Drug resistance phenotypes and genotypes in Mexico in representative gram-negative species: Results from the invar network

Elvira Garza-Gonzále1, Paola Bocanegra-Ibarias1, Miriam Bobadilla-del-Valle2, Luis Alfredo Ponce-de-León-Garduño3, Verónica Esteban-Kenel4, Jesús Silva-Sánchez5, Ulises Garza-Ramos3, Humberto Barrios-Camacho3, Luis Esau López-Jácome3, Claudia A. Colin-Castro4, Rafael Franco-Cendejas4, Samantha Flores-Treviño1, Rayo Morfin-Otero6, Fabian Rojas-Larios8, Juan Pablo Mena-Ramírez7, María Guadalupe Fong-Camargo7, Cecilia Teresita Morales-De-la-Peña9, Lourdes García-Mendoza10, Elena Victoria Choy-Chang11, Laura Karina Aviles-Benitez12, José Manuel Feliciano-Guzmán13, Eduardo López-Gutiérrez14, Mariana Gil-Veloz15, Juan Manuel Barajas-Magallón16, Efren Aguirre-Burciaga17, Laura Isabel López-Moreno18, Rebeca Thelma Martínez-Villarreal19, Jorge Luis Canizales-Oviedo20, Carlos Miguel Cetina-Uña21, Daniel Romero-Romero22, Fidencio David Bello-Pazos23, Nicolás Rogelio Eric Barlandas-Rendón24, Joyarib Yanelli Maldonado-Anicacio25, Enrique Bolado-Martínez26, Mario Galindo-Méndez27, Talia Perez-Vicelis28, Norma Alavez-Ramírez29, Braulio J. Méndez-Sotelo30, Juan Francisco Cabriales-Zavala31, Yrla Cittiali Nava-Pacheco32, Martha Irene Moreno-Méndez33, Ricardo García-Romo33, Aldo Rafael Silva-Gamín34, Ana Maria Avalos-Aguilera35, María Asunción Santiago-Calderón36, Maribel López-García37, María del Consuelo Velázquez-Acosta38, Dulce Isabel Cobos-Canul39, María del Rosario Vázquez-Larios40, Ana Elizabeth Ortiz-Porcayo41, Arel Elizabeth Guerrero-Núñez42, Jazmín Valero-Guzmán43, Alina Aracely Rosales-García44, Heidy Leticia Osto-Cantu45, Adrián Camacho-Ortí46

1 Hospital Universitario Dr. José E. González, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico, 2 Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Ciudad de México, Mexico, 3 Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico, 4 Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra, Ciudad de México, Mexico, 5 Hospital Civil de Guadalajara E Instituto de Patología Infecciosa, Guadalajara, Jalisco, Mexico, 6 Hospital Regional Universitario de Colima, Colima, Colima, Mexico, 7 Hospital General de Zona 21 Tepatitlán De Morelos, Centro Universitario de los Altos (CUALTSO), Universidad de Guadalajara, Tepatitlán de Morelos, Jalisco, Mexico, 8 Hospital General Regional No. 1, Chihuahua, Chihuahua, Mexico, 9 Hospital General con Especialidades Juan María de Saldivar, La Paz, Baja California Sur, Mexico, 10 Hospital Angeles Valle Oriente, Monterrey Nuevo León, Mexico, 11 Hospital General de Zona No. 1, Tapachula, Chiapas, Mexico, 12 Hospital Infantil de Morelia, Morelia, Michoacán, Mexico, 13 Hospital de Especialidades Pediatrías de Chiapas, Tuxtla Gutiérrez, Chiapas, Mexico, 14 Hospital Regional de Alta Especialidad de Oaxaca, San Bartolo Coyotepec, Oaxaca, Mexico, 15 Hospital Regional de Alta Especialidad del Bajío, Guanajuato, Guanajuato, Mexico, 16 Laboratorio Dipromi, Michoacán, Morelia, Mexico, 17 Hospital Regional Delicias, Delicias, Chihuahua, Mexico, 18 Galería Hospital, Cancún, Quintana Roo, Mexico, 19 Centro Universitario de Salud, Universidad Autónoma de Nuevo León. Laboratorio Victorice Guerrero, Monterrey Nuevo León, Mexico, 20 Centro Universitario de Salud, Universidad Autónoma de Nuevo León. Laboratorio Pueblo Nuevo, Monterrey Nuevo León, Mexico, 21 Hospital Materno Infantil Morelos, Chetumal Quintana Roo, Mexico, 22 Laboratorio de Análisis Bioquímico Clínicos “Louis Pasteur” Toluca, Estado de México, Mexico, 23 Hospital H+ Querétaro, Querétaro, Mexico, 24 Laboratorio Bioclin, Chilpancingo, Guerrero, Mexico, 25 Hospital general de Chilpancingo, Chilpancingo, Guerrero, Mexico, 26 Universidad de Sonora, Hermosillo, Sonora, Mexico, 27 Laboratorios Galindo SC, Oaxaca, Oaxaca, Mexico, 28 Hospital Regional “Bicentenario de la Independencia” ISSSTE, Tultitlán, Estado de México, Mexico, 29 Hospital General “Dr. Manuel Gea González”, Ciudad de México, Mexico, 30 Swiss Hospital, Monterrey Nuevo león, Mexico, 31 Hospital para el Niño Poblano, San Andrés Cholula, Puebla, Mexico, 32 Laboratorios del Centro, Zamora, Michoacán, Mexico, 33 Centenario Hospital Miguel Hidalgo, Aguascalientes, Aguascalientes, Mexico, 34 Hospital Ángeles Morelia, Morelia, Michoacán, Mexico, 35 Hospital General “Dr. Miguel Silva”, Morelia, Michoacán, Mexico, 36 Hospital General de Zona No 1 Dr. Demetrio Mayoral Pardo, Oaxaca, Oaxaca, Mexico, 37 Hospital de la Madre y Niño Guerrerense, Chilpancingo, Guerrero, Mexico, 38 Instituto Nacional de
Abstract

**Aim**

This report presents phenotypic and genetic data on the prevalence and characteristics of extended-spectrum β-lactamases (ESBLs) and representative carbapenemases-producing Gram-negative species in Mexico.

**Material and methods**

A total of 52 centers participated, 43 hospital-based laboratories and 9 external laboratories. The distribution of antimicrobial resistance data for *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, *Acinetobacter baumannii* complex, and *Pseudomonas aeruginosa* in selected clinical specimens from January 1 to March 31, 2020 was analyzed using the WHONET 5.6 platform. The following clinical isolates recovered from selected specimens were included: carbapenem-resistant *Enterobacteriaceae*, ESBL or carbapenem-resistant *E. coli*, and *K. pneumoniae*, carbapenem-resistant *A. baumannii* complex, and *P. aeruginosa*. Strains were genotyped to detect ESBL and/or carbapenemase-encoding genes.

**Results**

Among blood isolates, *A. baumannii* complex showed more than 68% resistance for all antibiotics tested, and among Enterobacteria, *E. cloacae* complex showed higher resistance to carbapenems. *A. baumannii* complex showed a higher resistance pattern for respiratory specimens, with only amikacin having a resistance lower than 70%. Among *K. pneumoniae* isolates, *bla*TEM, *bla*SHV, and *bla*CTX were detected in 68.79%, 72.3%, and 91.9% of isolates, respectively. Among *E. coli* isolates, *bla*TEM, *bla*SHV, and *bla*CTX were detected in 20.8%, 4.53%, and 85.7% isolates, respectively. For both species, the most frequent genotype was *bla*CTX-M-15. Among *Enterobacteriaceae*, the most frequently detected carbapenemase-encoding gene was *bla*NDM-1 (81.5%), followed by *bla*OXA-232 (14.8%) and *bla*OXA-181 (7.4%), in *A. baumannii* was *bla*OXA-24 (76%) and in *P. aeruginosa*, was *bla*IMP (25.3%), followed by *bla*GES and *bla*VIM (13.1% each).

**Conclusion**

Our study reports that NDM-1 is the most frequent carbapenemase-encoding gene in Mexico in Enterobacteriaceae with the circulation of the oxacillinase genes 181 and 232. KPC, in contrast to other countries in Latin America and the USA, is a rare occurrence. Additionally, a high circulation of ESBL *bla*CTX-M-15 exists in both *E. coli* and *K. pneumoniae*.
Introduction

National and local surveillance of drug resistance and the involved genotypes is fundamental to implementing adequate infection control measures [1, 2].

The prevalence of carbapenemases from Ambler class A, B, and D, cephalosporinas (AmpCs), is rapidly increasing among Gram-negative bacteria and is rapidly increasing among Gram-negative bacteria and is now widely distributed [3, 4].

Among class A, the most reported β-lactamases are the extended-spectrum β-lactamases (ESBLs) cefotaximase (CTX-M), temoneira (TEM), and sulfhydryl variable (SHV), along with the Klebsiella pneumoniae carbapenemase (KPC) [3, 4].

Class B metallo-β-lactamases include those enzymes that confer resistance to carbapenem antibiotics as the carbapenemases the imipenem (IMP), New Delhi metallo-β-lactamase (NDM), and those encoded by vimentin (VIM) [5]. Among class D β-lactamases, the most frequently reported oxacillinases (OXA) are those encoded by blaOXA-23-like, blaOXA-24-like, and blaOXA-58-like genes in Acinetobacter baumannii and by blaOXA-48-like especially in Enterobacteriaceae.

Some research groups from Mexico have published the drug resistance rates and involved genes for some Gram-negative bacteria, including A. baumannii, Pseudomonas aeruginosa, Enterobacter cloacae, K. pneumoniae, and Escherichia coli [6–9]. However, information available is limited, and nationwide studies are needed.

To contribute to the study of drug resistance in Mexico, the Network for the Research and Surveillance of Drug Resistance (Red Temática de Investigación y Vigilancia de la Farmacorre-sistencia INVIFAR, in Spanish) was created in 2018 and has reported an increase in drug resistance for several bacterial species, underlying the increase in carbapenem resistance for Enterobacter spp. and Klebsiella spp. [10, 11].

This report presents phenotypic and genetic data on the prevalence and characteristics of ESBL and carbapenemase-producing representative Gram-negative species in Mexico during the first trimester of 2020.

Materials and methods

Participating centers, data collection, and analysis

A total of 52 centers participated: 43 hospital-based laboratories and 9 external laboratories.

Identification and susceptibility test results from January 1 to March 31, 2020, from participating laboratories were deposited into the WHONET 5.6 platform and converted to the WHONET using the BacLink 2 tool. WHONET files were analyzed using macros to facilitate the revision, and only one strain per patient was included. The distribution of antimicrobial resistance for E. coli, K. pneumoniae, E. cloacae complex, A. baumannii complex, and P. aerugi-nosa was analyzed in clinical specimens such as urine, blood, and respiratory specimens. The results were scored according to the Clinical and Laboratory Standards Institute (CLSI) criteria in all laboratories [12].

Included isolates

Participating laboratories sent to the coordinating laboratory all recovered isolates with the following characteristics: carbapenem-resistant Enterobacteriaceae (any species); ESBL or carbapenem-resistant E. coli collected from urine or blood; ESBL or carbapenem-resistant K. pneumoniae recovered from urine, respiratory specimens (endotracheal and bronchoalveolar lavage), or blood; carbapenem-resistant A. baumannii complex and P. aeruginosa recovered.
from urine, respiratory specimens, or blood. Clinical isolates collected from January 1 to March 31, 2020, were included.

All identifications were confirmed at the coordinating laboratory using MALDI-TOF. After confirmation, phenotypic tests and genotyping tests were performed for each strain.

**Beta-lactamase identification and characterization in Enterobacteriaceae**

The ESBL phenotypic detection test was performed using the double disk method recommended by the CLSI for *E. coli* and *K. pneumonia* [12]. The molecular detection and characterization of ESBLs were performed for *bla*TEM, *bla*SHV, and *bla*CTX-M genes in selected isolates by PCR using previously described and newly designed primers (S1 Table) [13]. A selection of amplified products was sequenced.

Carbapenemase production in *Enterobacteriaceae* was detected using the CarbaNP test and modified carbapenem inactivation according to the CLSI [12].

For carbapenemase-encoding genes detection, *Enterobacteriaceae* were tested by PCR for *bla*KPC, *bla*GES, *bla*VIM, *bla*IMP, *bla*NDM-1, *bla*OXA-48-like, and chromosomal *ampC* genes as described [14–17].

All PCR products were sequenced using a Hitachi analyzer (Applied Biosystems, Hitachi High-Technologies Corporation, Tokyo, Japan). DNA sequences were aligned and edited using BioEdit software (Ibis Bioscience, Carlsbad, CA) and matched in a gene bank (www.ncbi.nlm.nih.gov/genbank).

**Carbapenemase assays in A. baumannii and P. aeruginosa**

For carbapenem-resistant *A. baumannii*, the *bla*OXA-23, *bla*OXA-24, *bla*OXA-51, *bla*OXA-58, *bla*VIM, *bla*IMP, *bla*NDM-type β-lactamase genes were screened using PCR as described elsewhere [18, 19]. For *P. aeruginosa*, the detection of carbapenemase-encoding genes *bla*KPC, *bla*GES, *bla*IMP, *bla*NDM, and *bla*VIM was performed by PCR as described previously [20–24].

**Ethics statement**

The local ethics committee of Hospital Civil de Guadalajara “Fray Antonio Alcalde,” Jalisco, Mexico) approved this study with reference number 129/17. Informed consent was waived by the ethics committee because no intervention was involved. All participating institutions agreed with the present study.

**Results**

**Participating centers, data, and collected strains**

In this study, 52 centers collected strains and sent them to the coordinating laboratory: 43 hospital-based laboratories and 9 external laboratories. The three-month identification and susceptibility data were obtained from 46 centers (37 hospital-based laboratories and 9 external laboratories). The centers were distributed across 19 Mexican states. The characteristics of hospital-based centers are shown in Table 1.

The results of drug susceptibility for 8,245 strains were included for analysis, and 2,243 clinical isolates were collected at the reference laboratory. A selection of 813 isolates (including isolates from each center and state) was included for phenotypic and genotypic analysis.

**Drug resistance**

Regarding urine isolates, resistance was higher than 55% for all antibiotics in *A. baumannii* complex. In *P. aeruginosa*, the lowest percentage of resistance was for piperacillin/tazobactam...
| Center                                                  | Type     | Hosp beds | ICU Beds |
|--------------------------------------------------------|----------|-----------|----------|
| Hospital General con Especialidades Juan María de Salvatierra | Pu Spe   | 120       | 18       |
| Bioclinisa, Hospital Ginequito                         | Pu M&Ch  | 93        | 26       |
| Centenario Hospital Miguel Hidalgo                     | Pu Pu    | 103       | 21       |
| Galenia Hospital                                        | Pr Spe   | 54        | 4        |
| Hospital Ángeles Morelia                               | Pr Spe   | 50        | 11       |
| Hospital Clínica Nova                                  | Pr Spe   | 44        | 4        |
| Hospital de Alta Especialidad de Veracruz              | Pu Spe   | 235       | 10       |
| Hospital de Especialidades Pediatrías de Chiapas       | Pu Ped   | 90        | 19       |
| Hospital General Ciudad Obregón                        | Pu Univ  | 156       | 5        |
| Hospital H+ Querétaro                                  | Pr Spe   | 33        | 5        |
| Hospital Infantil de Morelia “Eva Sámano de López Mateos” | Pu Ped   | 80        | 6        |
| Hospital Regional de Alta Especialidad del Bajío       | Pu Spe   | 184       | 29       |
| Hospital Regional Delicias                             | Pu Spe   | 67        | 8        |
| Hospital Regional Universitario de Colima              | Pu Univ  | 108       | 8        |
| Hospital Ángeles Valle Oriente                         | Pr Spe   | 71        | 21       |
| Hospital Civil de Guadalajara “Fray Antonio Alcalde”   | Pu Univ  | 1000      | 85       |
| Hospital de Especialidad Materno Infantil de León      | Pu M&Ch  | 16        | 70       |
| Hospital de Especialidades Pediatrías León             | Pu Ped   | 38        | 17       |
| Hospital de la Madre y Niño Guerrerense                | Pu M&Ch  | 30        | 10       |
| Hospital General “Dr. Manuel Gea González”             | Pu Gen   | 107       | 5        |
| Hospital General “Dr. Miguel Silva”                    | Pu Gen   | 300       | 14       |
| Hospital General “Dr. Raymundo Abarca Alarcón”        | Pu Gen   | 108       | 8        |
| Hospital General de Chetumal                           | Pu Gen   | 88        | 10       |
| Hospital General de Chilpancingo                       | Pu Gen   | 114       | 8        |
| Hospital General de Zona No 21                         | Pu Gen   | 73        | 9        |
| Hospital General del Estado “Dr. Ernesto Ramos Bours”  | Pu Univ  | 200       | 20       |
| Hospital General Regional No.1                         | Pu Gen   | 233       | 10       |
| Hospital General de Zona No 1                          | Pu Gen   | 180       | 22       |
| Hospital General de Zona No 1 “Dr. Demetrio Mayoral Pardo” | Pu Gen | 168    | 8        |
| Hospital Materno Infantil “Morelos”                    | Pu M&Ch  | 30        | 0        |
| Hospital Militar Regional de Especialidades de Mazatlán | Pu Spe   | 126       | 6        |
| Hospital para el Niño Poblano                          | Pu Ped   | 90        | 17       |
| Hospital Regional Bicentenario de la Independencia, ISSSTE | Pu Spe   | 206       | 8        |
| Hospital Regional de Alta Especialidad de Oaxaca       | Pu Spe   | 60        | 6        |
| Hospital Regional Monterrey ISSSTE Monterrey           | Pu Spe   | 141       | 25       |
| Hospital Universitario “Dr. José Eleuterio González”   | Pu Univ  | 670       | 46       |
| Instituto Materno infantil del Estado de México        | Pu M&Ch  | 115       | 30       |
| Instituto Nacional de Cancerología                     | Pu Spe   | 135       | 6        |
| Instituto Nacional de Cardiología “Ignacio Chávez”      | Pu Spe   | 249       | 28       |
| Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán” | Pu Spe | 170 | 14 |
| Instituto Nacional de Rehabilitación “Luis Guillermo Ibarra Ibarra” | Pu Spe | 228 | 20 |
| Sanatorio La Luz                                       | Pr Gen   | 30        | 3        |
| Swiss Hospital                                         | Pr Spe   | 55        |          |

Abbreviations: Ad, adults; Gen, general; M&Ch, mother and child; Pr, Private; Pu, Public; Ped, pediatrics; Spe, specialties; Univ.

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Meanwhile, carbapenem resistance was low in *E. coli* (<1%) but high in *E. cloacae* complex (10.9%) (Table 2). Also, 44.9% and 39.3% of *E. coli* and *K. pneumoniae*, respectively, were reported to be ESBLs producers.

Among blood isolates, *A. baumannii* showed more than 68% resistance for all antibiotics tested, and *P. aeruginosa* had 37.1% resistance to meropenem. Among *Enterobacteriaceae*, *E. cloacae* showed higher resistance to carbapenems (4.4% for meropenem), whereas *K. pneumoniae* and *E. coli* had more than 59% resistance for cefepime (Table 3).

Also, 60% and 49.3% of *E. coli* and *K. pneumoniae*, respectively, were reported to be ESBLs producers.

*A. baumannii* showed a higher resistance pattern in respiratory specimens, with only amikacin exhibiting a resistance less than 70%. In general, *K. pneumoniae* had higher resistance to antibiotics than *E. cloacae* (Table 4). Also, 47% of *K. pneumoniae* isolates were reported to be ESBLs producers.

### ESBL phenotype and genotype

A total of 1059 *E. coli* and 370 *K. pneumoniae* from selected specimens were received. A selection of isolates was evaluated for further analysis (including representative isolates from each center).

Among isolates selected for analysis, 173/215 *K. pneumoniae* and 419/425 *E. coli* were confirmed to be ESBLs using the double disk method. All were screened using PCR to detect ESBL-encoding genes *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX</sub>.

**Table 2. Percentage of resistant, intermediate, and susceptible gram-negative isolates collected from urine.**

| Antibiotic | A. baumannii complex | P. aeruginosa | K. pneumoniae | E. coli | E. cloacae complex |
|------------|----------------------|---------------|---------------|--------|-------------------|
| AMK        | n 151               | %R 31.8       | %I 4.0        | %S 64.2| n 306             | %R 2.0        | %I 0.0        | %S 98.0       |
| AMC        | ND                   | ND            | ND            | ND     | ND                | ND            | ND            | ND            |
| AMP        | ND                   | ND            | ND            | ND     | ND                | 43            | 44.2%         | 0.0%          |
| AZT        | ND                   | ND            | ND            | ND     | ND                | ND            | ND            | ND            |
| CAZ        | 41                   | 87.8%         | 0.0%          | 12.2%  | 148               | 33.8%         | 6.1%          | 60.1%         |
| FEP        | 47                   | 85.1%         | 0.0%          | 14.9%  | 207               | 31.9%         | 1.9%          | 66.2%         |
| FOX        | ND                   | ND            | ND            | ND     | 85                | 45.9%         | 1.2%          | 52.9%         |
| CIP        | 63                   | 82.5%         | 0.0%          | 17.5%  | 230               | 46.5%         | 1.3%          | 52.2%         |
| CTX        | 36                   | 58.3%         | 8.3%          | 33.4%  | ND                | ND            | ND            | ND            |
| GEN        | 65                   | 63.1%         | 4.6%          | 32.3%  | 234               | 30.8%         | 10.3%         | 58.9%         |
| IMP        | ND                   | ND            | ND            | ND     | 50                | 44.0%         | 2.0%          | 54.0%         |
| LVX        | ND                   | ND            | ND            | ND     | ND                | ND            | ND            | ND            |
| MEM        | 41                   | 82.9%         | 0.0%          | 17.1%  | 205               | 38.5%         | 5.9%          | 55.6%         |
| NIT        | ND                   | ND            | ND            | ND     | 242               | 2.1%          | 0.5%          | 97.4%         |
| NOR        | ND                   | ND            | ND            | ND     | 389               | 32.6%         | 30.6%         | 36.8%         |
| SAM        | ND                   | ND            | ND            | ND     | 111               | 39.6%         | 4.5%          | 55.9%         |
| TZP        | 28                   | 92.9%         | 0.0%          | 7.1%   | 73                | 20.5%         | 20.5%         | 59.0%         |
| SXT        | 39                   | 69.2%         | 0.0%          | 30.8%  | 384               | 53.6%         | 0.0%          | 46.4%         |

Abbreviations: AMC, amoxicillin-clavulanic acid; AMK, amikacin; AMP, ampicillin; AZT, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; GEN, gentamicin; IMP, imipenem; LVX, levofloxacin; MEM, meropenem; NIT, nitrofurantoin; NOR, norfloxacin; SAM, ampicillin-sulbactam; SXT, trimethoprim-sulfamethoxazole; TZP, piperacillin-tazobactam. R, resistant; I, intermediate; S, susceptible. ND, Not determined.

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(29.5%). Meanwhile, carbapenem resistance was low in *E. coli* (<1%) but high in *E. cloacae* complex (10.9%) (Table 2). Also, 44.9% and 39.3% of *E. coli* and *K. pneumoniae*, respectively, were reported to be ESBLs producers.

Among blood isolates, *A. baumannii* showed more than 68% resistance for all antibiotics tested, and *P. aeruginosa* had 37.1% resistance to meropenem. Among *Enterobacteriaceae*, *E. cloacae* showed higher resistance to carbapenems (4.4% for meropenem), whereas *K. pneumoniae* and *E. coli* had more than 59% resistance for cefepime (Table 3).

Also, 60% and 49.3% of *E. coli* and *K. pneumoniae*, respectively, were reported to be ESBLs producers.

*A. baumannii* showed a higher resistance pattern in respiratory specimens, with only amikacin exhibiting a resistance less than 70%. In general, *K. pneumoniae* had higher resistance to antibiotics than *E. cloacae* (Table 4). Also, 47% of *K. pneumoniae* isolates were reported to be ESBLs producers.
Among *K. pneumoniae* isolates, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX</sub> were detected in 119/173, 68.8%, 125/173, 72.3%, and 159/173, 91.9% of isolates, respectively, with 124/173 (71.7%) isolates carrying both *bla*<sub>SHV</sub> and *bla*<sub>CTX</sub>. A selection of ESBL PCR products were sequenced and most of the *bla*<sub>CTX-M</sub> genes were detected to be *bla*<sub>CTX-M-15</sub> (15/17, 88.23%) followed by

Table 3. Percentage of resistant, intermediate, and susceptible gram-negative isolates collected from blood.

| Antibiotic | A. baumannii complex | P. aeruginosa | K. pneumoniae | E. coli | E. cloacae |
|------------|----------------------|---------------|---------------|---------|-----------|
|            | n | %R | %I | %S | n | %R | %I | %S | n | %R | %I | %S | n | %R | %I | %S |
| AMK        | 20 | 70.0 | 5.0 | 25.0 | 54 | 16.7 | 3.7 | 79.6 | 79 | 2.5 | 1.3 | 96.2 | 136 | 2.9 | 0.7 | 96.4 |
| AMP        | ND | ND | ND | ND | ND | ND | ND | ND | 83 | 86.7 | 0.0 | 13.3 | ND | ND | ND | ND |
| CAZ        | 73 | 85.0 | 1.4 | 13.6 | 55 | 16.4 | 0.0 | 80.6 | 78 | 64.1 | 1.3 | 34.6 | 135 | 66.7 | 0.0 | 33.3 |
| FEP        | 76 | 83.0 | 0.0 | 17.0 | 71 | 15.5 | 11.3 | 73.2 | 102 | 59.8 | 0.0 | 40.2 | 197 | 61.9 | 0.5 | 37.6 |
| FOX        | ND | ND | ND | ND | 48 | 62.5 | 0.0 | 37.5 | 83 | 71.1 | 1.2 | 27.7 | ND | ND | ND | ND |
| CIP        | 90 | 83.0 | 0.0 | 17.0 | 68 | 14.7 | 5.9 | 79.4 | 106 | 32.1 | 13.2 | 54.7 | 180 | 67.8 | 0.6 | 32.2 |
| CTX        | 41 | 78.0 | 9.8 | 12.2 | 40 | 65.0 | 0.0 | 35.0 | 55 | 60.0 | 1.8 | 38.2 | ND | ND | ND | ND |
| MEM        | 75 | 81.0 | 1.3 | 17.7 | 70 | 12.9 | 8.6 | 78.5 | 113 | 38.1 | 0.9 | 61.0 | 201 | 44.3 | 2.0 | 53.7 |
| SAM        | 73 | 75.0 | 8.2 | 16.8 | 70 | 37.1 | 15.7 | 47.2 | 104 | 2.9 | 0.0 | 97.1 | 197 | 1.5 | 0.0 | 98.5 |
| SXT        | 47 | 75.0 | 0.0 | 25.0 | 50 | 56.0 | 0.0 | 44.0 | 81 | 63.0 | 0.0 | 37.0 | 23 | 30.4 | 0.0 | 69.6 |
| TZP        | 48 | 90.0 | 0.0 | 10.0 | 45 | 15.6 | 13.3 | 71.1 | 79 | 10.1 | 3.3 | 86.6 | 30 | 16.7 | 10.0 | 73.3 |

Abbreviations: AMK, amikacin; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; GEN, gentamicin; IMP, imipenem; LVX, levofloxacin; MEM, meropenem; NIT, nitrofurantoin; SAM, ampicillin/sulbactam; SXT, trimethoprim-sulfamethoxazole; TZP, piperacillin-tazobactam. R, resistant; I, intermediate; S, susceptible. ND, Not determined

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Table 4. Percentage of resistant, intermediate, and susceptible gram-negative isolates collected from respiratory specimens.

| Antibiotic | A. baumannii complex | P. aeruginosa | K. pneumoniae | E. coli | E. cloacae |
|------------|----------------------|---------------|---------------|---------|-----------|
|            | n | %R | %I | %S | n | %R | %I | %S | n | %R | %I | %S | n | %R | %I | %S |
| AMK        | 39 | 69.0 | 15.0 | 16.0 | 105 | 15.2 | 4.8 | 80.0 | 62 | 0.0 | 0.0 | 100.0 | 33 | 0.0 | 0.0 | 100.0 |
| AMC        | ND | ND | ND | ND | ND | ND | ND | ND | 16 | 18.8 | 18.8 | 62.5 | ND | ND | ND | ND |
| AMP        | ND | ND | ND | ND | ND | ND | ND | ND | 40 | 65.0 | 0.0 | 35.0 | 55 | 60.0 | 1.8 | 38.2 |
| CTX        | 68 | 79.0 | 7.4 | 13.6 | 113 | 38.1 | 0.9 | 61.0 | 201 | 44.3 | 2.0 | 53.7 | 47 | 21.3 | 0.0 | 78.7 |
| CAZ        | 130 | 89.0 | 1.5 | 9.5 | 105 | 23.8 | 12.4 | 63.8 | 62 | 48.4 | 1.6 | 50.0 | 33 | 27.3 | 0.0 | 72.7 |
| FEP        | 174 | 89.0 | 1.1 | 9.9 | 141 | 15.6 | 5.0 | 79.4 | 96 | 46.9 | 0.0 | 53.1 | 58 | 5.2 | 3.4 | 91.4 |
| FOX        | ND | ND | ND | ND | ND | ND | ND | ND | 31 | 45.2 | 0.0 | 54.8 | ND | ND | ND | ND |
| CIP        | 160 | 86.0 | 0.0 | 14.0 | 139 | 27.3 | 2.9 | 69.8 | 87 | 31.0 | 4.6 | 64.4 | 42 | 4.8 | 2.4 | 92.8 |
| GEN        | 163 | 73.0 | 7.4 | 20.0 | 146 | 16.4 | 8.2 | 75.4 | 104 | 34.6 | 1.0 | 64.4 | 57 | 5.3 | 0.0 | 94.7 |
| IPM        | 62 | 90.0 | 0.0 | 10.0 | 64 | 50.0 | 0.0 | 50.0 | 51 | 0.0 | 0.0 | 100.0 | 38 | 0.0 | 7.9 | 92.1 |
| LVX        | 65 | 92.0 | 0.0 | 8.0 | 31 | 3.2 | 6.5 | 90.3 | 43 | 20.9 | 2.3 | 76.8 | 25 | 4.0 | 0.0 | 96.0 |
| MEM        | 163 | 87.0 | 0.6 | 12.4 | 137 | 33.6 | 16.1 | 50.4 | 97 | 2.1 | 0.0 | 97.9 | 59 | 1.7 | 0.0 | 98.3 |
| SAM        | 131 | 81.0 | 5.3 | 14.0 | ND | ND | ND | ND | 68 | 48.5 | 2.9 | 48.6 | ND | ND | ND | ND |
| TZP        | 113 | 93.0 | 1.8 | 5.3 | 92 | 21.7 | 10.9 | 67.4 | 80 | 16.2 | 7.5 | 76.3 | 44 | 13.6 | 2.3 | 84.1 |
| SXT        | 82 | 79.0 | 0.0 | 21.0 | ND | ND | ND | ND | 71 | 50.7 | 0.0 | 49.3 | 40 | 17.5 | 0.0 | 82.5 |

Abbreviations: AMC, amoxicillin-clavulanic acid; AMK, amikacin; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; GEN, gentamicin; IMP, imipenem; LVX, levofloxacin; MEM, meropenem; NIT, nitrofurantoin; SAM, ampicillin-sulbactam; SXT, trimethoprim-sulfamethoxazole; TZP, piperacillin-tazobactam; TGC, tigecycline; TOB, tobramycin. R, resistant; I, intermediate; S, susceptible.

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Among the blaSHV gene, a great diversity was detected, including blaSHV-11, blaSHV-28, blaSHV-158, blaSHV-171, blaSHV-176, blaSHV-196, blaSHV-205, blaSHV-213, and blaSHV-228. Some of them with no evidence of ESBL activity (Table 5).

Among E. coli isolates, blaTEM, blaSHV, and blaCTX were detected in 87/419, 20.76%, 19/419, 4.53%, and 359/419, 85.68% of isolates, respectively, with 18 (4.29%) isolates carrying both blaSHV and blaCTX.

A selection of ESBL PCR products were sequenced and most of the blaCTX-M encoding genes were detected to be blaCTX-M-15 (32/34, 94.1%), followed by blaCTX-M-55 (17/34, 50.0%). Among the blaSHV gene, blaSHV-5 and blaSHV-11 (with reported ESBL activity), blaSHV-38 (with reported carbapenemase activity), and blaSHV-171 (with no report of ESBL activity) were detected (Table 5).

**Carbapenemase-encoding genes**

A total of 26 carbapenem-resistant Enterobacteriaceae isolates were received for genotyping (one of them with a subpopulation). Carbapenem-encoding genes were detected primarily in
*E. coli*, followed by *K. pneumoniae*. The most frequently detected carbapenemase-encoding gene was *blaNDM-1* (81.5%), followed by *blaOXA-232* (14.8%) and *blaOXA-181* (7.4%). One *K. pneumoniae* isolate was detected to harbor both *blaKPC* and *blaNDM-1* (Table 6).

A total of 102 carbapenem-resistant *A. baumannii* isolates were received, and the most frequent carbapenemase-encoding gene was *blaOXA-24* (76%), followed by *blaOXA-23* (18.5%). Other genes detected were *blaVIM* and *blaNDM* (Table 7). All the isolates were negative to *blaKPC*, *blaGES*, and *blaOXA-58*.

Regarding carbapenem-resistant *P. aeruginosa*, 93 isolates were received, and the carbapenemase-encoding genes most frequently detected were *blaIMP* (25.3%), *blaGES*, and *blaVIM* (13.1% each), with 44 (47.31%) isolates containing none of the screened carbapenemase-encoding genes (Table 8).

**Discussion**

This report presents phenotypic and genetic data on the frequency and characteristics of ESBL and representative carbapenemase-producing Gram-negative species in Mexico using strains collected from 52 centers in 19 Mexican states.

**Table 6. Distribution of carbapenemase-encoding genes among selected carbapenem-resistant Enterobacteriaceae.**

| Isolate | Specimen | Species                       | *blaKPC*-like | *blaOXA-48*-like | *blaNDM-1* | *blaIMP-2* | *blaCTXM-15* | ampC |
|---------|----------|-------------------------------|---------------|-----------------|------------|------------|-------------|-----|
| 53      | Blood    | Enterobacter cloacae          | -             | -               | +          | -          | +           | -   |
| 223     | Blood    | Enterobacter cloacae          | -             | -               | +          | -          | +           | -   |
| 255     | Blood    | Klebsiella pneumoniae         | -             | -               | +          | -          | +           | -   |
| 303     | Blood    | Serratia marcescens           | -             | -               | +          | -          | -           | -   |
| 463     | Blood    | Escherichia coli              | -             | -               | +          | +          | -           | -   |
| 489     | Catheter | Klebsiella pneumoniae         | -             | -               | +          | +          | -           | -   |
| 562     | Urine    | Providencia rettgeri          | -             | -               | +          | -          | -           | -   |
| 591     | Urine    | Klebsiella variicola          | -             | -               | +          | -          | +           | -   |
| 849     | Abscess  | Escherichia coli              | -             | *blaOXA-181*    | -          | -          | -           | -   |
| 850     | BAL      | Escherichia coli              | -             | -               | +          | -          | +           | -   |
| 851     | BAL      | Escherichia coli              | -             | *blaOXA-181*    | -          | -          | +           | -   |
| 853     | Blood    | Escherichia coli              | -             | -               | +          | -          | +           | -   |
| 854     | Urine    | Escherichia coli              | -             | -               | +          | -          | +           | -   |
| 861     | Wound    | Klebsiella oxytoca            | -             | -               | +          | -          | +           | -   |
| 882     | Urine    | Escherichia coli              | -             | -               | +          | -          | +           | -   |
| 891     | Urine    | Klebsiella pneumoniae         | *blaKPC-2*    | -               | +          | -          | -           | -   |
| 1202    | Blood    | Escherichia coli              | -             | *blaOXA-232*    | -          | -          | -           | -   |
| 1203    | Blood    | Klebsiella pneumoniae         | *blaKPC-2*    | -               | +          | -          | +           | -   |
| 1457    | BAL      | Escherichia coli              | -             | -               | +          | -          | +           | -   |
| 1627    | Urine    | Klebsiella variicola          | -             | -               | +          | +          | -           | -   |
| 2063    | No data  | Enterobacter xiangfangensis   | -             | -               | +          | -          | +           | +   |
| 2177    | Blood    | Escherichia coli              | -             | *blaOXA-232*    | -          | -          | +           | -   |
| 2178    | Urine    | Escherichia coli              | -             | *blaOXA-232*    | -          | -          | +           | -   |
| 2175–1  | Abscess  | Escherichia coli              | -             | *blaOXA-232*    | -          | -          | -           | -   |
| 2175–2  | Abscess  | Escherichia coli              | -             | *blaOXA-232*    | -          | -          | +           | -   |
| 1562–1  | Blood    | Escherichia coli              | -             | -               | +          | -          | +           | -   |
| 1562–2  | Blood    | Escherichia coli              | -             | -               | +          | -          | +           | -   |

Abbreviation: BAL, bronchoalveolar lavage.

*All strains were negative for *blaGES*, *blaIMP-1*, and *blaVIM*.*

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OXA-48-like carbapenemases are important causes of carbapenem resistance and are now the most common carbapenemase in some populations [25]. In Enterobacteriaceae, several variants of blaOXA-48 have been identified, with blaOXA-181 and blaOXA-232 being the two most common [26, 27]. Kinetic properties of these two enzymes had been measured, and both appear broadly similar to blaOXA-48 in their activity, with blaOXA-232 demonstrating better hydrolysis of penicillin [28]. In this study, blaOXA-181 and blaOXA-232 were detected in E. coli. At present, blaOXA-232 has been reported in Mexico in two single-center reports: in E. coli, carrying blaOXA-232 plus blaCTXM-15 [8] and in a case-control-control study in which the infection by blaOXA-232 strains was associated with the previous use of β-lactam/β-lactamase antibiotics (OR, 6.2) [29]. The OXA-181 variant has been associated with other carbapenemase genes, including blaNDM-1 and blaVIM-5 [30]. No previous reports of blaOXA-181 circulation in Mexico were identified in the literature.

Enterobacteriaceae-producing OXA-48-like enzymes are rapidly spreading, and thus, laboratory detection should be optimized. This enzyme has low-level hydrolytic activity against carbapenems and, thus, may not be detected [27]. As detected in this study, blaOXA-48-like genes can co-harbor genes encoding ESBL or AmpC enzymes, or both, which confers nonsusceptibility to aztreonam, extended-spectrum cephalosporins, and carbapenem agents and renders these genes a serious menace [31].

### Table 7. Distribution of carbapenemase-encoding genes among A. baumannii complex

| n  | blaOXA23 | blaOXA24 | blaVIM | blaNDM |
|----|---------|---------|--------|--------|
| 66 | -       | +       | -      | -      |
| 15 | +       | -       | -      | -      |
| 9  | -       | +       | -      | -      |
| 4  | -       | -       | +      | -      |
| 3  | +       | +       | -      | -      |
| 3  | -       | -       | -      | -      |
| 1  | -       | -       | +      | +      |
| 1  | +       | -       | +      | -      |

*All strains were negative for blaKPC and blaGES and positive for blaOXA31*

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### Table 8. Distribution of carbapenemase-encoding genes among P. aeruginosa carbapenem-resistant clinical isolates

| n  | Specimen | blaGES | blaVIM | blaIMP |
|----|----------|--------|--------|--------|
| 24 | Respiratory | -      | -      | -      |
| 10 | Blood    | -      | -      | -      |
| 10 | Urine    | -      | -      | -      |
| 23 | Urine    | -      | -      | +      |
| 5  | Urine    | +      | -      | -      |
| 5  | Respiratory | +      | -      | -      |
| 4  | Blood    | -      | +      | -      |
| 4  | Urine    | -      | +      | -      |
| 3  | Respiratory | -      | +      | -      |
| 2  | Blood    | +      | -      | -      |
| 1  | Urine    | -      | +      | +      |
| 1  | Blood    | -      | -      | +      |
| 1  | Urine    | +      | +      | -      |

*All strains were negative for blaKPC and blaNDM*

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Drug resistance phenotypes and genotypes in Mexico in representative gram-negative species
NDM has a worldwide distribution, with multiple reports in Asia and Europe since this enzyme was first described in 2007 [32–37]. However, it has remained uncommon in Enterobacteriaceae in America, with some reports in Canada, the United States, and Latin American countries [38–41]. In this study, the most frequently detected carbapenemase-encoding gene was bla_{NDM-1}. The NDM carbapenemase was first described in Mexico in 2013 [6], and since then, several reports have been published about it in the county [8, 41]. According to our report, NDM is now the most prevalent carbapenemase in Mexico. This study reports by Mexico the first NDM-1-positive Klebsiella variicola isolates considered an emerging pathogen in humans [42].

Within a few years, KPC producers became global as they were reported in America, Europe, and Asia [32, 43]. Interestingly, this enzyme has a lower frequency in Mexico when compared to other Latin American countries, as confirmed by our report [43]. KPC and NDM have received special attention due to limited therapeutic options and high mortality associated with infections caused by strains carrying genes that encode these enzymes [44].

A. baumannii isolates have resistance rates greater than 50.0% to carbapenems worldwide, and our results confirmed this resistance [45, 46]. In this study, we detected that the most frequent carbapenemase-encoding gene was bla_{OXA-24}, followed by bla_{OXA-23}. OXA-23 isolates have been primarily detected in Asia, Europe, the United States, Brazil, and South America, whereas OXA-24 has been reported in Europe, Asia, and North America [5, 47–51].

Among P. aeruginosa isolates, 44 out of 93 isolates did not contain any of the screened carbapenemase-encoding genes. The most frequent carbapenem resistance mechanism described in P. aeruginosa is the overexpression of efflux pumps and the loss of the Opr porin [52]. Less frequently, genes encoding carbapenemases have been described as an alternative mechanism, with GES variants and IMP, VIM, and NDM reported. In this study, we did not analyze the overexpression of efflux pumps and porins, but bla_{GES}, bla_{VIM}, and bla_{IMP} genes were detected in approximately half of the strains (49/93) (Table 8). Similar results were reported in Mexico with a prevalence of 36.2% of carbapenemases (IMP, VIM, and GES types) on P. aeruginosa clinical isolates. These genes have been reported to be chromosomally encoded on embedded class 1 integron arrays [53].

Besides carbapenemase-encoding genes, other important mechanisms conferring carbapenem resistance have been observed, including carbapenem hydrolysis by AmpCs in combination with ESBL enzymes, rendering carbapenem resistance to Gram-negative bacteria [54]. In our study, a high frequency of ESBL-producing Enterobacteriaceae was identified, with the AMPc-encoding gene detected in two strains (Enterobacter xiangfangensis (a member of the E. cloacae complex) and E. coli harboring both bla_{NDM-1}, bla_{CTX-M-15}, and ampC). The presence of AmpC/ESBL and the exact changes of the porins may significantly affect carbapenem resistance. Thus, these mechanisms need to be considered in future research.

The prevalence of bacterial isolates expressing the ESBL phenotype varies across different geographical regions, with rates from 10% to 58% [55]. ESBLs arise primarily due to mutations in the bla_{TEM}, bla_{SHV}, or bla_{CTX} genes, and at present, the CTX-M type is known to be the most frequent non-TEM, non-SHV ESBL [55]. In our study, 72.25% of ESBL-producing K. pneumoniae isolates and 85.7% of E coli isolates harbored bla_{CTX-M}, confirming the spread of this enzyme.

The presence of CTX-M-type enzymes is relevant because they are readily inhibited by all commercially available β-lactamase inhibitors, including avibactam, vaborbactam, and relebactam [56]; a valuable alternative therapy to the recommended ertapenem regimen.

In this study, the non-ESBL TEM-1 was frequently detected, and SHV was detected with no predominance of any subtype. Worldwide, the prevalence of TEM and SHV has diminished, mirroring the worldwide dissemination of isolates producing CTX-M-type -lactamases [57].
Some of the limitations of this study are that not all states in Mexico participated, and the analysis of porins was not included. Furthermore, we only included some bacterial species involved in ESBL production. Our network will continue to actively survey drug resistance and molecular mechanisms involved.

In conclusion, our report identifies NDM as the most frequent carbapenemase-encoding gene in *Enterobacteriaceae* Mexico with circulation of the oxacillinase genes 181 and 232. KPC, in contrast to other countries in Latin America and the USA, is a rare occurrence. Additionally, a high circulation of ESBL *bla* _CTX-M-15_ existed in *E. coli* and *K. pneumoniae*.

**Supporting information**

S1 Table. Primers used for genotyping of ESBs genes.

(DOCX)

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**Author Contributions**

**Conceptualization:** Luis Alfredo Ponce-de-León-Garduño, Luis Esau López-Jácome, Rafael Franco-Cendejas, Rayo Morfín-Otero, Adrián Camacho-Ortiz.

**Data curation:** Elvira Garza-González.

**Formal analysis:** Elvira Garza-González, Ulises Garza-Ramos, Fabian Rojas-Larios, Juan Pablo Mena-Ramírez, María Guadalupe Fong-Camargo, Cecilia Teresita Morales-De-la-Peña, Lourdes García-Mendoza, Elena Victoria Choy-Chang, Laura Karina Aviles-Benzite, José Manuel Feliciano-Guzmán, Eduardo López-Gutiérrez, Mariana Gil-Veloz, Juan Manuel Barajas-Magalhães, Efren Aguierre-Burciaga, Laura Isabel López-Moreno, Rebeca Thelma Martínez-Villarreal, Jorge Luis Canizales-Oviedo, Carlos Miguel Cetina-Umaña, Daniel Romero-Romero, Fidencio David Bello-Pazos, Nicolás Rogelio Eric Barlandas-Rendón, Joyarib Yanelli Maldonado-Anicacio, Enrique Bolado-Martínez, Mario Galindo-Méndez, Talía Pérez-Vicelis, Norma Alavez-Ramírez, Braulio J. Méndez-Sotelo, Juan Francisco Cabriales-Zavala, Yirla Citlali Nava-Pacheco, Martha Irene Moreno-Méndez, Ricardo García-Romó, Aldo Rafael Silva-Gamino, Ana María Avalos-Aguilera, Marí旭 Asunción Santiago-Calderón, Maribel López-García, María del Consuelo Velásquez-Acosta, Dulce Isabel Cobos-Canul, María del Rosario Vázquez-Larios, Ana Elizabeth Ortiz-Portocayo, Arely Elizabeth Guerrero-Núñez, Jazmín Valero-Guzmán, Alina Aracely Rosales-García, Heidy Leticia Ostos-Cantú.

**Funding acquisition:** Jesus Silva-Sánchez.

**Investigation:** Paola Bocanegra-Ibarias, Miriam Bobadilla-del-Valle, Luis Alfredo Ponce-de-León-Garduño, Verónica Esteban-Kenel, Jesus Silva-Sánchez, Ulises Garza-Ramos, Humberto Barrios-Camacho, Luis Esau López-Jácome, Claudia A. Colin-Castro, Rafael Franco-Cendejas, Samantha Flores-Treviño, Rayo Morfín-Otero, Fabian Rojas-Larios, Juan Pablo Mena-Ramírez, María Guadalupe Fong-Camargo, Cecilia Teresita Morales-De-la-Peña, Lourdes García-Mendoza, Elena Victoria Choy-Chang, Laura Karina Aviles-Benitez, José
Manuel Feliciano-Guzmán, Eduardo López-Gutiérrez, Mariana Gil-Veloz, Juan Manuel Barajas-Magallón, Efren Aguirre-Burciaga, Laura Isabel López-Moreno, Rebeca Thelma Martínez-Villarreal, Jorge Luis Canizales-Oviedo, Carlos Miguel Cetina-Umaña, Daniel Romero-Romero, Fidencio David Bello-Pazos, Nicolás Rogelio Eric Barlandas-Rendón, Joyarib Yanelli Maldonado-Anicacio, Enrique Bolado-Martínez, Mario Galindo-Méndez, Talaia Perez-Vicelis, Norma Alavez-Alvarez, Braulio J. Méndez-Soto, Juan Francisco Cabrera-Vázquez, Yirla Citlali Nava-Pacheco, Martha Irene Moreno-Méndez, Ricardo García-Romo, Aldo Rafael Silva-Gamiño, Ana María Avalos-Aguilera, María Asunción Santiago-Calderón, Maribel López-García, María del Consuelo Velázquez-Acosta, Dulce Isabel Cobos-Canul, María del Rosario Vázquez-Larios, Ana Elizabeth Ortiz-Porcayo, Arley Elizabeth Guerrero-Núñez, Jazmín Valero-Guzmán, Alina Aracely Rosales-García, Heidy Leticia Ostos-Cantú.

**Methodology:** Elvira Garza-González, Paola Bocanegra-Ilbarias, Miriam Bobadilla-del-Valle, Luis Alfredo Ponce-de-León-Garduño, Verónica Esteban-Kenel, Luis Esaú López-Jácome, Fabian Rojas-Larios, Juan Pablo Mena-Ramírez, María Guadalupe Fong-Camargo, Cecilia Teresita Morales-De-La-Peña, Lourdes García-Mendoza, Elena Victoria Choy-Chang, Laura Karina Aviles-Benitez, José Manuel Feliciano-Guzmán, Eduardo López-Gutiérrez, Mariana Gil-Veloz, Juan Manuel Barajas-Magallón, Efren Aguirre-Burciaga, Laura Isabel López-Moreno, Rebeca Thelma Martínez-Villarreal, Jorge Luis Canizales-Oviedo, Carlos Miguel Cetina-Umaña, Daniel Romero-Romero, Fidencio David Bello-Pazos, Nicolás Rogelio Eric Barlandas-Rendón, Joyarib Yanelli Maldonado-Anicacio, Enrique Bolado-Martínez, Mario Galindo-Méndez, Talaia Perez-Vicelis, Norma Alavez-Alvarez, Braulio J. Méndez-Soto, Juan Francisco Cabrera-Vázquez, Yirla Citlali Nava-Pacheco, Martha Irene Moreno-Méndez, Ricardo García-Romo, Aldo Rafael Silva-Gamiño, Ana María Avalos-Aguilera, María Asunción Santiago-Calderón, Maribel López-García, María del Consuelo Velázquez-Acosta, Dulce Isabel Cobos-Canul, María del Rosario Vázquez-Larios, Ana Elizabeth Ortiz-Porcayo, Arley Elizabeth Guerrero-Núñez, Jazmín Valero-Guzmán, Alina Aracely Rosales-García, Heidy Leticia Ostos-Cantú.

**Project administration:** Elvira Garza-González.

**Software:** Elvira Garza-González.

**Validation:** Elvira Garza-González.

**Writing – original draft:** Elvira Garza-González.

**Writing – review & editing:** Elvira Garza-González, Paola Bocanegra-Ilbarias, Miriam Bobadilla-del-Valle, Luis Alfredo Ponce-de-León-Garduño, Verónica Esteban-Kenel, Luis Esaú López-Jácome, Fabian Rojas-Larios, Juan Pablo Mena-Ramírez, María Guadalupe Fong-Camargo, Cecilia Teresita Morales-De-La-Peña, Lourdes García-Mendoza, Elena Victoria Choy-Chang, Laura Karina Aviles-Benitez, José Manuel Feliciano-Guzmán, Eduardo López-Gutiérrez, Mariana Gil-Veloz, Juan Manuel Barajas-Magallón, Efren Aguirre-Burciaga, Laura Isabel López-Moreno, Rebeca Thelma Martínez-Villarreal, Jorge Luis Canizales-Oviedo, Carlos Miguel Cetina-Umaña, Daniel Romero-Romero, Fidencio David Bello-Pazos, Nicolás Rogelio Eric Barlandas-Rendón, Joyarib Yanelli Maldonado-Anicacio, Enrique Bolado-Martínez, Mario Galindo-Méndez, Talaia Perez-Vicelis, Norma Alavez-Alvarez, Braulio J. Méndez-Soto, Juan Francisco Cabrera-Vázquez, Yirla Citlali Nava-Pacheco, Martha Irene Moreno-Méndez, Ricardo García-Romo, Aldo Rafael Silva-Gamiño, Ana María Avalos-Aguilera, María Asunción Santiago-Calderón, Maribel López-García, María del Consuelo Velázquez-Acosta, Dulce Isabel Cobos-Canul, María del Rosario Vázquez-Larios, Ana Elizabeth Ortiz-Porcayo, Arley Elizabeth Guerrero-Núñez, Jazmín Valero-Guzmán, Alina Aracely Rosales-García, Heidy Leticia Ostos-Cantú.

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