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Global spread of carbapenem-resistant *Acinetobacter baumannii*

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Objectives: We have investigated the molecular epidemiology and distribution of carbapenemase genes in 492 imipenem-non-susceptible *Acinetobacter baumannii* worldwide isolates (North and Latin America, Europe, Asia, South Africa and Australia).

Methods: MICs were determined by broth microdilution and Etest. The presence of carbapenemase-encoding genes was investigated by PCR. Molecular epidemiology was performed by repetitive sequence-based PCR (rep-PCR; DiversiLab), sequence-type multiplex PCR and PFGE.

Results: Imipenem non-susceptibility was associated with IS*Aba1* upstream of the intrinsic $bla_{\text{OXA-51-like}}$ or the acquired carbapenemase $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$ or $bla_{\text{OXA-58-like}}$. Isolates were grouped into eight distinct clusters including European clones I, II and III. European clone II was the largest (246 isolates) and most widespread group (USA, pan-Europe, Israel, Asia, Australia and South Africa).

Conclusions: The global dissemination of eight carbapenem-resistant lineages illustrates the success this organism has had in epidemic spread. The acquired OXA enzymes are widely distributed but are not the sole carbapenem resistance determinant in *A. baumannii*.

Keywords: oxacillinases, epidemiology, rep-PCR, tigecycline, IS*Aba1*

Introduction

Acinetobacter baumannii is a serious and emerging nosocomial pathogen. Initially regarded as of little clinical significance, it is now being isolated more frequently, particularly in intensive care settings where it is a cause of serious infections such as ventilator-associated pneumonia, bloodstream infection, urinary tract infection, meningitis and wound infection.[1] It affects mainly the severely immunocompromised, and is typically selected by prior antimicrobial therapy.[2] *A. baumannii* is rarely found on human skin, is not a normal environmental organism and its natural reservoir is unknown.[1] *A. baumannii* shares several characteristics with methicillin-resistant *Staphylococcus aureus* (MRSA): multidrug resistance; long-term survival on inanimate surfaces such as computer keyboards, pillows, curtains and other dry surfaces; and propensity for epidemic spread.[3,4] This longevity is thought to contribute to the clonal spread of isolates and to facilitate person-to-person transmission and environmental contamination. For the control of a hospital outbreak, strict adherence to infection control measures and sometimes even the closure of wards are required.[3]

A. baumannii infections are difficult to treat owing to innate and acquired antimicrobial resistance. Until recently, most isolates were susceptible to the carbapenems but there have been isolated reports of resistance since the early 1990s. Since then, the incidence of imipenem resistance has risen dramatically and is considered a global sentinel event.[5]

Carbapenem resistance in *A. baumannii* is mediated most often by oxacillinases (OXAs) and less frequently by metallo-β-lactamases (MBLs).[6] There are four main OXA subgroups associated with *A. baumannii*: the chromosomally located intrinsic OXA-51-like and the acquired OXA-23-like, OXA-40-like and OXA-58-like. OXAs exhibit such weak hydrolysis of carbapenems that they should not allow the development of resistance; however, they are sometimes associated with insertion elements that can increase expression of the carbapenemase.[7–9] In addition, the low outer membrane permeability of *A. baumannii* is a contributory factor towards carbapenem resistance.[10]

Compelling evidence suggests that *A. baumannii* is clonal in nature. Molecular typing of isolates obtained from various locations in the EU has shown the existence of three distinct clusters that have been termed pan-European clonal complexes I, II and III (EUI, II and III, respectively).[11,12] Isolates belonging to these clonal complexes have been found in nearly all European countries. There are limited data on the global epidemiology of *A. baumannii*. However, *A. baumannii* isolated from

repatriated British and US military personnel injured in Iraq have been shown to be indistinguishable from clinical isolates from the UK.[13,14]

In the present study, we have used DiversiLab, a semi-automated typing system based on repetitive sequence-based PCR (rep-PCR) for molecular typing. This method has been shown to be almost as discriminatory as fluorescent amplified fragment-length polymorphism (f-AFLP) and PFGE.[15,16] The aim of this study was to investigate the molecular epidemiology and distribution of carbapenem-hydrolysing enzymes in a global cohort of imipenem-resistant *A. baumannii* and to compare them with known epidemiological groups.

Material and methods

Study population

A global cohort of imipenem-susceptible ($n=23$) and non-susceptible ($n=492$) *A. baumannii* isolates were collected from 139 worldwide centres representing 32 countries as part of the larger Tigecycline Evaluation and Surveillance Trial (TEST) programme.[17] Identification of *A. baumannii* was confirmed by *gyrB* multiplex.[18] Control isolates representing European clonal complexes I–III were kindly provided by Dr Lenie Dijkshoorn, University of Leiden.

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed by broth microdilution according to the current CLSI guidelines.[19] The following antimicrobials were used: amikacin, amoxicillin/clavulanic acid, cefepime, ceftazidime, ceftriaxone, imipenem, levofloxacin, meropenem, minocycline, tigecycline and piperacillin/tazobactam. For tigecycline, the EUCAST clinical MIC breakpoints for Enterobacteriaceae (http://www.srga.org/eucastwt/MICTAB/MICtigecycline.htm) were used. Imipenem MICs were confirmed by Etest (AB Biodisk, Solna, Sweden). Imipenem MICs of $\leq$4 mg/L and $\geq$16 mg/L were interpreted as susceptible and resistant, respectively.[19]

Detection of carbapenemases

To detect the presence of the most common carbapenemases, multiplex PCR was performed with primers that anneal to $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$ and $bla_{\text{OXA-58-like}}$ carbapenemases and the MBLs IMP, VIM, SPM, GIM and SIM as previously described.[20,21] The presence of the insertion element IS*Aba1* upstream of $bla_{\text{OXA-51-like}}$ was investigated by PCR.[7]

Molecular typing

Epidemiological typing of isolates was performed by rep-PCR (DiversiLab System; bioMérieux, Nürtingen, Germany) following the manufacturer's instructions. Results were analysed with the DiversiLab software using the modified Kullback–Leibler statistical method to determine distance matrices and the unweighted pair group method with arithmetic averages (UPGMA) to create dendrograms. Isolates that clustered >95% were considered related.[15] Representative isolates belonging to pan-European *A. baumannii* clonal complexes I, II and III were included as controls. To confirm clustering by rep-PCR, two additional methods were used: PFGE and the identification of sequence groups by multiplex PCRs.[22,23] PFGE banding patterns were analysed visually.

Results

Antimicrobial susceptibility testing

Among the 515 *A. baumannii*, 471 isolates were confirmed to be resistant to imipenem, 21 were intermediate and 23 were susceptible. MICs for imipenem-non-susceptible isolates are shown in Table 1. Susceptibility to non-carbapenem β-lactams, including those with a β-lactamase inhibitor, was low, with ≤5% of isolates susceptible. Amikacin susceptibility was at 35% and only tigecycline and minocycline had high activity, with >80% of isolates recorded as susceptible.

Table 1. MIC distributions for 492 imipenem-non-susceptible *A. baumannii* isolates

	Cumulative percentage of isolates with MIC (mg/L)								
Drug	≤0.5	1	2	4	8	16	32	≥64	Percentage susceptible
IPM					4.3	12.6	25.2	100	0.0
MEM					2.1	100[a]			0.0
AMC						0.2	1	100	0.0
CRO					0.4	2.9	5.8	100	0.4
CAZ					1.7[b]	4	16.2	100	1.7
FEP				0.4	3.9	18	50	100	3.9
TZP		1.4	1.4	2	2.4	3.8	6.7	100	3.9
LVX		1.2	5.6	37.7	89.9	100[a]			5.6
AMK		0.4	5.8	13.7	24.3	35.7	51.9	100	35.7
MIN		56.8	70.7	82.7	95.4	99.1	100		82.7
TGC	46.5	82.7	95.3	100					82.7

IPM, imipenem; MEM, meropenem; AMC, amoxicillin/clavulanic acid; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin/tazobactam; LVX, levofloxacin; AMK, amikacin; MIN, minocycline; TGC, tigecycline.
[a]MIC ≥ 16 mg/L.
[b]MIC ≤ 8 mg/L.

Molecular typing

Results of the molecular analysis of all *A. baumannii* isolates using rep-PCR are shown in Table 2. Using a similarity index of ≥95% as the threshold, 91% of isolates clustered into eight distinct groups (Figure 1 and Table 2); 43 isolates did not cluster. Almost half the isolates clustered in one large group that included the EUII control strains. This group comprised isolates from Australia, China, Israel, Pakistan, pan-Europe, Singapore, South Africa, South Korea, Taiwan and the USA. Clusters including representatives of EUI and III (Table 2) also contain isolates from many countries outside Europe. Therefore we suggest that these are henceforth referred to as worldwide (WW) clonal lineages. WW1 (44 isolates from 17 centres) was as geographically widespread as WW2. WW3 isolates were predominantly from the USA but include isolates from South Africa and Spain. Within these clusters, we found evidence for the clonal spread of particular isolates: e.g. identical rep-PCR patterns were found between isolates from Honduras and Italy, and Singapore and Mexico (Figure 1). WW5, the second largest group, was composed almost entirely of isolates originating from North, Central and South America and could be considered a pan-American clone. Unclustered isolates comprised between one and four isolates originating from the same centre.

Sixty isolates representing a cross-section of each cluster were selected for PFGE analysis. A unique macrorestriction backbone was visible that confirmed rep-PCR clustering (data not shown). To confirm clustering of isolates with control strains belonging to the EU clonal lineages, sequence-group multiplex PCR was performed with 16, 42 and 23 randomly selected WW1, WW2 and WW3 isolates, respectively. In addition 8, 12, 6, 9 and 6 isolates from WW4, WW5, WW6, WW7 and WW8 were also tested. Based on allele variations in the *ompA*, *csuE* and $bla_{\text{OXA-51-like}}$ genes, WW2, WW1 and WW3 amplified gene products corresponding to previously defined sequence groups 1, 2 and 3 (EU clones II, I and III, respectively). The remaining WW isolates did not belong to these groups. WW4 had the same pattern as variable group 4 described by Towner *et al.*[24] (amplification of sequence group 2 $bla_{\text{OXA-51-like}}$ and *ompA*), WW5 and WW8 amplified only *ompA* from sequence group 1, WW6 amplified $bla_{\text{OXA-51-like}}$ and *ompA* from sequence group 1 and sequence group 2 *csuE*, and WW7 amplified from sequence group 1 $bla_{\text{OXA-51-like}}$ and either *ompA* or *csuE*.

Detection of carbapenemase genes

The *A. baumannii* isolates were investigated for the presence of OXA-type carbapenemases (Table 2). All isolates harboured $bla_{\text{OXA-51-like}}$. Three hundred and four isolates had, in addition, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$ or $bla_{\text{OXA-58-like}}$ genes. Of these isolates, 271 had an imipenem MIC ≥64 mg/L. Three isolates harboured both $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ genes. Five isolates harbouring $bla_{\text{OXA-58-like}}$ had imipenem MICs of 8 mg/L. We did not detect any MBL genes. Three isolates harboured the novel OXA, OXA-143.[25] IS*AbaI* was upstream of $bla_{\text{OXA-51-like}}$ in five $bla_{\text{OXA-40-like}}$ isolates, two $bla_{\text{OXA-58-like}}$ isolates, three $bla_{\text{OXA-23-like}}$ isolates and 183 isolates where an acquired carbapenem resistance gene was not detected, and includes seven carbapenem-susceptible isolates. Two isolates for which the imipenem MIC was >32 mg/L have an as yet uncharacterized resistance mechanism.

Table 2. Distribution of OXA-type genes in imipenem-non-susceptible *A. baumannii* isolates by epidemiological cluster

Epidemiological group (no. of centres)	No. of isolates	IPM resistance determinant (total no.)				Country of origin
		$bla_{\text{OXA-23-like}}$	$bla_{\text{OXA-40-like}}$	$bla_{\text{OXA-58-like}}$	IS*Aba1*-$bla_{\text{OXA-51-like}}$	
WW1 (EUI) (17)	44	21	0	19	5	AR, BG, CH, DE, ES, GR, IN, IT, PK, PL, PR, SG, US
WW2 (EUII) (75)	241	55	18	28	141	AU, AT, CN, DE, ES, GR, IE, IL, IT, KR, PK, PT, SG, TW, UK, US, ZA
WW3 (EUIII) (14)	23	1	0	1	21	ES, US, ZA
WW4 (11)	27	10	0	16	2	AR, CL, DE, HU, IN, PL, TR
WW5 (26)	80	50	20	10	5	AR, CO, DE, ES, MX, US, VE
WW6 (2)	12	0	0	4	8	HN, IT
WW7 (8)	13	8	0	5	0	AR, BG, CH, CO, MX, SG, VE
WW8 (4)	9	1	0	8	0	BG, ES, FR, TR
Unrelated (28)	43[a]	14	0	18	11	AR, AU, BG, BR, CL, CO, ES, IN, IT, MX, PL, SG, TW, US, VE
Total	492	160	38	109	193	

WW, worldwide; AR, Argentina; AT, Austria; AU, Australia; BG, Bulgaria; BR, Brazil; CH, Switzerland; CL, Chile; CN, China; CO, Columbia; DE, Germany; ES, Spain; FR, France; GR, Greece; HN, Honduras; HU, Hungary; IE, Ireland; IL, Israel; IN, India; IT, Italy; KR, South Korea; MX, Mexico; PK, Pakistan; PL, Poland; PR, Puerto Rico; PT, Portugal; SG, Singapore; TR, Turkey; TW, Taiwan; UK, United Kingdom; US, United States; VE, Venezuela; ZA, South Africa.

[a]Three $bla_{\text{OXA-143}}$ isolates.

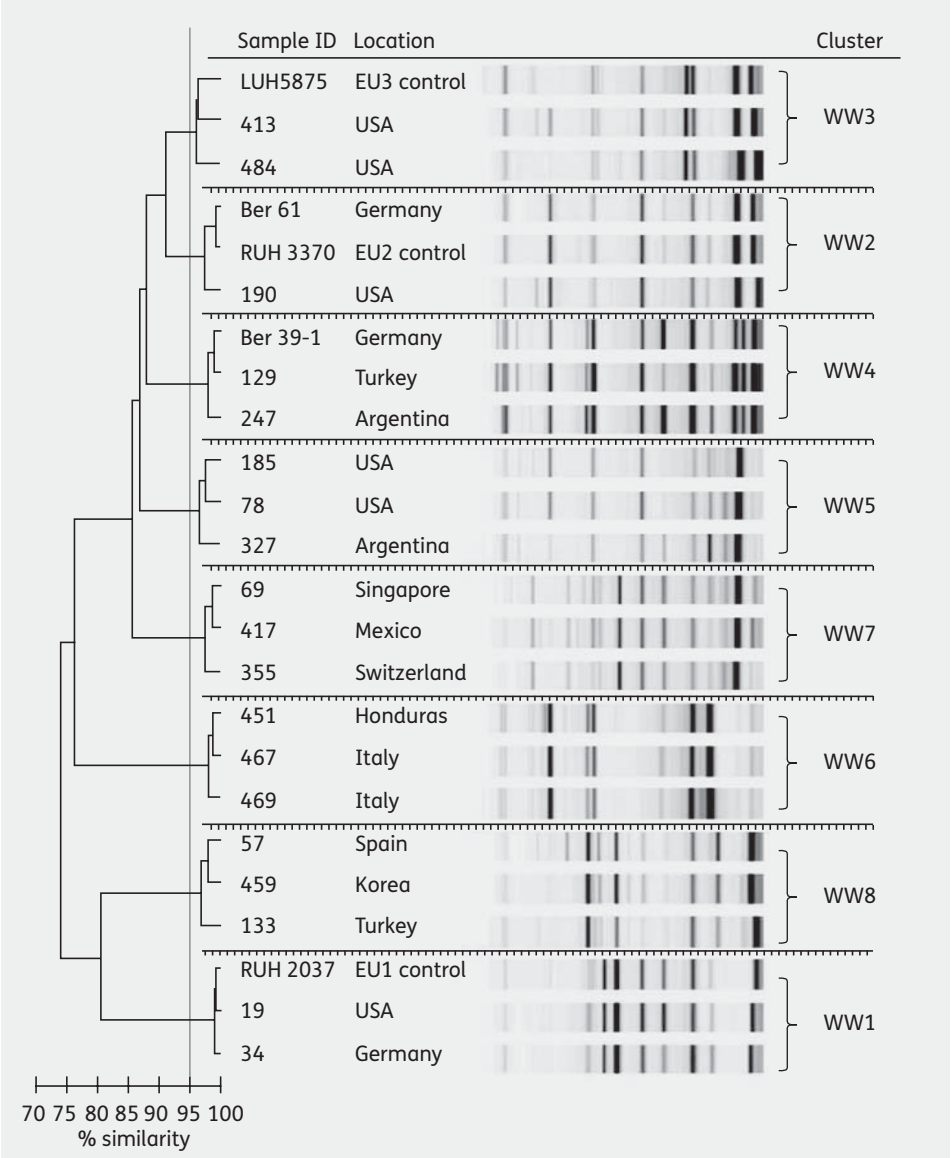

Figure 1. rep-PCR analysis. Dendrogram and computer-generated image of rep-PCR banding patterns showing representatives of the eight worldwide (WW) clusters and their country of isolation.

We found examples of identical rep-PCR patterns associated with isolates from different geographical locations harbouring $bla_{\text{OXA-23-like}}$ or $bla_{\text{OXA-58-like}}$ genes. Figure 1 shows isolates Ber 39-1 (Germany) and 129 (Turkey) sharing the same rep-PCR pattern with both harbouring $bla_{\text{OXA-58-like}}$, likewise isolates 417 (Mexico) and 355 (Switzerland). However, there were also identical rep-PCR patterns with different resistance genes; isolates 19 (USA) and 34 (Germany) harboured $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$, respectively.

Discussion

It is now some 13 years since the first description of European clonal lineages I and II,[11] and 16 years since the first acquired OXA was found.[26] The spread of acquired OXAs are well documented.[13,24,27,28] The incidence of imipenem resistance is rising, with figures from the Health Protection Agency UK website (http://www.hpa.org.uk) showing imipenem-resistant *A. baumannii* rising from 7% to 24% of bacteraemia isolates for the years 2003–07 while ciprofloxacin resistance rates remained stable at 30%.

The role of these European lineages in the spread of carbapenem resistance has only recently been addressed. Where studies have included defined control strains it has been shown that the majority of carbapenem-resistant *A. baumannii* from European countries belonged to EUI and EUII and harboured $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$ or $bla_{\text{OXA-58-like}}$ genes.[24,27–29] In the Czech Republic it is mainly EUII isolates associated with IS*AbaI*-$bla_{\text{OXA-51}}$ that were found in isolates for which the imipenem MIC was >16 mg/L.[30] Imipenem-resistant isolates recovered from American and British casualties repatriated from the Iraq conflict were

associated with OXA-23 and OXA-58, some of which clustered with EUI and EUII.[13,31] In the present study, we have described carbapenem-resistant *A. baumannii* isolated from 139 centres in 32 countries, with almost half of them (17 countries, 75 centres, 241 isolates) clustering with EUII control strains. Identification of European clonal lineages I–III was confirmed by sequence-type PCR.[23] All bar two carbapenem-resistant isolates not in possession of an acquired OXA had the IS*Aba1* element adjacent to $bla_{\text{OXA-51-like}}$. Expression of $bla_{\text{OXA-51-like}}$ has been shown to be higher in carbapenem-resistant isolates with IS*AbaI* upstream of $bla_{\text{OXA-51-like}}$.[32]

We found several examples of identical rep-PCR patterns associated with isolates from different continents harbouring $bla_{\text{OXA-23-like}}$ or $bla_{\text{OXA-58-like}}$ genes, suggesting clonal spread of a resistant organism. Resistance determinants were not associated with a particular cluster; rather each cluster contained more than one resistance gene type. This suggests that carbapenem resistance has developed after or during the spread of the clonal lineages.

In the present study we have used three molecular typing methods but have focused on rep-PCR for the main body of work. Rep-PCR was chosen for its ease of use, high throughput and discriminatory power that has been shown to be comparable to that of PFGE.[15] From our large global cohort, we found eight distinct clusters, three of which contain representative isolates belonging to European clonal lineages I, II and III with a marked predominance of EUII. In addition, based on rep-PCR and the sequence type by multiplex PCR, we described four new clonal clusters. Sequencing of the alleles *ompA*, *csuE* and $bla_{\text{OXA-51-like}}$ may further delineate the clusters, especially WW5 and WW8, which had similar profiles.[29] As these clusters contain isolates from multiple geographical locations, we feel it is prudent to refer to these as WW clonal lineages.

The origin of these clonal lineages is unknown. Our data suggest that it is likely that clusters have originated in at least eight distinct loci and then spread into new locations, possibly by transfer of patients.[28,33] Given that clonal spread is likely responsible for the majority of organisms presented in this study, *A. baumannii* infections may indicate a serious infection-control problem. The global dissemination of at least eight clonal lineages illustrates the success this organism has had in acquiring carbapenem resistance and represents a serious challenge to both patients and health professionals alike.

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Transparency declarations

No conflicts of interest to declare.

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