

Origin of OXA-23 Variant OXA-239 from a Recently Emerged Lineage of *Acinetobacter baumannii* International Clone V

Lucia Graña-Miraglia,^a Benjamin A. Evans,^d Luis E. López-Jácome,^b Melissa Hernández-Durán,^b Claudia Adriana Colín-Castro,^b Patricia Volkow-Fernández,^c Miguel A. Cevallos,^a Rafael Franco-Cendejas,^b Santiago Castillo-Ramírez^a

^aPrograma de Genómica Evolutiva, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico

^bDivisión de Infectología, Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra, Mexico City, Mexico

^cInstituto Nacional de Cancerología, Mexico City, Mexico

^dNorwich Medical School, University of East Anglia, Norfolk, United Kingdom

Lucia Graña-Miraglia and Benjamin A. Evans shared first authorship; author order was determined by drawing straws. Rafael Franco-Cendejas and Santiago Castillo-Ramírez contributed equally to this study; author order was determined by reverse seniority.

ABSTRACT Over the last few decades, carbapenemase-producing *Acinetobacter baumannii* has become a major cause of nosocomial infections all over the world. However, the genome identity of lineages of this species in Latin America has not been studied as much as in developed countries. Here, through a population genomics approach considering the whole genomes of 148 isolates (almost 40 from Mexico and Honduras), we describe the recent emergence of the lineage sequence type 758 (ST758), which belongs to the international clone V and has spread out to Canada, Mexico, Honduras, and Colombia. Notably, this lineage was found to coexist with other *A. baumannii* lineages in hospitals in Mexico and Honduras. Isolates from this lineage show considerable variation in antibiotic resistance profiles, but most of them are resistant to carbapenems. Moreover, we found a variety of acquired oxacillinase (OXA) families within this lineage and tracked the very recent inception, and subsequent horizontal transmission, of the OXA-239 carbapenemase. This work highlights the urgent need to investigate recently emerged lineages of this species in Latin America and elsewhere, as these might harbor novel antibiotic resistance genes.

IMPORTANCE *A. baumannii* is a major cause of nosocomial infections all over the world. Although many isolates from developed countries have been studied in terms of their genome sequence, isolates from Latin America have been much less studied. In this study, using a population genomics approach considering the whole genomes of 148 isolates, we describe the recent emergence of the lineage ST758 endemic to Latin America and the inception of the OXA-239 carbapenemase. Our study highlights the urgent need to investigate recently emerged lineages of this species in Latin America and elsewhere, as these might harbor novel antibiotic resistance genes.

KEYWORDS evolutionary biology, infectious diseases, population genomics, *Acinetobacter baumannii*, OXA β -lactamases, OXA-239, molecular epidemiology, IC5, OXA, genome analysis, international clones

The constant overuse of antibiotics has made many bacterial infections harder to treat, and the global dissemination of antibiotic-resistant bacteria is currently one of the major public health concerns. In this regard, over the last decades, the Gram-negative opportunistic pathogen *Acinetobacter baumannii* has become a major cause of nosocomial infections with antibiotic-resistant phenotypes all over the world (1). This species consists of several, well-differentiated lineages, and many of them show

Citation Graña-Miraglia L, Evans BA, López-Jácome LE, Hernández-Durán M, Colín-Castro CA, Volkow-Fernández P, Cevallos MA, Franco-Cendejas R, Castillo-Ramírez S. 2020. Origin of OXA-23 variant OXA-239 from a recently emerged lineage of *Acinetobacter baumannii* international clone V. *mSphere* 5:e00801-19. <https://doi.org/10.1128/mSphere.00801-19>.

Editor Ana Cristina Gales, Escola Paulista de Medicina/Universidade Federal de São Paulo

Copyright © 2020 Graña-Miraglia et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Santiago Castillo-Ramírez, iago@ccg.unam.mx.

Received 1 November 2019

Accepted 9 December 2019

Published 8 January 2020

multidrug resistance (MDR) phenotypes (2). However, the most studied lineages are the three international clones (the European clones I, II, and III) sampled mainly in Asia, Europe, and the United States (3).

The traditional antimicrobial chemotherapy options against *A. baumannii* are carbapenems, but the constant use of them has been an important factor driving the appearance and dissemination of carbapenem-resistant *A. baumannii* strains worldwide. This resistance is mainly due to the production of carbapenem-hydrolyzing class D beta-lactamases, a particular group of oxacillinases (OXAs). The intrinsic OXA-51 family of β -lactamases has been found in all isolates of this species and is located in the chromosome, whereas acquired OXA families (OXA-23, OXA-40, OXA-58, OXA-143, and OXA-235) have been found in plasmids and occasionally also in the chromosome (4). Among these acquired families, OXA-23 seems to be the most prevalent around the world (5). OXA-239 is a variant of OXA-23, which has been recently described and only found in Mexico (6, 7).

Whole-genome sequencing (WGS) has become an essential tool to decipher the molecular evolution and phylogeography of bacterial pathogens (8–13). Furthermore, due to the very dynamic genome of *A. baumannii*, commonly used genotyping strategies (such as multilocus sequence typing [MLST]) do not accurately reveal the genetic relationships of the isolates under study (14). Thus, WGS is the only viable way to establish the true evolutionary relationships of the clones within this species. Additionally, WGS has been of paramount importance to study the transmission dynamics of antibiotic resistance genes (ARGs) in many bacterial populations (11, 15, 16). A considerable amount of information in terms of genomic sequences has been generated for *A. baumannii* isolates from Europe and the United States, yet very little is known about developing countries. Furthermore, although it is thought that international clones I to III (IC1 to IC3) cause most of the infections in the world, previous studies suggest that this might not be the case for Latin America (3, 17–21). In particular, IC4 and IC5 have been documented in this region (17, 20), and very recently, IC7 was also reported (22).

It is worth mentioning that the genome identity of lineages of this species in Latin America has not been studied as much as in developed countries. To solve this, we recently carried out a few studies. In the first study, we conducted WGS of 8 isolates from Mexico's National Institute of Oncology (INCAN) collected from 2011 to 2013 (19), whereas in the second study, we sequenced 16 isolates from 5 hospitals in Honduras (23). Here, to extend those two previous data sets and to put together the most extensive data set considering isolates from North America (Canada and Mexico) and Central America (Honduras), we sequenced another 10 isolates from the National Institute of Rehabilitation (INR), which is a tertiary hospital in Mexico City, Mexico. To properly understand the phylogenetic relationships of the Mexican and Honduran isolates, we also included a global data set of publicly available genomes representing many sequence types (STs) and the major international clones. We found an endemic clade (ST758) that recently emerged from within the IC5 and that has considerable variation in terms of antibiotic resistance genes. Moreover, we determined that OXA-239, an OXA-23 variant, very recently emerged within this clade.

RESULTS AND DISCUSSION

Novel lineages of *A. baumannii*. Among the almost 150 genomes considered for this study, 38 were either recently published by us or were sequenced in this study (see Table S1 in the supplemental material). These 38 isolates were collected from 2011 to 2016 in 6 hospitals located in 3 different cities in Mexico and Honduras. The maximum likelihood (ML) phylogeny (Fig. 1) shows that 20 of the isolates from Mexico and three from Honduras cluster in one clade (external cycle, Fig. 1). All but 3 isolates from this clade are ST758, as per the Oxford MLST scheme, and these 3 exceptions belong to ST1091, a single-locus variant (SLV) of ST758; this clade clusters with an ST758 Canadian strain (AB030) previously described (24). Both ST1091 and ST758 belong to clonal complex 636 (CC636), which is part of international clone V (IC5) (Fig. 2). According to

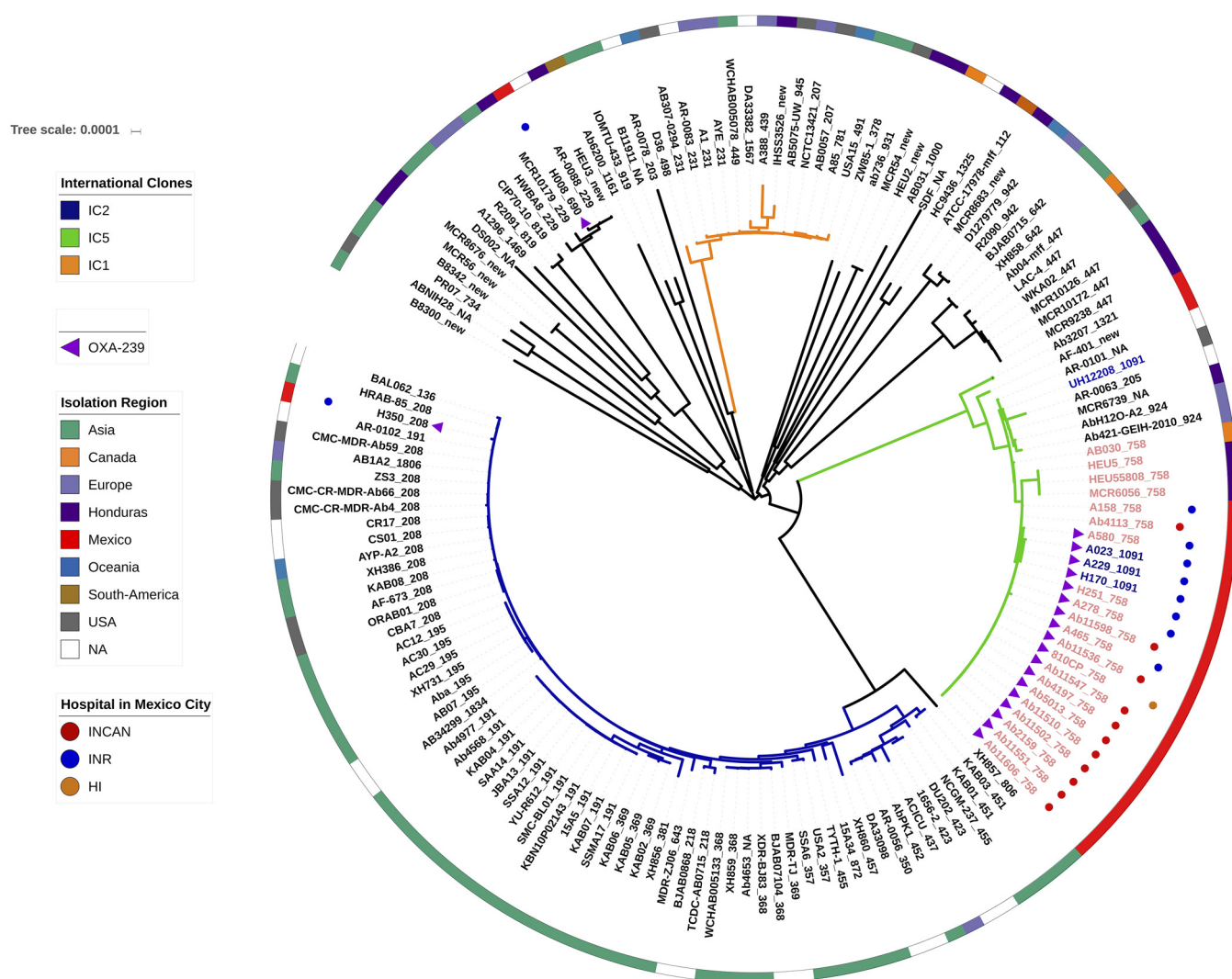


FIG 1 ML phylogeny showing the relationships among all the isolates. The external circle provides the geographic location of the isolates, when available. Colored dots next to some strains denote the Mexican hospitals. International clones I and II are identified by orange and blue branches, respectively, whereas green branches indicate international clone V. Pink isolates show the ST758 lineage, while the three blue isolates (A023, A029, and H170) clustering within the pink isolates belong to ST1091. The presence of the *bla*_{OXA-239} gene is marked with purple triangles. The scale bar gives the number of substitutions per site.

the PubMLST database, several studies have reported isolates from this ST in Canada (24), Mexico and Honduras (19, 23), and even Colombia (25). Of note, ST758 and ST1091 correspond to ST156 under the MLST Pasteur scheme. Then, given that previous publications considering Latin American isolates (20, 25, 26) have used the MLST Pasteur scheme, we constructed an eBURST diagram using the Pasteur scheme, and a partial view highlighting ST156 is shown in Fig. 2. This analysis shows that ST156 is an SLV of ST79 (Fig. 2) and that both STs are part of the CC79 that corresponds to IC5 (left-hand side, Fig. 2). IC5 has been found in North, Central, and South America and has been considered a Pan-American clone; notably, it very recently has been described in southeastern Europe as well (27). However, there are 11 Honduran isolates and two Mexican isolates that are assigned to other distantly related clades within the tree, demonstrating that several clones coexist in these countries. Regarding the newly sequenced isolates from the INR (blue dots, Fig. 1), we found not only isolates assigned to ST758 but also one isolate (H008) belonging to ST690 and another isolate (H350) assigned to ST208; these three STs are distantly related. We note that a similar pattern applies to some Honduran isolates; for instance, the Mario Catarino Rivas Hospital has more than 5 different STs (some of them are new STs) (Table S1) that are located on

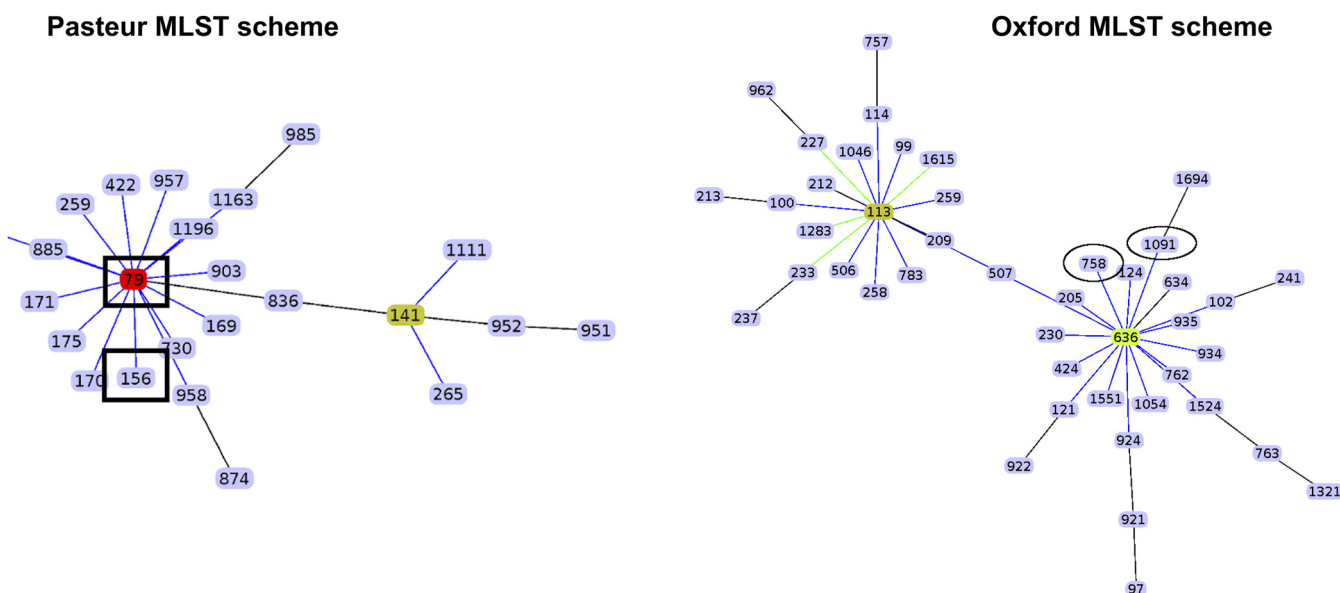


FIG 2 Partial eBURST diagrams for Pasteur and Oxford MLST schemes. Only IC5 is shown for both schemes. Rectangles in the Pasteur scheme show the ST found in some Mexican and Honduran strains (ST156) and the most frequent ST found in South America (ST79, in red), whereas the ovals under the Oxford scheme denote the STs found in some Mexican and Honduran strains (ST758 and ST1091).

distant branches in the phylogeny. Hence, it seems that distantly related lineages could coexist in the very same hospital. The ST758 lineage has very little genetic variation, as judged by the low nucleotide diversity ($\pi = 0.000187$), and this could imply a very recent emergence of that clade; therefore, a molecular dating analysis was conducted to obtain an estimate for the emergence of this clade. According to the analysis, this clade emerged very recently in early 2008 (confidence interval, late 2007 to late 2008). This estimate is compatible with the collection dates of the Mexican and Honduran isolates, as none of them were collected before 2010. Taken together, these results imply that several lineages are circulating in hospitals from Mexico and Honduras; the ST758 clade seems to have emerged some 11 years ago and during that period of time has been identified in Central America (Honduras) and North America (Mexico and Canada). On a side note, as noted previously (14), we observed some issues with the MLST genotyping; e.g., ST758 is not a monophyletic group, as three ST1091 isolates (A023, A229 and H170) cluster within the ST758 isolates.

Antibiotic resistance profiles. Antimicrobial susceptibility tests on the isolates from the INR were conducted, and a high variation in the phenotypic antibiotic resistance profiles among the isolates was noted, but high rates of resistance to many of the antibiotics tested were also found (Table 1). Whereas for the aminoglycosides gentamicin and amikacin we found sensitive, intermediately resistant, and even resistant isolates, for ciprofloxacin, all but one of the isolates were resistant. However, most of these isolates were resistant to doripenem, imipenem, and meropenem, as all but two isolates had MICs higher than 16 mg/liter for all three antibiotics (Table 1). We did not find any isolate resistant to colistin. Clearly, all of these isolates are MDR, as they were resistant to at least one antibiotic in at least three different antibiotic classes. Therefore, although these newly sequenced isolates came from the same hospital (sampled over just 5 years), they present different antibiotic resistance profiles; however, all of the strains were MDR. Then, an *in silico* prediction of the antibiotic resistance gene profiles considering all the isolates within the clade ST758 (Fig. 3) was conducted. All isolates carried the intrinsic *bla*_{OXA-51}-like gene (*bla*_{OXA-65} in all cases). Regarding the acquired OXA genes, we noted two isolates with genes from the *bla*_{OXA-40}-like gene family; isolate UH12208 contained OXA-24, whereas isolate A158, collected in Mexico, contained OXA-72 (Fig. 3). Most of the isolates (all but three) had genes belonging to the

TABLE 1 Antimicrobial susceptibility profiles of the isolates collected from the National Institute of Rehabilitation

Strain	MIC (mg/liter) (resistance breakpoint) for ^a :													AMP-sulbactam (≥32/16)
	AMK (≥64)	GEN (≥16)	CAZ (≥32)	CEP (≥32)	CIP (≥4)	LVX (≥8)	DOR (≥8)	IPM (≥8)	MEM (≥8)	CST (≥4)	TZP (≥128/4)	MIN (≥16)		
A023	8	16	>64	64	64	8	32	64	64	0.125	>128/4	0.25	16/8	
A158	16	8	>64	16	8	8	>32	32	64	0.5	128/4	0.25	8/4	
A580	16	8	64	64	64	8	64	32	32	0.5	128/4	0.25	64/32	
A229	64	8	>64	>64	>8	8	32	64	32	1	>128/4	0.125	32/16	
H251	>64	64	16	>64	>8	8	>64	>64	64	0.5	32/4	0.062	1/0.5	
H350	64	64	64	64	64	8	32	32	16	0.25	128/4	4	16/8	
H170	8	4	>64	>64	64	8	16	32	32	0.125	128/4	0.062	32/16	
A465	16	8	>64	>64	>8	16	64	64	64	1	>128/4	0.25	16/8	
H008	16	32	>64	64	64	8	8	8	8	0.125	128/4	0.5	16/8	
A278	16	16	>64	>64	64	4	32	32	32	0.25	128/4	0.125	16/8	

^aUnderlining indicates intermediate resistance, and bold type denotes resistance. Intermediate and resistant phenotypes were defined as per the CLSI 2018 guidelines. AMK, amikacin; GEN, gentamicin; CAZ, ceftazidime; CEP, cefepime; CIP, ciprofloxacin; LVX, levofloxacin; DOR, doripenem; IPM, imipenem; MEM, meropenem; CST, colistin; TZP, piperacillin-tazobactam; MIN, minocycline; AMP, ampicillin.

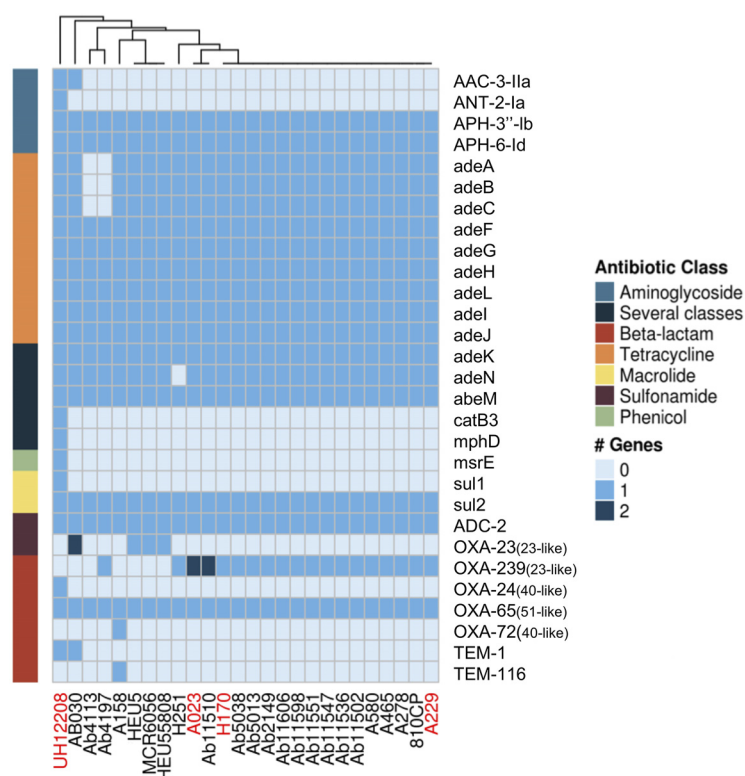


FIG 3 *In silico* prediction of antibiotic resistance genes for some strains from ST758 (in black text) and ST1091 (in red text). A heat map shows how many times a gene is present in the strains considered. The different antibiotic resistance classes are color coded. Strains are grouped according to a hierarchical clustering analysis (cladogram on the top).

*bla*_{OXA-23}-like gene family. Interestingly, the most predominant gene of the *bla*_{OXA-23}-like gene family was *bla*_{OXA-239}, a recently described variant to date only described in Mexico (7). Collectively, our data suggest that these isolates might not only be carbapenem resistant but also MDR showing variation in their resistance profiles. Furthermore, it is very likely that *bla*_{OXA-23}-like genes could be functioning as the main carbapenem-resistant determinant.

Natural history of *bla*_{OXA-239}. Then, the evolutionary history of the *bla*_{OXA-239} gene was inferred. We first conducted a BLAST search and did not find any exact match (100% amino acid identity) other than the previously described isolates from Mexico. Notably, none of the isolates having the gene *bla*_{OXA-239} were collected before 2010. Hence, the dates of collection of the isolates having *bla*_{OXA-239} potentially suggest a very recent emergence of this variant. To corroborate this, we conducted another molecular dating analysis using the *bla*_{OXA-239} sequences and their closest *bla*_{OXA} genes. According to this analysis, *bla*_{OXA-239} emerged in early 2010, which completely aligns with the dates of collection of the isolates having this gene. Then, to explore the phylogenetic relationship of *bla*_{OXA-239} with other members of the *bla*_{OXA-23}-like gene family, we constructed an ML phylogeny (Fig. S1) considering all the members of the *bla*_{OXA-23}-like gene family. The closest sequence to *bla*_{OXA-239} was the *bla*_{OXA-469} gene (Fig. S1) that also has only been described in Mexico, sampled in 2012 (6, 7), and it is associated with ST771. OXA-239 and OXA-469 differ only at the amino acid position 250 (M in OXA-239 and I in OXA-469), and both OXA-239 and OXA-469 had three amino acid substitutions (S109L, D222N, and P225S) compared to OXA-23. These changes have been proposed to be responsible for stabilizing the enzyme and for increasing the enzyme's substrate profile to include cephalosporins and monobactams, at the expense of a decreased ability to hydrolyze carbapenems (28).

To establish which transposon carries the *bla*_{OXA-239} gene, we located the contig from each strain that carried the *bla*_{OXA-23-like} gene and analyzed the upstream and downstream regions (Fig. S2). The *bla*_{OXA-239} gene is most likely carried on Tn2008, as some strains do have IS*Aba1* upstream of the *bla*_{OXA-239} gene. However, this could only be confidently established in the two strains, namely, Ab11510 and 810CP, which were sequenced using the PacBio technology. Remarkably, we did not only find *bla*_{OXA-239} in ST758 isolates but also in two other unrelated lineages, ST690 and ST208 (purple triangles, Fig. 1), indicating that this gene may have been subject to horizontal gene transfer between lineages, as all three of these lineages were found in the same hospital, INR (Fig. 1). Furthermore, for two strains, we recorded two copies of *bla*_{OXA-239} (Fig. 3, A023 and Ab11510); in the case of A023, one of the copies is chromosomally located and the other is in a plasmid, whereas for Ab11510, both copies reside in the chromosome. All in all, these results suggest that the *bla*_{OXA-239} gene has recently emerged in different lineages in Latin America.

Conclusions. In summary, we have described the recent emergence of an IC5 lineage of *A. baumannii* that contains a very recently emerged OXA-23-like gene. Importantly, in terms of public health, our study calls attention to the fact that recently emerged lineages should be under surveillance, as these could be reservoirs of new antibiotic resistance genes.

MATERIALS AND METHODS

Bacterial isolates, DNA sequencing, and antimicrobial susceptibility testing. For this study, we have gathered one of the most extensive data sets for this species in terms of the diversity of lineages. This data set has 148 genomes and covers more than 50 STs (Table S1). For this study, we sequenced 10 isolates from the INR collected between 2011 and 2015 and sampled from different sources (urine culture, bloodstream, biopsy specimen tissue, endotracheal aspirate, etc.). Samples were isolated and inoculated following standard procedures. The isolates were sequenced using an Illumina MiSeq platform with a 2 × 250-bp configuration. The genome sequencing was conducted at the Instituto Nacional de Medicina Genómica (<https://www.inmegen.gob.mx/>) in Mexico City. Genomes were assembled with Velvet v1.2.10 (29) and SPAdes v3.11.0 (30), and the best assembly (fewest contigs and largest *N*₅₀) was chosen in each case. The antimicrobial susceptibility tests were performed by broth microdilution following the recommendations of CLSI M07-A10 (31) for 14 antibiotics (Table 1).

Phylogenetic analysis and molecular dating analysis. A maximum likelihood (ML) phylogeny was performed on the concatenated alignment of 388 nonrecombinant single-gene families (SGFs). Using Roary (32), we identified 766 SGFs whose sequences were at least 90% identical and aligned at least 80% of their lengths. These SGFs were aligned using PRANK (33), and 383 SGFs had evidence of recombination, as per PhiPack (34) (*P* < 0.05), and were discarded. The appropriate evolutionary model for the phylogenetic reconstruction was chosen with ModelTest (<https://github.com/ddarriba/modeltest>), which was GTR+R+I, and the tree was built with RaxML with 100 bootstrap replicates (35). We also carried out another ML phylogeny of the OXA-23 carbapenemase family. The *bla*_{OXA-23} sequence from strain A85 (NCBI RefSeq accession number [NC_025109.1](https://www.ncbi.nlm.nih.gov/nuccore/NC_025109.1)) was used as a query in a BLASTN search against the nucleotide database of the NCBI. We chose hits with more than 90% nucleotide identity (and more than 80% of alignment coverage) since lower parameters recovered other OXA families, and 213 sequences (29 different OXA-23 alleles) were retrieved (Table S2). The sequences were aligned with PRANK, and RaxML was used for the phylogenetic reconstruction. We visualized and annotated the trees using iTOL (36).

We ran two molecular dating analyses by means of BEAST 2 (37), one for the concatenated alignment of the nonrecombinant SGF and the other for the *bla*_{OXA-239} gene. The former analysis considered only the ST758 isolates plus the ST1091 isolates that fall within the clade and the two ST924 isolates as an outgroup. In both analyses, the HKY site model with empirical frequencies and a strict clock with a constant population coalescent were set. We ran the analysis for 80,000,000 generations sampling every 40,000 steps. The molecular dating analysis for the *bla*_{OXA-239} gene considered all of the *bla*_{OXA-239} gene sequences plus the *bla*_{OXA-469} gene and *bla*_{OXA-255} gene sequences. This analysis was run for 100,000,000 steps sampling every 10,000 generations. We calibrated the clocks using the dates of collection of the isolates. Both molecular dating analyses were run twice to ensure consistency and made sure that the effective sampling size of the likelihood of the tree and the coalescent model was above 200.

In silico prediction of the antibiotic resistance genes and eBURST analysis. We carried out BLASTP searches for all of the genes present in the genomes against the Comprehensive Antibiotic Resistance Database (CARD) (38). We chose in each case only the best hit, requiring more than 70% amino acid identity between the query and the target and created a matrix of counts, where we have the genomes in the database and the number of times a certain genetic determinant of resistance was present. For the newly sequenced strains, the absence of a gene was corroborated by aligning the reads against a reference gene sequence to avoid possible negative results due to a fragmented assembly. Resistance profiles of the genomes were visualized using ComplexHeatmap in R. All of the MLST profiles under the Pasteur and the Oxford schemes were downloaded from the PubMLST database (39), and we

employed those profiles to construct an eBURST diagram by means of the goeBURST program (40), using the default settings. Contigs that harbor the OXA-23 allele were uploaded to the SimpleSynteny program (41), which allows the generation of visuals for comparative genome analysis. Eight strains were compared for the sake of clarity, and Ab11510 and 810CP were included as references because they have closed genomes (Fig. S2).

Ethics information. This study was performed using publicly available genomes and clinical isolates collected at the INR. Regarding the clinical isolates, personal information about the patients is not provided in order to guarantee their anonymity and confidentiality.

Data availability. The sequenced genomes were submitted to the NCBI under the BioProject number [PRJNA355850](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA355850), and the accession numbers for the newly sequenced isolates are provided in Table S1.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, PDF file, 0.1 MB.

FIG S2, PDF file, 0.3 MB.

TABLE S1, XLS file, 0.1 MB.

TABLE S2, XLS file, 0.1 MB.

ACKNOWLEDGMENTS

We are very grateful to Alfredo José Hernández Álvarez and Victor Manuel del Moral Chávez for their excellent job managing our servers and installing some of the software required for this study. We thank the reviewers for their comments on the manuscript.

This study was supported by CONACyT Ciencia Básica 2016 (grant no. 284276) and “Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica PAPIIT” (grant no. IN206019) to S.C.R. L.G.M. was a doctoral student from the Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM), and also received a CONACyT doctoral fellowship (no. 585414).

We declare no conflicts of interest.

REFERENCES

1. Peleg AY, Seifert H, Paterson DL. 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 21:538–582. <https://doi.org/10.1128/CMR.00058-07>.
2. Xie R, Zhang XD, Zhao Q, Peng B, Zheng J. 2018. Analysis of global prevalence of antibiotic resistance in *Acinetobacter baumannii* infections disclosed a faster increase in OECD countries. *Emerg Microbes Infect* 7:31. <https://doi.org/10.1038/s41426-018-0038-9>.
3. Higgins PG, Dammhayn C, Hackel M, Seifert H. 2010. Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 65:233–238. <https://doi.org/10.1093/jac/dkp428>.
4. Evans BA, Amyes SG. 2014. OXA β -lactamases. *Clin Microbiol Rev* 27: 241–263. <https://doi.org/10.1128/CMR.00117-13>.
5. Mugnier PD, Poirel L, Naas T, Nordmann P. 2010. Worldwide dissemination of the *bla*_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. *Emerg Infect Dis* 16:35–40. <https://doi.org/10.3201/eid1601.090852>.
6. Gonzalez-Villoria AM, Tamayo-Legorreta E, Garza-Ramos U, Barrios H, Sanchez-Perez A, Rodriguez-Medina N, Uribe-Avina N, Cevallos MA, CRAB Study Group, Silva-Sanchez J. 2016. A multicenter study in Mexico finds *Acinetobacter baumannii* clinical isolates belonging to clonal complexes 636^B (113^B) and 92^B harboring OXA-72, OXA-239, and OXA-469. *Antimicrob Agents Chemother* 60:2587–2588. <https://doi.org/10.1128/AAC.02042-15>.
7. Tamayo-Legorreta EM, Garza-Ramos U, Barrios-Camacho H, Sanchez-Perez A, Galicia-Paredes A, Meza-Chavez A, Silva-Sanchez J. 2014. Identification of OXA-23 carbapenemases: novel variant OXA-239 in *Acinetobacter baumannii* ST758 clinical isolates in Mexico. *New Microbes New Infect* 2:173–174. <https://doi.org/10.1002/nmi2.60>.
8. Becker NS, Margos G, Blum H, Krebs S, Graf A, Lane RS, Castillo-Ramírez S, Sing A, Fingerle V. 2016. Recurrent evolution of host and vector association in bacteria of the *Borrelia burgdorferi* sensu lato species complex. *BMC Genomics* 17:734. <https://doi.org/10.1186/s12864-016-3016-4>.
9. Castillo-Ramírez S, Fingerle V, Jungnick S, Straubinger RK, Krebs S, Blum H, Meinel DM, Hofmann H, Guertler P, Sing A, Margos G. 2016. Trans-Atlantic exchanges have shaped the population structure of the Lyme disease agent *Borrelia burgdorferi* sensu stricto. *Sci Rep* 6:22794. <https://doi.org/10.1038/srep22794>.
10. Challagundla L, Reyes J, Rafiqullah I, Sordelli DO, Echaniz-Aviles G, Velazquez-Meza ME, Castillo-Ramírez S, Fittipaldi N, Feldgarden M, Chapman SB, Calderwood MS, Carvajal LP, Rincon S, Hanson B, Planet PJ, Arias CA, Diaz L, Robinson DA. 2018. Phylogenomic classification and the evolution of clonal complex 5 methicillin-resistant *Staphylococcus aureus* in the Western Hemisphere. *Front Microbiol* 9:1901. <https://doi.org/10.3389/fmicb.2018.01901>.
11. Ezewudo MN, Joseph SJ, Castillo-Ramírez S, Dean D, Del Rio C, Didelot X, Dillon J-A, Selden RF, Shafer WM, Turingan RS, Unemo M, Read TD. 2015. Population structure of *Neisseria gonorrhoeae* based on whole genome data and its relationship with antibiotic resistance. *PeerJ* 3:e806. <https://doi.org/10.7717/peerj.806>.
12. Joseph SJ, Cox D, Wolff B, Morrison SS, Kozak-Muiznieks NA, Frace M, Didelot X, Castillo-Ramírez S, Winchell J, Read TD, Dean D. 2016. Dynamics of genome change among *Legionella* species. *Sci Rep* 6:33442. <https://doi.org/10.1038/srep33442>.
13. Joseph SJ, Marti H, Didelot X, Castillo-Ramírez S, Read TD, Dean D. 2015. Chlamydiae genomics reveals interspecies admixture and the recent evolution of *Chlamydia abortus* infecting lower mammalian species and humans. *Genome Biol Evol* 7:3070–3084. <https://doi.org/10.1093/gbe/evv201>.
14. Castillo-Ramírez S, Graña-Miraglia L. 2019. Inaccurate multilocus sequence typing of *Acinetobacter baumannii*. *Emerg Infect Dis* 25: 186–187. <https://doi.org/10.3201/eid2501.180374>.
15. Frisch MB, Castillo-Ramírez S, Petit RA, Farley MM, Ray SM, Albrecht VS, Limbago BM, Hernandez J, See I, Satola SW, Read TD. 2018. Invasive methicillin-resistant *Staphylococcus aureus* USA500 strains from the US emerging infections program constitute three geographically distinct lineages. *mSphere* 3:e00571-17. <https://doi.org/10.1128/mSphere.00571-17>.
16. Holden MTG, Hsu L-Y, Kurt K, Weinert LA, Mather AE, Harris SR, Strommenger B, Leyer F, Witte W, de Lencastre H, Skov R, Westh H, Zemlickova H, Coombs G, Kearns AM, Hill RLR, Edgeworth J, Gould I, Gant V, Cooke

- J, Edwards GF, McAdam PR, Templeton KE, McCann A, Zhou Z, Castillo-Ramirez S, Feil EJ, Hudson LO, Enright MC, Balloux F, Aanensen DM, Spratt BG, Fitzgerald JR, Parkhill J, Achtman M, Bentley SD, Nubel U. 2013. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. *Genome Res* 23:653–664. <https://doi.org/10.1101/gr.147710.112>.
17. Cieslinski JM, Arend L, Tuon FF, Silva EP, Ekermann RG, Dalla-Costa LM, Higgins PG, Seifert H, Pilonetto M. 2013. Molecular epidemiology characterization of OXA-23 carbapenemase-producing *Acinetobacter baumannii* isolated from 8 Brazilian hospitals using repetitive sequence-based PCR. *Diagn Microbiol Infect Dis* 77:337–340. <https://doi.org/10.1016/j.diagmicrobio.2013.07.018>.
18. Graña-Miraglia L, Lozano L, Castro-Jaimes S, Cevallos MA, Volkow P, Castillo-Ramírez S, Graña-Miraglia L, Lozano L, Castro-Jaimes S, Cevallos MA, Volkow P, Castillo-Ramírez S. 2016. First genome sequence of a Mexican multidrug-resistant *Acinetobacter baumannii* isolate. *Genome Announc* 4:e00156-16. <https://doi.org/10.1128/genomeA.00156-16>.
19. Graña-Miraglia L, Lozano LF, Velázquez C, Volkow-Fernández P, Pérez-Oseguera Á, Cevallos MA, Castillo-Ramírez S. 2017. Rapid gene turnover as a significant source of genetic variation in a recently seeded population of a healthcare-associated pathogen. *Front Microbiol* 8:1817. <https://doi.org/10.3389/fmicb.2017.01817>.
20. Stietz MS, Ramírez MS, Vilacoba E, Merkier AK, Limansky AS, Centrón D, Catalano M. 2013. *Acinetobacter baumannii* extensively drug resistant lineages in Buenos Aires hospitals differ from the international clones I–III. *Infect Genet Evol* 14:294–301. <https://doi.org/10.1016/j.meegid.2012.12.020>.
21. Opazo-Capurro A, San Martín I, Quezada-Aguiluz M, Morales-León F, Domínguez-Yévenes M, Lima CA, Esposito F, Cerdeira L, Bello-Toledo H, Lincopan N, González-Rocha G. 2019. Evolutionary dynamics of carbapenem-resistant *Acinetobacter baumannii* circulating in Chilean hospitals. *Infect Genet Evol* 73:93–97. <https://doi.org/10.1016/j.meegid.2019.04.022>.
22. Cerezales M, Xanthopoulou K, Wille J, Bustamante Z, Seifert H, Gallego L, Higgins PG. 2019. *Acinetobacter baumannii* analysis by core genome multi-locus sequence typing in two hospitals in Bolivia: endemicity of international clone 7 isolates (CC25). *Int J Antimicrob Agents* 53: 844–849. <https://doi.org/10.1016/j.ijantimicag.2019.03.019>.
23. López-Leal G, Zuniga-Moya JC, Castro-Jaimes S, Graña-Miraglia L, Pérez-Oseguera Á, Reyes-García HS, Gough-Coto SD, Pavón-Madrid R, Bejarano SA, Ferrera A, Castillo-Ramírez S, Cevallos MA. 2019. Unexplored genetic diversity of multidrug-and extremely drug-resistant *Acinetobacter baumannii* isolates from tertiary hospitals in Honduras. *Microb Drug Resist* 25:690–695. <https://doi.org/10.1089/mdr.2018.0311>.
24. Loewen PC, Alsaadi Y, Fernando D, Kumar A. 2014. Genome sequence of an extremely drug-resistant clinical isolate of *Acinetobacter baumannii* strain AB030. *Genome Announc* 2:e01035-14. <https://doi.org/10.1128/genomeA.01035-14>.
25. Vanegas JM, Higuera LF, Vargas CA, Cienfuegos AV, Rodríguez ÉA, Roncancio GE, Jiménez JN. 2015. Carbapenem-resistant *Acinetobacter baumannii* causing osteomyelitis and infections of skin and soft tissues in hospitals of Medellín, Colombia. *Biomedica* 35:522–530. (In Spanish.) <https://doi.org/10.7705/biomedica.v35i4.2572>.
26. Levy-Blitchtein S, Roca I, Plasencia-Rebata S, Vicente-Taboada W, Velásquez-Pomar J, Muñoz L, Moreno-Morales J, Pons MJ, del Valle-Mendoza J, Vila J. 2018. Emergence and spread of carbapenem-resistant *Acinetobacter baumannii* international clones II and III in Lima, Peru. *Emerg Microbes Infect* 7:119. <https://doi.org/10.1038/s41426-018-0127-9>.
27. Higgins PG, Hrenovic J, Seifert H, Dekic S. 2018. Characterization of *Acinetobacter baumannii* from water and sludge line of secondary wastewater treatment plant. *Water Res* 140:261–267. <https://doi.org/10.1016/j.watres.2018.04.057>.
28. Harper TM, June CM, Taracila MA, Bonomo RA, Powers RA, Leonard DA. 2018. Multiple substitutions lead to increased loop flexibility and expanded specificity in *Acinetobacter baumannii* carbapenemase OXA-239. *Biochem J* 475:273–288. <https://doi.org/10.1042/BCJ20170702>.
29. Zerbino D, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
30. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
31. Clinical and Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 10th ed. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, PA.
32. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, Fookes M, Falush D, Keane JA, Parkhill J. 2015. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 31:3691–3693. <https://doi.org/10.1093/bioinformatics/btv421>.
33. Löytynoja A. 2014. Phylogeny-aware alignment with PRANK. *Methods Mol Biol* 1079:155–170. https://doi.org/10.1007/978-1-62703-646-7_10.
34. Bruen TC, Philippe H, Bryant D. 2006. A simple and robust statistical test to detect the presence of recombination. *Genetics* 172:2665–2681. <https://doi.org/10.1534/genetics.105.048975>.
35. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>.
36. Letunic I, Bork P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res* 44:W242–W245. <https://doi.org/10.1093/nar/gkw290>.
37. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* 10:e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>.
38. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN. 2016. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res* 45:D566–D573. <https://doi.org/10.1093/nar/gkw1004>.
39. Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 3:124. <https://doi.org/10.12688/wellcomeopenres.14826.1>.
40. Francisco AP, Bugalho M, Ramirez M, Carrico JA. 2009. Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach. *BMC Bioinformatics* 10:152. <https://doi.org/10.1186/1471-2105-10-152>.
41. Veltri D, Wight MM, Crouch JA. 2016. SimpleSynteny: a Web-based tool for visualization of microsynteny across multiple species. *Nucleic Acids Res* 44:W41–W45. <https://doi.org/10.1093/nar/gkw330>.