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Acinetobacter baumannii is Able to Gain and Maintain a Plasmid Harbouring In35 Found in *Enterobacteriaceae* Isolates From Argentina

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Abstract The aim of this study was to determine the presence of $bla_{\text{CTX-M-2}}$ in our *A. baumannii* population and their putative role as an alternative mechanism of resistance to third-generation cephalosporins in this species. The $bla_{\text{CTX-M-2}}$ gene is widespread among the *Enterobacteriaceae* isolates from our country; however, it was not found in 76 isolates *A. baumannii* non-epidemiologically related clinical isolates resistant to third-generation cephalosporins isolated since 1982 in hospitals from Buenos Aires City. A plasmid isolated from *Proteus mirabilis* that possesses the complex class 1 integron In35::IS*CR1*::$bla_{\text{CTX-M-2}}$ was used to transform the natural competent *A. baumannii* clinical strain A118. PCR, plasmid extraction, DNA restriction, and susceptibility test confirmed that A118 could gain and maintain the plasmid possessing In35::IS*CR1*::$bla_{\text{CTX-M-2}}$, the genetic platform where the $bla_{\text{CTX-M-2}}$ gene is dispersing in Argentina.

Introduction

Acinetobacter baumannii is considered an important pathogen in our hospital environment, and is well known for its capacity to acquire different mechanisms of antibiotic resistance by horizontal transfer of genes [6, 10]. ß-lactam antibiotics, usually carbapenems, are the most used antibiotics in the treatment of *A. baumannii* infections worldwide [5, 6, 10]. Among the third-generation cephalosporins, cefotaxime (CTX) can exhibit susceptibility in some strains in vitro [11], but it is considered not active in vivo for the treatment of *A. baumannii* infections.

We have previously shown the spreading of the complex class 1 integron In35::IS*CR1*::$bla_{\text{CTX-M-2}}$ among the multidrug-resistant *Enterobacteriaceae* isolates from Argentina [1]. It was also shown that this structure is usually localized in plasmids [1, 4, 14], explaining at least one of the reasons of the widespread distribution of the extended spectrum ß-lactamase $bla_{\text{CTX-M-2}}$, conferring resistance to CTX, ceftazidime (CAZ), and cefepime (FEP) [1, 4]. Until now, there are few reports describing the nosocomial transmission of $bla_{\text{CTX-M-2}}$ in *A. baumannii* isolates, one in Japan and the other from Bolivian hospitals [2, 8].

In this study, we investigated the role of $bla_{\text{CTX-M-2}}$ as another molecular mechanism of resistance to expanded-spectrum cephalosporins and its putative acquisition by *A. baumannii* clinical isolates from our country.

Materials and Methods

Bacterial Strains and Plasmids

Seventy-six *A. baumannii* non-epidemiologically related clinical isolates collected since 1982 from Buenos Aires City hospitals and resistant to CTX, CAZ, and/or FEP were included. The natural competent *A. baumannii* strain A118, which is a clinical strain [13], susceptible to CTX, CAZ, and FEP, was used in the transformation assays.

The pDCMSR1 plasmid extracted from the strain *Proteus mirabilis* Prm9 isolated in 1994 in a hospital of Buenos Aires City was used in the transformation assay. This plasmid was analyzed by PCR reactions, restriction DNA (*Hin*dIII, *Xba*I and *Bam*HI), and conjugation mating

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studies, showing that the pDCMSR1 is a 25 Kb mobilizable plasmid.

Transformation Assays, Plasmid Extraction and Stability

Natural competency assays were carried out adding 100 ng of plasmid DNA pDCMSR1 to a mix containing 50 µl of fresh LB broth and 50 µl of culture in stationary phase and incubated during 1 h at 37°C. Then, the mix was plated on LB agar plates containing 12 mg/l of CTX. Colonies were subcultured in liquid medium and plasmid DNA was extracted using the QIAfilter midi kit (QIAGEN). Stability of the transforming plasmids and confirmation of their extrachromosomal nature in colonies that conserved the resistance was determined as described by Tolmasky et al. 2000 [13, 15].

Antimicrobial Susceptibility Tests

Minimal inhibitory concentration (MICs) of ß-lactam antibiotics, CTX, CAZ, and FEP, were determined using the broth microdilution method, as recommended by the CLSI [3].

DNA Techniques

PCR reaction for *intI1*, $bla_{\text{CTX-M-2}}$, and IS*CR1* genes were performed following the manufacturer's instructions (Promega, Madison, WI). Integron PCR cartography was also done as previously described [12]. Sequencing of amplicons with ABI Prism 3100 BioAnalyzer equipment and sequence analysis with Blast V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/) were performed [9, 12].

Results and Discussion

The *intI1* gene was present in seventeen (22%) *A. baumannii* isolates, while $bla_{\text{CTX-M-2}}$ and IS*CR1* genes were not found among the 76 *A. baumannii* isolates analyzed.

To discern if the results exposed above are the consequence of the inability of *A. baumannii* to acquire plasmids harboring $bla_{\text{CTX-M-2}}$, we used the pDCMSR1 plasmid, which carries a complex class 1 integron In35::IS*CR1*::-$bla_{\text{CTXM-2}}$ to transform the natural competent *A. baumannii* A118 strain [13]. This plasmid was previously found widespread in several *P. mirabilis* clones isolated during 1994 to 1996 in several hospitals from Buenos Aires City (Data not shown).

The stability assays showed that the plasmid is stable maintained after 40 generations in A118. The extrachromosomal nature of the plasmid was also verified by plasmid extraction. The obtained MICs results (Table 1) showed the expression of the $bla_{\text{CTX-M-2}}$ gene in the Ab118::pDCMSR1 strain, which is in concordance with a phenotype of resistance to CTX in *A. baumannii* (Table 1) [3]. In addition, the acquisition of pDCMSR1 affects the susceptibility to other ß-lactams showing an increase in the MIC to CAZ and FEP of 3 dilutions comparing to the isolates without plasmid (Table 1).

Table 1 Minimal inhibitory concentration (MIC) to CTX, CAZ, and FEP of the different strains used in the study

Strain	Source	MIC (mg/l) CTX	CAZ	FEP
A118	Blood culture	8	1.5	2
A118pDCMSR1	This work	32	4	16
Pr9	Urine	128	0.24	8
Ec J53pDCMSR1	This work	>16	4	8

A: *Acinetobacter baumannii*, Pr: *Proteus*, Ec: *Escherichia coli*

The minimal inhibitory concentration (MIC) to cefotaxime (CTX), ceftazidime (CAZ) and cefepime (FEP) were performed following CLSI recommendations

Acinetobacter baumannii and several *Enterobacteriaceae* species can share the same habitats, such as the respiratory tract of nosocomial patients and they can also survive in air, environment, and staff-hands [6, 7]. However, the widespread In35::IS*CR1*::$bla_{\text{CTX-M-2}}$ plasmid-harboured from our country since 1989 was not found in any isolate of our *A. baumannii* population.

Our findings exposed that *A. baumannii* can easily acquire and maintain a large multidrug resistance plasmid circulating in the *Enterobacteriaceae* nosocomial environment, the pDCMSR1, sustaining its faculty to gain different mechanisms of antibiotic resistance and gene capture.

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References

1. Arduino SM, Catalano M, Orman BE, Roy PH, Centrón D (2003) Molecular epidemiology of orf513-bearing class 1 integrons in multiresistant clinical isolates from Argentinean hospitals. Antimicrob Agents Chemother 47:3945–3949
2. Celenza G, Pellegrini C, Caccamo M, Segatore B, Amicosante G, Perilli M (2006) Spread of *bla*(CTX-M-type) and *bla*(PER-2) beta-lactamase genes in clinical isolates from Bolivian hospitals. J Antimicrob Chemother 57:975–978
3. Clinical and Laboratory Standards Institute (CLSI) (2006) Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard M7–A7. Clinical and Laboratory Standards Institute, Wayne

4. Di Conza J, Ayala JA, Power P, Mollerach M, Gutkind G (2002) Novel class 1 integron (InS21) carrying *bla*CTX-M-2 in *Salmonella enterica* serovar infantis. Antimicrob Agents Chemother 46:2257–2261
5. Garnacho-Montero J, Amaya-Villar R (2010) Multiresistant *Acinetobacter baumannii* infections: epidemiology and management. Curr Opin Infect Dis 23:332–339
6. Gordon NC, Wareham DW (2010) Multidrug-resistant *Acinetobacter baumannii*: mechanisms of virulence and resistance. Int J Antimicrob Agents 35:219–226
7. Morgan DJ, Liang SY, Smith CL, Johnson JK, Harris AD, Furuno JP, Thom KA, Snyder GM, Day HR, Perencevich EN (2010) Frequent multidrug-resistant *Acinetobacter baumannii* contamination of gloves, gowns, and hands of healthcare workers. Infect Control Hosp Epidemiol 31:716–721
8. Nagano N, Nagano Y, Cordevant C, Shibata N, Arakawa Y (2004) Nosocomial transmission of CTX-M-2 beta-lactamase-producing *Acinetobacter baumannii* in a neurosurgery ward. J Clin Microbiol 42:3978–3984
9. Orman BE, Pineiro SA, Arduino S, Galas M, Melano R, Caffer MI, Sordelli DO, Centrón D (2002) Evolution of multiresistance in nontyphoid salmonella serovars from 1984 to 1998 in Argentina. Antimicrob Agents Chemother 46:3963–3970
10. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA (2007) Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 51:3471–3484
11. Predari SKS, Gutierrez MA, De Paulis A, Guelfand L, García DC (1991) In vitro activity of 16 antibiotics and sulbactam against *Acinetobacter calcoaceticus* phenotype A1. J Chemother 4S: 20–25
12. Quiroga MP, Andres P, Petroni A, Soler Bistue AJ, Guerriero L, Vargas LJ, Zorreguieta A, Tokumoto M, Quiroga C, Tolmasky ME, Galas M, Centrón D (2007) Complex class 1 integrons with diverse variable regions, including *aac(6′)-Ib-cr*, and a novel allele, *qnrB10*, associated with IS*CR1* in clinical enterobacterial isolates from Argentina. Antimicrob Agents Chemother 51: 4466–4470
13. Ramírez MS, Don M, Merkier AK, Bistue AJ, Zorreguieta A, Centrón D, Tolmasky ME (2010) Naturally competent *Acinetobacter baumannii* clinical isolate as a convenient model for genetic studies. J Clin Microbiol 48:1488–1490
14. Roy Chowdhury P, Ingold A, Vanegas N, Martinez E, Merlino J, Merkier AK, Castro M, Gonzalez Rocha G, Borthagaray G, Centrón D, Bello Toledo H, Marquez CM, Stokes HW (2011) Dissemination of multiple drug resistance genes by class 1 integrons in *Klebsiella pneumoniae* isolates from four countries: a comparative study. Antimicrob Agents Chemother 55:3140–3149
15. Tolmasky ME, Colloms S, Blakely G, Sherratt DJ (2000) Stability by multimer resolution of pJHCMW1 is due to the Tn*1331* resolvase and not to the *Escherichia coli* Xer system. Microbiology 146(Pt 3):581–589