MICROBIAL DRUG RESISTANCE
Volume 29, Number 6, 2023

DOI: 10.1089/mdr.2022.0226

EPIDEMIOLOGY

Open camera or QR reader and scan code to access this article and other resources online.

Carbapenemase-Encoding Genes and Colistin Resistance in Gram-Negative Bacteria During the COVID-19 Pandemic in Mexico: Results from the Invifar Network

Ulises Garza-Ramos,[1] Jesús Silva-Sánchez,[1] Luis Esaú López-Jácome,[2] Melissa Hernández-Durán,[2] Claudia Adriana Colín-Castro,[2] Alejandro Sánchez-Pérez,[1] Jonathan Rodríguez-Santiago,[1] Rayo Morfín-Otero,[3] Eduardo Rodriguez-Noriega,[3] María-del-Consuelo Velázquez-Acosta,[4] María Del Rosario Vázquez-Larios,[5] José Manuel Feliciano-Guzmán,[6] Fabián Rojas-Larios,[7] Alfredo Ponce-De-Leon,[8] Margarita Lozano-Garcia,[9] Elena Victoria Choy-Chang,[10] Eduardo López-Gutiérrez,[11] Aarón Molina-Jaimes,[12] Mariana Gil-Veloz,[13] Reyna Edith Corte-Rojas,[14] Ismelda López-Ovilla,[15] Jose Luis Ramirez-Mis,[16] Dora Elia Rodríguez-Balderas,[17] Alejandro Molina-Chavarria,[18] Cecilia Padilla-Ibarra,[19] Maria Angelina Quevedo-Ramos,[20] Christian Daniel Mireles-Dávalos,[21] Nadia Rodríguez-Medina,[1] Daira Rubio-Mendoza,[22] Carlos Córdova-Fletes,[22] Flora Cruz-López,[23] Dilva Angelina Becerra-Montejano,[24] Roberto Mercado-Longoria,[22] Rebeca Thelma Martínez-Villarreal,[25] Nicolás Rogelio Eric Barlandas-Rendón,[26] Juan Pablo Mena-Ramírez,[27] Carlos Antonio Couoh-May,[28] Margarita Alcaraz-Espejel,[29] César Adame-Alvarez,[30] Lourdes Hernández-Vicente,[31] and Elvira Garza-González[22]

[1]Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico.
[2]Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra, Ciudad de México, Mexico.
[3]Hospital Civil de Guadalajara Fray Antonio Alcalde, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico.
[4]Instituto Nacional de Cancerología, Ciudad de México, Mexico.
[5]Instituto Nacional de Cardiología Ignacio Chávez, Ciudad de Mexico, Mexico.
[6]Hospital de Especialidades Pediátricas, Tuxtla Gutiérrez, Chiapas, Mexico.
[7]Laboratorio de Ecología y Microbiología, Facultad de Medicina, Universidad de Colima, Colima, Mexico.
[8]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Ciudad de Mexico, Mexico.
[9]Hospital General Dr. Manuel Gea González, Ciudad de México, Mexico.
[10]Hospital General de Zona No.1 Nueva Frontera, Tapachula, Chiapas, Mexico.
[11]Hospital Regional de Alta Especialidad de Oaxaca, San Bartolo Coyotepec, Oaxaca, Mexico.
[12]Hospital Regional de Alta Especialidad Bicentenario de la Independencia, Tultitlán de Mariano Escobedo, Estado de México, Mexico.
[13]Hospital Regional de Alta Especialidad del Bajío, Leon, Guanajuato, Mexico.
[14]Hospital para el Niño Poblano, San Andrés Cholula, Puebla, Mexico.
[15]Hospital Dr. Gilberto Gomez Maza, Tuxtla Gutiérrez, Chiapas, Mexico.
[16]Hospital Galenia, Cancun, Quintana Roo, Mexico.
[17]Hospital del Niño Dr. Federico Gómez Santos, Saltillo, Coahuila, Mexico.
[18]Centro médico Dr. Ignacio Chávez, Hermosillo, Sonora, Mexico.
[19]Hospital General del Estado Dr. Ernesto Ramos Bours, Hermosillo, Sonora, Mexico.
[20]Hospital General León, Leon, Guanajuato, Mexico.
[21]Instituto Nacional de Enfermedades Respiratorias ''Ismael Cosío Villegas'', Ciudad de México, Mexico.
[22]Facultad de Medicina, Hospital Universitario Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico.
[23]Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico.
[24]Hospital General Dr. Miguel Silva, Morelia, Michoacán, Mexico.
[25]Centro Universitario de Salud, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico.
[26]Laboratorio BIOCLIN SA DE CV, Chilpancingo, Guerrero, Mexico.
[27]Hospital General de Zona N. 21 IMSS, Centro Universitario de los Altos CUALTOS, Tepatitlán de Morelos, Jalisco, Mexico.
[28]Hospital General Dr. Agustín O'Horan, Mérida, Yucatán, Mexico.
[29]Sanatorio la luz, Morelia, Michoacan, Mexico.
[30]Hospital Centenario Miguel Hidalgo, Aguascalientes, Mexico.
[31]Hospital General Dr. Raymundo Abarca Alarcón, Chilpancingo de los Bravo, Guerrero, Mexico.

In this study, we report the carbapenemase-encoding genes and colistin resistance in *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* in the second year of the COVID-19 pandemic. Clinical isolates included carbapenem-resistant *K. pneumoniae*, carbapenem-resistant *E. coli*, carbapenem-resistant *A. baumannii*, and carbapenem-resistant *P. aeruginosa*. Carbapenemase-encoding genes were detected by PCR. Carbapenem-resistant *K. pneumoniae* and carbapenem-resistant *E. coli* isolates were analyzed using the Rapid Polymyxin NP assay. *mcr* genes were screened by PCR. Pulsed-field gel electrophoresis and whole-genome sequencing were performed on representative isolates. A total of 80 carbapenem-resistant *E. coli*, 103 carbapenem-resistant *K. pneumoniae*, 284 carbapenem-resistant *A. baumannii*, and 129 carbapenem-resistant *P. aeruginosa* isolates were recovered. All carbapenem-resistant *E. coli* and carbapenem-resistant *K. pneumoniae* isolates were included for further analysis. A selection of carbapenem-resistant *A. baumannii* and carbapenem-resistant *P. aeruginosa* strains was further analyzed (86 carbapenem-resistant *A. baumannii* and 82 carbapenem-resistant *P. aeruginosa*). Among carbapenem-resistant *K. pneumoniae* and carbapenem-resistant *E. coli* isolates, the most frequent gene was bla_{NDM} (86/103 [83.5%] and 72/80 [90%], respectively). For carbapenem-resistant *A. baumannii*, the most frequently detected gene was $bla_{\text{OXA-40}}$ (52/86, 60.5%), and for carbapenem-resistant *P. aeruginosa*, was bla_{VIM} (19/82, 23.2%). For carbapenem-resistant *A. baumannii*, five indistinguishable pulsotypes were detected. Circulation of *K. pneumoniae* New Delhi metallo-β-lactamase (NDM) and *E. coli* NDM was detected in Mexico. High virulence sequence types (STs), such as *K. pneumoniae* ST307, *E. coli* ST167, *P. aeruginosa* ST111, and *A. baumannii* ST2, were detected. Among *K. pneumoniae* isolates, 18/101 (17.8%) were positive for the Polymyxin NP test (two, 11.0% positive for the *mcr-1* gene, and one, 5.6% with disruption of the *mgrB* gene). All *E. coli* isolates were negative for the Polymyxin NP test. In conclusion, *K. pneumoniae* NDM and *E. coli* NDM were detected in Mexico, with the circulation of highly virulent STs. These results are relevant in clinical practice to guide antibiotic therapies considering the molecular mechanisms of resistance to carbapenems.

Keywords: COVID-19, drug resistance, NDM, carbapenem resistance

Introduction

Since patients with COVID-19 (coronavirus disease 2019) may acquire secondary coinfections, they commonly receive antimicrobial therapy, including carbapenems.[1,2] Several reports have described an increase in carbapenem-resistant gram-negative organisms, especially *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*.[3,4]

Drug resistance is generated by various mechanisms, including decreased expression of outer membrane protein, expression of efflux pumps, and/or expression of β-lactamases as extended-spectrum β-lactamases (ESBL) and carbapenemases.[5] Carbapenemases belong to Ambler classes A, B, or D and are chromosomal or plasmidic.[6] Ambler class A (*e.g.*, *K. pneumoniae* carbapenemase [KPC]) is most frequently observed in Enterobacterales. Class B carbapenemases (metallo-β-lactamases [MBLs], including Verona integron-encoded MBLs [VIM] and imipenemase enzymes [IMP]) are most frequently observed in *P. aeruginosa* and Enterobacterales,[7] and the New Delhi MBL [NDM] carbapenemase has been detected in *K. pneumoniae* and *E. coli* and to a lesser extent in other bacterial species.[8] Class D carbapenemases, which include oxacillinases (OXA) and OXA type-related enzymes, are most commonly observed in Enterobacterales and *A. baumannii*.[9–11]

Among emerging carbapenem resistance genes, bla_{NDM} has been considered to be a significant challenge due to its ability to hydrolyze a wide range of β-lactams, spread rapidly, and confer resist most available treatments.[12] Currently, 24 NDM variants have been characterized from more than 60 species of 11 bacterial families[12] and are usually transferred by plasmids.[7,13] Based on available data, the worldwide highest frequency of NDM-producing bacteria is reported across Asia, America, Africa, and Europe.[14]

The presence of carbapenem-resistant strains of *K. pneumoniae*, *E. coli*, *A. baumannii*, and *P. aeruginosa* has led to the reuse of old antibiotics that were considered too toxic for clinical use, such as the family of antimicrobial lipopeptides polymyxins (colistin and polymyxin B).[15–17] Regrettably, the overuse of colistin in human and animal medicine has led to the emergence of colistin-resistant pathogens.[18] Different mechanisms of colistin resistance have been characterized, including intrinsic, mutational, and transferable mechanisms.[19] Up to 2019, Mexico was ranked fifth among countries with the highest reports of carbapenemase-producing and colistin-resistant (mediated by chromosomal mechanisms) *K. pneumoniae* in the Americas.[20] This study aimed to report the carbapenemase-encoding genes in *K. pneumoniae*, *E. coli*, *A. baumannii*, and *P. aeruginosa* bacterial species from medical centers of Mexico in the second year of the COVID-19 pandemic, as well as to identify the colistin resistance in these bacterial isolates.

Materials and Methods

Study design, data collection, and analysis

In this study, clinical isolates and susceptibility data from 23 participating centers (21 hospital-based laboratories and 2 external laboratories) were collected from January 1, 2021, to August 31, 2021.

Clinical isolates included carbapenem-resistant *K. pneumoniae*, carbapenem-resistant *E. coli*, carbapenem-resistant *A. baumannii*, and carbapenem-resistant *P. aeruginosa.*

Carbapenem resistance was determined according to the Clinical and Laboratory Standards Institute (CLSI) criteria.[21] All identifications were confirmed using matrix-assisted laser desorption/ionization - time-of-flight. Isolates from all wards were included, and only the first isolate per patient was considered for analysis.

Susceptibility test results, including ESBL production, included *K. pneumoniae*, *E. coli*, *A. baumannii*, and *P. aeruginosa* isolates collected from blood, urine, and respiratory specimens (bronchial lavage and tracheal aspirates). Each laboratory tested susceptibilities using routine methods, including some instruments (VITEK 2, BioMérieux; Phoenix Automated Microbiology System, Becton-Dickinson; MicroScan WalkAway, Siemens Healthcare Diagnostics; and Sensititre, TREK Diagnostic Systems, Inc.) or the disk diffusion method. Data were collected and deposited into the WHONET 2021 platform and converted to the WHONET format using the BacLink 2 tool.

Carbapenemase phenotypic assays

All clinical isolates were received at the coordinating laboratory. Nonsusceptibility to carbapenems was confirmed using the disk diffusion test according to the CLSI criteria.[21] All carbapenem-nonsusceptible *A. baumannii* and *P. aeruginosa* were included for further analysis.

K. pneumoniae and *E. coli* strains categorized as carbapenem resistant or intermediate were tested to determine the presence of carbapenemases using carbapenem inactivation tests.[21] Only positive strains for these tests were included for further analysis.

Detection of carbapenemase genes

For carbapenemase-encoding genes detection, Enterobacterales isolates were evaluated by PCR for bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}, and bla_{GES} genes, as previously described.[22–26]

Carbapenem-resistant *A. baumannii* and carbapenem-resistant *P. aeruginosa* were evaluated by PCR for bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{GES}, $bla_{OXA-23-like}$, $bla_{OXA-40-like}$ (former $bla_{OXA-24-like}$), and bla_{OXA-58} using primers designed for this study (Table 1). DNA was extracted using the boil lysis method. For each reaction, 0.2 mM of each dNTP (Invitrogen), 1 U Taq DNA polymerase with ThermoPol (New England BioLabs), and 10 pmol of each primer were added for a 25 μL reaction mixture. PCR conditions were 1 cycle at 95°C for 1 min; 30 cycles at 95°C for 30 sec, 56°C for 30 sec (52°C only for IMP), and 68°C for 40 sec; and a final cycle at 68°C for 6 min. PCR products were visualized using a 1% agarose gel.

Phenotypic and genotypic screening of colistin nonsusceptibility

All *K. pneumoniae* and *E. coli* isolates were analyzed using the Rapid Polymyxin NP assay.[27] For clinical isolates positive for the assay, the minimal inhibitory concentration (MIC) for colistin was determined as recommended by the joint CLSI–European Committee on Antimicrobial Susceptibility Testing (EUCAST) polymyxin breakpoints working group. The plasmid-borne *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5* genes were screened by PCR using generic primers.[28] The *mgrB* gene and its promoter region were amplified with the following primers: Pr_mgrB_F, CCAT AAGATAGCCACCAAG′ and mgrB_ext_R, TTAAGAA GGCCGTGCTATCC.[29] The PCR products were sequenced using the Sanger method.

Clonal relatedness and multilocus sequence typing

The clonal relatedness of clinical isolates and those recovered was determined through pulsed-field gel electrophoresis (PFGE) of representative isolates for each bacterial species.[30] For *K. pneumoniae*, 55 isolates were selected; for *E. coli*, 24 isolates; for *A. baumannii*, 56 isolates; and *P. aeruginosa*, 55 isolates. The results were analyzed using GelCompar II software (Applied Maths, Kortrijk, Belgium). Band patterns were interpreted according to the similarity percentage represented using a dendrogram derived from UPGMA and Dice coefficients (band position tolerance and optimization were set at 0.7% and 0.65%, respectively) and according to the Tenover criteria.[31]

Whole-genome sequencing and sequence type determination

Whole-genome sequencing was performed in five selected carbapenem-resistant isolates from each species to detect

Table 1. Primers Used for the Detection of Carbapenemase-Encoding Genes for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

Gene	*Sequence (5′→3′)*	*PCR product, bp*
bla_{NDM}	F-GGCGGAATGGCTCATCACGA R-CGCAACACAGCCTGACTTTC	635
bla_{VIM}	F-AGTGGTGAGTATCCGACAG R-ATGAAAGTGCGTGGAGAC	485
bla_{IMP}	F-GTGATGCGTCYCCAAYTTCACT R-GGAATAGAGTGGCTTAATTCT	435
bla_{GES}	F-TCATTCACGCHCTATTVCTGGCA R-CTATTTGTCCGTGCTCAGG	857
bla_{OXA-23}	F-GATCGGATTGGAGAACCAGA R-ATTTCTGACCGCATTTCCAT	320
bla_{OXA-40}	F-GGAATTCCATGAAAAAATTTATACTTCC R-CGGGATCCCGTTAAATGATTCCAAGATTTTCTAGCG	405
bla_{OXA-58}	F-CTCAATCATCGATCAGAA R-ACCCACATACCAACCCAC	380

sequence type (ST). Strains were selected from different centers included in this study. DNA was extracted using the DNeasy Kit (Qiagen, Germany) according to the manufacturer's instructions. Sequencing was performed using the Illumina (MiSeq) platform. Quality-based trimming was performed with the Trim Galore software, and *de novo* assembly was completed with SPAdes v3.12.0. The draft genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline.

The multilocus sequence typing (MLST) for *K. pneumoniae*, *E. coli*, *A. baumannii*, and *P. aeruginosa* was determined *in silico*.

Ethics statement

The local ethics committee of Hospital Civil de Guadalajara ''Fray Antonio Alcalde'' (Jalisco, Mexico) approved this study (Reference No. 129/17). The ethics committee waived informed consent. All participating institutions agreed with the present study.

Results

Collected isolates

A total of 596 carbapenem-resistant or carbapenem-intermediate isolates were recovered: 103 (17.3%) *K. pneumoniae*, 80 (13.4%) *E. coli*, 284 (47.6%) *A. baumannii*, and 129 (21.7%) *P. aeruginosa.* All *K. pneumoniae* and *E. coli* isolates were included for further analysis. A selection of *A. baumannii* and *P. aeruginosa* strains was further analyzed (86 *A. baumannii*, 82 *P. aeruginosa*), considering the inclusion of clinical isolates from all centers that participated in the study.

Carbapenemase-encoding genes

Among *K. pneumoniae* isolates, the most frequent carbapenemase-encoding gene was bla_{NDM} ($n=86$), followed by bla_{KPC} ($n=11$) (Table 2); other carbapenemase-encoding genes detected were bla_{VIM} ($n=5$) and bla_{IMP} ($n=1$), and four isolates were negative for all genes screened. Among *E. coli* isolates, the most frequent carbapenemase-encoding gene was bla_{NDM} ($n=72$), followed by bla_{VIM} ($n=9$), bla_{KPC} ($n=1$), bla_{IMP} ($n=1$), and bla_{GES} ($n=1$), and 10 isolates were negative for all genes screened. Among *A. baumannii* isolates, the most frequent carbapenemase-encoding gene was bla_{OXA-40} ($n=52$), followed by bla_{OXA-23} ($n=20$) and bla_{OXA-58} ($n=1$), and four isolates were negative for all genes screened (Table 3).

Table 2. Distribution of Genes Encoding Carbapenem Resistance in *Klebsiella pneumoniae* and *Escherichia coli*

n	bla_{NDM}	bla_{KPC}	bla_{VIM}	bla_{IMP}	bla_{OXA-48}	bla_{GES}
K. pneumoniae ($n=103$)						
83	+	−	−	−	−	−
9	−	+	−	−	−	−
4	−	−	−	−	−	−
3	+	−	+	−	−	−
1	−	+	−	+	−	−
1	−	+	+	−	−	−
1	−	−	+	−	−	−
1	−	−	−	−	+	−
E. coli ($n=80$)						
64	+	−	−	−	−	−
6	+	−	+	−	−	−
3	−	−	+	−	−	−
3	−	−	−	−	+	
1	+	−	−	+	−	−
1	−	+	−	−	−	−
1	+	−	−	−	−	+
1	−	−	−	−	−	−

+, positive; −, negative.

Among *P. aeruginosa* isolates, the most frequent carbapenemase-encoding gene was bla_{VIM} ($n=19$), followed by bla_{GES} ($n=8$) and bla_{IMP} ($n=7$), and 48 isolates were negative for all genes screened (Table 3). Regarding *K. pneumoniae*, five strains carried two carbapenemases (three carried bla_{NDM} and bla_{VIM}, one carried bla_{KPC} and bla_{IMP}, and one carried bla_{KPC} and bla_{VIM}). Regarding *E. coli*, eight strains carried two carbapenemases (six carried bla_{NDM} and bla_{VIM}, one bla_{NDM} and bla_{IMP}, and one carried bla_{NDM} and bla_{GES}).

Clonal relatedness and MLST

PFGE analyzed by the Tenover criteria indicated that for *K. pneumoniae, E. coli*, and *P. aeruginosa*, no indistinguishable isolates were detected; clusters were detected (5 for *K. pneumoniae* and 11 for *P. aeruginosa*).

For *A. baumannii*, five indistinguishable pulsotypes were detected: A ($n=3$) with subtype A1 ($n=1$); G ($n=2$) with subtypes G1 ($n=1$), G2 ($n=1$), G3 ($n=1$), and G4 ($n=1$); H ($n=2$) with seven subtypes H1 ($n=1$), H2 ($n=1$), H3 ($n=2$), H4 ($n=1$), H5 ($n=1$), H6 ($n=1$), H7 ($n=2$); K ($n=2$) with subtype K1 ($n=1$); and N ($n=4$) with subtypes N1 ($n=3$) and N2 ($n=1$) (Supplementary Fig. S1A–D).

Five strains, from each species, selected from different centers were included in this study for MLST. Among *K. pneumoniae* isolates, ST307 ($n=2$; NDM-1 and KPC-3), ST1876 ($n=2$; NDM-1), and ST4839 ($n=1$; NDM-1) were detected. Among *E. coli* isolates, ST167 ($n=4$; NDM-5) and ST361 ($n=1$; NDM-5) were detected. Among *A. baumannii* isolates, two strains corresponded to ST369 ($n=2$; bla_{OXA-40}) (Oxford system) or ST2 (Pasteur system), two strains corresponded to ST208 ($n=2$; bla_{OXA-40}) (Oxford system) or ST2 (Pasteur system), and one was ST1694 ($n=1$; bla_{OXA-40}) (Oxford system) or ST422 (Pasteur system). Among *P. aeruginosa* isolates, ST111 ($n=1$), ST274 ($n=1$), ST983 ($n=1$), ST309 ($n=1$), and ST260 ($n=1$) were detected.

Colistin nonsusceptibility analysis

Among *K. pneumoniae* isolates, 101 isolates were evaluated, of which 83 were negative for the Polymyxin NP test and 18 were positive (Table 4). Two isolates were positive for the *mcr-1* gene, and in one isolate, disruption of the *mgrB* gene by an insertion sequence was identified. No other colistin resistance mechanism could be identified in the present study. The colistin MIC was 4 μg/mL for *mcr-1*-encoding isolates and 32 μg/mL for the *mgrB*-disrupted isolate. The MIC range was 8–32 μg/mL for the other colistin-resistant isolates. All *E. coli* isolates were negative for the Rapid Polymyxin NP test (Table 4).

Table 3. Distribution of Genes Encoding Carbapenem Resistance in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

n	bla_{NDM}	bla_{VIM}	bla_{IMP}	bla_{GES}	bla_{OXA-23}	bla_{OXA-40}	bla_{OXA-58}
A. baumannii ($n=86$)							
52	−	−	−	−	−	+	−
20	−	−	−	−	+	−	−
13	−	−	−	−	−	−	−
1	−	−	−	−	−	−	+
P. aeruginosa ($n=82$)							
48	−	−	−	−	−	−	−
19	−	+	−	−	−	−	−
8	−	−	−	+	−	−	−
7	−	−	+	−	−	−	−

+, positive; -, negative.

Drug resistance patterns in the included centers

Among clinical isolates collected from blood, carbapenem resistance was the highest for *A. baumannii* (88.8% for meropenem) (Supplementary Table S1 and Fig. 1). For *P. aeruginosa*, resistance to ceftazidime and piperacillin–tazobactam was 23.8% and 23.2%, respectively. In this study, 63.7% of *K. pneumoniae* isolates were detected to be ESBL producers.

Among respiratory isolates, *A. baumannii* had resistance rates higher than 86% for antibiotics evaluated (88.9% for meropenem). Carbapenem resistance in *K. pneumoniae* was near 10% (Supplementary Table S2 and Fig. 1). Among *K. pneumoniae* isolates, 49.1% were ESBL producers.

For clinical isolates collected from urine, *A. baumannii* had resistance rates up to 91.5% for imipenem (Supplementary Table S3 and Fig. 1). For *E. coli*, carbapenem resistance was lower than 1.5% and resistance rates higher than 50% were detected for cephalosporins and quinolones. Among *K. pneumoniae* isolates, 45.4% were ESBL producers.

Discussion

The COVID-19 pandemic has redefined the hospital microbiota and the distribution of genes encoding drug resistance, particularly the carbapenemase-encoding genes. In this study, we identified the genes encoding carbapenemases in clinically relevant gram-negative bacteria in Mexican centers during the COVID-19 pandemic in 2021 and detected that the most frequent carbapenemase-encoding gene was bla_{NDM} in both *K. pneumoniae* and *E. coli*. Regarding this carbapenemase-encoding gene, a systematic review reported that *K. pneumoniae* bla_{NDM} strains were as frequent as 20.1% in Europe, 9.0% in America, 5.6% in Africa, and 0.4% in Oceania, with variants bla_{NDM-1}, bla_{NDM-5}, bla_{NDM-4}, and bla_{NDM-7} being more frequently reported.[14]

In Mexico, bla_{NDM-1} was first reported in 2013 in a clinical isolate of *Providencia rettgeri*.[32] Since this first case, several reports have been published,[33–35] including a recent multicenter report that included clinical isolates from January to March 2020 (just before the COVID-19 pandemic in Mexico), in which bla_{NDM-1} was detected in *K. pneumoniae* (4/4) and *E coli* (10/15).[36] In the present study, 103 *K. pneumoniae* ($n=86$; 83.5% bla_{NDM}) and 80 *E. coli* ($n=72$; 90% bla_{NDM}) isolates were collected from 23 centers over 8 months during the COVID-19 pandemic, indicating an increase in circulation of this carbapenemase-encoding gene in Enterobacterales in Mexico.

K. pneumoniae ST258 is widely recognized as an antibiotic-resistant, high-risk clonal lineage.[37] In addition, *K. pneumoniae* ST307 is now considered a lineage with the potential to become a clinically relevant epidemic clone,[38–41] with several outbreak reports in clinical settings[42–45] and the community.[46]

K. pneumoniae ST307 often carries transferable resistance-conferring genes against carbapenems, including bla_{KPC-3} and bla_{NDM-1}.[38,47] In the present study, two of the five *K. pneumoniae* strains were detected as ST307. These strains harbored the bla_{KPC-3} gene and bla_{NDM-1}, underlying

Table 4. Results from Rapid Polymyxin NP Test, Colistin Minimal Inhibitory Concentration, and Genes Involved in Colistin Resistance

n	*Rapid Polymyxin NP*	*Colistin MIC, mg/L*	mcr-1[a]	mgrB *genotype*
Klebsiella pneumoniae				
83	−	Not determined	Not determined	Not determined
11	+	32	−	Wild type
3	+	16	−	Wild type
1	+	32	−	IS insertion
1	+	8	−	Wild type
2	+	4	+	Wild type
Escherichia coli				
76	−	Not determined	Not determined	Not determined

[a]All isolates were negative for *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5* genes.
MIC, minimal inhibitory concentration; +, positive; −, negative.

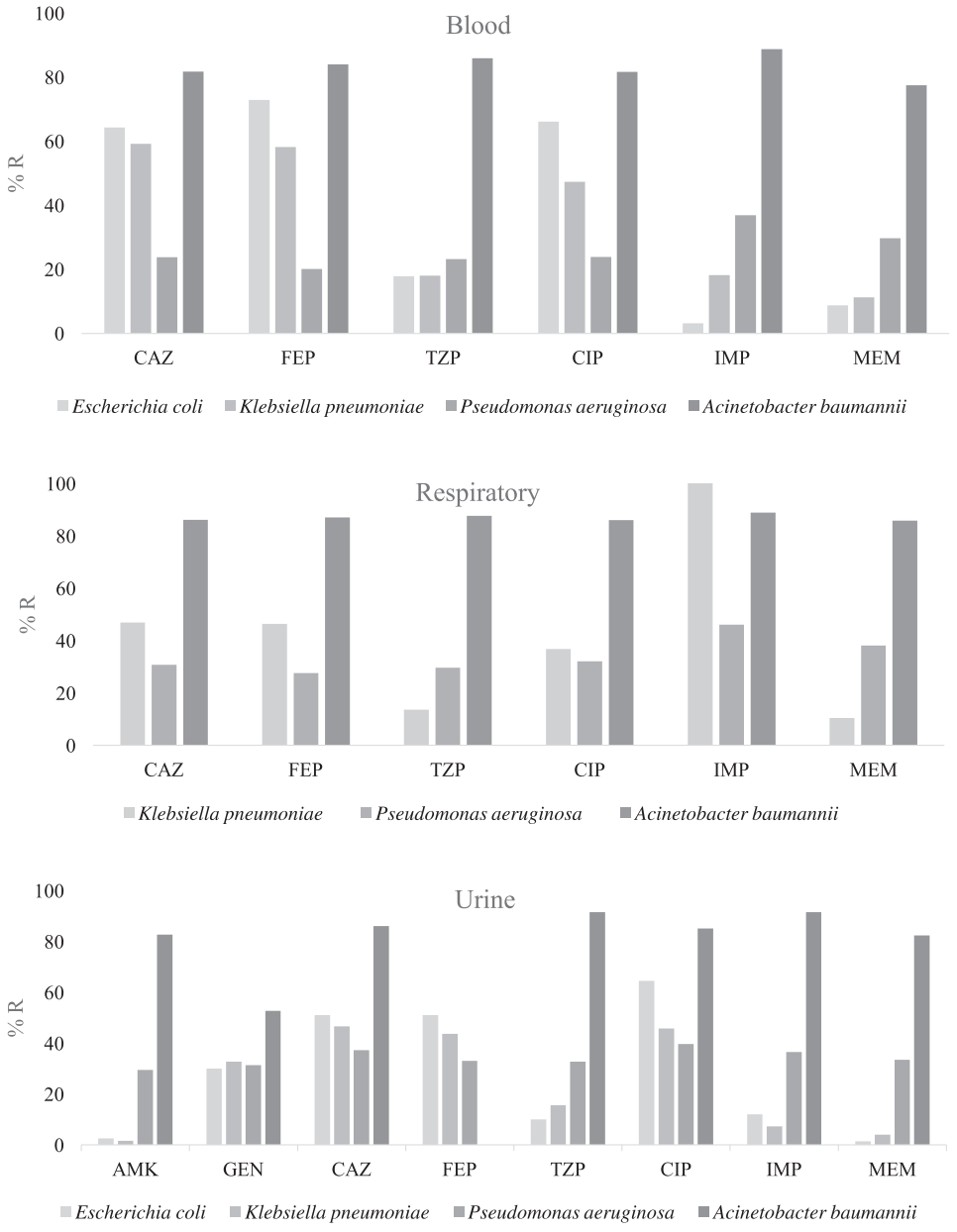

FIG. 1. Distribution of antibiotic resistance for samples collected from blood, respiratory specimens, and urine.

its potential health threat due to multiple drug resistance. The presence of *K. pneumoniae* ST307 was reported in 2019 in Mexico,[45] but no other reports have been published about this lineage in this country. Recently, 38 strains of *K. pneumoniae* clinical isolates collected from 2010 to 2012 were sequenced in a Mexican pediatric hospital, and 24 different STs were identified. The most prevalent STs were ST76 ($n=6$), followed by ST70 ($n=4$). One ST4839 was detected, but there was no detection of ST307.[48]

Among *A. baumannii* isolates, the most frequent genes detected were $bla_{\text{OXA-40}}$, followed by $bla_{\text{OXA-23}}$. Genetic lineages of *A. baumannii* have been studied using two MLST schemes with three genes in common, as reported by Bartual et al (Oxford scheme)[49] and by Diancourt et al (Pasteur scheme).[50] It has been reported that MLST analysis based on the Pasteur scheme is more appropriate than the Oxford scheme for population biology and epidemiological studies.[51] In our study, four strains were detected to be ST2 according to the Pasteur scheme (two ST369 and two ST208, according to the Oxford scheme). Previous reports have indicated that *A. baumannii* ST2 is predominant in human samples in Germany[52] and is widely distributed in Pakistan and Iran.[53–55] *A. baumannii* ST2 has been related to multidrug resistance, including colistin resistance in South Africa.[56] In Mexico, the most frequently reported is ST156 (27.27%, 24/88).[57]

MLST is the standard method for epidemiological surveys on *P. aeruginosa* outbreaks worldwide,[58] with outbreaks by ST235 and ST357 strains reported in many countries.[59,60] In the present study, no predominant ST was detected among the five strain types (ST111, ST260, ST274, ST983, ST309) for *P. aeruginosa*, but the presence of ST111 is quite relevant because it is a major international high-risk *P. aeruginosa* clone, in addition to ST175 and ST235.

P. aeruginosa ST111 has been described as a high-risk strain resistant to multiple antibiotics, including

carbapenem, and has been associated with outbreaks in health care settings worldwide.[61–65] Furthermore, the ST111 clone has been described to have increased capacity to form a biofilm, and this virulence factor may contribute to clonal dominance.[66]

Regarding *E. coli*, the most frequent carbapenemase-encoding gene was bla_{NDM} (bla_{NDM-5}), and ST167 was the most frequent lineage detected (associated with NDM-5). NDM-5 had been primarily identified in multidrug-resistant *E. coli* in the United Kingdom and has been reported to have more activity over carbapenems than NDM-1.[4] The ST167 clonal lineage is common among NDM-5-producing *E. coli* isolates, being identified in many different countries including China, Italy, Egypt, Switzerland, and Germany.[67–71]

In light of the COVID-19 pandemic, the carbapenem resistance rate for *K. pneumoniae* has been reported to be 75.5% in China.[72] Previous studies in Mexico showed a low frequency of carbapenem resistance in Enterobacterales, with meropenem resistance near 3% in *E. coli* and 12.5% in *K. pneumoniae*.[73] However, carbapenem resistance increased immediately after the COVID-19 pandemic, with values as high as 21.4% in *K. pneumoniae* recovered from blood.[74] In the present study, resistance rates for imipenem and meropenem were 18.3% and 11.3%, respectively, in *K. pneumoniae* recovered from blood.

Colistin resistance had previously been identified in NDM-1-producing *K. pneumoniae* in Mexico.[20] Probably, the overuse of colistin in infections by bacteria resistant to carbapenems and the circulation of NDM-1-producing *K. pneumoniae* have favored the emergence of these potentially pandrug-resistant strains.

Although *mgrB* gene modification is the main mechanism of colistin resistance in *K. pneumoniae* in the Americas,[20] we only identified this mechanism in one isolate. Further characterization is necessary to understand the other mechanisms of colistin resistance identified in this study. However, we also identified two *K. pneumoniae* isolates carrying the *mcr-1* gene; this represents the second report of *mcr-1* in clinical isolates in Mexico[75] and the first report in *K. pneumoniae*.

Distribution of clones was observed in the species included in the study; this distribution of clones is observed by the hospital and by bacterial species. For A. *baumannii*, several clones were detected, with isolates from each of the clone distributed on the same center

In the case of *P. aeruginosa*, many clones were identified but with a reduced number of isolates in each clone, as in *E. coli*. Therefore, we consider that the dissemination of carbapenemase-encoding genes in the bacterial species included in this study could be due to clonality and possibly to the dissemination of mobile genetic elements such as plasmids that contribute to their dissemination.

There are limitations to our study. First, there is insufficient information from all states from Mexico; thus, we were not able to assess the frequency of carbapenem resistance encoding genes on a truly national scale, and second, some antibiotic resistance mechanisms remained unidentified.

Conclusions

We detected a circulation of *K. pneumoniae* NDM and *E. coli* NDM in centers in Mexico. We identified high virulence ST types, such as *K. pneumoniae* ST307, *E. coli* ST167, *P. aeruginosa* ST111, and *A. baumannii* ST2 (Pasteur). Resistance to colistin was identified in carbapenemase-producing *K. pneumoniae* isolates, with nonidentified molecular mechanisms being the main one, followed by the *mcr-1* gene. These results are relevant in clinical practice to guide antibiotic therapies considering the molecular mechanisms of resistance to carbapenems and will contribute to optimize antibiotic stewardship programs.

Acknowledgment

The authors thank Lucy Acevedo for technical support.

Authors' Contributions

U.G.-R., J.S.-S., L.E.L.-J., M.H.-D., C.A.C.-C., A.S.-P., and J.R.-S. conducted molecular analysis, and reviewed and edited the article. R.M.-O., E.R.-N., M.-d.-C.V.-A., M.D.R.V.-L., J.M.F.-G., F.R.-L., A.P.-D.-L., M.L.-G., E.V.C.-C., E.L.-G., A.M.-J., M.G.-V., R.E.C.-R., I.L.-O., J.L.R.-M., D.E.R.-B., A.M.-C., C.P.-I., M.A.Q.-R., C.D.M.-D., N.R.-M., D.R.-M., C.C.-F., F.C.-L., D.A.B.-M., R.M.-L., R.T.M.-V., N.R.E.B.-R., J.P.M.-R., C.A.C.-M., M.A.-E., C.A.-A., and L.H.-V. isolated and provided the strains with routine phenotypic data, and reviewed and edited the article. E.G.-G. conceptualization, conducted molecular, data analysis, and drafted the article.

Disclosure Statement

No competing financial interests exist.

Funding Information

No funding was received for this article.

Supplementary Material

Supplementary Figure S1
Supplementary Table S1
Supplementary Table S2
Supplementary Table S3

References

1. Langford BJ, So M, Raybardhan S, et al. Bacterial co-infection and secondary infection in patients with COVID-19: A living rapid review and meta-analysis. Clin Microbiol Infect 2020;26(12):1622–1629; doi: 10.1016/j.cmi.2020.07.016
2. Lansbury L, Lim B, Baskaran V, et al. Co-infections in people with COVID-19: A systematic review and meta-analysis. J Infect 2020;81(2):266–275; doi: 10.1016/j.jinf.2020.05.046
3. Palacios-Baena ZR, Giannella M, Manissero D, et al. Risk factors for carbapenem-resistant Gram-negative bacterial infections: A systematic review. Clin Microbiol Infect 2021;27(2):228–235; doi: 10.1016/j.cmi.2020.10.016
4. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: The impact and evolution of a global menace. J Infect Dis 2017;215(Suppl 1):S28–S36; doi: 10.1093/infdis/jiw282
5. Wong D, Nielsen TB, Bonomo RA, et al. Clinical and pathophysiological overview of *Acinetobacter* infections: A

century of challenges. Clin Microbiol Rev 2017;30(1):409–447; doi: 10.1128/CMR.00058-16
6. Miriagou V, Cornaglia G, Edelstein M, et al. Acquired carbapenemases in Gram-negative bacterial pathogens: Detection and surveillance issues. Clin Microbiol Infect 2010; 16(2):112–122; doi: 10.1111/j.1469-0691.2009.03116.x
7. Rolain JM, Parola P, Cornaglia G. New Delhi metallo-beta-lactamase (NDM-1): Towards a new pandemia? Clin Microbiol Infect 2010;16(12):1699–1701; doi: 10.1111/j.1469-0691.2010.03385.x
8. Nori P, Szymczak W, Puius Y, et al. Emerging co-pathogens: New Delhi metallo-beta-lactamase producing Enterobacterales infections in New York City COVID-19 patients. Int J Antimicrob Agents 2020;56(6):106179; doi: 10.1016/j.ijantimicag.2020.106179
9. Ambler RP. The structure of beta-lactamases. Philos Trans R Soc Lond B Biol Sci 1980;289(1036):321–331; doi: 10.1098/rstb.1980.0049
10. Hall BG, Barlow M. Revised Ambler classification of {beta}-lactamases. J Antimicrob Chemother 2005;55(6): 1050–1051; doi: 10.1093/jac/dki130
11. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2011;17(10):1791–1798; doi: 10.3201/eid1710.110655
12. Hammoudi Halat D, Ayoub Moubareck C. The current burden of Carbapenemases: Review of significant properties and dissemination among Gram-negative bacteria. Antibiotics (Basel) 2020;9(4):186; doi: 10.3390/antibiotics9040186
13. Cui X, Zhang H, Du H. Carbapenemases in Enterobacteriaceae: Detection and antimicrobial therapy. Front Microbiol 2019;10:1823; doi: 10.3389/fmicb.2019.01823
14. Safavi M, Bostanshirin N, Hajikhani B, et al. Global genotype distribution of human clinical isolates of New Delhi metallo-β-lactamase-producing *Klebsiella pneumoniae*: A systematic review. J Glob Antimicrob Resist 2020; 23:420–429; doi: 10.1016/j.jgar.2020.10.016
15. Jeannot K, Bolard A, Plésiat P. Resistance to polymyxins in Gram-negative organisms. Int J Antimicrob Agents 2017; 49(5):526–535; doi:10.1016/j.ijantimicag.2016.11.029
16. Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. Curr Med Res Opin 2015;31(4): 707–721; doi: 10.1185/03007995.2015.1018989
17. Zayyad H, Eliakim-Raz N, Leibovici L, et al. Revival of old antibiotics: Needs, the state of evidence and expectations. Int J Antimicrob Agents 2017;49(5):536–541; doi: 10.1016/j.ijantimicag.2016.11.021
18. Mlynarcik P, Kolar M. Molecular mechanisms of polymyxin resistance and detection of mcr genes. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2019; 163(1):28–38; doi: 10.5507/bp.2018.070
19. Gogry FA, Siddiqui MT, Sultan I, et al. Current update on intrinsic and acquired colistin resistance mechanisms in Bacteria. Front Med (Lausanne) 2021;8:677720; doi: 10.3389/fmed.2021.677720
20. Rodríguez-Santiago J, Cornejo-Juárez P, Silva-Sánchez J, et al. Polymyxin resistance in Enterobacterales: Overview and epidemiology in the Americas. Int J Antimicrob Agents 2021;58(5):106426; doi: 10.1016/j.ijantimicag.2021.106426
21. CLSI. M100-S30. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Clinical and Laboratory Standars Institute: Wayne, PA; 2020.
22. Bogaerts P, Rezende de Castro R, de Mendonça R, et al. Validation of carbapenemase and extended-spectrum β-lactamase multiplex endpoint PCR assays according to ISO 15189. J Antimicrob Chemother 2013;68(7):1576–1582; doi: 10.1093/jac/dkt065
23. Hong SS, Kim K, Huh JY, J et al. Multiplex PCR for rapid detection of genes encoding class A carbapenemases. Ann Lab Med 2012;32(5):359–361; doi: 10.3343/alm.2012.32.5.359
24. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. Clin Microbiol Rev 2007;20(3):440–458; doi: 10.1128/CMR.00001–07
25. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 2002;40(6): 2153–2162; doi: 10.1128/jcm.40.6.2153–2162.2002
26. Brink AJ, Coetzee J, Corcoran C, et al. Emergence of OXA-48 and OXA-181 carbapenemases among Enterobacteriaceae in South Africa and evidence of in vivo selection of colistin resistance as a consequence of selective decontamination of the gastrointestinal tract. J Clin Microbiol 2013;51(1):369–372; doi: 10.1128/JCM.02234-12
27. Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in Enterobacteriaceae. Emerg Infect Dis 2016;22(6):1038–1043; doi: 10.3201/eid2206.151840
28. Rodríguez-Santiago J, Rodríguez-Medina N, Tamayo-Legorreta EM, et al. Molecular and genomic insights of *mcr-1*-producing *Escherichia coli* isolates from piglets. Antibiotics (Basel) 2022;11(2):157; doi: 10.3390/antibiotics11020157
29. Cannatelli A, D'Andrea MM, Giani T, et al. In vivo emergence of colistin resistance in *Klebsiella pneumoniae* producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP *mgrB* regulator. Antimicrob Agents Chemother 2013;57(11):5521–5526; doi: 10.1128/AAC.01480-13
30. Kaufmann ME. Pulsed-field gel electrophoresis. Methods Mol Med 1998;15:33–50; doi: 10.1385/0-89603-498-4:33
31. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol 1995;33(9):2233–2239.
32. Barrios H, Garza-Ramos U, Reyna-Flores F, et al. Isolation of carbapenem-resistant NDM-1-positive *Providencia rettgeri* in Mexico. J Antimicrob Chemother 2013;68(8): 1934–1936; doi: 10.1093/jac/dkt124
33. Bocanegra-Ibarias P, Garza-González E, Morfín-Otero R, et al. Molecular and microbiological report of a hospital outbreak of NDM-1-carrying Enterobacteriaceae in Mexico. PLoS One 2017;12(6):e0179651; doi: 10.1371/journal.pone.0179651
34. Aquino-Andrade A, Merida-Vieyra J, Arias de la Garza E. Carbapenemase-producing Enterobacteriaceae in Mexico: Report of seven non-clonal cases in a pediatric hospital. BMC Microbiol 2018;18(1):38; doi: 10.1186/s12866-018-1166-z
35. Garza-González E, Bocanegra-Ibarias P, Rodríguez-Noriega E, et al. Molecular investigation of an outbreak associated with total parenteral nutrition contaminated with NDM-producing *Leclercia adecarboxylata*. BMC Infect Dis 2021;21(1):235; doi: 10.1186/s12879-021-05923-0
36. Garza-González E, Bocanegra-Ibarias P, Bobadilla-Del-Valle M, et al. Drug resistance phenotypes and genotypes in Mexico in representative gram-negative species: Results

from the infivar network. PLoS One 2021;16(3):e0248614; doi:10.1371/journal.pone.0248614
37. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998;11(4):589–603; doi: 10.1128/CMR.11.4.589
38. Villa L, Feudi C, Fortini D, et al. Diversity, virulence, and antimicrobial resistance of the KPC-producing. Microb Genom 2017;3(4):e000110; doi: 10.1099/mgen.0.000110
39. Wyres KL, Holt KE. *Klebsiella pneumoniae* population genomics and antimicrobial-resistant clones. Trends Microbiol 2016;24(12):944–956; doi: 10.1016/j.tim.2016.09.007
40. Schaufler K, Nowak K, Düx A, et al. Clinically relevant ESBL-producing. Front Microbiol 2018;9:150; doi: 10.3389/fmicb.2018.00150
41. Long SW, Olsen RJ, Eagar TN, et al. Population genomic analysis of 1,777 extended-spectrum beta-lactamase-producing. mBio 2017;8(3):e00489-17; doi: 10.1128/mBio.00489-17
42. Baek EH, Kim SE, Kim S, et al. Successful control of an extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* ST307 outbreak in a neonatal intensive care unit. BMC Infect Dis 2020;20(1):166; doi: 10.1186/s12879-020-4889-z
43. Kim JO, Song SA, Yoon EJ, et al. Outbreak of KPC-2-producing Enterobacteriaceae caused by clonal dissemination of *Klebsiella pneumoniae* ST307 carrying an IncX3-type plasmid harboring a truncated Tn4401a. Diagn Microbiol Infect Dis 2017;87(4):343–348; doi: 10.1016/j.diagmicrobio.2016.12.012
44. Boonstra MB, Spijkerman DCM, Voor In 't Holt AF, et al. An outbreak of ST307 extended-spectrum beta-lactamase (ESBL)-producing. Infect Control Hosp Epidemiol 2020;41(1):31–36; doi: 10.1017/ice.2019.304
45. Bocanegra-Ibarias P, Garza-González E, Padilla-Orozco M, et al. The successful containment of a hospital outbreak caused by NDM-1-producing *Klebsiella pneumoniae* ST307 using active surveillance. PLoS One 2019;14(2):e0209609; doi: 10.1371/journal.pone.0209609
46. Lu B, Lin C, Liu H, et al. Molecular characteristics of *Klebsiella pneumoniae* isolates from outpatients in Sentinel Hospitals, Beijing, China, 2010–2019. Front Cell Infect Microbiol 2020;10:85; doi: 10.3389/fcimb.2020.00085
47. Carattoli A, Bertini A, Villa L, et al. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 2005;63(3):219–228; doi: 10.1016/j.mimet.2005.03.018
48. Flores-Valdez M, Ares MA, Rosales-Reyes R, et al. Whole genome sequencing of pediatric. Front Microbiol 2021;12:711577; doi: 10.3389/fmicb.2021.711577
49. Bartual SG, Seifert H, Hippler C, et al. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii.* J Clin Microbiol 2005;43(9):4382–4390; doi: 10.1128/JCM.43.9.4382–4390.2005
50. Diancourt L, Passet V, Nemec A, et al. The population structure of *Acinetobacter baumannii:* Expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 2010;5(4):e10034; doi:10.1371/journal.pone.0010034
51. Gaiarsa S, Batisti Biffignandi G, Esposito EP, et al. Comparative analysis of the two. Front Microbiol 2019;10:930; doi:10.3389/fmicb.2019.00930
52. Wareth G, Linde J, Hammer P, et al. Molecular characterization of German. Pathogens 2021;10(6):60690; doi: 10.3390/pathogens10060690
53. Ejaz H, Ahmad M, Younas S, et al. Molecular epidemiology of extensively-drug resistant. Infect Drug Resist 2021;14:1931–1939; doi: 10.2147/IDR.S310478
54. Abdollahi A, Aliramezani A, Salehi M, et al. Co-infection of ST2. BMC Infect Dis 2021;21(1):927. doi: 10.1186/s12879-021-06642-2
55. Meshkat Z, Salimizand H, Amini Y, et al. Detection of efflux pump genes in multiresistant *Acinetobacter baumannii* ST2 in Iran. Acta Microbiol Immunol Hung 2021;68(2):113–120; doi: 10.1556/030.2021.01314
56. Nogbou ND, Ramashia M, Nkawane GM, et al. Whole-genome sequencing of a colistin-resistant. Antibiotics (Basel) 2022;11(5):594; doi: 10.3390/antibiotics11050594
57. Mancilla-Rojano J, Ochoa SA, Reyes-Grajeda JP, et al. Molecular epidemiology of *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex isolated from children at the Hospital Infantil de México Federico Gómez. Front Microbiol 2020;11:576673; doi: 10.3389/fmicb.2020.576673
58. Curran B, Jonas D, Grundmann H, et al. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa.* J Clin Microbiol 2004;42(12):5644–5649; doi:10.1128/JCM.42.12.5644–5649.2004
59. Yoo JS, Yang JW, Kim HM, et al. Dissemination of genetically related IMP-6-producing multidrug-resistant *Pseudomonas aeruginosa* ST235 in South Korea. Int J Antimicrob Agents 2012;39(4):300–304; doi: 10.1016/j.ijantimicag.2011.11.018
60. Hrabák J, Cervená D, Izdebski R, et al. Regional spread of *Pseudomonas aeruginosa* ST357 producing IMP-7 metallo-β-lactamase in Central Europe. J Clin Microbiol 2011;49(1):474–475; doi: 10.1128/JCM.00684-10
61. Cabot G, Ocampo-Sosa AA, Domínguez MA, et al. Genetic markers of widespread extensively drug-resistant *Pseudomonas aeruginosa* high-risk clones. Antimicrob Agents Chemother 2012;56(12):6349–6357; doi: 10.1128/AAC.01388-12
62. Oliver A, Mulet X, López-Causapé C, et al. The increasing threat of *Pseudomonas aeruginosa* high-risk clones. Drug Resist Updat 2015;21–22:41–59; doi: 10.1016/j.drup.2015.08.002
63. Kossow A, Kampmeier S, Willems S, et al. Control of multidrug-resistant *Pseudomonas aeruginosa* in allogeneic hematopoietic stem cell transplant recipients by a novel bundle including remodeling of sanitary and water supply systems. Clin Infect Dis 2017;65(6):935–942; doi: 10.1093/cid/cix465
64. Molina-Mora JA, Chinchilla-Montero D, García-Batán R, et al. Genomic context of the two integrons of ST-111 *Pseudomonas aeruginosa* AG1: A VIM-2-carrying old-acquaintance and a novel IMP-18-carrying integron. Infect Genet Evol 2021;89:104740; doi: 10.1016/j.meegid.2021.104740
65. Papagiannitsis CC, Verra A, Galani V, et al. Unravelling the features of success of VIM-producing ST111 and ST235. Microorganisms 2020;8(12):1884; doi: 10.3390/microorganisms8121884
66. Zhang L, Tan FC, Strasfeld L, et al. Long-term dominance of Carbapenem-non-susceptible. Front Cell Infect Microbiol 2022;12:904602; doi: 10.3389/fcimb.2022.904602

67. Li X, Fu Y, Shen M, et al. Dissemination of bla_{NDM-5} gene via an $IncX_3$-type plasmid among non-clonal *Escherichia coli* in China. Antimicrob Resist Infect Control 2018;7:59; doi: 10.1186/s13756-018-0349-6
68. Giufrè M, Errico G, Accogli M, et al. Emergence of NDM-5-producing *Escherichia coli* sequence type 167 clone in Italy. Int J Antimicrob Agents 2018;52(1):76–81; doi: 10.1016/j.ijantimicag.2018.02.020
69. Ramadan H, Gupta SK, Sharma P, et al. Circulation of emerging NDM-5-producing *Escherichia coli* among humans and dogs in Egypt. Zoonoses Public Health 2020; 67(3):324–329; doi: 10.1111/zph.12676
70. Endimiani A, Brilhante M, Bernasconi OJ, et al. Employees of Swiss veterinary clinics colonized with epidemic clones of carbapenemase-producing *Escherichia coli.* J Antimicrob Chemother 2020;75(3):766–768; doi: 10.1093/jac/dkz470
71. Chakraborty T, Sadek M, Yao Y, et al. Cross-border emergence of *Escherichia coli* producing the Carbapenemase NDM-5 in Switzerland and Germany. J Clin Microbiol 2021;59(3):e02238-20; doi: 10.1128/JCM.02238-20
72. Li J, Wang J, Yang Y, et al. Etiology and antimicrobial resistance of secondary bacterial infections in patients hospitalized with COVID-19 in Wuhan, China: A retrospective analysis. Antimicrob Resist Infect Control 2020; 9(1):153; doi: 10.1186/s13756-020-00819-1
73. Garza-González E, Morfín-Otero R, Mendoza-Olazarán S, et al. A snapshot of antimicrobial resistance in Mexico. Results from 47 centers from 20 states during a six-month period. PLoS One 2019;14(3):e0209865; doi: 10.1371/journal.pone.0209865
74. López-Jácome LE, Fernández-Rodríguez D, Franco-Cendejas R, et al. Increment antimicrobial resistance during the COVID-19 pandemic: Results from the Invifar Network. Microb Drug Resist 2021; doi: 10.1089/mdr.2021.0231
75. Merida-Vieyra J, De Colsa-Ranero A, Arzate-Barbosa P, et al. First clinical isolate of *Escherichia coli* harboring mcr-1 gene in Mexico. PLoS One 2019;14(4):e0214648; doi: 10.1371/journal.pone.0214648

Address correspondence to:
Elvira Garza-González, PhD
Facultad de Medicina
Hospital Universitario Dr. José Eleuterio González
Universidad Autónoma de Nuevo León
Avenida Francisco I. Madero S/N
Colonia Mitras Centro
Monterrey, NL CP 64460
Mexico

E-mail: elvira_garza_gzz@yahoo.com