Short Communication

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ISCR2 and IS26: two insertion sequences highly dispersed among *Acinetobacter* spp. clinical strains

Abstract

The aim of this work is to study the dispersion of two ISs, IS*CR2* and IS*26*, which are known to contribute in the acquisition of resistance determinants and genome plasticity, among a collection of hundred and seventy-four *Acinetobacter* spp. clinical isolates. PCR amplification reactions using total DNA were performed to search IS*CR2*, IS*26*, and different antibiotic resistance genes (*tet*(B), *aphA6, sul2, floR, dfr9*) previously described in the genetic context of IS*CR2*.

Among *A. baumannii*, positive amplification for IS*CR2* was obtained in 66% of the included isolates and most of them (93%) were positive for IS*26* amplification. The platform *tet*(B)::IS*CR2* was found in 57% of the IS*CR2* positive isolates and only 14 gave negative amplification for *tet*(B). In these isolates positive amplifications of genes that were previously described associated to IS*CR2* -such as, *aphA6, sul2, floR,* and *dfr9*- were observed. When searching these ISs in the *non-baumannii Acinetobacter* studied-collection, two isolates were positive for IS*CR2* and two isolates for IS*26*. No positive results were obtained for *tet*(B), but the presence of *sul2, floR* and *dfr9* was found. Our results exposed a great dispersion of IS*CR2* as well as IS*26* among *Acinetobacter* spp. clinical isolates reinforcing the idea that the ISs play a crucial role in the plasticity and evolution towards drug resistance in this genus.

Keywords: *Acinetobacter* spp., IS*CR2*, IS*26*, insertion sequences, minimum inhibitory

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Introduction

Acinetobacter are gram-negative, *coccobacilli*, non-fermenting aerobes and can cause a variety of nosocomial infections.[1–4] Currently forty-one distinctive *Acinetobacteracinetobacter*.html)]. Among this genus, *A. baumannii* is responsible for the majority of nosocomial infections and has an intrinsic ability to acquire and to develop antibiotic resistance determinants to all available antibiotics to treat it.[5] Patient outcomes associated with extensively drug-resistant *A. baumannii* infections can be deadly. The CDC reports 500 deaths annually due to infections with multidrug-resistant *Acinetobacter* (CDC). Recently due to advances in technologies used to identify bacterial species, other members of the *Acinetobacter* genus have been recovered in the clinical setting and have demonstrated resistance to different antibiotics.[3,6]

Insertion sequences (ISs) are important for the acquisition and dissemination of antimicrobial resistance determinants, and can also contribute to resistance by insertional inactivation of proteins, such as transcriptional regulators or outer membrane protein.[6,7] More than 30 different types of ISs have been reported in *Acinetobacter* spp.[8] supporting the idea that ISs play a crucial role in the evolution of this genus and that they have contributed to the development of the multidrug-resistant phenotype observed in this genus.

Among the ISs described in *A. baumannii*, most of the studies referred to IS*Aba1*, IS*Aba125* and IS*Aba3* since they are associated with $bla_{\text{OXA-like}}$ carbapenemases.[7] Among other ISs that have been not deeply studied, we found IS*CR2*. IS*CR* are recognized as powerful elements that can capture and mobilize antibiotic resistance genes, as well as, array extended clusters of antibiotic resistance genes on plasmids or on chromosomes.[9] Twenty-one members of the IS*CR* family have been described[10] and most of them are related with antibiotic resistance.[11] IS*CR2* constitutes the second group of IS*CR* elements and it has been found in many species such as *Vibrio cholerae, Shigella flexneri, Salmonella enterica, Escherichia coli, Klebsiella pneumoniae, Aeromonas salmonicida, Pasteurella piscicida*. IS*CR2* have been described on numerous plasmids carrying trimethoprim, tetracycline, chloramphenicol and sulphonamides resistance gene[9,12] and also within resistance island.[13–17]

Another important IS is IS*26*, which was recognized to play an important role in the acquisition and dissemination of genes that confer resistance to many different classes of antibiotics. It has been reported that IS*26* plays a relevant role in the genomic plasticity observed in *A. baumannii*. They are involved in building transposons carrying different resistance genes in the fusion of additional transposons into resistance regions and also as a contributor of the variability observed among the genomic resistance island.[18]

While conducting a surveillance exploring the dispersion of tetracycline resistance determinants among extensively-drug resistance (XDR) *A. baumannii* strains, we found a high prevalence of the genetic platform *tet*(B)::IS*CR2* among minocycline resistant strains.[19] IS*CR2* has been also described in *Acinetobacter* spp. associated with bla_{VEB1}, suggesting its involvement in the acquisition and the mobilization of a β-lactamase.[1] Moreover, it was found as part of transposon and in AbaR-like resistant island close to tetracycline and aminoglycosides resistance genes[13–15] The aim of the present work was to further explore the dispersion of this particular insertion sequence (IS*CR2*) among a collection of hundred and seventy-five XDR *Acinetobacter* spp. clinical isolates in order to expose its prevalence and its potential role as an important IS among XDR isolates. Moreover, we also explore the presence IS*26* since is known to have an important role in the plasticity of this species.

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A total of 174 *Acinetobacter* spp. isolates were recovered from a variety of clinical sites and samples from individual patients including blood, urine, and respiratory tract, among others, from 12 different hospitals during 2010-2014. One hundred and sixty-four were *A. baumannii*, while the remaining ten were a novel species, *Acinetobacter* spp. A47 strain,[2] *A. pitti, A. radioresistans, A. johnsonii, A. ursingii, A. guilloiae. A. lwoffi, A. haemolyticus, A. soli and A. junii*.

The species of the isolates were confirmed by MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) and rpoB amplification and sequencing. The resistance profiles of the isolates to ampicilin, ampicilin/sulbactam, piperacillin/tazobactam, cefalotin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, nalidixic acid, ciprofloxacin, nitrofuratoin, colistin and trimetroprim/sulfamethoxazole were determined by disk diffusion according to Clinical Laboratory Standards Institute (CLSI) guidelines or using the VITEK 2 System (bioMerieux, Marcy, L'Etoile, France) employing the panel AST-082 (GNS susceptibility card) and the minimum inhibitory concentration (MIC) results were interpreted using the CLSI categories.

We extracted total DNA and used it to perform PCR amplification reactions according to the manufacturer's instructions (Promega, Madison, Wisconsin). Specific primers for IS*CR2* (IS*CR2*F: AAGAATTTCTCCAATGCGGG and IS*CR2*R: GCGGCTCCTTTTCCGACAAC) and IS*26* (IS*26*F: GCTGGCTGAACGCGGAGandIS*26*R:ATACCTTTGATGGTGGC) were used. Moreover, we searched different antibiotic resistance genes (*tet*(B), *aphA6, sul2, floR, dfr9*) previously described in the genetic context of IS*CR2* and we confirm its association in the required cases.[9]

To confirm the association of antibiotic resistance genes (*tet*(B), *aphA6, sul2, floR, dfr9*) with IS*CR2* several PCR products were sequenced after purifying the DNA by using the Wizard SV Gel and PCR clean-up System kit according to the manufacturer's directions (Promega, USA). Sequencing was performed on both DNA strands, using an ABI Prism 3100 Bio Analyzer equipment. The nucleotide sequences were analyzed using the Blast V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/).

Conclusion

All *A. baumannii* isolates were categorized as extreme drug-resistant (XDR) according to Magiorakos et al.,[20] being resistant to carbapenems and all antibiotics tested except colistin, and in some cases minocycline. Among *non-baumannii Acinetobacter* isolates, six were categorized as multi drug-resistant (MDR), and three were susceptible to all antibiotics tested (Table 1).

Table 1 Minimum inhibitory concentrations (MICs) of antimicrobial agents in non-*baumannii Acinetobacter* isolates

Isolate	MIC (mg/L)°																
	AMP	**SAM**	**TZP**	**CEP**	**FOX**	**CTX**	**CAZ**	**CEF**	**IPM**	**MEM**	**AMK**	**GEN**	**NAL**	**CIP**	**NIT**	**COL**	**SXT**
47	≤2	≤2	≤4	≥64	≤4	≤1	≤1	≤1	≤1	≤0.25	≤2	≤1	4	≤0.25	≥512	≤0.5	≤20
23	≥32	≤2	16	≥64	ND	8	4	2	4	≥16	16	≤1	≤2	≤0.25	≥512	≤0.5	160
350	16	≤2	≥128	≥64	ND	32	≥64	8	≤0.25	0.5	≤2	≤1	16	≤0.25	≥512	≤0.5	≤20
306	≤2	≤2	≤4	≤2	≤4	≤1	≤1	≤1	≤1	≤0.25	≤2	≤1	8	≤0.25	32	2	≤20
7	8	≤2	8	≥64	ND	8	8	2	≤0.25	≤0.25	≤2	≤1	4	≤0.25	256	≤0.5	≤20
181	>16	>16/8	>64/4	ND	>16	ND	>16	>16	>8	>8	≤8	≤2	≤8	≤0.125	>128	≤1	1/19
407	>16	≤4/2	≤4/4	ND	>16	ND	16	2	≤1	≤1	≤8	≤2	≤8	0.5	>128	≤1	≤0.5/9.5
432	≤4	≤4/2	≤4/4	ND	8	ND	8	≤1	≤1	≤1	≤8	≤2	≤8	≤0.125	>128	≤1	1/19
476	≤4	≤4/2	≤4/4	ND	>16	ND	4	4	≤1	≤1	≤8	≤2	≤8	≤0.125	>128	≤1	>2/38

°Bold indicates resistance.

AMP: Ampicilin; SAM: Ampicilin/Sulbactam; TZP: Piperacillin/Tazobactam; CEP: Cefalotin; FOX: Cefoxitin; CTX: Cefotaxime; CAZ: Ceftazidime; CEF: Cefepime; IPM: Imipenem; MEM: Meropenem; AMK: Amikacin; GEN: Gentamicin; NAL: Nalidixic Acid; CIP: Ciprofloxacin; NIT: Nitrofuratoin; COL: Colistin; SXT: trimetroprim/sulfamethoxazole; ND, no determinated.

Surprisingly, in the *A. baumannii* isolates we obtained positive results for the amplification of IS*CR2* in 108 out of 164 (66%). The distribution of IS*26* was also high among our isolates since 93% (153/164) of the isolates resulted positive for the amplification of this IS. This last result confirms the previous suggested role of IS*26* as a ubiquitous and highly disperse IS.[21] Among the 164 *A. baumannii* isolates included in this study 102 of the isolates were harboring both IS. As we previously found a high association of IS*CR2* with *tet*(B) in our *A. baumannii* population, we decided to investigate the presence of the previously reported platform *tet*(B)::IS*CR2*.[19] We obtained positive results for the association of *tet*(B) with the IS*CR2* element in 94 *A. baumannii* isolates (Figure 1). Moreover, 14 of the isolates that were positive for IS*CR2* and IS*26* gave negative amplification for *tet*(B). In these 14 isolates we investigated for the presence of other genes (*aphA6, sul2, floR, dfr9*) that were previously described associated to IS*CR2*. We found that all this isolates were positive for *sul2*, and in most (13/14) of the cases other genes were also present. We found that four isolates were positive for *sul2* and *dfr9*, five were positive for *floR* apart of *sul2*, one was positive for *aphA6, sul2* and *dfr9* and another was positive for *floR, sul2* and *dfr9* (Figure 1). We were able to determine the association of IS*CR2* with genes different than *tet*(B) only in one isolate, we were able to link IS*CR2* with *floR*. Moreover, among IS*CR2* negative isolates, we found three *tet*(B) positive isolates that were also IS*26* negative.

On the other hand, in the *non-baumannii Acinetobacter* isolates we obtained positives results for IS*CR2* in two isolates, one of which was positive for *sul2* and IS*26* while the other was positive for *sul2* and *floR*. Positives results for IS*26* were found in two isolates, for *sul2* in five isolates, for *aphA6* in two isolates, and for *floR* in 1 isolate. No positive results were found for *tet*(B).

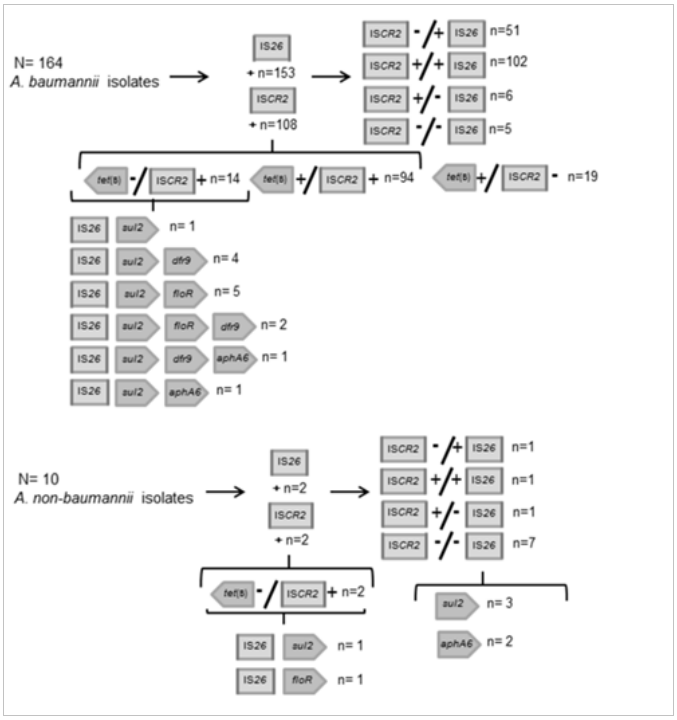

Figure 1 Diagram showing the dispersion of IS*CR2*, IS26and the most prevalent genes associated to IS*CR2* among *A. baumannii* and *A. non baumannii* isolates.

This study shows a great dispersion of IS*CR2* as well as IS*26* among *Acinetobacter* spp. clinical isolates reinforcing the idea that ISs plays a crucial role in the plasticity and evolution towards drug resistance in this genus. The association of IS*CR2* with *tet*(B) is the predominant platform among the *A. baumannii* isolates studied. However, IS*CR2* is also present in other *Acinetobacter* spp. isolates where *tet*(B) is not present. We plan to perform further studies to find out the genetic environment of the IS*CR2* positive isolates where *tet*(B) is not present. The high prevalence of IS*CR2* and IS*26* in our *Acinetobacter* spp. isolates allow us to suggest that this element is successfully been maintained is this species, giving a tool that could be involved in the acquisition and dissemination of resistance traits.

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Conflict of interest

The author declares no conflict of interest.

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Figure 1 Diagram showing the dispersion of ISCR2, IS26and the most prevalent genes associated to ISCR2 among A. baumannii and A. non baumannii isolates.

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