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PROGRAMA ACADÉMICO DE MEDICINA

**Emergence and spread of carbapenem-resistant
Acinetobacter baumannii international clones II and
III in Lima, Perú**

TRABAJO DE SUFICIENCIA PROFESIONAL

PARA OPTAR POR EL TÍTULO DE MÉDICO-CIRUJANO

Autor:

Saul Levy Blitchtein (0000-0001-5673-5477)

Asesora:

Juana Del Valle Mendoza (0000-0002-6011-5040)

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A mis padres y a mi hermana,

Agradecimientos:

A mis padres por su constante apoyo.

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A todos los participantes que desde diferentes áreas de la ciencia y lugares del mundo contribuyeron a la realización del presente trabajo.

La science ne connaît aucun pays, parce que la connaissance appartient à l'humanité et est la torche qui illumine le monde. (Luis Pasteur)

Resumen

Introducción: *Acinetobacter baumannii* es el principal patógeno en la lista de la Organización Mundial de la Salud de resistencia antibiótica. Este ha surgido como patógeno a nivel mundial debido a la expansión de clonas epidémicas internacionales (CI) productoras de carbapenemasas de clase D (tipo OXA). Durante la última década, sin embargo, los reportes de la CI-I en Latinoamérica son escasos e inexistentes para las CI-II y CI-III.

Objetivo: Este estudio evalúa la resistencia fenotípica, la presencia de mecanismos moleculares de resistencia a carbapenemos y la relación clonal de 80 muestras clínicas de *A. baumannii* recolectados desde Febrero de 2014 y Abril de 2016 en dos hospitales de tercer nivel en Lima.

Materiales y métodos: La identificación de especies se realizó por espectrometría de masas (MALDI-TOF MS), la susceptibilidad antimicrobiana por difusión de discos y E-test, excepto para colistina, que fue determinado por microdilución en caldo de cultivo e interpretado según las guías del CLSI. Para tigeciclina se utilizó las guías EUCAST para Enterobacterias. Los genes de resistencia a carbapenémicos se detectaron por PCR y secuenciación. La relación clonal se estudió por electroforesis de campo pulsado (PFGE) y MLST con el esquema Pasteur.

Resultados: La mayoría de las cepas eran resistentes a carbapenémicos (97.5%) y la susceptibilidad se encontraba elevada únicamente para colistina (95%). La electroforesis de campo pulsado identificó dos clonas principales en ambos hospitales: la clona D comprendiendo 51 aislamientos (61.3%) asociada con la secuencia-tipo 2 (ST2) portadora de OXA-72 y la clona F de 13 aislamientos (16.3%), asociada con

ST79 y portadora de OXA-72. Estas eran endémicas en al menos un hospital. Se identificaron cepas ST1 y ST3 productoras de OXA-23, representando aislamientos esporádicos. Resulta resaltante la presencia de la nueva variante OXA-253 de la OXA-143 con una nueva secuencia de inserción (IS*Aba47*).

Conclusión: Mientras las líneas clonales predominantes de *A. baumannii* en Latinoamérica se relacionan con ST79, ST25, ST15 y ST1 productoras de OXA-23, en el estudio se reporta el surgimiento de ST2 altamente resistentes (CI-II) productoras de OXA-72 y la primera identificación de ST3 (CI-III) en Latinoamérica, ambas representando un serio riesgo para la salud pública a nivel mundial.

Palabras clave: *Acinetobacter baumannii*; resistencia antimicrobiana; mecanismos moleculares de resistencia; clonas epidémicas

Abstract

Background: Carbapenem-resistant *Acinetobacter baumannii* is the top-ranked pathogen in the World Health Organisation priority list of antibiotic-resistant bacteria. It emerged as a global pathogen due to the successful expansion of a few epidemic lineages, or international clones (IC), producing acquired class-D carbapenemases (OXA-type). During the last decade, however, reports regarding IC-I isolates in Latin America are scarce and non-existent for IC-II and IC-III isolates.

Objective: This study evaluates the phenotypic resistance patterns, the presence of carbapenem-resistance mechanisms and the clonal relatedness of 80 non-duplicate clinical samples of *A. baumannii* collected from February 2014 through April 2016 at two tertiary care hospitals in Lima.

Methods and materials: Species identification was performed by MALDI-TOF MS, antimicrobial susceptibility testing was analysed by disk diffusion and E-test for all antibiotics but colistin, which was determined by broth microdilution, and interpreted according to CLSI guidelines for *Acinetobacter* spp. Tigecycline results were interpreted following EUCAST guidelines for Enterobacteriaceae. Carbapenemase genes were detected by PCR and Sanger DNA sequencing. The clonal relatedness was examined by pulsed-field gel electrophoresis (PFGE) and MLST with the Pasteur scheme.

Results: Almost all isolates were carbapenem-resistant (97.5%), and susceptibility only remained high for colistin (95%). PFGE showed two main clusters spread between both hospitals: cluster D containing 51 isolates (63.8%) associated with sequence type 2

(ST2) and carrying OXA-72, and cluster F containing 13 isolates (16.3%) associated with ST79 and also carrying OXA-72. ST2 and ST79 were endemic in at least one of the hospitals. International clone I (IC-I) ST1 and IC-III (ST3) OXA-23-producing isolates were also identified. They accounted for sporadic hospital isolates. Interestingly, two isolates carried the novel OXA-253 variant of OXA-143 together with an upstream novel insertion sequence (*ISAb₄₇*).

Conclusion: While the predominant *A. baumannii* lineages in Latin America are linked to ST79, ST25, ST15, and ST1 producing OXA-23 enzymes, we report the emergence of highly-resistant ST2 (IC-II) isolates in Peru producing OXA-72 and the first identification of ST3 isolates (IC-III) in Latin America, both considered a serious threat to public health worldwide.

Key words: *Acinetobacter baumannii*; antibiotic resistance; molecular mechanisms of resistance; epidemic clones.

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1. Introduction

Acinetobacter baumannii is an opportunistic nosocomial pathogen responsible for a broad range of nosocomial infections¹, including ventilator-associated pneumonia and bacteraemia (35-52% mortality)^{1,2}, as well as skin and soft tissue infections, endocarditis, urinary tract infections and meningitis^{1,3}. Nosocomial isolates of this bacterium are often resistant to most currently available antibiotics. Carbapenem-resistant *A. baumannii* has recently been considered the most critical pathogen for public health, topping the global priority list of antibiotic-resistant bacteria published by the World Health Organization⁴. *Acinetobacter baumannii* strains can develop resistance to all the antibiotics available⁵. Outbreaks caused by multidrug-resistant (MDR), extensively drug-resistant (XDR) and even pan-drug-resistant (PDR) strains having been reported worldwide⁶⁻⁹. In the past decade, resistance rates have been rising, and recent reports show a steady increase in carbapenem resistance among *A. baumannii* strains^{8,10-13}.

Resistance to carbapenems in *A. baumannii* is usually mediated by the expression of carbapenem-hydrolysing class D β -lactamases, also known as OXA-type carbapenemases, although the expression of different class B metallo- β -lactamases (MBLs), or even *Klebsiella pneumoniae* carbapenemase (KPC) enzymes, has also been reported.⁵ The OXA-type enzymes described in *A. baumannii* belong to six different families, namely the intrinsic OXA-51 oxacillinase family, which is usually chromosomally encoded but has rarely been reported in plasmids¹⁴, and the acquired OXA-23, OXA-24, OXA-58, OXA-143, and OXA-235 families⁵. Although the population structure of *A. baumannii* strains is quite diverse, there seems to be a clonal spread of a few epidemic lineages that predominate over the rest¹⁵. In particular, the

international clones I-III account for most *A. baumannii* infections worldwide and are usually associated with the production of either OXA-23-like, OXA-24-like, or OXA-58-like enzymes¹⁵.

Our knowledge of the epidemiology and antibacterial susceptibility profiles of *A. baumannii*, however, is still incomplete in many parts of the world, including many countries in Latin America. The present study was designed to evaluate the phenotypic resistance patterns, the presence of carbapenem-resistance mechanisms, and the clonal relatedness of *A. baumannii* isolates circulating in Lima, Peru.

2. Methods and materials

1. Samples

This study included 80 consecutive clinical isolates of *A. baumannii* collected from different inpatients at two tertiary care hospitals (HNAL and INEN) from February 2014 through April 2016 in Lima, Peru. Only one isolate from each patient was included in the study.

The samples were obtained from blood, bronchial aspirate, soft tissues, cerebrospinal fluid, and urine, all of which were initially identified as *Acinetobacter* spp. by the BD Phoenix™ Automated Microbiology System (BD Biosciences, USA) and later identified to the species level by matrix-assisted laser desorption ionisation–time of flight mass spectrometry (Bruker, Germany) as described previously¹⁶.

2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was assessed by disc diffusion on Mueller-Hinton agar plates in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines for the following antimicrobials: ampicillin-sulbactam, piperacillin-tazobactam, cefotaxime, ceftazidime, cefepime, gentamicin, amikacin, levofloxacin, doxycycline, tetracycline, meropenem, imipenem, and trimethoprim-sulfamethoxazole. Susceptibility to colistin was assessed by broth microdilution as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group¹⁷. The MIC values of selected strains were also determined by gradient diffusion (E-test, bioMérieux, Sweden) for the following antimicrobials: imipenem, meropenem, cefotaxime, ceftazidime, cefepime, gentamicin, amikacin, tigecycline, ciprofloxacin and levofloxacin. The MICs were interpreted according to CLSI clinical breakpoints and expert rules for *Acinetobacter*¹⁷, except for tigecycline, which was interpreted using the EUCAST breakpoints and rules for Enterobacteriaceae (Version 8.0,

January 2018)¹⁸. *Escherichia coli* ATCC 25922 and *A. baumannii* ATCC 19606 were used as quality control strains.

All the isolates were categorised as MDR, XDR or PDR according to the following ad hoc definitions; MDR, non-susceptible to at least one antimicrobial agent from three classes tested; XDR, non-susceptible to all antimicrobial agents tested but two or fewer; PDR, resistant to all antimicrobial agents tested⁷.

3. Detection of carbapenem resistance genes

The presence of the following carbapenemase-encoding genes was screened by PCR: *bla*_{KPC}, for serine class A carbapenemases;¹⁹ *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{SPM}, and *bla*_{SIM} for class B MBLs;²⁰ and *bla*_{OXA-51-like}, *bla*_{OXA-23-like}, *bla*_{OXA-24-like}, *bla*_{OXA-58-like}, *bla*_{OXA-143-like}, and *bla*_{OXA-235-like}, for class D oxacillinases²¹⁻²³. Amplification products were purified from agarose gels (SpinPrepTM Gel DNA Kit, San Diego, USA) and further sent for Sanger sequencing (Macrogen, Korea) whenever necessary. The genetic identity of *bla*_{OXA} genes was determined upon pairwise sequence alignment with reference sequences retrieved from <http://www.lahey.org/Studies/>.

The presence of IS sequences flanking the *bla*_{OXA} genes was studied by PCR using specific primers as well as by inverse PCR and primer walking whenever needed. IS structures were annotated manually using BLASTn and the NCBI bacterial and ISFinder databases^{24,25}.

4. Molecular typing

Pulsed-field gel electrophoresis (PFGE) was performed as described previously¹¹, using genomic digestions with the *Apa*I restriction enzyme and a CHEF-DRIII system (Bio-Rad Laboratories). Molecular patterns were analysed with the InfoQuestTM FP v.5.4 software

(Bio-Rad laboratories) and the unweighted pair group method with arithmetic mean to create dendrograms based on Dice's similarity coefficient. Using bandwidth tolerance and optimisation values set at 1.5% and 1%, respectively, isolates were considered to belong to the same PFGE cluster (pulsotype) if their Dice similarity index was $\geq 85\%$ ²⁶.

MLST was performed using the Pasteur scheme for *A. baumannii*²⁷. The allele sequences and STs of selected strains were identified and retrieved from the PubMLST *A. baumannii* MLST database (<http://pubmlst.org/abaumannii/>). The population structure of STs was evaluated using the goeBURST software (<http://www.phyloviz.net/goeburst/>).

3. Results

1. Bacterial Isolates

A total of 80 non-redundant *A. baumannii* isolates were recovered from blood (n=59, 73.8%), bronchial aspirate (n=15, 18.8%), soft tissue (n=2, 2.5%), cerebrospinal fluid (n=2, 2.5%), and urine samples (n=2, 2.5%) of different patients admitted at two tertiary care hospitals in Lima. Fifty-three isolates were recovered at the Instituto Nacional de Enfermedades Neoplásicas (INEN) from February 2014 through April 2016, and the remaining 27 isolates originated from inpatients at the Hospital Nacional Arzobispo Loayza (HNAL) from July through October 2015.

2. Antibiotic susceptibility

Overall, antimicrobial susceptibility testing by disc diffusion reported high resistance rates to most of the antibiotics tested. More specifically, resistance to both imipenem and meropenem was as high as 97.5%, with only two isolates being susceptible to carbapenems, one from each hospital. The non-susceptibility rates to ceftazidime, cefotaxime,

piperacillin/tazobactam and levofloxacin were 98.8%, 97.5% to cefepime, 93.8% to trimethoprim-sulfamethoxazole, 80% to tetracycline, 76.3% to gentamicin, 63.8% to ampicillin/sulbactam, 62.5% to doxycycline, 61.3% to amikacin and 57.5% to tobramycin. Susceptibility only remained high for colistin (95%); the colistin minimal inhibitory concentrations (MICs) ranged from 0.25 mg/L to 16 mg/L. The colistin MIC₅₀ and MIC₉₀ were of 1 mg/L and 2 mg/L, respectively.

All the isolates were resistant to at least one antibiotic from three different classes. One isolate (1.2%) was resistant to all the antimicrobial agents tested and was therefore classified as PDR. Thirty-seven isolates (46.3%) presented an XDR phenotype, most being susceptible to colistin alone, and the remaining 42 isolates (52.5%) were therefore considered MDR.

Antibiotic susceptibility testing by gradient diffusion of selected strains showed elevated MIC values of carbapenems (>32 mg/L) in all the strains producing acquired class D carbapenemases as well as elevated MIC values of cephalosporins, aminoglycosides and quinolones, in good agreement with previous data obtained by disc diffusion (Table 1). Interestingly, three strains presented MIC values of tigecycline of 4 mg/L and therefore should be considered resistant according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for Enterobacteriaceae¹⁸.

3. Detection of carbapenem-resistance genes

The presence of genes encoding both intrinsic and acquired class D carbapenemases, KPC, and MBLs was investigated by polymerase chain reaction (PCR). None of the isolates were positive for the genes encoding KPC or MBLs, but the intrinsic *bla*_{OXA-51}-like gene was detected in all the samples. In two isolates, this gene was the only carbapenemase detected,

although it could not be associated with the presence of an upstream *ISAbal* element, in good agreement with the carbapenem susceptible phenotype of these two isolates.

The *bla*_{OXA-24}-like gene, however, was predominant and present in 65 isolates (81.3%) collected from both hospitals, whereas 11 isolates (13.8%) carried the *bla*_{OXA-23}-like gene, and 2 isolates (2.5%) proved positive in the multiplex PCR for *bla*_{OXA-143}-like gene. Overall, there were no substantial differences between the two hospitals regarding the proportion of isolates carrying different acquired class D carbapenemases (Table 2).

4. Pulsed-field gel electrophoresis

The clonal relatedness was initially studied by pulsed-field gel electrophoresis (PFGE), as described, which allowed the classification of all the isolates into nine different clusters, or pulsotypes (A-I) (Figure 1). Two major clusters disseminating between the two healthcare settings were identified; pulsotypes D and F, which contained 51 (63.8%) and 13 (16.3%) isolates, respectively. All the isolates in these two clusters were associated with the carriage of OXA-24-like carbapenemases except for one isolate within pulsotype F with no acquired carbapenemases that was susceptible to carbapenems. OXA-23-like producing *A. baumannii* were mostly grouped in pulsotype G (n=6, 7.5%), containing isolates recovered from both hospitals, and pulsotype A, with only 4 isolates (5%) that were recovered from INEN. Pulsotype I contained only two isolates also recovered from INEN, but one isolate carried an OXA-23-like enzyme, while the other carried an OXA-143-like oxacillinase. Pulsotypes B, C, E, and H contained singletons (1.3%) producing either OXA-24-like (pulsotypes E and H), OXA-143-like (pulsotype B) or no acquired OXA-like enzymes (pulsotype C), the latter

isolate being susceptible to carbapenems. Figure 2 shows the temporal and spatial distributions of all the clones.

5. Multilocus sequence typing analysis and OXA sequencing of selected strains

Eighteen isolates were selected for further characterisation on the grounds of their clustering in the PFGE dendrogram, susceptibility profiles, and carriage of acquired OXA-type carbapenemases (Table 1).

Multilocus sequence typing (MLST) analysis using the Pasteur scheme of selected isolates showed full agreement among isolates clustered within the same pulsotype and ST designation (Table 1). Isolates from the major PFGE cluster D as well as the singleton from pulsotype E were assigned to ST2 (ST2), which belongs to clonal complex 2 (CC2) and to international clone II. Isolates from the PFGE cluster F, both isolates from pulsotype I and the singletons B and C were assigned to ST79, which is included in CC79, international clone V²⁸. Isolates from pulsotype A were assigned to ST1, belonging to CC1 and international clone I, and those of pulsotype G to ST3, belonging to CC3, international clone III. The H singleton belonged to ST108, which is not assigned to any CC or international clone (Figure 3).

Sequencing of the acquired class D oxacillinase genes in all the selected isolates revealed the presence of the OXA-72 variant in ST2, ST79 and ST108 isolates bearing a *bla*_{OXA-24}-like gene as well as the presence of the novel OXA-253 variant in the two isolates with a *bla*_{OXA-143}-like allele. Likewise, sequencing of the intrinsic class D oxacillinase gene identified specific OXA-51-like variants associated with each ST: ST1 isolates presented the *bla*_{OXA-69} gene, ST2 was

associated with *bla*_{OXA-66}, ST3 isolates carried the *bla*_{OXA-71} gene, ST79 was linked with *bla*_{OXA-65}, and ST108 had the *bla*_{OXA-132} variant (Table 1).

Of note, sequence analysis of the genetic structures surrounding the *bla*_{OXA} alleles identified the presence of insertion sequence *IS**Aba1* in reverse orientation upstream from the *bla*_{OXA-23} gene in all ST1 and ST3 isolates, although we were not able to detect the presence of a downstream *IS**Aba1* copy, suggesting a Tn2008-like structure in these isolates²⁹. Additionally, a novel insertion sequence was identified in forward orientation upstream from *bla*_{OXA-253} in the two strains carrying this OXA enzyme. To provide an attribution number to the new IS, its nucleotide sequence was submitted to ISFinder, the reference center for bacterial insertion sequences (<http://www-is.biotoul.fr>)²⁴, and it was designated as *IS**Aba47*. This novel mobile element showed 82.5% identity at the nucleotide level with *IS**Aba9* and was related to the IS982 family. The downstream region flanking *bla*_{OXA-253}, however, resembled that previously described by Girlich *et al.*³⁰ A 2,334 bp sequence containing the *bla*_{OXA-253} gene together with upstream and downstream flanking regions (which include *IS**Aba47*) from strain 33 was also submitted to GenBank and can be retrieved under accession number MH347317.

4. Discussion

Over the last decades we have witnessed the emergence of MDR *A. baumannii* isolates worldwide, which has been associated with the rapid spread of a few carbapenem-resistant epidemic lineages producing acquired OXA-type carbapenemases^{1,15}. To date, however, there are only a few studies regarding the epidemiology and carbapenem susceptibility of *A. baumannii* in Latin America, and this is the first study providing such data from Peru.

Interestingly, the predominant *A. baumannii* lineages reported in Latin America so far are different from those reported in other parts of the world, as international clones II and III are currently absent in this region. International clone II was reported in Brazil in the past, when ST2 isolates producing OXA-23 were described from 1999 through 2003, but it disappeared in 2004^{31,32}. Instead, predominant clones are linked to ST79 (international clone V), ST25 (international clone VII), ST15, and, to a lesser extent, ST1 (international clone I), all of which are mainly associated with the production of OXA-23 enzymes^{28,33-40}.

Nevertheless, there are a few reports describing OXA-72-producing *A. baumannii* isolates in Latin America, and overall, these isolates are considered less prevalent⁴⁰⁻⁴³, or at least they were until recently. In 2017 Pagano et al.⁴⁴ described the emergence of CC79 and CC15 *A. baumannii* isolates carrying *bla*_{OXA-72} in Brazil and warned of the potential dissemination of these epidemic lineages. Likewise, Nuñez Quezada et al.⁴⁵ reported an outbreak in Ecuador in 2017 caused by OXA-72-producing *A. baumannii*⁴⁵, although the ST of these isolates was not investigated.

The data presented in this study show extremely high resistance rates (>97%) and MIC levels (>32 mg/L) of imipenem and meropenem as well as the predominance of the MDR phenotype among *A. baumannii* isolates recovered from two tertiary hospitals in the capital city of Peru. Fortunately, the majority (95%) of isolates still remain susceptible to colistin, as opposed to several studies reporting increasing rates of colistin resistant *A. baumannii* isolates in different countries⁸. During the study period, carbapenem resistance in both settings was linked to the widespread dissemination of a major clone producing OXA-72 and belonging to ST2, the founder ST of the epidemic international clone II that has spread globally (Figure

3)¹⁵. A second group of highly clonal strains producing OXA-72 and belonging to ST79, international clone V, was also identified in both hospitals. In addition, we report the presence of a few sporadic clones producing OXA-23 and linked to international clones I and III (ST1 and ST3, respectively).

International clonal lineages have traditionally been associated with the carriage of specific genetic variants of the intrinsic *bla*_{OXA-51} gene^{34,38,39}. This correlation is also shown in our study and supports the genetic relatedness of these isolates with epidemic international lineages. The *bla*_{OXA-65}, *bla*_{OXA-66}, *bla*_{OXA-69}, and *bla*_{OXA-71} genes were detected in isolates belonging to ST79, ST2, ST1 and ST3, respectively. We also identified the *bla*_{OXA-132} variant in a ST108 isolate that does not belong to any known CC. Interestingly, a single ST108 isolate carrying *bla*_{OXA-132} was reported in Lebanon in 2014, and *bla*_{OXA-132} was also present in ST15 isolates in Portugal as well as in a ST197 isolate from Saudi Arabia^{37,46,47}.

The temporal and spatial distributions of the isolates (Figure 2) show that the OXA-72-producing ST2 clone was present in both centres throughout the study period and was likely endemic in INEN, while OXA-72-producing ST79 isolates were sporadic during 2014 and also became endemic at INEN during the second half of 2015. Unfortunately, the collection period of *A. baumannii* isolates from HNAL was too short to extrapolate endemicity, but as many as 17 ST2 isolates were recovered from July through September 2015, suggesting at least an outbreak situation (Figure 2). Patients are frequently referred between the two hospitals because INEN gathers most patients with neoplastic diseases. That might explain the presence of shared high-risk clones. Of note, 29 out of 37 XDR isolates were OXA-72-

producing ST2 isolates. The single PDR isolate, however, was an OXA-72-producing ST79 isolate (Figure 1).

We would also like to highlight the identification of two different ST79 isolates carrying an OXA-253 variant from the OXA-143 family. OXA-253 was first described from an ST79 *A. baumannii* isolate in Honduras, and to date, only a few additional studies have reported this enzyme in *A. baumannii* isolates from different STs in Brazil^{30,48,49}. In addition, the genetic environment of *bla*_{OXA-253} in this study resembled that previously described by Girlich *et al.*³⁰, except for the presence of a novel insertion sequence (*IS**Aba47*) in the upstream region. *IS**Aba47* might have a role in the mobilization and expression of this gene, as it is already the case for the *bla*_{OXA-23}, *bla*_{OXA-58} and *bla*_{OXA-235} genes encoding likewise acquired OXA-type carbapenemases that typically present different flanking *IS**Aba* sequences⁵. To our knowledge, this is the first time that the presence of an IS is reported to flank a gene belonging to the *bla*_{OXA-143} family.

Conclusions: In summary, while the emergence of carbapenem resistant *A. baumannii* in Latin America has been associated with the spread of OXA-23-producing ST1, ST15, ST25 and ST79 clonal lineages³⁸, the results of our study reveal the dissemination of OXA-72-producing XDR clones of *A. baumannii* in Peru as well as the identification of the epidemic international clones II and III, which were previously absent in this region. The findings presented here are extremely worrisome and should warrant the implementation of infection control measures as well as national and international surveillance measures in the region to contain the further spread of what could be considered nosocomial pandemic lineages of XDR *A. baumannii*.

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Author contributions

Collected the samples: SL, SP-R, WV-T, JV-P. Conceived and designed the experiments: IR, LM, JdVM, JV. Performed the experiments: SL, IR, SP-R, LM, JM-M MJP. Analysed the data: SL, IR, SP-R, WV-T, JV-P, JM-M, MJP, JdVM, JV. Wrote the paper: SL, IR, JdVM, JV. All authors critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Notes

Conflict of Interests

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare that they have no conflicts of interest.

Ethics approval

This study was performed on clinical laboratory isolates. The authors had no contact or interaction with the patients. Personal information of the patients was not collected, to guarantee anonymity and confidentiality. Ethics approval was obtained from the Committee of the Instituto de Investigación Nutricional (IIN), Lima, Peru.

Footnotes

These authors contributed equally: Saúl Levy-Blitchtein, Ignasi Roca.

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Annex

Table 1. Antimicrobial susceptibility and molecular characterisation of selected *A. baumannii* isolates from two tertiary hospitals in Lima, Peru

Strain	Source	MIC (mg/L)											PT	ST	aOXA	iOXA
		IP	MP	CAZ	FEP	CTX	AK	GM	COL ^a	TGC	CiP	LEV				
3	INEN	>32	>32	>256	192	>32	48	>256	0.5	4	>32	>32	A	1	23	69
29	INEN	>32	>32	>256	256	>32	96	>256	1	4	>32	>32	A	1	23	69
33	INEN	>32	>32	>256	48	>32	32	2	0.5	4	>32	>32	B	79	253	65
54	HNAL	0.38	0.75	4	16	>32	96	3	1	2	>32	>32	C	79	-----	65
21	INEN	>32	>32	128	16	>32	>256	>256	2	2	>32	>32	D	2	72	66
34	INEN	>32	>32	>256	24	>32	3	2	0.5	3	>32	>32	D	2	72	66
47	INEN	>32	>32	192	24	>32	>256	>256	0.5	3	>32	32	D	2	72	66
55	HNAL	>32	>32	192	24	>32	>256	>256	0.5	3	>32	>32	D	2	72	66
69	HNAL	>32	>32	>256	24	>32	>256	>256	0.5	2	>32	>32	D	2	72	66
79	HNAL	>32	>32	>256	24	>32	>256	>256	0.5	2	>32	>32	D	2	72	66
42	INEN	>32	>32	>256	24	>32	>256	>256	0.5	2	>32	>32	E	2	72	66

4	INEN	>32	>32	256	24	>32	16	>256	0.5	1.5	>32	>32	F	79	72	65
32	INEN	0.5	1	>256	24	>32	64	4	0.5	2	>32	32	F	79	-----	65
56	HNAL	>32	>32	96	24	>32	32	256	1	1.5	>32	32	F	79	72	65
37	INEN	>32	>32	96	32	>32	16	0.38	1	0.25	>32	32	G	3	23	71
60	HNAL	>32	>32	96	64	>32	32	0.38	1	0.5	>32	>32	G	3	23	71
61	HNAL	>32	>32	>256	16	>32	48	>256	1	1	>32	>32	H	108	72	132
53	INEN	>32	>32	>256	32	>32	24	2	0.5	2	>32	24	I	79	253	65

MIC: minimum inhibitory concentration; INEN: Instituto Nacional de Enfermedades Neoplásicas; HNAL: Hospital Nacional Arzobispo Loayza; IP: imipenem; MP: meropenem; CAZ: ceftazidime; FEP: cefepime; CTX: cefotaxime; AK: amikacin; GM: gentamicin; COL: colistin; TGC: tigecycline; CiP: ciprofloxacin; LEV: levofloxacin; PT: pulsotype; ST: sequence type. aOXA: acquired OXA-type; iOXA: intrinsic OXA-type; ^a Colistin MIC was determined by broth microdilution.

Table 2. Number and percentages of *A. baumannii* isolates from each participant centre carrying different acquired *bla*_{OXA} genes

OXA-type (acquired)	INEN		HNAL		Total	
	N	%	N	%	N	%
<i>bla</i> _{OXA-24}	43	81.1	22	81.5	65	81.3
<i>bla</i> _{OXA-23}	7	13.2	4	14.8	11	13.8
<i>bla</i> _{OXA-143}	2	3.8	0	0	2	2.5
None	1	1.9	1	3.7	2	2.5
Total	53		27		80	

N: number of isolates; %: Percentage of isolates; INEN: Instituto Nacional de Enfermedades Neoplásicas; HNAL: Hospital Nacional Arzobispo Loayza.

Figure1

Figure 2

[illegible]

Figure 1. PFGE dendrogram of *A. baumannii* isolates from two tertiary hospitals in Lima showing: carriage of intrinsic (iOXA) and acquired (aOXA) *bla*_{OXA} variants; susceptibility (S) or resistance (R) to carbapenems (Carb); categorisation as MDR, XDR or PDR (RStatus); pulsotype (PT); and sequence type (ST). Isolates in red were selected as representative of each clonal group. Braces indicate classification to the corresponding international clones I-V (IC). Isolates were included in the same pulsotype if their Dice similarity index is $\geq 85\%$. Coloured squares indicate production of distinct families of acquired OXA-type enzymes: Blue: OXA-23; Green: OXA-24; Black: OXA-143; Magenta: None. INEN: Instituto Nacional de Enfermedades Neoplásicas; HNAL: Hospital Nacional Arzobispo Loayza. MDR: multidrug resistant; XDR: extensively drug resistant; PDR; pan-drug resistant.

Figure 2. Temporal and spatial distribution of *A. baumannii* isolates recovered from patients at two tertiary hospitals in Lima, Peru. Stacked columns show the number of isolates from each pulsotype/sequence type (PT/ST) recovered at each institute over a three-months period. INEN: Instituto Nacional de Enfermedades Neoplásicas; HNAL: Hospital Nacional Arzobispo Loayza.

Figure 3. Partial snapshot of the population structure of *A. baumannii* according to MLST and using goeBURST (PHYLOViZ). Blue colour links show SLVs. Founder STs are highlighted in yellow. Reddish circles indicate isolates grouped into a particular international clone (IC). The black arrow points at ST108 and its SLV ST112.