genes revealed Ser-83 to Leu and Ser-80 to Phe amino acid changes in isolates resistant to ciprofloxacin. Resistance to ciprofloxacin was mainly due to target site mutations in the *gyrA* and *parC* with a small contribution by the efflux pumps.

Discussion

This work analyzed the genetic environment and the multi-drug resistance profile of *A. baumannii* isolated in Cochabamba, Bolivia, as there are few surveys that study the multi-drug resistant profile of clinical isolates in Bolivia. Multi-drug resistance is certainly of great concern. A previous study by Celenza *et al.* [17] reported no carbapenem resistance in any of the isolates studied. The current investigation showed that carbapenem resistance is manifested largely by the presence of the OXA-58

carbapenemase. Surprisingly, the PCR results for the *bla*_{PER-2} and *bla*_{CTX-M-2} genes were negative. These results contradict those of a study conducted in Bolivia in 2006 by Celenza *et al.* [17] in which the presence of these genes was reported. We therefore conclude that the presence of *ISAbal* upstream of the *bla*_{ADC} gene has substituted the *bla*_{PER-2} and *bla*_{CTX-M-2} genes to confer high levels of resistance to ceftazidime and provide a stable mechanism of cephalosporin resistance to the bacterium.

The presence of the *bla*_{OXA-58} gene has been observed in other Latin American countries. These results support the worldwide spread of the *bla*_{OXA-58} gene, one of the major causes of carbapenem resistance [27]. Ciprofloxacin resistance in the isolates was largely determined by target site mutations with partial contribution by the efflux pumps. Each strain had at least two aminoglycoside modifying enzymes which together contributed to high levels of resistance to gentamicin. The high proportion of strains with resistance genes to the major groups of antibiotics used to control *A. baumannii* found in this study is of great concern as clonal spread has been observed among the three hospitals where these isolates were isolated. Mobilization of *bla*_{OXA-58-like} carbapenemase genes has often been found to be associated with insertion sequences or transposons, and the potential of spread via plasmid motors is very high. The OXA-23-like and OXA-58-like enzymes have been found in Brazil, Venezuela, Colombia, Chile, Bolivia and Argentina; however, OXA-58-like enzymes are most frequently identified in South America [28]. The spread of plasmid-borne carbapenemases can occur inter- or intra-specifically in hospital environments, so it is important to develop control strategies to prevent infections caused by carbapenem-resistant *A. baumannii*. This strategy should help in the prevention of nosocomial spread of infections, especially among immuno-compromised patients.

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References

1. Bergogne-Bérézin E, Towner KJ (1996) *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev* 9: 148-165.

2. Vila J, Martí S, Sánchez-Céspedes J (2007) Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. J Antimicrob Chemother 59: 1210-1215.
3. Valentine SC, Contreras D, Tan S, Real LJ, Chu S, Xu HH (2008) Phenotypic and molecular characterization of *Acinetobacter baumannii* clinical isolates from nosocomial outbreaks in Los Angeles County, California. Antimicrob Agents and Chemother 46: 2499-2507.
4. Noppe-Leclercq I, Wallet F, Haentjens S, Courcol R, Simonet M (1999) PCR detection of aminoglycoside resistance genes: a rapid molecular typing method for *Acinetobacter baumannii*. Res Microbiol 150: 317-322.
5. Mugnier PD, Poirel L, Nordmann P (2009) Functional analysis of insertion sequence IS*Aba1*, responsible for genomic plasticity of *Acinetobacter baumannii*. J Bacteriol 191: 2414-2418.
6. Corvec S, Poirel L, Naas T, Drugeon H, Nordmann P (2007) Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene *bla*_{OXA-23} in *Acinetobacter baumannii*. Antimicrob Agents Chemother 51: 1530-1533.
7. Héritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P (2005) Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob Agents Chemother 49: 4174-4179.
8. La Scola B, Gundi VAKB, Khamis A, Raoult D (2006) Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. J Clin Micro 44: 827-832.
9. Andrews JM, and the British Society for Antimicrobial Chemotherapy (BSAC) (2010) Methods for Antimicrobial Susceptibility Testing Version 9.1. Birmingham.
10. Higgins PG, Lehmann M, Seifert H (2010) Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents 35: 305.
11. Héritier C, Dubouix A, Poirel L, Marty N, Nordmann P (2005) A nosocomial outbreak of *Acinetobacter baumannii* isolates expressing the carbapenem-hydrolyzing oxacillinase OXA-58. J Antimicrob Chemother 55: 115-118.
12. Poirel L and Nordmann P (2006) Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene *bla*_{OXA-58} in *Acinetobacter baumannii*. Antimicrob Agents Chemother 50: 1442-1448.
13. Lopes BS, Evans BA, Amyes SGB (2012) The disruption of the *bla*_{OXA-51-like} gene by IS*Aba16* and the activation of the *bla*_{OXA-58} gene leading to carbapenem resistance in *Acinetobacter baumannii* Ab244. J Antimicrob Chemother 67: 59-63.
14. Mussi MA, Limansky AS, Viale AM (2005) Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: Natural insertional inactivation of a gene encoding a member of a novel family of β -Barrel Outer Membrane Proteins. Antimicrob Agents Chemother 49: 1432-1440.
15. Yamane K, Wachino J, Suzuki S, Shibata N, Kato H, Shibayama K, Kimura K, Kai K, Ishikawa S, Ozawa Y, Konda T, Arakawa Y (2007) 16S rRNA methylase-producing, gram-negative pathogens, Japan. Emerg Infect Dis 13: 642-646.
16. Doi Y, Ghilardi AC, Adams J, de Oliveira Garcia D, Paterson DL (2007) High prevalence of metallo-beta-lactamase and 16S rRNA methylase coproduction among imipenem-resistant *Pseudomonas aeruginosa* isolates in Brazil. Antimicrob Agents Chemother 51: 3388-3390.
17. Celenza G, Pellegrini C, Caccamo M, Segatore B, Amicosante G, Perilli M (2006) Spread of *bla*_{CTX-M-type} and *bla*_{PER-2} β -lactamase genes in clinical isolates from Bolivian hospitals. J Antimicrob Chemother 57: 975-978.
18. Moubareck C, Brémont S, Conroy MC, Courvalin P, Lambert T (2009) GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. Antimicrob Agents Chemother 53: 3579-3581.
19. Ruiz M, Martí S, Fernandez-Cuenca F, Pascual A, Vila J (2007) Prevalence of IS*Aba1* in epidemiologically unrelated *Acinetobacter baumannii* clinical isolates. FEMS Microbiol Lett 274: 63-66.
20. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SG, Livermore DM (2006) Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents 27: 351-353.
21. Lin L, Ling BD, Li XZ (2009) Distribution of the multidrug efflux pump genes, *adeABC*, *adeDE* and *adeJK*, and class I integron genes in multiple-antimicrobial-resistant clinical isolates of *Acinetobacter baumannii*-*Acinetobacter calcoaceticus* complex. Int J Antimicrob Agents 33: 27-32.
22. Sevillano E, Fernandez E, Bustamante Z, Zabalaga S, Rosales I, Umanan A, Gallego L (2012) Emergence and Clonal Dissemination of Carbapenem-Hydrolyzing OXA-58-producing *Acinetobacter baumannii* isolates in Bolivia. J Med Micro 61: 80-84.
23. Shaw KJ, Rather PN, Hare RS, Miller GH (1993) Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev 57: 138-163.
24. Magnet S, Courvalin P, Lambert T (2001) Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in *Acinetobacter baumannii* strain BM4454. Antimicrob Agents Chemother 45: 3375-3380.
25. Ruzin A, Keeney D, Bradford PA (2007) AdeABC multidrug efflux pump is associated with decreased susceptibility to tigecycline in *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex. J Antimicrob Chemother 59: 1001-1004.
26. Marchand I, Damier-Piolle L, Courvalin P, Lambert T (2004) Expression of the RND-Type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the AdeRS two-component system. Antimicrob Agents Chemother 48: 3298-3304.
27. Coelho J, Woodford N, Afzal-Shah M, Livermore D (2006) Occurrence of OXA-58-like carbapenemases in *Acinetobacter* spp. collected over 10 years in three continents. Antimicrob Agents Chemother 50: 756-758.
28. Opazo A, Domínguez M, Bello H, Amyes SG, González-Rocha G (2012) OXA-type carbapenemases in *Acinetobacter baumannii* in South America. J Infect Dev Ctries 6: 311-316.

Corresponding author

Professor S. G. B. Amyes
 Medical Microbiology, University of Edinburgh
 Chancellor's Building, 49 Little France Crescent
 Edinburgh EH16 4SB Scotland
 Telephone: 0131 242 6652
 Fax: 0131 242 6611
 Email: s.g.b.amyes@ed.ac.uk

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