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RESEARCH NOTE

The sequences of seven class D β-lactamases isolated from carbapenem-resistant *Acinetobacter baumannii* from four continents

S. Brown and S. G. B. Amyes

Medical Microbiology, Edinburgh University Medical School, Edinburgh, UK

ABSTRACT

Carbapenem resistance associated with class D β-lactamases is an increasing problem in *Acinetobacter baumannii*. Most enzymes of this class reported so far belong to two subgroups, 1 and 2; however, a novel class D carbapenemase (OXA-51) has been reported recently which shares 56% and < 63% amino-acid identity with subgroups 1 and 2, respectively, and which belongs to a third subgroup. This study describes a further seven novel subgroup 3 β-lactamases in carbapenem-resistant *A. baumannii* isolates from four continents.

Keywords *Acinetobacter baumannii*, β-lactamase, carbapenemase, class D β-lactamase, OXA carbapenenases, resistance

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Acinetobacter baumannii is an opportunistic nosocomial pathogen associated with a range of infections, particularly in ventilated patients in intensive therapy units [1,2]. An alarming trend has been the emergence of multiresistant isolates that are resistant to one or more of the carbapenems, with the result that pan-drug resistance is now emerging [3]. β-Lactamase-mediated carbapenem resistance is being reported with increasing frequency. Some isolates from Asia, southern Europe and the UK have IMP and VIM molecular class B metallo-β-lactamases [4–7], but carbapenem-hydrolysing β-lactamases that belong to class D have also been described. The first class D carbapenemase (OXA-23) [8] was detected in an imipenem-resistant isolate from Edinburgh in 1985 [9] that belongs to subgroup 1, along with OXA-27 (Singapore) [10] and OXA-49 (China) (GenBank accession number AY288523). OXA-24, -25, -26 and -40, identified in isolates from Spain, Belgium, France and Portugal, differ by one or two amino-acids, and belong to subgroup 2 [10–13]. The two subgroups share 60% amino-acid homology, but all of these enzymes possess a tyrosine-to-phenylalanine substitution in the conserved Y-G-N motif of the class D protein [14]. A novel class D carbapenemase (OXA-51) has been characterised recently in two distinct clones of *A. baumannii* from Argentina. OXA-51 shares 56% and 61–62% amino-acid homology with subgroups 1 and 2, respectively, and thus represents a new subgroup of carbapenemases in *Acinetobacter* [15]. The present report describes a further seven novel class D β-lactamases belonging to this new subgroup in carbapenem-resistant *A. baumannii* isolates collected worldwide.

Multiresistant *A. baumannii* clinical isolates were collected between 1996 and 2000 from centres in Argentina, South Africa, Hong Kong, Spain, Singapore and Turkey. Ten isolates from Argentina included in this study belonged to two clones (designated as pulsed-field gel electrophoresis genotypes B and C) that were identified previously in an outbreak of carbapenem resistant isolates from two hospitals in Buenos Aires (39th Interscience Conference on Antimicrobial Agents and Chemotherapy, abstract 1463); clones B and C were distinct from two other clones of carbapenem-resistant *A. baumannii* that produced the OXA-51 carbapenemase [15]. The isolates from Turkey included six related multiresistant *A. baumannii* isolates (designated as pulsed-field gel electrophoresis genotype D) that were collected from the surgical intensive care unit of Dokuz Eylul University Hospital in Izmir (39th Interscience Conference on Antimicrobial Agents and Chemotherapy, abstract 1463). The remaining isolates in this study included two from South Africa collected from the Chris Hani Baragwanath Hospital, Soweto, three isolates from the KK Women's and Children's Hospital, Singapore, two isolates from a hospital clinic in Barcelona, and two isolates from the Prince of Wales Hospital, Hong Kong. Clinical *A. baumannii* strains

Corresponding author and reprint requests: S. Brown, Medical Microbiology, Edinburgh University Medical School, Teviot Place, Edinburgh, EH8 9AG, UK
E-mail: Susan.Brown@ed.ac.uk

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789 (carbapenem-susceptible) and 788 (OXA-51 producer) were included as controls for PCR analysis.

Imipenem and meropenem MICs were determined according to published guidelines [16,17]. DNA was extracted from isolates for PCR analysis by boiling one to three colonies in 100 μL of sterile ultra-pure water for 10 min and centrifuging briefly. PCRs (50-μL final volumes) contained 2 μL of DNA extract, 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.8 mM dNTPs and 1.25 U of *Pfu* DNA polymerase (Promega, Southampton, UK). To determine the presence of bla_{OXA}-51-related sequences, primers designed to amplify the OXA-51 gene [15] were used. PCR conditions on a Techne Px2 Gradient Thermal Cycler (Thermo Hybaid, Ashford, UK) comprised 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 50°C for 1 min and 72°C for 2 min, with a final extension period of 72°C for 10 min. Purified amplicons from three separate PCRs were sequenced in both directions on an ABI Prism Automated Sequencer (Perkin-Elmer, Beaconsfield, UK). Protein sequences were aligned by hierarchical clustering [18].

The isolates studied had imipenem and meropenem MICs of 4–128 mg/L, suggesting that additional factors, such as outer-membrane impermeability, may also be involved in the carbapenem resistance of these isolates. Sequencing of an amplified 1000-bp product identified a gene with an 825-bp open reading frame that encoded a protein with 274 amino-acids. Analysis of the protein from Argentinian strains 884 (clone B) and 790 (clone C) identified a novel β-lactamase (designated OXA-65) that shared 98% amino-acid identity with OXA-51. A further six novel sequences with 97–98% amino-acid homology with OXA-51 (Fig. 1) were identified, namely OXA-64 in isolate 889 (South Africa), OXA-66 in isolates 806 (Spain), 811 (Hong Kong) and 864 (Singapore), OXA-68 in isolate 809 (Spain), OXA-69 in isolates 877, 881 (both Singapore) and 823 (clone D, Turkey), OXA-70 in isolate 812 (Hong Kong), and OXA-71 in isolate 888 (South Africa).

Comparison of these enzymes with OXA-51 identified between three and eight amino-acid substitutions (Table 1). All had an A → G change at nucleotide position 13, resulting in a threonine-to-alanine substitution at amino-acid 5. In addition, bla_{OXA-71} had an A → C change at the third position in this codon (Table 1). Each of these

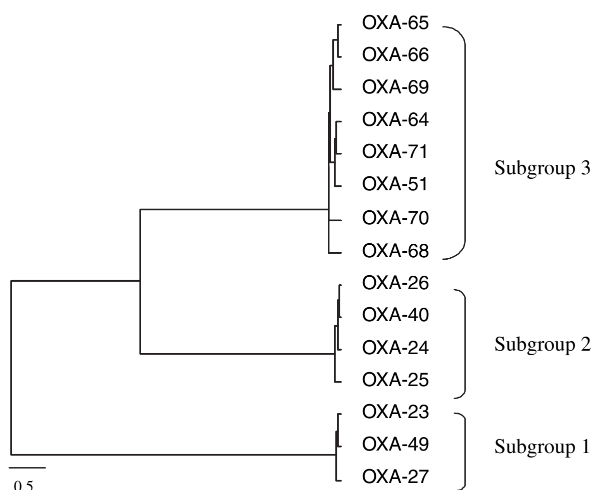

Fig. 1. Relationship of subgroup 3 to other OXA-type carbapenemases found in *Acinetobacter* spp. Analysis was performed with BioNumerics v. 2.5 (Applied Maths BVBA, Sint-Martens-Latem, Belgium). A gap penalty of 3 was applied.

Table 1. Amino-acid changes detected in subgroup 3 carbapenemases related to OXA-51

Amino-acid position (related to OXA-51)	Amino-acid change[a]	OXA
5	Thr → Ala	
	(ACA) → (**G**CA)	− 64, − 65, − 66, − 68, − 69, − 70, − 71
	(ACA) → (**G**C**C**)	− 71
24	Thr → Ser	
	(ACT) → (**T**CT)	− 68
36	Glu → Asp	
	(GAA) → (GA**C**)	− 69
36	Glu → Lys	
	(GAA) → (**A**AA)	− 70
36	Glu → Val	
	(GAA) → (G**T**A)	− 66
38	Ala → Gly	
	(GCA) → (G**G**A)	− 64
48	Val → Ala	
	(GTA) → (G**C**A)	− 64, − 65, − 66, − 68, − 69, − 70, 71
57	Gln → His	
	(CAA) → (CA**T**)	− 69
96	Ala → Thr	
	(GCA) → (**A**CA)	− 71
105	Asp → Asn	
	(GAC) → (**A**AC)	− 70
107	Gln → Lys	
	(CAA) → (**A**AA)	− 65, − 66
107	Gln → Glu	
	(CAA) → (**G**AA)	− 69
117	Asp → Asn	
	(GAC) → (**A**AC)	− 68, − 69
146	Lys → Asn	
	(AAG) → (AA**T**)	− 68, − 70
194	Pro → Gln	
	(CCA) → (C**A**A)	− 65, − 66, − 68, − 69, − 70
195	Lys → Glu	
	(AAA) → (**G**AA)	− 68
198	Asp → His	
	(GAT) → (**C**AT)	− 70
225	Asp → Asn	
	(GAC) → (**A**AC)	− 65, − 66, − 68, 69

[a]Codons are in parentheses. Nucleotide changes are in bold.

genes also had a T → C change at nucleotide postion 143, causing a valine-to-alanine substitution at amino-acid 48. As observed with OXA-51, all enzymes had a valine-to-isoleucine substitution in the S-X-V class D motif (amino-acids 127–129 in OXA-51) and, in contrast to carbapenemases in subgroups 1 and 2 [8,10–12], a tyrosine of the Y-G-N motif (amino-acids 153–155) was also retained (data not shown). However, subgroup 3 enzymes were similar to those in subgroup 2 in that the K-T-G motif (amino-acids 217–219 in OXA-51) was replaced by K-S-G [10–12]. Identical silent changes were present at 11 nucleotide positions in at least two different OXA genes (data not shown).

In conclusion, this study identified seven novel OXA-type β-lactamases related to OXA-51 in carbapenem-resistant *A. baumannii* isolates from six countries, with two of these enzymes found in isolates from more than one continent. Carbapenemases in subgroup 2 have been identified so far only in strains from Europe [10–13,19], and OXA-23 is the only enzyme in subgroup 1 to have been found in more than one country (GenBank accession number AY288523) [20,21]. Consequently, subgroup 3 now represents the largest and most diverse collection of class D carbapenemases in *A. baumannii*, and may become the major mechanism of carbapenem resistance in this species.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

The sequence data reported in this study are deposited in the GenBank nucleotide database under accession numbers AY750907 (OXA-64), AY750908 (OXA-65), AY750909 (OXA-66), AY750910 (OXA-68), AY750911 (OXA-69), AY750912 (OXA-70) and AY750913 (OXA-71).

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RESEARCH NOTE

Prevalence of *Acinetobacter baumannii* and other *Acinetobacter* spp. in faecal samples from non-hospitalised individuals

L. Dijkshoorn[1], E. van Aken[1], L. Shunburne[2], T. J. K. van der Reijden[1], A. T. Bernards[3], A. Nemec[4] and K. J. Towner[2]

[1]Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands, [2]Department of Microbiology, University Hospital, Queen's Medical Centre, Nottingham, UK, [3]Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands and [4]Centre of Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Republic

ABSTRACT

In total, 226 individuals from the community were investigated for faecal carriage of *Acinetobacter* spp. by broth enrichment culture, followed by growth on blood agar and/or Leeds Acinetobacter Medium (LAM). *Acinetobacter baumannii* was isolated on both LAM and blood agar from one of 100 specimens in the UK and one of 126 specimens in The Netherlands. The predominant species were *Acinetobactor johnsonii* and genomic sp. 11, which were cultured from 22 and five specimens, respectively. *A. baumannii* did not seem to be widespread in the faecal flora of individuals in the community.

Corresponding author and reprint requests: L. Dijkshoorn, Department of Infectious Diseases, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands
E-mail: L.Dijkshoorn@lumc.nl

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Acinetobacter baumannii is an increasingly important nosocomial pathogen which can cause major outbreaks of infection. The organism is often acquired nosocomially, but can be introduced initially by patients admitted from other hospitals [1]. Certain strains (clones) have been shown to be distributed between hospitals in wide geographical areas [2–4]. However, the origin of a suddenly appearing epidemic *A. baumannii* strain in a hospital is often unknown, and it remains possible that *A. baumannii* has a reservoir in the non-hospitalised human population, from which strains can be introduced into a hospital. The present study investigated the occurrence of *A. baumannii* in faecal specimens from non-hospitalised individuals in order to determine whether there is a significant intestinal reservoir from which spread to hospitals could occur. The study was performed in Nottingham (UK) and Leiden (The Netherlands), thus allowing comparison of populations at two locations. In Nottingham, the investigation focused exclusively on *A. baumannii*, while in Leiden, the prevalence of other *Acinetobacter* spp. was also investigated.

In total, 226 individuals from the community were investigated. One faecal specimen from each individual was received from general practitioners for investigation of parasites or microbial pathogens by the diagnostic laboratories at Leiden University Medical Center and University Hospital, Nottingham. A sterile cotton swab was used to sample the faeces, and the material taken up on the swab was transferred to flasks containing 20 mL of acetate mineral medium [5]. The samples were mixed to resuspend the faecal material, and then incubated with vigorous aeration for up to 48 h at 30°C to enrich for *Acinetobacter* spp. Following incubation, the cultures were streaked on to Leeds Acinetobacter

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