1 Evolutionary dynamics of carbapenem-resistant Acinetobacter baumannii

2 circulating in Chilean hospitals

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| 17 | Running Head: Carbapenem-resistant A. baumannii in Chilean Hospitals |
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24 ABSTRACT

| 25 | We analyze the evolutionary dynamics of ninety carbapenem-resistant Acinetobacter |
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| 26 | baumannii (CRAB) isolates collected between 1990 and 2015 in Chile. CRAB were |
| 27 | identified at first in an isolate collected in 2005, which harbored the ISAba1-bla _{OXA-69} |
| 28 | arrangement. Later, OXA-58- and OXA-23-producing A. baumannii strains emerged in |
| 29 | 2007 and 2009, respectively. This phenomenon was associated with variations in the |
| 30 | epidemiology of OXA-type carbapenemases, linked to nosocomial lineages belonging to |
| 31 | ST109 (CC1), ST162 (CC79), ST15 (CC15) and ST318 (CC15). |
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47 Carbapenem-resistant Acinetobacter baumannii (CRAB) has been deemed a critical-48 priority pathogen by the World Health Organization (WHO) (1). It is normally involved in infections acquired in the intensive care units (ICUs), and is commonly resistant to several 49 antibiotics, including carbapenems (2). Accordingly, OXA-type carbapenemases (OTCs) 50 are the main resistance mechanism to carbapenems in A. baumannii (3). While OXA-51-51 52 like carbapenemases are chromosomally encoded, the remaining OTCs (OXA-23-like, -24-53 like, -58-like and -143-like) are frequently plasmid encoded (4, 5). OXA-51-like enzymes can mediate resistance to carbapenems if they are overexpressed when the ISAba1 element 54 is present upstream of the *bla*_{OXA-51-like} gene (6). CRAB outbreaks are commonly associated 55 to the three predominant clonal complexes (CCs) CC109/1, CC118/2 and CC187/3 56 57 (University of Oxford/Institute Pasteur MLST schemes) (7). Although, the clonal complex CC113/CC79 has been predominant in South America; CC104/CC15, CC110/ST25 and 58 59 CC109/CC1 are also present in this region (8).

60 The aim of this study was to investigate the evolutionary dynamics of CRAB in61 Chilean hospitals, where this pathogen has an endemic status.

Ninety non-repetitive *A. baumannii* isolates recovered between 1990 and 2015 were included. They were collected in hospitals from nine different cities throughout Chile, in which the greatest distance between two cities is 2,433 km, representing over 50% of the length of the country.

Antibiotic susceptibility tests were performed to carbapenems, cephalosporins, aminoglycosides, ampicillin/sulbactam, piperacillin/tazobactam, ciprofloxacin, and tetracycline (9). Imipenem (IPM) and meropenem (MEM) MICs were determined following the CLSI guidelines (9). Colistin-resistance was screened using the

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70 SuperPolymyxin media (10). Multidrug-resistant (MDR), extensively-drug resistant (XDR)

and pandrug-resistant (PDR) phenotypes were defined as previously described (11, 12).

Genetic relatedness was determined by pulsed-field gel electrophoresis (PFGE) as described earlier (13). Groups with at least three genetically related isolates (>87% similarity) were designated as major PFGE clusters (14). Single-locus $bla_{OXA-51-like}$ sequence-based typing (SBT) was carried out as described previously (15). Isolates representative of the main PFGE clusters were subjected to whole-genome sequencing (WGS), and sequence types (STs) were determined (Pasteur's scheme) as published earlier (16).

OTCs genes were screened by multiplex-PCR (17), whereas $bla_{OXA-51-like}$ alleles were investigated by PCR and sequencing. IS*Aba1-bla*_{OXA-51-like} array was examined by conventional PCR (18). CarbAcinetoNP test was performed on all carbapenem nonsusceptible isolates that were negative for bla_{OXA} genes (19).

The comprised isolates were grouped into three different periods: P1 (1990-1999, 83 n=27), P2 (2000-2009, n=30), and P3 (2010-2015, n=33). Consequently, carbapenem 84 resistance was confirmed in 56 (62%) isolates, being identified for the first time in 2005 in 85 a strain (A329, P2) carrying the ISAba1-bla_{OXA-69} array (Figure 1). XDR, MDR or PDR 86 profiles were displayed by 51 (57%), 28 (31%) and 3 (3%) isolates, respectively. 87 Furthermore, 65 (72%) isolates were non-susceptible to amikacin, whereas 64 (71%) were 88 non-susceptible to gentamicin. Additionally, 32 (36%) isolates exhibited resistance to 89 90 ampicillin-sulbactam, and 4 (3.6%) were colistin-resistant.

Further, *bla*_{OXA-58} (30%) and *bla*_{OXA-23} (30%) genes were more prevalent and were
associated with highest carbapenems MICs (Figure 1). The ISA*ba1-bla*_{OXA-219} array was
observed in 14 of 56 (25%) CRAB isolates. In this regard, OXA-58-producing isolates

seems to have emerged in 2007, whereas ISAba1-OXA-219 and OXA-23 producers arose
in 2009, being disseminated among different hospitals.

As expected, no OTC producers were identified in P1. Otherwise, eleven OXA-58-,
seven OXA-23-, and four ISAba1-bla_{OXA-51}-like-positive CRAB isolates were detected in
P2 (Figure 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 ISAba1, n= 15)- and OXA-58 (n= 4)-positive isolates (Figure 1).
Interestingly, the CRAB isolate A223 was negative for both CarbAcinetoNP and OTCs
PCR. Thus carbapenem-resistance could be mediated by a different mechanism (2).

Four major clusters (I – IV) were identified by PFGE (Figure 1). Cluster I included four carbapenem-susceptible isolates from P1, while cluster II comprised CRABs from 2015 that harbored the OXA-23-like (n=3) and ISAba1-bla_{OXA-219} array (n=5), which were collected from two hospitals separated by >1000 km (Figure 1). Cluster III contained three CRABs carrying $bla_{OXA-23-like}$ genes and the OXA-51-like variants OXA-51 and OXA-69. Finally, cluster IV included three isolates from three different cities, comprising a single OXA-58-like-producing CRAB (Figure 1). Four isolates were non-typeable.

Fifteen bla_{OXA-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) (Figure 1). They are not associated to the three predominant international clones (ICs). Furthermore, isolates from PFGE cluster I corresponded to ST109, whereas those from clusters II and III belonged to ST15 and ST162, respectively (Figure 1). In cluster IV, two isolates from P1 belonged to ST109, whereas a single isolate (A462) from P3 corresponded to ST318, which is part of the CC15. In Chile, CRAB has been responsible for about 26% of ventilator-associated pneumonia (VAP) in hospitalized adults (20), whereas carbapenem-resistance rates are above 66% (21). 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 (22). Although the rate of colistin resistance was 3.6%, this percentage is higher than the previously published in 2012 (1.4%) (22), 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-69} array identified in 2005, where ISs play an essential role in the regulation of this resistance (23).

129 Concerning to acquired OTCs, OXA-58-like-producing isolates seem to have emerged in 2007, whereas OXA-23-like producers arose later (3, 24). Significantly, after 130 2010 a new change in the molecular epidemiology of circulating OTCs was observed, 131 132 where OXA-23 producers have been predominant and widely disseminated along the 133 country. Additionally, we detected the replacement of certain carbapenem-susceptible 134 clones present in P1, by carbapenem-resistant linages that began to emerge in the late 2000s. SBT revealed that the CRAB isolates were not related to the major ICs (I-III). The 135 main OXA-51-like variants present were OXA-219, OXA-67 and OXA-51. Of these, 136 137 OXA-51 has been associated with the CC15 (15), previously detected in Europe, Pakistan and South America, which is considered as a high-risk clone (25). In South America, this 138 CC is categorized as epidemic in Brazil (26), which suggests the dissemination of resistant 139 140 clones through the region. Otherwise, OXA-67 and OXA-219 are related to less prevalent

ICs (15). Interestingly, OXA-219 was originally identified in 2012 from a single isolate
from Chile, being related to the worldwide (WW) clone 4 (27), associated to the ISAba1-*bla*_{OXA-219} array. These results suggest the presence of an endemic lineage (WW4, OXA219) coexisting with a regional lineage (ST15) in Chile (8, 28), which has been described in
Brazil (29) and Ecuador (28).
Other identified lineages included ST109 (CC1), ST162 (CC79), and ST318

146 Other identified lineages included S1109 (CC1), S1162 (CC79), and S1318 147 (CC15). ST109 has been originally identified in Sweden (30), whereas ST162 and ST318 148 have been described in Brazil (29, 31). These findings reaffirm that the major lineages 149 present in the region are different to those globally spread (8). However, ICII and III have 150 been lately identified in Peru (32), which might have an important impact on the local 151 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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1 Figure 1. Dendrogram generated after restriction with ApaI enzyme for 86/90 typed A.

- 2 baumannii isolates. The black dotted line represents 87% similarity. I to IV denote the
- 3 major PFGE groups characterized according to the criteria described in the manuscript.
- 4 MDR: multidrug-resistant; XDR: extensively-drug resistant; PDR: pandrug-resistant; OTC:
- 5 OXA-type carbapenemase; ST: Sequence type; COL: colistin. Isolates typified by MLST.

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|-----|-----------------|---|--|-------------------|--------------------|--------------------------|-------------------------|--|------------|-----------------|---------------|----------------------------------|
| | | | <u> </u> | Aumder a A254 | 2009-BY-N 2009 | Santiago | International 0XA-67 | CXA-58-like | PDR XDR | 16 32 | a 16 | R S |
| | | | | A343 | 2005 | Sanuago Talcahuano | OXA-55 | OXA-98-like | ADR | 32 0.5 | 2 | 5 |
| | | | i I | A539 | 2015 | Temuco | OXA-219 | ISAba1-OXA-51-like | XDR | 16 | 18 | 5 |
| | | | i | A281 | 2008 | Santiago | OXA-67 | OXA-58-like | XDR | 16 | 8 | \$ |
| | | i | <u>. </u> | A90-59 A90-33 | 1990 1990 | Sentingo Valparaiso | OXA-400 OXA-111 | | MÓR | 1 0.125 | 7 0.25 | 8 9 |
| | | | | A408 | 2012 | Temuco | OXA-106 | OXA-23-lika | XDR | 16 | 16 | 3 |
| | | | <u> </u> | A407 | 2012 | Temuce | OXA-106 | OXA-23-like | XDR | 16 | 16 | 9 |
| | | | Ļ | A235 | 2008 | Santiago | OXA-67 | OXA-58-like | XDR | 16 | 4 | 8 |
| | | | ¦ | A233 A559 | 2008 2015 | Santiago La Sausan | OXA-57 OXA-59 | OXA-55-like OXA-23-like | XDR XDR | 16 32 | 5 64 | 5 S |
| | | | | A380 | 2015 | La Serena Sontiogo | OXA-87 | OXA-58-like | XDR | 18 | 8 | 3 |
| | | _ | <u>; </u> | A231 | 2008 | Santiago | OXA-51 | OXA-58-like | XDR | 16 | ß | 3 |
| | | | | A93-05 | 1993 | Concepción | OXA-400 | | MDR | 1 | 2 | 3 |
| | | | | A97-02 A90-103 | 1997 1990 | Concepción Tolor | 0XA-67 0XA-86 | | MDR | 2 0.25 | 2 | ŝ |
| | | | 1 | A90-105 A90-47 | 1990 | Taica Sentiago | OXA-50 OXA-57 | | MDR MDR | 0.25 | 1 | \$ \$ |
| Π | 1 | | · | A93-03 🔴 | 1993 | Concepción | OXA-87 | | MDR | 0.5 | 1 | 8 |
| | • | | ;ſ── ╹ ──── | A97-08 | 1997 | Grincapolun | QXA-87 | | MDR | 0.25 | 1 | ន |
| | ST109 | | ┥└──── | A93-78 | 1993 | Talca | OXA-67 | | MDR | 0.5 | 1 | 9 |
| 4 | | | | A97-11 🌑 A417 | 1897 2012 | Concepción Santiago | OXA-400 OXA-67 | OXA-58-like | MDR PDR | 0.25 | 8 | S R |
| | | | ;C | A418 | 2012 | Santiago | OXA-57 | OXA-55-like | XDR | 16 | 4 | R |
| | | ∥ | | A560 | 2015 | La Serenz | OXA-51 | OXA-23-like | XDR | 64 | 128 | 5 |
| | | | | A554 | 2015 | La Serena Sentrere | OXA-51 | OXA-23-like | XDR | 32 | 16 8 | S |
| | | | 1 | A419 A93-76 | 2012 1993 | Santiago Talas | OXA-219 OXA-258 | ISAba1-OXA-51-like | XDR | 8 0.25 | • | R |
| | | | <u> </u> | A535 | 2014 | Los Angeles | OXA-219 | ISAba1-QXA-51-like | XDR | 8 | 8 | 9 |
| | | | | A9D-67 | 1990 | Temuco | OXA-400 | | | 0.5 | 1 | 3 |
| | | | <u>.</u> | A90-68 | 1990 | Temuco | OXA-400 | | MDR | 0.25 | 1 | 9 |
| | | | 1 | A93-02 A97-08 | 1993 1997 | Cancepción Cancepción | OXA-400 OXA-400 | | MDR MDR | 1 | 1 | 5 5 |
| | | | · | A232 | 2008 | Santiago | OXA-67 | OXA-58-like | XDR | a | 4 | s |
| | | | | A362 | 2007 | Santiogo | OXA-219 | ISAba1-OXA-51-like | XOR | 18 | 32 | з |
| + | | | · | A331 | 2006 | Taleahuane | OXA-69 | ISAba1-OXA-51-like | XDR | 16 | 4 | 3 |
| | | | i I | A546 🌑 A545 | 2015 2015 | Temuco Temuco | OXA-219 OXA-219 | ISAba1-OXA-51-ike ISAba1-OXA-51-ike | XDR XDR | 4 8 | 9 5 | 9 5 |
| | 11 | | | A543 | 2015 | Temuco | OXA-219 | ISAba1-OXA-51-like | XDR | 8 | 8 | s |
| 1 | ST15 | | ┢────┤└── | A542 | 2015 | Temuco | OXA-219 | ISAba1-OXA-51-like | XDR | 4 | 4 | 5 |
| 11 | | | | A541 🌰 | 2015 | Temuco | OXA-219 | ISAbs1-OXA-51-like | XDR | 18 | 16 | 8 |
| | | | | A562 💭 | 2015 2015 | La Serena La Serena | OXA-51 OXA-51 | OXA-23-like OXA-23-like | XOR XDR | 32 32 | 16 16 | 9 |
| | | | | A556 | 2015 | La Serena | OXA-61 | OXA-23-like | XDR | 32 | 32 | s |
| T | | | | A93-79 | 1993 | Taica | OXA-71 | | MDR | 0.5 | 2 | \$ |
| | | | 1 | A93-80 | 1993 | Taica | OXA-71 | - | MDR | 1 | 1 | 5 |
| | | | <u> </u> | A369 A353 | 2009 2009 | Temuco Temuco | OXA-51 OXA-219 | OXA-23-like ISAba1-OXA-51-like | XDR XDR | 64 8 | 32 8 | \$ \$ |
| | | | | A341 | 2005 | Talsahuano | OXA-59 | | NDR | 4 | 4 | 8 |
| Г | | | · | A356 🌰 | 2009 | Temuce | OXA-51 | OXA-23-like | XDR | 64 | 32 | 3 |
| | | ST162 | ∦ | A352 🌰 A355 | 2009 | Татисе | OXA-69 | OXA-23-like | XDR | 64 | 32 | 9 |
| Щ- | + | + | | A350 A360 | 2009 | Temuco | OXA-51 OXA-51 | OXA-23-like OXA-58-like | XDR XDR | 32 | 32 | 5 5 |
| | | ┥└───┤└─── | ;L | A367 | 2007 | Santiago | OXA-51 | OXA-58-like | XDR | 15 | 16 | s |
| | | | <u> </u> | A361 | 2007 | Santiago | OXA 61 | OXA 52 like | XDR | 18 | 125 | S |
| | | | ! | A544 | 2016 | Temuco | OXA-219 | ISAba1-OXA-51-like | XDR | 4 | 4 | 8 |
| | | | · · · · · · · · · · · · · · · · · · · | A93-91 A549 | 1984 2015 | Santiago Santiago | OXA-217 OXA-51 | | MDR | 0.125 1 | 1 | 3 |
| | - | | ; | A354 | 2009 | Temuco | OXA-132 | OXA-23-like | XDR | 32 | 32 | 3 |
| | | | <u> </u> | A364 | 2007 | Santiago | OXA-51 | OXA-58-like | XDR | 16 | 4 | 6 |
| | | — I · · · · · · · · · · · · · · · · · · | ! | A357 A551 | 2009 | Temuco Sections | OXA-51 | OXA-23-like | XDR XDR | 32 64 | 32 32 | \$ 5 |
| | | | | A551 A463 | 2015 2011 | Sentiago Santiago | OXA-51 OXA-51 | OXA-23-like OXA-23-like | XDR | 64 64 | 32 | 8 5 |
| | | | i | A222 | 2006 | Tajaahuano | QXA-65 | | MOR | 1 | 2 | 9 |
| | Ц | | i | A336 | 2005 | Talcahuano | OXA 65 | | MDR | 1 | 2 | 3 |
| | | | <u> </u> | A552 A90-26 | 2015 | Santiago Concención | OXA-120 OXA-58 | | | 1 0.25 | 1 | 5 6 |
| | | | | A90-25 A209 | 1990 2001 | Concepción Iquique | OXA-58 OXA-37 | | MDR | 2 | 1 2 | 5 5 |
| | | | i | A223 | 2008 | Tainahuano | OXA-59 | | XDR | 18 | 16 | s |
| | | | ī | A329 | 2005 | Tolcahuano | OXA-65 | ISAba1-OXA-51-like | XDR | 18 | 32 | 5 |
| | | | <u> </u> | A93-75 A90-44 | 1993 1990 | Talca Talca | OXA-87 OXA-400 | | MDR MDR | 0.125 | 1 | s s |
| | | | r | A93-72 | 1993 | Taica Taica | OXA-400 | | MDR | 0.25 | 1 | 3 |
| | | | <u> </u> | A547 | 2015 | Temuco | OXA-219 | ISAba1-OXA-51-like | XDR | 8 | 4 | \$ |
| | | | L | A564 | 2015 | La Serens | OXA-51 | OXA-58-like | XDR | 32 | 8 | 5 |
| | | | <u> </u> | A550 A563 | 2015 2016 | Santiago La Serena | OXA-219 OXA-51 | ISAba1-OXA-51-like OXA-23-like | XDR XDR | 8 32 | 5 16 | 5 S |
| | | | | A93 01 | 2018 1993 | La screna Concepción | OXA 87 | and a second and the last | MDR | 0.5 | 1 | \$ \$ |
| r | | ST162 + ST318 | i | A90-41 🌰 | 1990 | Vaiparaiso | OXA-57 | | MOR | 1 | 1 | 2 |
| | IV | | ; | A93-04 | 1993 | Concepción | OXA-67 | | MDR | 1 | 1 | s |
| | | | • | A462 A93-08 | 2011 1993 | Santiago Concepción | OXA-219 OXA-67 | OXA-58-like | XDR MDR | 16 0.5 | 9 1 | 9 \$ |
| | | <u> </u> | · | A93-08 A540 | 2015 | Cancepcion Temuco | OXA-37 OXA-219 | ISAbs1-OXA-51-like | XDR | 0.5 8 | 4 | 8 8 |
| | | | ;L | A535 | 2014 | Los Angeles | OXA-219 | ISAba1-OXA-51-like | XDR | e. | 8 | s |
| | | | ; ; | A534 | 2014 | Los Angeles | OXA-219 | ISAba1-OXA-51-lke | XDR | 8 | 8 | 3 |
| | | | · | A538 | 2014 | Los Angeles | QXA-219 | ISAba1-QXA-S1-like | XDR | a | 9 | 3 |
| | | | | | | | | | | | | |