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Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program

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***Objectives*: The aim of this study was to evaluate the occurrence and dissemination of acquired carbapenem-hydrolysing class D β-lactamase (class D carbapenemase)- and metallo-β-lactamase (MBL)-encoding genes among *Acinetobacter* spp. isolates recovered from medical centres in the Asia-Pacific (APAC) region.**

***Methods*: During 2006–07, 41 medical centres located in 10 countries in the APAC region forwarded to a central monitoring site 544 *Acinetobacter* spp. isolates, which were tested for susceptibility by the reference broth microdilution method. Isolates non-susceptible to imipenem or meropenem (MIC $\geq$8 mg/L) were screened for OXA-23-, OXA-24/40-, OXA-58- and MBL-encoding genes and confirmed by sequencing. Clonality was assessed by ribotyping and PFGE.**

***Results*: Polymyxins (99.1% susceptible) and tigecycline (98.9% susceptible) were the most active antimicrobial agents tested. Among the isolates, 230 (42.3%) were non-susceptible to imipenem or meropenem, and class D carbapenemase- or MBL-encoding genes were detected in 162 (70.4%). *bla*$_{\text{OXA-23}}$ was found in isolates recovered from six countries, while *bla*$_{\text{OXA-24/40}}$ and *bla*$_{\text{OXA-58}}$ were less common. Several isolates harboured more than one class D carbapenemase, and MBL-encoding genes were detected in one *Acinetobacter johnsonii* from the Philippines (*bla*$_{\text{IMP-4}}$) and one *Acinetobacter baumannii* from Korea (*bla*$_{\text{VIM-2}}$). Overall, clonal dissemination was noted within medical centres; however, genetic relatedness was also noted among class D carbapenemase-producing *A. baumannii* isolates recovered from different countries.**

***Conclusions*: This study shows a high distribution of class D carbapenemase-encoding genes, mainly *bla*$_{\text{OXA-23}}$, in *Acinetobacter* spp. isolates. In addition, clonal dissemination among medical centres located in different countries in the APAC region, previously documented in many regions of Europe, emphasizes the epidemic potential of these bacteria.**

Keywords: class D carbapenemase, clonal dissemination, acquired, resistance

Introduction

Acinetobacter spp. are opportunistic pathogens, frequently associated with immunosuppressed patients as well as those with serious underlying diseases or subjected to invasive procedures and treated with broad-spectrum antimicrobial agents.[1] During the past decade, several reports have described the association of these pathogens with numerous outbreaks,[2] mainly in intensive care units. This corresponds with the emergence of resistance to nearly all clinically used classes of antimicrobial agents on a worldwide scale.[3] As such, carbapenems are important therapeutic options for treating infections caused by *Acinetobacter* spp.; however, these antimicrobial agents may be hydrolysed by Ambler class B metallo-β-lactamases (MBLs) as well as by carbapenem-hydrolysing class D β-lactamases (class D carbapenemase).[4]

Six groups of acquired MBL-encoding genes have been identified;[5] however, only *bla*$_{\text{IMP}}$- and *bla*$_{\text{VIM}}$-like, and more

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recently bla_{SIM-1}, have been reported in *Acinetobacter* spp.[4] IMP variants have been described in several Far Eastern countries, including Japan, China (Hong Kong), South Korea and Australia, while VIM variants have rarely been detected in those nations.[4] Conversely, acquired class D carbapenemase-encoding genes have been more frequently detected in *Acinetobacter* spp.[1] and are clustered in three major subfamilies, bla_{OXA-23}-, bla_{OXA-24}- and bla_{OXA-58}-types.[6]

Given the presence of these resistance determinants within mobile elements and the genetic potential of *Acinetobacter* spp. for acquiring foreign DNA from the environment,[1] intensive surveillance to assist infection control interventions has become very important to minimize the dissemination of resistant isolates and/or resistance genes. The objective of this surveillance study was to evaluate the occurrence and dissemination of acquired class D carbapenemase- and MBL-encoding genes among *Acinetobacter* spp. isolates recovered from medical centres in the Asia-Pacific (APAC) region.

Materials and methods

Bacterial isolates

During 2006–07, 41 medical centres located in 10 countries in the APAC region were recruited to participate in the SENTRY Antimicrobial Surveillance Program. A total of 544 isolates were consecutively collected from bloodstream, respiratory tract, skin and skin structure and urinary tract infections, according to defined protocols, from Republic of China (182 isolates), India (132), Indonesia (67), Thailand (48), Korea (47), Taiwan (24), Singapore (21), Australia (9), Hong Kong (8) and the Philippines (6). Only isolates responsible for documented infections were included in the study; one per patient episode. Species identification was performed by standard biochemical tests and by use of the Vitek system (bioMérieux, Hazelwood, MO, USA), when necessary. Additionally, species identification was confirmed by sequencing of the 16S ribosomal RNA gene on those isolates showing negative PCR results for $bla_{OXA-51/69}$-like.[6]

Antimicrobial susceptibility testing

All isolates were tested for susceptibility and the results were interpreted as described previously.[3] Tigecycline MIC results were interpreted according to the breakpoints for Enterobacteriaceae approved by the United States Food and Drug Administration (USA-FDA; breakpoint for susceptibility, $\leq$2 mg/L; breakpoint for resistance, $\geq$8 mg/L).[3] *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality control purposes; all results were within published ranges.

Class D carbapenemase- and MBL-encoding genes detection

Isolates non-susceptible to imipenem or meropenem (MIC, $\geq$8 mg/L) were screened using primers able to detect and distinguish alleles encoding three subfamilies of acquired class D carbapenemase (bla_{OXA-23}-like, $bla_{OXA-24/40}$-like and bla_{OXA-58}-like) and the intrinsic subgroup of $bla_{OXA-51/69}$-like (*Acinetobacter baumannii*) genes in a multiplex PCR assay format.[6] MBL screening was performed using generic primers able to detect VIM- and IMP-like, SPM-1-, GIM-1- and SIM-1-encoding genes in a multiplex real-time platform.[7] Positive controls for all screened genes were run simultaneously. Amplicons were sequenced on both strands. The nucleotide sequences and deduced amino acid sequences were analysed using the Lasergene software package (DNASTAR, Madison, WI, USA) and compared with the sequences available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

Molecular typing

Clinical isolates recovered within each medical centre that harboured carbapenemase-encoding genes were typed using the Riboprinter™ Microbial Characterization system (DuPont Qualicon, Wilmington, DE, USA). Isolates showing identical ribotypes were further characterized by PFGE, as described previously.[8] Gel pattern analysis was carried out using the GelCompar II software (Applied Math, Kortrijk, Belgium). Percentage similarities were identified on a dendrogram derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. Band position tolerance and optimization were set at 1.3% and 0.5%, respectively.[8] Isolates showing a similarity coefficient of $>$80% were considered as genetically related for this study. The PFGE pattern was determined based on the number of the medical centre followed by a capital letter (A, B, C). Isolates were assigned with the same PFGE pattern when the similarity coefficient was 100%. When this coefficient was in between 80% and 100%, the isolates were assigned as a subtype or variant of the major type, which was designated with the same capital letter followed by an Arabic number (e.g. C1, C2, C3).

Results

Bacterial isolates and antimicrobial susceptibility profile

A total of 544 *Acinetobacter* spp. isolates were recovered during 2006–07 from medical centres located in the APAC region. The vast majority were identified as *A. baumannii* (94.1%), followed by a lower prevalence of *Acinetobacter junii* (2.8%), *Acinetobacter lwoffii* (1.8%), *Acinetobacter haemolyticus* (0.4%), *Acinetobacter johnsonii* (0.2%), *Acinetobacter calcoaceticus* (0.2%) and *Acinetobacter radioresistens* (0.2%). Two isolates could not be identified to the species level (0.4%). Polymyxins (99.1% susceptible) and glycylcyclines (tigecycline; 98.9% of isolates showed MIC $\leq$2 mg/L) were the most active antimicrobial agents tested, followed by the carbapenems, imipenem and meropenem (52.0% and 51.3% susceptible, respectively). The remaining antimicrobials evaluated showed activity at $\leq$41.2%. Among the isolates, 230 (42.3%) were non-susceptible (MIC $\geq$8 mg/L) to imipenem or meropenem, and this resistance phenotype was most common in isolates recovered from Singapore (95.2%), Korea (87.0%), Taiwan (62.5%), Thailand (59.2%) and Hong Kong (50.0%).

Class D carbapenemase- and MBL-encoding genes

Among the carbapenem non-susceptible isolates, class D carbapenemase- and MBL-encoding genes were detected in 70.0% (160 isolates) and 0.8% of the isolates (two isolates), respectively. bla_{OXA-23} was the most common gene, which accounted for 95.0% of the class D carbapenemase-encoding genes detected, followed by a lower occurrence of bla_{OXA-58} (11.9%) and $bla_{OXA-24/40}$ (5.6%). bla_{OXA-23} was found in isolates distributed among six nations (Table 1), which included all nations participating in the SENTRY Program (APAC), except

Table 1. Molecular epidemiology data from class D carbapenemase-producing *Acinetobacter* spp. isolates at each medical centre

Enzyme	Bacterial isolate	No. of isolates	Medical centre	Clones[a] (no. of isolates)	Country
OXA-23	*A. baumannii*	4	204	A (4)	Hong Kong
		20	217	A (16), B (2), C (2)	Singapore
		6	218	A (5), B (1)	China
		14	224	A (10), B (1), C (1), D (1), E (1)	Korea
		9	225	A (2), B (4), C (3)	Korea
		15	226	A (1), B (4), C (1), D (3), E (1), F (1), G (1), H (1), I (1), J (1)	Thailand
		8	227	A (1), B (4), C (3)	Thailand
		9	231	A (6), A1 (1), A2 (1), A3 (1)	China
		1	238	A (1)	China
		7	243	A (6), B (1)	India
		28	248	A (2), B (2), C (1), D (1), E (1), F (1), G (8), G1 (1), H (2), I (1), K (1), L (7)	India
		4	252	A (1), B (2), C (1)	India
		9	246	A (9)	India
	A. lwoffii	1	246	ND[b] (1)	India
	A. junii	1	246	ND[b] (1)	India
OXA-24/40	*A. baumannii*	2	223	A (2)	Taiwan
		2	226	A (2)	Thailand
		1	242	A (1)	Indonesia
OXA-58	*A. baumannii*	2	232	A (2)	China
OXA-58	*A. calcoaceticus*	1	234	ND[b] (1)	China
OXA-23 and -58	*A. baumannii*	11	231	A (10), A1 (1)	China
OXA-133 and -58	*A. radioresistens*	1	251	ND[b] (1)	India
OXA-24/40 and -58	*A. baumannii*	1	226	A (1)	Thailand
OXA-23, -24/40 and -58	*A. baumannii*	3	226	A (3)	Thailand

[a]Number of clones carrying class D carbapenemase-encoding genes within each participating medical centre.
[b]ND, not determined; *Acinetobacter* species other than *baumannii*.

Australia and Indonesia; while $bla_{OXA-24/40}$ was detected only in isolates recovered from Taiwan, Thailand and Indonesia, and bla_{OXA-58} was detected in isolates recovered from China, India and Thailand. Several isolates harbouring more than one class D carbapenemase-encoding gene were found in China (bla_{OXA-23} plus bla_{OXA-58}) and Thailand ($bla_{OXA-24/40}$ plus bla_{OXA-58}, and bla_{OXA-23} plus $bla_{OXA-24/40}$ plus bla_{OXA-58}; Table 1), including an *A. radioresistens* clinical isolate recovered from India harbouring bla_{OXA-58} in addition to the intrinsic bla_{OXA-23}-like.[9] Sequencing results of this intrinsic gene revealed the presence of a novel variant, named $bla_{OXA-133}$ (accession no. EU571228). MBL-encoding genes were detected in one *A. johnsonii* recovered from the Philippines (bla_{IMP-4}) and one *A. baumannii* recovered from Korea (bla_{VIM-2}).

Molecular typing

Clonal dissemination of bla_{OXA-23}-carrying *A. baumannii* was noted in medical centres 204, 217, 218, 224, 231, 243 and 246; while bla_{OXA-23}-carrying *A. baumannii* from the remaining centres showed greater genetic diversity (Table 1). Among $bla_{OXA-24/40}$-carrying *A. baumannii* recovered from Taiwan and Thailand, dissemination of related and unrelated clinical isolates was noted, respectively. Additionally, clonal spread was observed among isolates showing multiple bla_{OXA} genes [bla_{OXA-23}- and bla_{OXA-58}- carrying *A. baumannii* recovered from China (site 231) and bla_{OXA-23}-, $bla_{OXA-24/40}$- and bla_{OXA-58}-carrying *A. baumannii* recovered from Thailand (site 226)].

Figure 1 shows a dendrogram containing representative isolates belonging to each dominant clone within each medical site and according to carbapenemase production. bla_{OXA-23}-carrying *A. baumannii* representative isolates belonging to clones ACB-252-B and ACB-248-G recovered from different medical sites in India were considered genetically related (similarity coefficient of 82.8%). Similar findings were noted with bla_{OXA-23}-carrying *A. baumannii* belonging to clones ACB-218-A and ACB-238-A recovered from two medical sites located in Beijing, China (similarity coefficient of 86.7%). $bla_{OXA-24/40}$-carrying *A. baumannii* belonging to clone ACB-223-A isolated from Taiwan clustered with bla_{OXA-23}-carrying *A. baumannii* belonging to clones ACB-225-B and ACB-217-A recovered from Korea and Singapore (similarity coefficient $\geq$85.7%; Figure 1), respectively, and these isolates also showed the same ribogroup. In addition, the representative isolate harbouring

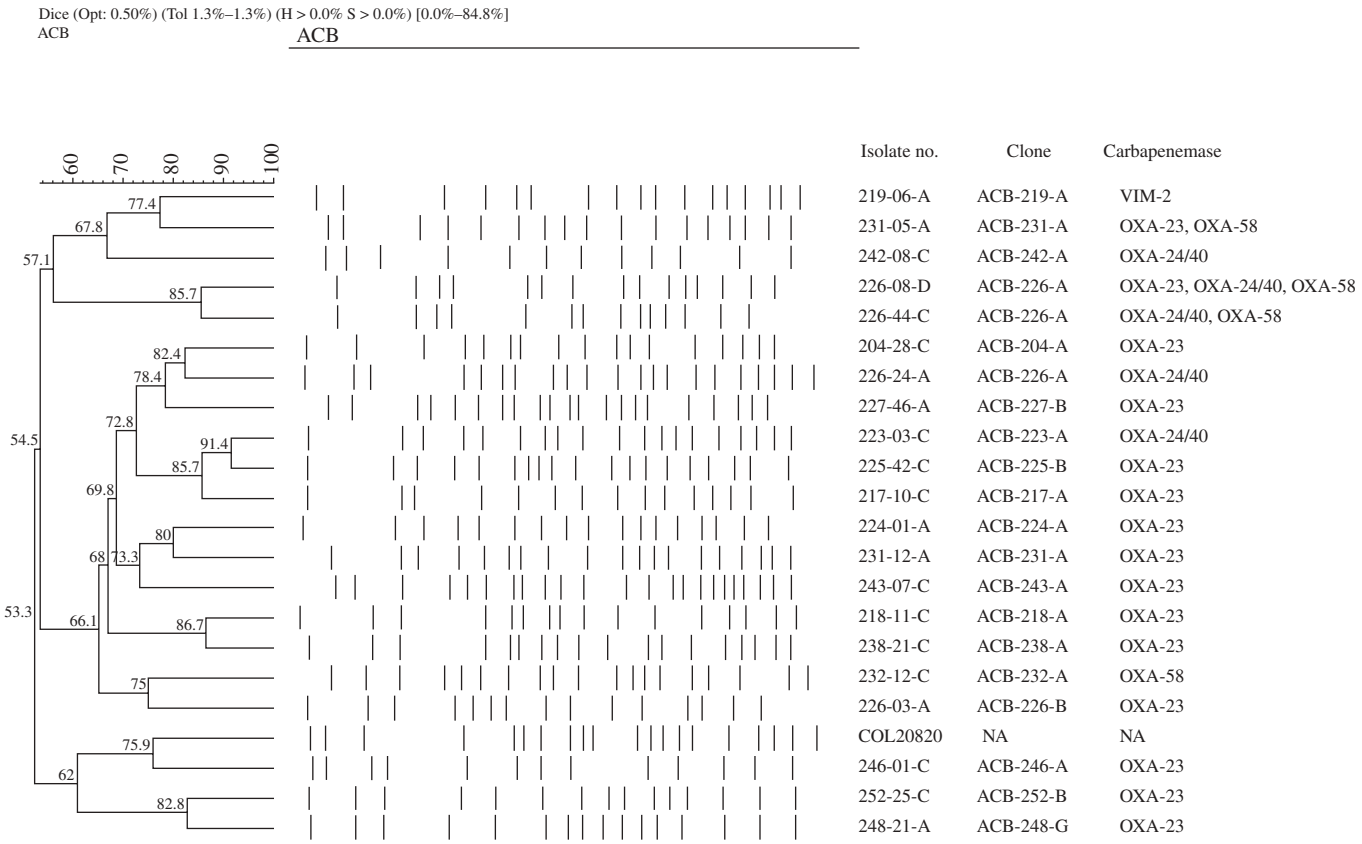

Figure 1. PFGE profile of *Apa*I-digested genomic DNA from representative isolates belonging to the dominant clones within each medical institution and according to carbapenemase production. Band position tolerance and optimization were set at 1.3% and 0.5%, respectively. Isolates showing a similarity coefficient of >80% were considered as genetically related for this study. Isolate number, clone designation and carbapenemase detected are shown, as well as the PFGE profile of *A. baumannii* COL 20820 strain for comparison purposes. NA, not applicable.

$bla_{\text{OXA-24/40}}$ from Thailand (226-24-A) clustered with the representative isolate harbouring $bla_{\text{OXA-23}}$ from Hong Kong (similarity coefficient of 82.4%; Figure 1). The isolate harbouring $bla_{\text{OXA-24/40}}$ and $bla_{\text{OXA-58}}$ from Thailand was clustered with OXA-23-, OXA-24/40- and OXA-58-producing *A. baumannii* recovered from the same medical site (ACB-226-A; Figure 1). Genetic relatedness was not found among other minor clones.

Discussion

Overall, this study showed low susceptibility rates to most of the clinically available antimicrobial agents for the treatment of infections caused by *Acinetobacter* spp., except for polymyxin B and the novel glycylcycline, tigecycline, which may represent valuable antimicrobial options for the treatment of infections caused by these isolates.[3] Among those isolates showing a resistant phenotype towards imipenem, 75.4% harboured at least one acquired class D carbapenemase-encoding gene, while among those isolates showing intermediate resistance towards this drug, only 11.1% harboured an acquired gene (data not shown). Therefore, the high prevalence of class D carbapenemase-encoding genes among the clinical isolates from the APAC region appears responsible for low susceptibility rates for imipenem [$P < 0.001$; OR = 0.04 (0.01–0.20)].

Recently, Zhou *et al.*[2] reported that 94.2% of imipenem-resistant *A. baumannii* isolates from China harboured $bla_{\text{OXA-23}}$, while Wang *et al.*[10] described that 97.7% and 4.1% of the imipenem-resistant *Acinetobacter* spp. isolates harboured $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58}}$, respectively. In this study, bla_{OXA}-carrying *Acinetobacter* spp. represented 68.2% (30 of 44) of imipenem-resistant isolates from China. Among these isolates, 90.0% (27 of 30) and 46.6% (14 of 30) harboured $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58}}$, respectively. However, among the isolates harbouring $bla_{\text{OXA-23}}$, 40.7% (11 of 27) also possessed $bla_{\text{OXA-58}}$, suggesting that the epidemiology of class D carbapenemase-encoding genes has significantly changed in *Acinetobacter* spp. clinical isolates recovered from China.

Two clusters (ACB-252-B and ACB-248-G) considered genetically related (similarity coefficient of 82.8%) were noted in two different cities in India. However, clonal dissemination within each medical centre was predominantly observed, suggesting that the isolates from this study mostly belonged to local clones causing sporadic or endemic outbreaks within each medical institution. Previously published reports have shown clonal dissemination of OXA-23-producing *A. baumannii* among several medical centres located in different cities in China,[2,10] which contrasts with our findings. In this study, only the clones ACB-218-A and ACB-238-A, considered genetically related, were noted in different institutions from China (similarity coefficient of 86.7%); however, these medical centres were located in the same city (Beijing). These contrasting results may have been due to the fact that the carbapenem-resistant isolates from this study were recovered from a smaller number of medical centres

and cities other than those included in those previous studies.[2,10] Interestingly, genetically related isolates were observed among medical centres located in different countries [Taiwan, Singapore and Korea (similarity coefficient $\geq$85.7%), and Hong Kong and Thailand (similarity coefficient of 82.4%)], suggesting these isolates derived from a common ancestor. Furthermore, these clones may have been spreading in this region as previously documented for many regions in Europe, where the European clones I and II have become epidemic since 1980.[11]

bla$_{IMP-4}$ has been previously detected in *Acinetobacter* spp. and *Citrobacter youngae* from China, and subsequently, *bla*$_{IMP-4}$-carrying *P. aeruginosa* emerged and this resistance gene rapidly disseminated among several Gram-negative pathogens in an Australian hospital setting.[5] Although the prevalence of MBL genes was very low in our study, the detection of a *bla*$_{IMP-4}$- carrying *A. johnsonii* recovered from a patient in the Philippines underscores the continued spread of this gene in the geographic region. This highlights the importance of active hospital surveillance to avoid possible infection outbreaks by such organisms.[5] Furthermore, there have been only a few detections of VIM variants in *Acinetobacter* spp. in the APAC region, predominantly represented by the occurrence of *bla*$_{VIM-2}$ in South Korea,[4] and our study highlights the continued detection of this determinant in that country, albeit at low frequency.

The presence of multiple class D carbapenemase-encoding genes in single isolates was noted in China, India and Thailand (Table 1). Additionally, the detection of an *A. radioresistens* clinical isolate carrying the intrinsic *bla*$_{OXA-23}$-like and *bla*$_{OXA-58}$ emphasizes (i) the natural occurrence of class D carbapenemase-encoding genes among *Acinetobacter* species,[4,9] (ii) the ability for acquiring and/or exchanging foreign DNA,[1] and (iii) the diversity of resistance genes in the hospital environment. These findings also provide strong evidence that the hospital environment has become the main reservoir of these resistance determinants,[9] which may facilitate DNA exchange among endemic nosocomial pathogens.

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References

1. Perez F, Hujer AM, Hujer KM *et al.* Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2007; **51**: 3471–84.

2. Zhou H, Yang Q, Yu YS *et al.* Clonal spread of imipenem-resistant *Acinetobacter baumannii* among different cities of China. *J Clin Microbiol* 2007; **45**: 4054–7.

3. Mendes RE, Fritsche TR, Sader HS *et al.* Increased antimicrobial susceptibility profile among polymyxin-resistant *Acinetobacter baumannii* clinical isolates. *Clin Infect Dis* 2008; **46**: 1324–6.

4. Poirel L, Pitout JD, Nordmann P. Carbapenemases: molecular diversity and clinical consequences. *Future Microbiol* 2007; **2**: 501–12.

5. Rossolini GM. Acquired metallo-β-lactamases: an increasing clinical threat. *Clin Infect Dis* 2005; **41**: 1557–8.

6. Woodford N, Ellington MJ, Coelho JM *et al.* Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006; **27**: 351–3.

7. Mendes RE, Kiyota KA, Monteiro J *et al.* Rapid detection and identification of metallo-β-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. *J Clin Microbiol* 2007; **45**: 544–7.

8. Seifert H, Dolzani L, Bressan R *et al.* Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; **43**: 4328–35.

9. Poirel L, Figueiredo S, Cattoir V *et al.* *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. *Antimicrob Agents Chemother* 2008; **52**: 1252–6.

10. Wang H, Guo P, Sun H *et al.* Molecular epidemiology of clinical isolates of carbapenem-resistant *Acinetobacter* spp. from Chinese hospitals. *Antimicrob Agents Chemother* 2007; **51**: 4022–8.

11. van Dessel H, Dijkshoorn L, van der Reijden T *et al.* Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. *Res Microbiol* 2004; **155**: 105–12.