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Short communication

Evolutionary dynamics of carbapenem-resistant *Acinetobacter baumannii* circulating in Chilean hospitals

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ABSTRACT

We analyze the evolutionary dynamics of ninety carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates collected between 1990 and 2015 in Chile. CRAB were identified at first in an isolate collected in 2005, which harbored the IS*Aba1*-bla_{OXA-69} arrangement. Later, OXA-58- and OXA-23-producing *A. baumannii* strains emerged in 2007 and 2009, respectively. This phenomenon was associated with variations in the epidemiology of OXA-type carbapenemases, linked to nosocomial lineages belonging to ST109, ST162, ST15 (CC15) and ST318 (CC15).

1. Introduction

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) has been deemed a critical-priority pathogen by the World Health Organization (WHO) (2017). It is normally involved in infections acquired in the intensive care units (ICUs), and is commonly resistant to several antibiotics, including carbapenems (Peleg et al., 2008). Accordingly, OXA-type carbapenemases (OTCs) are the main resistance mechanism to carbapenems in *A. baumannii* (Opazo et al., 2012). While OXA-51-like carbapenemases are chromosomally encoded, the remaining OTCs (OXA-23-like, −24-like, −58-like and −143-like) are frequently plasmid encoded (Evans et al., 2013; Evans and Amyes, 2014). OXA-51-like enzymes can mediate resistance to carbapenems if they are over-expressed when the IS*Aba1* element is present upstream of the $bla_{OXA-51-like}$ gene (Turton et al., 2006). CRAB outbreaks are commonly associated to the three predominant clonal complexes (CCs) CC109/1, CC118/2 and CC187/3 (University of Oxford/Institute Pasteur MLST schemes) (Martins et al., 2016). Although, the clonal complex CC113/CC79 has been predominant in South America; CC104/CC15, CC110/ST25 and CC109/CC1 are also present in this region (Higgins et al., 2010a, 2010b).

The aim of this study was to investigate the evolutionary dynamics of CRAB in Chilean hospitals, where this pathogen has an endemic status.

2. Materials and methods

Ninety non-repetitive *A. baumannii* isolates recovered between 1990 and 2015 in Chile from pathological products of nosocomial infections were included. They were collected in 13 hospitals from nine different cities throughout Chile, in which the greatest distance between two cities is 2433 km, representing over 50% of the length of the country. The set of isolates included both carbapenem-susceptible and –resistant strains which were provided directly by the laboratories of each hospital. Species-identification was carried out by multiplex-PCR according to Chen et al. (2007).

Antibiotic susceptibility tests were performed to carbapenems, cephalosporins, aminoglycosides, ampicillin/sulbactam, piperacillin/tazobactam, ciprofloxacin, and tetracycline (Clinical and Laboratory Standards Institute, 2017). Imipenem (IPM) and meropenem (MEM) minimum inhibitory concentrations (MICs) were determined by the broth microdilution method following the CLSI guidelines (Clinical and

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Laboratory Standards Institute, 2017). Colistin-resistance was screened using the SuperPolymyxin media (Nordmann et al., 2016). CarbAcinetoNP test was performed on all carbapenem non-susceptible isolates that were negative for bla_{OXA} genes (Dortet et al., 2014). Multidrug-resistant (MDR), extensively-drug resistant (XDR) and pandrug-resistant (PDR) phenotypes were defined as previously described (Magiorakos et al., 2012; Manchanda and Sinha Sanchaita, 2010).

Genetic relatedness was determined by pulsed-field gel electrophoresis (PFGE) as described earlier (Seifert et al., 2005). Groups with at least three genetically related isolates (> 87% similarity) were designated as major PFGE clusters (Al-Sultan et al., 2015). Single-locus $bla_{OXA\text{-}51\text{-}like}$ sequence-based typing (SBT) was carried out as described previously (Pournaras et al., 2014), in order to characterize the predominant lineage associated to a specific $bla_{OXA\text{-}51\text{-}like}$ allele. Isolates representative of the 4 main PFGE clusters (n = 10) were subjected to whole-genome sequencing (WGS) using the MiSeq Illumina platform (2 × 250 bp paired end reads) with libraries prepared by the NexteraXT kit (Illumina). From these data, we determined the Pasteur's scheme sequence types (STs) and acquired resistance genes using the bioinformatic tools available at the Center for Genomic Epidemiology (CGE) server (http://www.genomicepidemiology.org/).

The population structure based on the MLST data was determined by the goeBURST software (http://www.phyloviz.net/goeburst/).

All CRABs were screened for the OTCs genes $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$ and $bla_{OXA\text{-}143\text{-}like}$ by multiplex-PCR (Higgins et al., 2010a, 2010b), while $bla_{OXA\text{-}51\text{-}like}$ alleles were investigated by PCR and sequencing. In addition, the IS*Aba1*-$bla_{OXA\text{-}51\text{-}like}$ array in CRABs was examined by conventional PCR (Ruiz et al., 2007). Moreover, the metallo-β-lactamases (MβLs) genes bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM} and bla_{GIM} we screened by multiplex-PCR (Poirel et al., 2011).

3. Results and discussion

The comprised isolates (n = 90) were grouped into three different periods: P1 (1990–1999, n = 27), P2 (2000–2009, n = 30), and P3 (2010–2015, n = 33). Consequently, carbapenem resistance was confirmed in 56 (62%) isolates, being identified for the first time in 2005 in a strain (A329, P2) carrying the IS*Aba1*-$bla_{OXA\text{-}69}$ array (Fig. 1). XDR, MDR or PDR profiles were displayed by 51 (57%), 28 (31%) and 3 (3%) isolates, respectively. Furthermore, 65 (72%) isolates were non-susceptible to amikacin, whereas 64 (71%) were non-susceptible to gentamicin. Additionally, 32 (36%) isolates exhibited resistance to ampicillin-sulbactam, and 4 (3.6%) were colistin-resistant, since they were able to grow on the SuperPolymyxin media. Three colistin-resistant isolates were recovered in 2014, and one on 2008. Interestingly, the CRAB isolate A223 was negative for CarbAcinetoNP.

Of all 56 CRAB isolates, $bla_{OXA\text{-}58}$ (n = 17; 30%) and $bla_{OXA\text{-}23}$ (n = 17; 30%) genes were more prevalent and were associated with highest carbapenems MICs, whereas MβLs were not detected on the isolates (Fig. 1). In addition, IS*Aba1*-$bla_{OXA\text{-}51\text{-}like}$ arrangement was identified in 19 isolates (34%). Specifically, the IS*Aba1*-$bla_{OXA\text{-}219}$ array was observed in 14 of 56 (25%) CRAB isolates. A single isolate (A223) was negative for any carbapenemases, including OTC and MBLs. These findings were corroborated by the WGS performed on the selected isolates (Fig. 1), where the same carbapenemases-genes were detected by ResFinder. In this regard, OXA-58-producing isolates seems to have emerged in 2007, whereas IS*Aba1*-OXA-219 and OXA-23 producers arose in 2009, being disseminated among different hospitals.

As expected, no OTC producers were identified in P1. Otherwise, twelve OXA-58-, seven OXA-23-, and four IS*Aba1*-$bla_{OXA\text{-}51}$-like-positive CRAB isolates were detected in P2 (Fig. 1). In P3, a change in the molecular epidemiology of circulating OTCs was observed, where OXA-23 producers (n = 11) were predominant, followed by OXA-51-like (associated with IS*Aba1*, n = 15)- and OXA-58 (n = 4)-positive isolates (Fig. 1). Moreover, the CRAB isolate A223 was negative for OTCs and/or MBLs, in concordance with the CarbAcinetoNP result, suggesting that carbapenem-resistance in this isolate might be mediated by a different mechanism (Peleg et al., 2008).

Four major clusters (I - IV) were identified by PFGE (Fig. 1). Cluster I included three isolates from three different cities, comprising a single OXA-58-like-producing CRAB. Cluster II included four carbapenem-susceptible isolates from P1. Moreover, cluster III comprised CRABs from 2015 that harbored the OXA-23-like (n = 3) and IS*Aba1*-$bla_{OXA\text{-}219}$ array (n = 5), which were collected from two hospitals separated by > 1000 km (Fig. 1). Finally, cluster IV contained three CRABs carrying $bla_{OXA\text{-}23\text{-}like}$ genes and the OXA-51-like variants harbored by these isolates were OXA-51 and OXA-69.

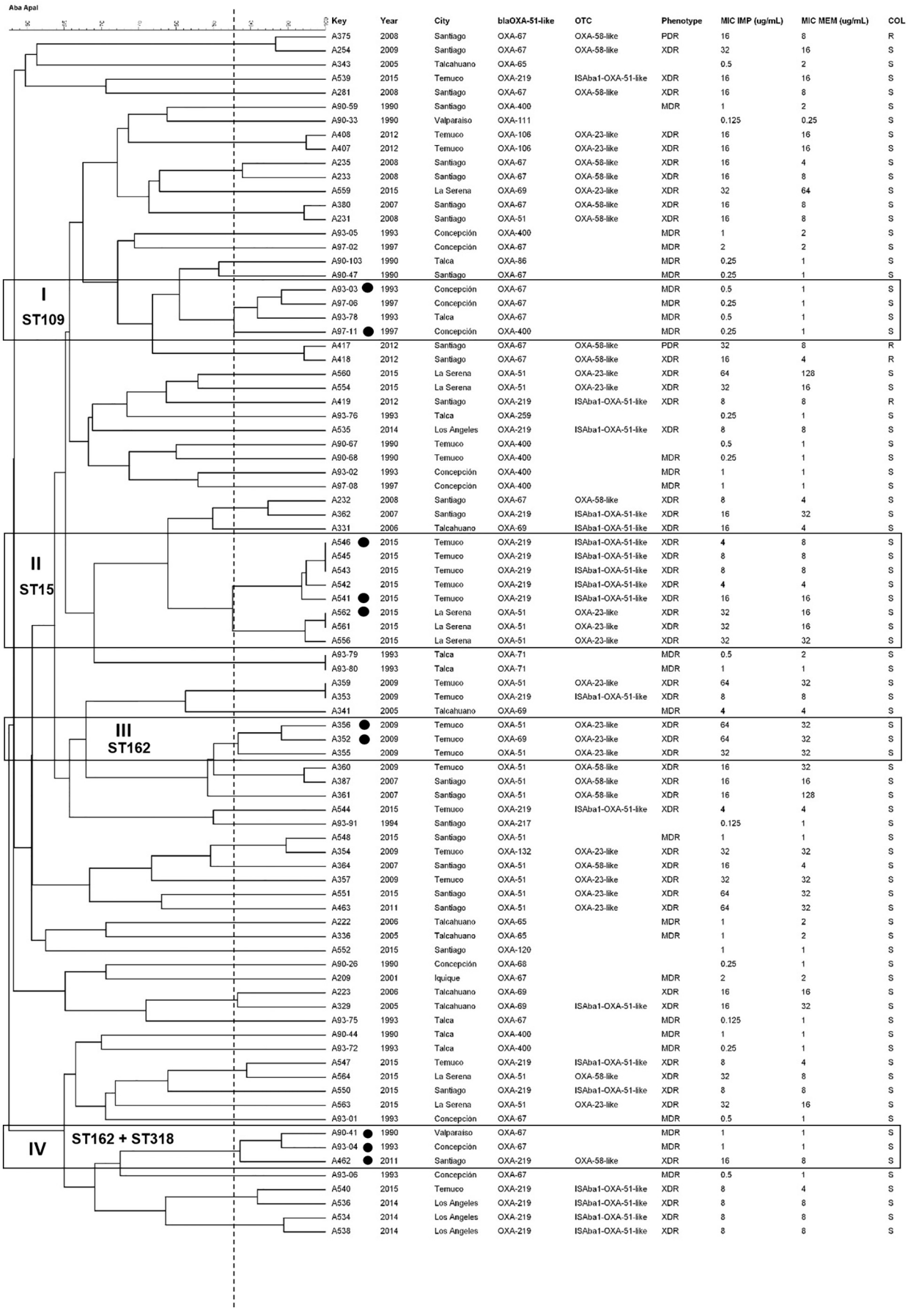
Fifteen $bla_{OXA\text{-}51}$-like variants were identified from SBT, where most prevalent alleles were OXA-51 (n = 21), OXA-67 (n = 20) and OXA-219 (n = 18) (Fig. 1). Relevantly, they are not associated to the three predominant CCs, since CCs I, II and III harbored the OXA-69, OXA-66 and OXA-71, respectively (Pournaras et al., 2014). In PFGE cluster I, two isolates from P1 belonged to ST162, whereas a single isolate (A462) from P3 corresponded to ST318, which is part of the CC15 (Fig. 2). Moreover, cluster II includes two isolates from P1 which are part of ST109 (Fig. 1). Finally, those isolates from clusters III and IV belonged to ST15 and ST162, respectively. As shown in fig. 2, these STs are not related with the major GCs 1–3, which are predominant worldwide. Interestingly, ST318 is a SLVs of ST15, both part of CC15 (Fig. 2). A relevant limitation of our study is that we determined the STs of the major PFGE clusters defined previously, thus non-related isolates were not included in this analysis. Due to this, other STs might be unnoticed, which could be associated to the CCs disseminated globally, thus a larger study of the endemic STs is needed.

In Chile, CRAB has been responsible for about 26% of ventilator-associated pneumonia (VAP) in hospitalized adults (Otaíza et al., 2014), whereas carbapenem-resistance rates are above 66% (Instituto de Salud Publica de Chile (ISPCH), 2015). Our results reveal the evolutionary dynamics of CRAB in the country, focusing on the major carbapenem resistance genes and lineages circulating in hospital settings in a period of 25 years.

Worryingly, XDR isolates were predominant in our collection, including resistance to aminoglycosides and ampicillin/sulbactam, in concordance with previous reports in the country (Cifuentes et al., 2014). Although the rate of colistin resistance was 3.6%, this percentage is higher than the previously published in 2012 (1.4%) (Cifuentes et al., 2014), representing an alarming increase to be considered CRAB has been increasing lately worldwide, and our results reveal that initially in Chile it was related to the IS*Aba1*-$bla_{OXA\text{-}69}$ array identified in 2005, where ISs play an essential role in the regulation of this resistance (Perez et al., 2007).

Concerning to acquired OTCs, OXA-58-like-producing isolates seem to have emerged in 2007, whereas OXA-23-like producers arose later (Opazo et al., 2012; Rodríguez et al., 2018). Significantly, after 2010 a new change in the molecular epidemiology of circulating OTCs was observed, where OXA-23 producers have been predominant and widely disseminated along the country. In addition, MβLs were not detected on CRABs analyzed, which correlates with the data available in Chile, where these enzymes have been not detected in *A. baumannii*, and in general possess a low prevalence in the country (Escandón-Vargas et al., 2017).

Additionally, we detected the replacement of certain carbapenem-susceptible clones present in P1, by carbapenem-resistant lineages that began to emerge in the late 2000s. SBT revealed that the CRAB isolates were not related to the major CCs (1–3). The main OXA-51-like variants present were OXA-219, OXA-67 and OXA-51. Of these, OXA-51 has been associated with the CC15 (Pournaras et al., 2014), previously detected in Europe, Pakistan and South America, which is considered as a high-risk clone (Karah et al., 2012). In South America, this CC is categorized as epidemic in Brazil (Pagano et al., 2017), which suggests the dissemination of resistant clones through the region. Otherwise,



Key	Year	City	blaOXA-51-like	OTC	Phenotype	MIC IMP (ug/mL)	MIC MEM (ug/mL)	COL
A375	2008	Santiago	OXA-67	OXA-58-like	PDR	16	8	R
A254	2009	Santiago	OXA-67	OXA-58-like	XDR	32	16	S
A343	2005	Talcahuano	OXA-65			0.5	2	S
A539	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	16	16	S
A281	2008	Santiago	OXA-67	OXA-58-like	XDR	16	8	S
A90-59	1990	Santiago	OXA-400		MDR	1	2	S
A90-33	1990	Valparaiso	OXA-111			0.125	0.25	S
A408	2012	Temuco	OXA-106	OXA-23-like	XDR	16	16	S
A407	2012	Temuco	OXA-106	OXA-23-like	XDR	16	16	S
A235	2008	Santiago	OXA-67	OXA-58-like	XDR	16	4	S
A233	2008	Santiago	OXA-67	OXA-58-like	XDR	16	8	S
A559	2015	La Serena	OXA-69	OXA-23-like	XDR	32	64	S
A380	2007	Santiago	OXA-67	OXA-58-like	XDR	16	8	S
A231	2008	Santiago	OXA-51	OXA-58-like	XDR	16	8	S
A93-05	1993	Concepción	OXA-400		MDR	1	2	S
A97-02	1997	Concepción	OXA-67		MDR	2	2	S
A90-103	1990	Talca	OXA-86		MDR	0.25	1	S
A90-47	1990	Santiago	OXA-67		MDR	0.25	1	S
A93-03 ●	1993	Concepción	OXA-67		MDR	0.5	1	S
A97-06	1997	Concepción	OXA-67		MDR	0.25	1	S
A93-78	1993	Talca	OXA-67		MDR	0.5	1	S
A97-11 ●	1997	Concepción	OXA-400		MDR	0.25	1	S
A417	2012	Santiago	OXA-67	OXA-58-like	PDR	32	8	R
A418	2012	Santiago	OXA-67	OXA-58-like	XDR	16	4	R
A560	2015	La Serena	OXA-51	OXA-23-like	XDR	64	128	S
A554	2015	La Serena	OXA-51	OXA-23-like	XDR	32	16	S
A419	2012	Santiago	OXA-219	ISAba1-OXA-51-like	XDR	8	8	R
A93-76	1993	Talca	OXA-259			0.25	1	S
A535	2014	Los Angeles	OXA-219	ISAba1-OXA-51-like	XDR	8	8	S
A90-67	1990	Temuco	OXA-400			0.5	1	S
A90-68	1990	Temuco	OXA-400		MDR	0.25	1	S
A93-02	1993	Concepción	OXA-400		MDR	1	1	S
A97-08	1997	Concepción	OXA-400		MDR	1	1	S
A232	2008	Santiago	OXA-67	OXA-58-like	XDR	8	4	S
A362	2007	Santiago	OXA-219	ISAba1-OXA-51-like	XDR	16	32	S
A331	2006	Talcahuano	OXA-69	ISAba1-OXA-51-like	XDR	16	4	S
A546 ●	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	4	8	S
A545	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	8	8	S
A543	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	8	8	S
A542	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	4	4	S
A541 ●	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	16	16	S
A562 ●	2015	La Serena	OXA-51	OXA-23-like	XDR	32	16	S
A561	2015	La Serena	OXA-51	OXA-23-like	XDR	32	16	S
A556	2015	La Serena	OXA-51	OXA-23-like	XDR	32	32	S
A93-79	1993	Talca	OXA-71		MDR	0.5	2	S
A93-80	1993	Talca	OXA-71		MDR	1	1	S
A359	2009	Temuco	OXA-51	OXA-23-like	XDR	64	32	S
A353	2009	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	8	8	S
A341	2005	Talcahuano	OXA-69		MDR	4	4	S
A356 ●	2009	Temuco	OXA-51	OXA-23-like	XDR	64	32	S
A352 ●	2009	Temuco	OXA-69	OXA-23-like	XDR	64	32	S
A355	2009	Temuco	OXA-51	OXA-23-like	XDR	32	32	S
A360	2009	Temuco	OXA-51	OXA-58-like	XDR	16	32	S
A387	2007	Santiago	OXA-51	OXA-58-like	XDR	16	16	S
A361	2007	Santiago	OXA-51	OXA-58-like	XDR	16	128	S
A544	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	4	4	S
A93-91	1994	Santiago	OXA-217			0.125	1	S
A548	2015	Santiago	OXA-51		MDR	1	1	S
A354	2009	Temuco	OXA-132	OXA-23-like	XDR	32	32	S
A364	2007	Santiago	OXA-51	OXA-58-like	XDR	16	4	S
A357	2009	Temuco	OXA-51	OXA-23-like	XDR	32	32	S
A551	2015	Santiago	OXA-51	OXA-23-like	XDR	64	32	S
A463	2011	Santiago	OXA-51	OXA-23-like	XDR	64	32	S
A222	2006	Talcahuano	OXA-65		MDR	1	2	S
A336	2005	Talcahuano	OXA-65		MDR	1	2	S
A552	2015	Santiago	OXA-120			1	1	S
A90-26	1990	Concepción	OXA-68			0.25	1	S
A209	2001	Iquique	OXA-67		MDR	2	2	S
A223	2006	Talcahuano	OXA-69		XDR	16	16	S
A329	2005	Talcahuano	OXA-69	ISAba1-OXA-51-like	XDR	16	32	S
A93-75	1993	Talca	OXA-67		MDR	0.125	1	S
A90-44	1990	Talca	OXA-400		MDR	1	1	S
A93-72	1993	Talca	OXA-400		MDR	0.25	1	S
A547	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	8	4	S
A564	2015	La Serena	OXA-51	OXA-58-like	XDR	32	8	S
A550	2015	Santiago	OXA-219	ISAba1-OXA-51-like	XDR	8	8	S
A563	2015	La Serena	OXA-51	OXA-23-like	XDR	32	16	S
A93-01	1993	Concepción	OXA-67		MDR	0.5	1	S
A90-41 ●	1990	Valparaiso	OXA-67		MDR	1	1	S
A93-04 ●	1993	Concepción	OXA-67		MDR	1	1	S
A462 ●	2011	Santiago	OXA-219	OXA-58-like	XDR	16	8	S
A93-06	1993	Concepción	OXA-67		MDR	0.5	1	S
A540	2015	Temuco	OXA-219	ISAba1-OXA-51-like	XDR	8	4	S
A536	2014	Los Angeles	OXA-219	ISAba1-OXA-51-like	XDR	8	8	S
A534	2014	Los Angeles	OXA-219	ISAba1-OXA-51-like	XDR	8	8	S
A538	2014	Los Angeles	OXA-219	ISAba1-OXA-51-like	XDR	8	8	S

(caption on next page)

Fig. 1. Dendrogram generated after restriction with ApaI enzyme for 86/90 typed A. baumannii isolates. The black dotted line represents 87% similarity. I to IV denote the major PFGE groups characterized according to the criteria described in the manuscript. MDR: multidrug-resistant; XDR: extensively-drug resistant; PDR: pandrug-resistant; OTC: OXA-type carbapenemase; ST: Sequence type; COL: colistin. ● Isolates typified by MLST.

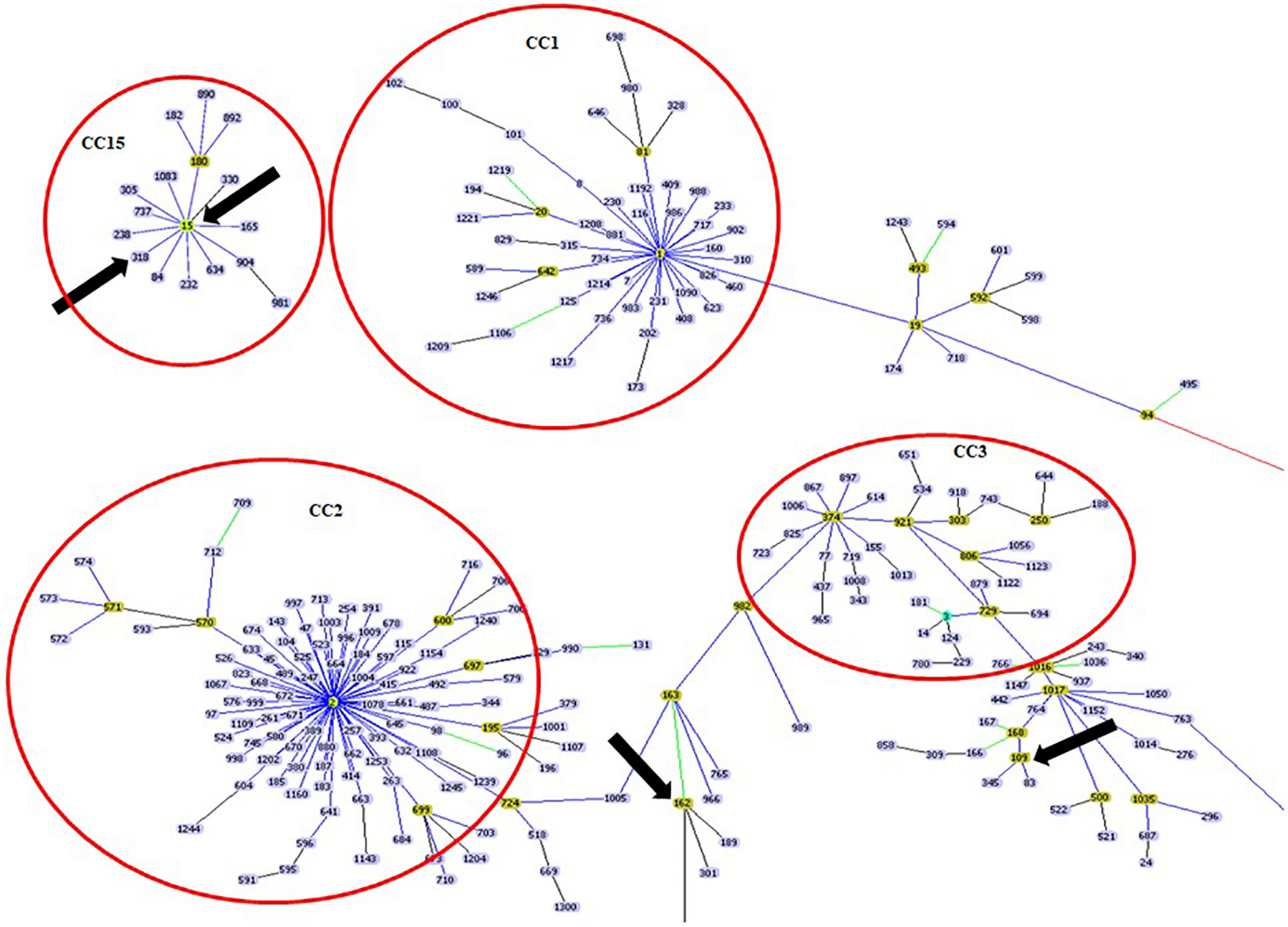


Fig. 2. Population structure of *A. baumannii* according to according to MLST. Blue lines represent single-locus variants (SLVs). Founders STs are highlighted in yellow. Red circles indicate the three main clonal complexes (CCs 1-3) and CC15. Black arrows identifies ST15, ST138, ST109 and ST162. The analysis was performed using goeBURST (PHYLOViZ).

OXA-67 and OXA-219 are related to less prevalent CCs (Pournaras et al., 2014). Interestingly, OXA-219 was originally identified in 2012 from a single isolate from Chile, being related to the worldwide (WW) clone 4 (Zander et al., 2012), associated to the IS*Aba1-bla*$_{\text{OXA-219}}$ array. These results suggest the presence of an endemic lineage (WW4, OXA-219) coexisting with a regional lineage (ST15) in Chile (Higgins et al., 2010a, 2010b; Rodriguez et al., 2016), which has been described in Brazil (Chagas et al., 2014) and Ecuador (Rodriguez et al., 2016).

In addition to ST15 (CC15), other identified lineages included ST162, ST318 (CC15), and ST109. The latter has been originally identified in Sweden (Hamidian et al., 2017), whereas ST162 and ST318 have been described in Brazil (Camargo et al., 2016; Chagas et al., 2014). These findings reaffirm that the major lineages present in the region are different to those globally spread (Higgins et al., 2010a, 2010b), and confirm that CC15, which encompasses ST15 and ST318, is one of the most predominant lineages in South America. Despite the above, CC2 and 3 have been recently identified in Peru (Levy-blitchtein et al., 2018), which might have an important impact on the local epidemiology.

In conclusion, our study provides data about evolutionary dynamics of CRAB circulating in Chilean hospitals, which were linked to particular lineages as well as to the emergence of specific OTCs, whereas colistin resistance deserves an urgent attention to strengthen surveillance.

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Conflict of interest statement

None to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.04.022.

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