function. Sequence analysis was conducted using the ORF Finder and the BLASTp functions at NCBI. For each isolate, multiresistance gene cluster-carrying contigs ranging in size between 9613 bp and 9690 bp were obtained. They comprised the region from the *aadE* gene to downstream of *lnu*(B). A comparison with the 17577 bp sequence of the cluster located on the multiresistance plasmid pV7037 (GenBank accession number JX560992) revealed a single nucleotide exchange (A→C) in the MRSA CC398 isolate at position 9474 in the non-coding region between *spw* and *orf4*. An additional nucleotide exchange (A→G), which, however, did not change the amino acid sequence, was seen in all 12 isolates at position 10071 within *orf4*. All CC9 isolates carried a transposase gene downstream of the gene *lnu*(B). The CC398 isolate exhibited at this position two genes coding for a resolvase and a nucleotidyltransferase with greatest amino acid identities of 99.5% and 100%, respectively, with the corresponding proteins from *Enterococcus faecium* Aus0085 plasmid p3 (GenBank accession number NC_021988.1). In all cases, the repeatedly negative results of conjugation, protoplast transformation and electrotransformation experiments suggested that the cluster was located in the chromosomal DNA, as previously seen for the majority of the Chinese porcine MRSA CC9 isolates and the Spanish MRSA ST398 and MSSA ST9 isolates of human origin.[1,2]

In conclusion, a large part of the pV7037-associated multiresistance gene cluster, including the novel resistance genes *lsa*(E) and *spw*, was identified in MRSA CC9 isolated from different food samples and a butcher in Hong Kong as well as from a single MRSA CC398 of human origin in Germany. These and previous observations suggest that a multiresistance gene cluster, closely related to the one that has recently been detected in enterococci of human and porcine origin,[4] has been acquired by MRSA/MSSA CC9 and CC398 several times on different occasions in Europe and Asia.

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Transparency declarations

S. M. and R. E. are employees of Alere Technologies GmbH, the company that manufactures the microarrays used for this study. S. M. and R. E. do not own stock or options in the company. All other authors: none to declare.

References

1 Lozano C, Aspiroz C, Saenz Y *et al*. Genetic environment and location of the *lnu*(A) and *lnu*(B) genes in methicillin-resistant *Staphylococcus aureus* and other staphylococci of animal and human origin. *J Antimicrob Chemother* 2012; **67**: 2804–8.

2 Li B, Wendlandt S, Yao J *et al*. Detection and new genetic environment of the pleuromutilin–lincosamide–streptogramin A resistance gene *lsa*(E) in methicillin-resistant *Staphylococcus aureus* of swine origin. *J Antimicrob Chemother* 2013; **68**: 1251–5.

3 Wendlandt S, Li B, Ma Z *et al*. Complete sequence of the multi-resistance plasmid pV7037 from a porcine methicillin-resistant *Staphylococcus aureus*. *Vet Microbiol* 2013; **166**: 650–4.

4 Li X-S, Dong W-C, Wang X-M *et al*. Presence and genetic environment of pleuromutilin–lincosamide–streptogramin A resistance gene *lsa*(E) in enterococci of human and swine origin. *J Antimicrob Chemother* 2014; **69**: 1424–6.

5 Wendlandt S, Lozano C, Kadlec K *et al*. The enterococcal ABC transporter gene *lsa*(E) confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2013; **68**: 473–5.

6 Wendlandt S, Li B, Lozano C *et al*. Identification of the novel spectinomycin resistance gene *spw* in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* of human and animal origin. *J Antimicrob Chemother* 2013; **68**: 1679–80.

7 Wendlandt S, Kadlec K, Feßler AT *et al*. Resistance phenotypes and genotypes of methicillin-resistant *Staphylococcus aureus* isolates from broiler chickens at slaughter and abattoir workers. *J Antimicrob Chemother* 2013; **68**: 2458–63.

8 Monecke S, Coombs G, Shore AC *et al*. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS One* 2011; **6**: e17936.

9 Schwarz S, Silley P, Simjee S *et al*. Assessing the antimicrobial susceptibility of bacteria obtained from animals. *J Antimicrob Chemother* 2010; **65**: 601–4.

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Emergence of genetically unrelated NDM-1-producing *Acinetobacter pittii* strains in Paraguay

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Sir,

The New Delhi metallo-β-lactamase (NDM-1) was initially identified in *Escherichia coli* and *Klebsiella pneumoniae* isolates in Sweden, from a patient previously hospitalized in India.[1] To date, NDM producers in Latin America have been scarce, and associated with species of Enterobacteriaceae from Guatemala, Mexico, Colombia and Brazil, although in Honduras it was reported in *Acinetobacter baumannii*.[2–6] Here, we report two genetically unrelated NDM-1-producing *Acinetobacter pittii* isolates identified in Paraguay.

Since 1996, the Pan American Health Organization (PAHO) has supported a regional surveillance system, the Antimicrobial Resistance Surveillance Network in Latin America (ReLAVRA), that includes 794 laboratories from 20 Latin American countries, including their respective reference laboratories.[7] This network provides reliable, timely and reproducible microbiological data in order to improve patient care. A regional protocol for the detection of carbapenemases has been harmonized and implemented through ReLAVRA. Briefly, metallo-β-lactamase (MBL) production is suspected in isolates that exhibit decreased susceptibility to carbapenems (CLSI criteria) and a positive synergy test result between a disc containing 10 μg of imipenem and a disc containing 750 μg of EDTA plus 1900 μg of sodium thioglycolate.[8]

During 2012, following the ReLAVRA algorithm, the National Health Laboratory of Paraguay confirmed an MBL phenotype in two *Acinetobacter* spp. isolates recovered from a single hospital. This phenotype had not previously been observed in *Acinetobacter* spp. from Paraguay. The first case was a 7-year-old patient admitted in July because of acute encephalitis. After 2 months of hospitalization, an *Enterobacter cloacae* extended-spectrum β-lactamase producer and *Acinetobacter* M15274 were recovered from the CSF obtained through a ventricular shunt. The patient received multiple treatment regimens, including trimethoprim/sulfamethoxazole, ciprofloxacin and amikacin, which resulted in clinical and microbiological cure. The patient died after 4 months of hospitalization due to non-infectious causes. The second case was a 2-year-old patient with a diagnosis of acute lymphocytic leukaemia who was admitted in November. Two weeks later the patient developed sepsis, and *Acinetobacter* M15373 was isolated from a blood culture. The patient showed clinical improvement after treatment with meropenem plus amikacin and was discharged alive after 50 days. Both patients were hospitalized in the same oncology ward but 4 months apart from each other. Remarkably, the patients had no history of travelling.

Table 1. Antimicrobial susceptibility (MICs in mg/L)[a] of NDM-producing *A. pittii* clinical isolates and *A. baumannii* and *E. coli* transconjugant and recipient strains

	Clinical isolates		Transconjugant strains[b]		Recipient strains	
	A. pittii M15274	*A. pittii* M15373	*A. baumannii* M17176	*E. coli* M15694	*A. baumannii* ATCC 19606	*E. coli* J53
Imipenem[c]	>256	>256	>256	1	0.25	0.06
Imipenem/EDTA[d]	0.12	0.12	0.25	0.12	0.25	0.06
Meropenem[c]	>256	>256	>256	0.5	1	0.015
Meropenem/EDTA[d]	0.12	0.25	0.5	0.015	1	0.015
Ertapenem[c]	>256	>256	>256	2	1	0.25
Ampicillin/sulbactam	>16	4	>16	>16	≤1	≤1
Piperacillin/tazobactam	>64	>64	>64	>64	8	≤4
Aztreonam[c]	32	32	32	≤0.03	32	≤0.03
Cefotaxime[c]	>256	>256	>256	32	16	≤0.015
Ceftazidime[c]	>256	>256	>256	>256	8	≤0.06
Cefepime[c]	>256	>256	>256	16	16	0.015
Cefoxitin	>32	>32	>32	>32	≤8	≤8
Gentamicin	≤1	≤1	8	≤1	8	≤1
Amikacin	≤2	≤2	≤2	≤2	≤2	≤2
Nalidixic acid	16	≤2	4	≤2	4	≤2
Ciprofloxacin	1	≤0.25	1	≤0.25	1	≤0.25
Trimethoprim/sulfamethoxazole	≤2	≤2	>256	≤2	>256	≤2
Nitrofurantoin	>256	>256	>256	≤16	>256	≤16
Colistin	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Tigecycline[c]	0.06	0.06	0.25	0.25	0.015	0.015

[a]Antimicrobial susceptibility testing according to CLSI standards.
[b]Transconjugant strains of *A. pittii* M15373.
[c]MICs were determined using agar dilution; MICs of other antibiotics were determined using the Vitek 2C (AST-N082 card).
[d]EDTA at a fixed concentration of 0.4 mM. The bla_{VIM-11}-producing *Pseudomonas aeruginosa* M5109 was used for quality control purposes.

Strains were submitted to the Regional Reference Laboratory (Servicio Antimicrobianos, INEI, ANLIS 'Dr Carlos G. Malbrán') for further characterization. Strains were identified as *A. pittii* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF; Bruker, Germany); the 10 most probable database matches were all consistent with *A. pittii*. Antimicrobial susceptibility testing revealed an identical resistance profile in both *A. pittii* isolates, except for ampicillin/sulbactam and quinolones (Table 1). EDTA reduced the carbapenem MICs by at least three dilutions in both strains, suggesting the presence of MBLs (Table 1). The modified Hodge test gave negative results with meropenem but was positive (weakly) with imipenem.

In both isolates, PCR screening followed by DNA sequencing detected the presence of bla_{NDM-1}. PCRs targeting other β-lactamase genes (bla_{VIM}, bla_{IMP}, bla_{SPM}, bla_{KPC}, bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, $bla_{OXA-143}$, bla_{CTX-M}, bla_{SHV} and bla_{TEM}) were negative. Sequencing of a 2.5 kb fragment surrounding bla_{NDM-1} from both strains revealed 100% identity to the sequence reported for *Acinetobacter lwoffii*, where IS*Aba125* was located upstream of bla_{NDM-1}, followed by Δ*trpF* and *tat*.[9,10] The genes that follow to the 3′ end, Δ*groES*, *groEL*, IS*CR27* and the second IS*Aba25* were PCR mapped, revealing a Tn*125* composite transposon as previously reported in *A. pittii*.[11]

ApaI PFGE studies revealed that the *A. pittii* isolates were not clonally related (>10 bands of difference in the macrorestriction pattern). Multilocus sequence typing (MLST) was performed according to the MLST Database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). M15274 and M15373 displayed unique and novel sequence types (STs), designated ST320 and ST321, respectively. M15274 and M15373 shared only the *rplB* allele, confirming that they were genetically unrelated. Furthermore, these STs branched with other reported isolates belonging to the *A. pittii* genomic species, confirming the MALDI-TOF results.

The bla_{NDM-1} gene was transferred by biparental conjugation to either sodium azide-resistant *E. coli* J53 or *A. baumannii* ATCC 19606 from M15373 but not from M15274, conferring non-susceptibility to carbapenems on the recipient strain (Table 1). S1 nuclease digestion showed that both *A. pittii* and the transconjugant strains harboured a single plasmid of ∼54 kb that hybridized with the bla_{NDM} probe. Plasmids gave negative results for all the Inc groups when assessed by PCR replicon typing.[12]

Additionally, further characterization of 23 contemporary carbapenem-resistant *Acinetobacter* spp. isolated in the same institution from November 2012 to March 2013 (clinical strains and patient swab samples) revealed a lack of MBL production and matched *A. baumannii* by MALDI-TOF.

A. pittii has recently been recognized as a key organism for the dissemination of NDM, since it has been associated with the dispersal of this carbapenemase in such diverse scenarios as food of animal origin and sewage, and has been responsible for both sporadic human infection and large outbreaks in hospital units.[10,13,14] Until now, non-*baumannii Acinetobacter* spp. expressing NDM have not been described in the American continent. These *A. pittii* clinical isolates are the first characterized NDM-1 producers from Paraguay. The origin of NDM-1-positive *A. pittii* in Paraguay remains unclear, since no history of travel to the suggested reservoirs of NDM was established for either patient. Our finding of bla_{NDM-1} on plasmids of identical 54 kb size in *A. pittii* strains with a heterogeneous clonal background suggests that bla_{NDM-1} most likely spread by the transfer of plasmids in *A. pittii*. Recent published data suggest that *A. pittii* could act as a potential NDM reservoir for Enterobacteriaceae based on the ease of plasmid transfer to *E. coli*,[11] as observed in one of the isolates reported here. Despite this ability, no further cases of NDM producers have so far been observed in the hospital.

In conclusion, the emergence of NDM in *A. pittii* constitutes a public health concern in Latin America, highlighting the relevance of an integrated surveillance of carbapenemase producers.

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Transparency declarations

None to declare.

References

1 Yong D, Toleman MA, Giske CG *et al*. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother* 2009; **53**: 5046–54.

2 Pasteran F, Albornoz E, Faccone D *et al*. Emergence of NDM-1-producing *Klebsiella pneumoniae* in Guatemala. *J Antimicrob Chemother* 2012; **67**: 1795–7.

3 Barrios H, Garza-Ramos U, Reyna-Flores F *et al*. Isolation of carbapenem-resistant NDM-1-positive *Providencia rettgeri* in Mexico. *J Antimicrob Chemother* 2013; **68**: 1934–6.

4 Escobar Pérez JA, Olarte Escobar NM, Castro-Cardozo B *et al*. Outbreak of NDM-1-producing *Klebsiella pneumoniae* in a neonatal unit in Colombia. *Antimicrob Agents Chemother* 2013; **57**: 1957–60.

5 Waterman PE, McGann P, Snesrud E *et al*. Bacterial peritonitis due to *Acinetobacter baumannii* sequence type 25 with plasmid-borne New Delhi metallo-β-lactamase in Honduras. *Antimicrob Agents Chemother* 2013; **57**: 4584–6.

6 D'Alincourt Carvalho-Assef A, Pereira1 P, Mattos Albano R *et al*. Isolation of NDM-producing *Providencia rettgeri* in Brazil. *J Antimicrob Chemother* 2013; **68**: 2956–7.

7 Schmunis G, Salvatierra-Gonzalez S. Birth of a public surveillance system: PAHO combats the spread of antimicrobial resistance in Latin America. *The APUA Newsletter* 2006; **24**: 6–8.

8 Lee K, Lim Y, Yong D *et al*. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; **41**: 4623–9.

9 Hu H, Hu Y, Pan Y *et al*. Novel plasmid and its variant harboring both a bla_{NDM-1} gene and type IV secretion system in clinical isolates of *Acinetobacter lwoffii*. *Antimicrob Agents Chemother* 2012; **56**: 1698–702.

10 Fu Y, Du X, Ji J *et al*. Epidemiological characteristics and genetic structure of bla_{NDM-1} in non-*baumannii Acinetobacter* spp. in China. *J Antimicrob Chemother* 2012; **67**: 2114–22.

11 Bogaerts P, Huang T, Rezende de Castro R *et al*. Could *Acinetobacter pittii* act as an NDM-1 reservoir for Enterobacteriaceae? *J Antimicrob Chemother* 2013; **68**: 2414–5.

12 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

13 Yang J, Chen Y, Jia X *et al*. Dissemination and characterization of NDM-1-producing *Acinetobacter pittii* in an intensive care unit in China. *Clin Microbiol Infect* 2012; **18**: E506–13.

14 Zhang R, Hu YY, Yang XF *et al*. Emergence of NDM-producing non-*baumannii Acinetobacter* spp. isolated from China. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 853–60.

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Detection of pan drug-resistant *Acinetobacter baumannii* in Germany

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Sir,

In December 2013, a carbapenem-resistant *Acinetobacter baumannii* was recovered from routine skin and rectum swabs from a patient during admission screening. The patient had been on a repatriation transport to Australia after previous hospitalization in Greece due to Waterhouse–Friderichsen syndrome as a result of severe meningococcal septicaemia. At the stop-over in Germany he presented with a decreased level of consciousness, multiple decubitus ulcers (grade IV) and reduced general condition and was admitted to the Internal Medicine Intensive Care Unit at the Hospital of the Johann Wolfgang von Goethe University in Frankfurt. Infection parameters were elevated, although a definite infection focus could not be established. Empirical anti-infective therapy with colistin, meropenem, linezolid, fosfomycin and caspofungin was initiated. The patient recovered after 1 week and could be transferred.

Susceptibility testing of the *A. baumannii* isolate using the Etest method revealed non-susceptibility to all antibiotic groups where breakpoints have been defined for *Acinetobacter* spp. by either EUCAST or CLSI (penicillins/β-lactamase inhibitors, extended-spectrum cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, tetracyclines, folate pathway inhibitors and polymyxins), including colistin, with an MIC of 128 mg/L (Table 1).[1,2] The *A. baumannii* isolate was thus categorized as pan drug-resistant (PDR) in accordance with the guidelines defined by the international expert committee of the European Centre for Disease Prevention and Control (ECDC) and CDC.[3] First reports of PDR *A. baumannii* emerged in 2001 and a nosocomial outbreak with PDR *A. baumannii* in 2002 was reported from Spain.[4,5] However, in many other studies the term PDR was used inappropriately since important antibiotics such as colistin and

Table 1. Antibiotic susceptibility of *A. baumannii* isolated from a colonized patient admitted at the Hospital of the Johann Wolfgang von Goethe University in Frankfurt, Germany, in December 2013

Antimicrobial category	Antimicrobial agent	MIC (mg/L)	Susceptibility[a]
Penicillins+β-lactamase inhibitors	ampicillin/sulbactam[b]	>256	R
	piperacillin/tazobactam[b]	>256	R
	ticarcillin/clavulanic acid[b]	>256	R
Extended-spectrum cephalosporins	cefotaxime	>32	R
	ceftriaxone	>32	R
	ceftazidime	>256	R
	cefepime	>256	R
Carbapenems	imipenem	>32	R
	meropenem	>32	R
	doripenem	>32	R
Fluoroquinolones	ciprofloxacin	>32	R
	levofloxacin	>32	R
Aminoglycosides	gentamicin	>256	R
	amikacin	>256	R
	tobramycin	>256	R
	netilmicin	>256	R
Tetracyclines	tigecycline	8	R
	tetracycline	>256	R
	doxycycline	128	R
	minocycline	8	I
Folate pathway inhibitors	trimethoprim/sulfamethoxazole	>32	R
Polymyxins	polymyxin B	16	R
	colistin	128	R
Others	chloramphenicol	>256	R
	fosfomycin	>256	R

R, resistant; I, intermediately susceptible.
[a]MICs were interpreted according to breakpoints for *Acinetobacter* spp. set by EUCAST and CLSI.[1,2] MICs of tigecycline, chloramphenicol and fosfomycin were interpreted according to breakpoints for Enterobacteriaceae set by EUCAST.
[b]β-Lactamase inhibitors sulbactam, tazobactam and clavulanic acid were used at a fixed concentration of 4, 4 and 2 mg/L, respectively, as recommended by EUCAST.