



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



ELSEVIER

Contents lists available at ScienceDirect

## American Journal of Infection Control

journal homepage: [www.ajicjournal.org](http://www.ajicjournal.org)

## Major Article

## ESKAPE bacteria characterization reveals the presence of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* outbreaks in COVID-19/VAP patients

Miguel Ángel Loyola-Cruz MSc<sup>a,b</sup>, Emilio Mariano Durán-Manuel MSc<sup>a</sup>, Clemente Cruz-Cruz MSc<sup>a</sup>, Laura Margarita Márquez-Valdelamar MSc<sup>c</sup>, Juan Carlos Bravata-Alcántara MSc<sup>a</sup>, Iliana Alejandra Cortés-Ortíz MSc<sup>a</sup>, Mónica Alethia Cureño-Díaz MPH<sup>a</sup>, Gabriela Ibáñez-Cervantes PhD<sup>a</sup>, Verónica Fernández-Sánchez PhD<sup>a</sup>, Graciela Castro-Escarpulli PhD<sup>b,\*</sup>, Juan Manuel Bello-López PhD<sup>a,\*\*</sup>

<sup>a</sup> Hospital Juárez de México, Mexico City, Mexico

<sup>b</sup> Laboratorio de Investigación Clínica y Ambiental, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico

<sup>c</sup> Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, México

## Key Words:

Bacteria ESKAPE  
Ventilator associated pneumonia  
COVID-19  
Clonal dispersion  
ERIC-PCR

## A B S T R A C T

**Introduction:** A decrease of detection of outbreaks by multidrug-resistant bacteria in critical areas has been reduced due to COVID-19 pandemic. Therefore, molecular epidemiological surveillance should be a primary tool to reveal associations not evident by classical epidemiology. The aim of this work was to demonstrate the presence of hidden outbreaks in the first wave of the COVID-19 pandemic and to associate their possible origin.

**Methods:** A population of 96 COVID-19 patients was included in the study (April to June 2020) from Hospital Juárez de México. Genetic identification and antimicrobial susceptibility testing of VAP causative agents isolated from COVID-19 patients was performed. Resistance phenotypes were confirmed by PCR. Clonal association of isolates was performed by analysis of intergenic regions obtained. Finally, the association of clonal cases of VAP patients was performed by timelines.

**Results:** ESKAPE and non-ESKAPE bacteria were identified as causative agents of VAP. ESKAPE bacteria were classified as MDR and XDR. Only *A. baumannii* and *P. aeruginosa* were identified as clonally distributed in 13 COVID-19/VAP patients. Time analysis showed that cross-transmission existed between patients and care areas.

**Conclusions:** *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were involved in outbreaks non-detected in COVID-19/VAP patients in the first wave of COVID-19 pandemic.

© 2022 Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved.

\* Address correspondence to Graciela Castro Escarpulli, PhD, Prolongación de Carpio y, Calle Plan de Ayala s/n, Santo Tomás, 11340 Mexico City, Mexico. \*\* Address correspondence to Juan Manuel Bello-López, PhD, Instituto Politécnico Nacional 5160, Magdalena de las Salinas, 07360 Mexico City, Mexico.

E-mail addresses: [chelacastro@hotmail.com](mailto:chelacastro@hotmail.com) (G. Castro-Escarpulli), [juanmanuelbello81@hotmail.com](mailto:juanmanuelbello81@hotmail.com) (J.M. Bello-López).

Financial support: This study is part of the project "CONACyT 313771: Análisis del efecto del ozono sobre SARS-CoV-2 como alternativa de producto desinfectante en equipos de protección del personal de salud de alta demanda" and funding of the 022 budget (year 2021) of the "Hospital Juárez de México." This study was approved by Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional (SIP 20211351, 20220818).

Declaration of Competing Interest: The authors declare no conflict of interest.

The COVID-19 pandemic has accumulated a high number of deaths,<sup>1</sup> where it has been estimated that approximately 80% of patients admitted to intensive care units (ICU) require mechanical ventilation, which is a risk factor for the development of ventilator-associated pneumonia (VAP).<sup>2,3</sup> Patient-associated comorbidities are another risk factor that increases the likelihood of acquiring VAP, complicating the clinical condition of COVID-19 patients and directly impacting the mortality.<sup>2,4-6</sup> Among the causative agents of VAP in COVID-19 patients are those belonging to the ESKAPE group.<sup>7-9</sup> This acronym is composed of bacteria such as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., among others.<sup>10,11</sup> These

bacteria have been recognised as pathogens in critically ill and immunocompromised individuals, and may be associated with resistance to multiple antibiotics.<sup>5,12,13</sup> The hospital environment play an important role in the development of Healthcare-Associated Infections (HAI) including VAP.<sup>14,15</sup> This is because these microorganisms have been isolated from inert surfaces, medical devices, and healthcare personnel, suggesting that the nosocomial environment is an important reservoir of pathogens.<sup>16</sup> The spread of bacteria in ICUs often occurs through cross-transmission events mediated by healthcare personnel.<sup>17,18</sup> This phenomenon has been demonstrated in previous studies by using molecular Biology assays and case associations, where it was observed that 20%–40% of pathogens identified in patients and medical devices were genetically related.<sup>19,20</sup> These results allowed speculation that the common source of contamination could be the hands of healthcare personnel who previously handled medical devices or patients with active infections. The use of other molecular tools, which have advantages over other techniques, have allowed the identification of cross-transmission in ICUs of COVID-19 patients. The analysis of intergenic regions, demonstrated the clonal dispersion of *A. baumannii* on inert surfaces, healthcare personnel, and on the skin of COVID-19 patients.<sup>16</sup> In contrast, the application of ERIC-PCR identified that mechanical ventilators of COVID-19 patients play an important role as reservoirs of pathogens and could be involved in the occurrence of undetected outbreaks.<sup>21,22</sup> This is why it has been suggested that a molecular approach in the COVID-19 pandemic is necessary for the containment of outbreaks associated by antibiotic-resistant bacteria.<sup>16</sup> Since *A. baumannii* has been recognised as the main causative agent of VAP in *Hospital Juárez de México (HJM)*, it is necessary to implement epidemiological surveillance at this pathogen. This will be done through genetic identification of VAP causative agents and identification of clones by analysis of intergenic regions of *A. baumannii* and other pathogens circulating in ICUs of COVID-19 patients. Implications on the identification of ESKAPE pathogen clones causing VAP in COVID-19 patients and the use of adequate disinfection process are analysed and discussed.

## MATERIAL AND METHODS

### Ethical considerations

The institutional Committee of Research, Ethics, and Biosafety from *HJM* approved the protocol under the registration number HJM 002/211 in accordance with the Regulation of the General Health Law on Research for Health ([http://www.conbioetica.mexico.salud.gob.mx/descargas/pdf/normatividad/normatinacional/10\\_NAL\\_Reglamento\\_de\\_Investigacion.pdf](http://www.conbioetica.mexico.salud.gob.mx/descargas/pdf/normatividad/normatinacional/10_NAL_Reglamento_de_Investigacion.pdf)).

### Description of the Hospital Juárez de México in the COVID-19 era

Derived from the pandemic by the SARS-CoV-2 virus, the *HJM* was converted to care COVID-19 patients. For this purpose, a total of 160 beds were distributed in seven areas, and multidisciplinary 15 health workers medical staff (morning, evening and night shifts), was involved in the care of each critical COVID-19 patient. Empirical treatments for VAP were ceftriaxone (1–2 g per 24 h) and due to the national shortage of colistin, the final treatments were meropenem (1 g per 24 h), imipenem (500 mg per 6–8 h), and piperacillin/tazobactam (4 g per 6 h). Finally, as an HAI control measures, towels impregnated with H<sub>2</sub>O<sub>2</sub> (Oxivir Tb, CA) were used as disinfection method.

### Study population of COVID-19 patients with VAP

A population consisting of 96 COVID-19 patients was included in the study during the period between April to June 2020 (first wave).

These patients met the inclusion criteria for suspected VAP (fever acquired after 48 h of intubation, purulent pulmonary secretions, leukocytosis, and infiltrates on chest X-ray).<sup>23–25</sup> In aseptic conditions, COVID-19 patients were sampled through tracheal aspirates and were transported at 4°C to the research laboratory for their microbiological culture. Additionally, demographic data was obtained from medical records in order to describe COVID-19 patient population.

### Isolation of ESKAPE bacteria from COVID-19 patients with VAP

Prior to bacteriological isolations, tracheal aspirates were handled under a biosafety level 2 cabinet according to laboratory safety with the operator wearing a coverall protective gown. Tracheal aspirates were cultured on selective MacConkey and Mannitol Salt agar (Becton Dickinson & Co., Franklin Lakes, NJ) and were incubated aerobically at 37°C for 24–48 h. Subsequently, bacterial strains were purified in LB agar, were growth in LB-broth, then frozen in glycerol (50%) and stored at –70°C for future experiments.

### DNA extraction

For molecular biology assays, total DNA from strains was isolated and purified by using the DNeasy Blood & Tissue Kit (QIAGEN, Venlo, The Netherlands). Integrity of genomic DNA was visualized on horizontal 0.8% agarose gels and were used as templates in PCR assays.

### Genetic identification of ESKAPE bacteria

Amplification reactions were performed in a T100 Thermal cycler (Bio-Rad, Germany) Polymerase chain reactions of the 16S rRNA gene were performed with universal primers 27F and 1492R by using the conditions recommended by DeSantis et al., (2007)<sup>26</sup> (Table 1). Amplicons were analysed on horizontal 1.5% agarose gels by using 1 × Tris-Borate-EDTA buffer (TBE). PCR products were purified and sequenced by the Biology Institute of *Universidad Nacional Autónoma de México (UNAM)* by using a DNA Analyzer 3730xL (Applied Biosystems, Forrest City, CA). Nucleotide sequences were compared with the nucleotide sequence database (GenBank) by means of the Blast algorithm (<http://blast.ncbi.nlm.nih.gov>), using parameters of coverage (>80%) and identity (90%).

### MDR, XDR, and PDR classification of ESKAPE bacteria isolated from COVID-19 patients

The classification of MDR (multidrug-resistant), XDR (extensively drug-resistant) and PDR (pandrug-resistant) of ESKAPE bacteria isolated from COVID-19 patients, were through of susceptibility/resistance assays, according to “*Consenso latinoamericano para definir, categorizar y notificar patógenos multirresistentes, con resistencia extendida o panresistentes*”<sup>27</sup> and CLSI (2021),<sup>28</sup> respectively. *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923 were used as controls. Results were inferred as susceptible, intermediate, or resistant by measuring the diameter of the inhibition zone. The frequency of antibiotic resistance was calculated and represented in percentages (%) and MDR, XDR, and PDR classification was done.

### Carbapenemase production and their relationship with their genotype in ESKAPE bacteria

#### Carbapenemases detection by mCIM assay

ESKAPE bacteria were subjected to the modified carbapenem inactivation method (mCIM) according to the method recommended by Pierce et al., (2017)<sup>29</sup> In brief, two 1- $\mu$ L loopfuls of ESKAPE bacteria

**Table 1**  
Primers used in this study

Primer	Molecular target	Sequence (5'→3')	Size (bp)	Reference
27F	16S rRNA	AGAGTTTGATCMTGGCTCAG	1492	26
1492R		TACGGYTACCTGTACGACTT		
oxa-23-F	<i>oxa23</i>	GATGTGTCATAGTATTCGTCG	1065	34
oxa-23-R		TCACAACAACATAAAGCACTG		
adeA-F	<i>adeA</i>	TCTGCAATATGCAACAGTTC	236	16
adeA-R		GGCTATTGGTAATACGAACG		
adeB-F	<i>adeB</i>	TTAACGATAGCGTTGTAACC	541	
adeB-R		TGAGCAGACAATGGAATAGT		
adeC-F	<i>adeC</i>	AGCCTGCAATTACATCTCAT	560	
adeC-R		TGGCACTTCACTATCAATAC		
AdeR-F	<i>adeR</i>	GCGTCAGATTAAGCAAG	447	
AdeR-F		ACTACGATATTGGCGACATT		
AdeS-F	<i>adeS</i>	AGTGAAGTTAGGTCAAGTT	544	
AdeS-R		TTGGTTAGCCACTGTTATCT		
ERIC1R	Intergenic consensus	ATGTAAGCTCCTGGGGATTCA	Variable	35
ERIC2		AAGTAAGTGACTGGGGTGAGC		
MultilMP_for	<i>blaIMP</i>	TTGACACTCCATTACDG	139	31
MultilMP_rev		GATYGAGAATTAAGCCACYCT		
MultiVIM_for	<i>blaVIM</i>	GATGGTGTGGTGCAGATA	390	
MultiVIM_rev		CGAATGCGCAGCACCAG		
NDM-Fm	<i>blaNDM</i>	GGTTGGCGAT CTGGTTTC	621	32
NDM-Rm		CGG AATGGCTCATCACGATC		

from an overnight blood agar plate were emulsified in 2 mL trypticase soy broth. Subsequently, a 10- $\mu$ g MEM disk (BD, Brea, CA) was immersed in each suspension and incubated at 37°C for 4 h. Furthermore, a Mueller-Hinton (MH) was massive plated with *E. coli* ATCC 25922 (MEM<sup>S</sup>) suspension adjusted to 0.5 McFarland nephelometer. Finally, MEM disks were removed from the bacterial suspension and were deposited on MH plate with indicator MEM<sup>S</sup> strain. MH plates were incubated at 37°C for 18–24 h and the zones of inhibition were measured as for the routine disk diffusion method. *Klebsiella pneumoniae* *bla*<sub>NDM-1</sub> was used as positive control.<sup>30</sup>

#### Molecular screening to confirm carbapenemase production in ESKAPE bacteria

The genetic resistance background in ESKAPE bacteria (only carbapenemase-producer) that confer resistance to  $\beta$ -lactams (previously detected by mCIM assay), RT-PCR assays were performed to detect metallo  $\beta$ -lactamases and serine  $\beta$ -lactamases genes, by using CRE ELITE MGB (Torino, Italy) kit according to the manufacturer's manual. Isolates with positive PCR in first step were subjected to second PCR assays in order to detect specific carbapenemase genes (*bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>).<sup>31,32</sup>

#### Resistance to $\beta$ -lactams (*bla*<sub>OXA</sub>) in *Acinetobacter baumannii* population

Once detected the phenotype of resistance to  $\beta$ -lactams in *A. baumannii* strains, a member of *bla*<sub>OXA</sub> subfamily (*bla*<sub>OXA-23</sub>) in these population ( $n = 14$ ) was detected by end-point PCR. Amplicons were purified, sequenced, and analysed by nucleotide alignment by using UGENE (42.0). Primers used for this purpose are shown in Table 1.

#### Molecular screening to detect *mcr* gene in ESKAPE bacteria

To confirm the absence of colistin resistance in ESKAPE bacteria, RT-PCR assays were performed to detect *mcr-1* and *mcr-2* genes, using COLISTIN-R ELITE MGB (Torino, Italy) kit according to Girlich et al. (2019).<sup>33</sup>

#### Assembly of AdeABC operon and regulators genes AdeRS in *A. baumannii*

Full operon AdeABC encoding efflux pumps and their regulator genes AdeRS were amplified by end-point PCR in *A. baumannii* strains. Detection was performed under amplification strategy of conserved genes as follows: a first PCR reaction was performed to amplify the

*AdeA* gene encoding a protein forming a dimeric complex that anchors in the periplasmic region of the cell. Once a positive amplification to first molecular target was performed, a second reaction to amplify *adeB* gene (encoding an intermembrane protein) was carried out. Finally, a third reaction was performed to amplify the *AdeC* gene (encoding an extramembrane protein). Additionally, *AdeR* and *AdeS* genes encoding a regulator protein and activating protein kinase respectively were amplified.<sup>16</sup> Sequences of primers used for AdeABC operon amplification are shown in Table 1.

#### Relation of Efflux pump AdeABC and MDR in *A. baumannii*

The possible relation between the MDR phenotype and the presence of detected efflux pumps was analysed as follows. Strains were tested for resistance before and after exposure to the efflux pump inhibitor with Phe-Arg- $\beta$ -naphthylamide (PAbN; Sigma, St. Louis, MO). This inhibitor was supplemented in the culture medium at a final concentration of 20  $\mu$ g/mL. Significant inhibition was defined as a 4-fold or greater increase to the halo of inhibition in the presence of PAbN and the antibiotic tested.<sup>16</sup>

#### Molecular typing of ESKAPE members by ERIC-PCR

ESKAPE bacteria was subjected to molecular typing by ERIC-PCR, by using the primers ERIC1R and ERIC2<sup>35</sup> (Table 1). The total reaction volume was 50  $\mu$ L and consisted of molecular Biology grade water, 1  $\times$  PCR buffer, 20 nM MgCl<sub>2</sub>, 25 mM deoxyribonucleotides phosphate, 100 pM of each primer, 3 units of Taq DNA polymerase (Thermo Scientific, Foster City, CA, USA) and 300 ng of template genomic DNA. Cycling conditions were as follows: pre-denaturation at 95°C for 7 s, denaturation at 90°C for 30 s, annealing at 58°C for 1 min, and extension at 65°C for 8 min, with a final extension at 68°C for 16 min at the end for 30 cycles. Genetic profiles were run in 1  $\times$  TBE buffer, pH 8.3, and separated in horizontal electrophoresis in 1.5% agarose gels, visualized, photographed under UV illumination, and analysed by intra-gel pattern comparison by using ImageLab 5.2.1. To confirm the reproducibility of ERIC-PCR assays three times was done. Tenover criteria was used in order to established the clonal relationship between isolates with the same genus and specie.<sup>36</sup> Finally, graphical relationship was performed through distance matrix by using a linear semilogarithmic method. The dendrograms were constructed using the UPGMA algorithm, with the Dice similarity index.

**Table 2**  
Distribution of COVID-19/VAP patients by sex, age groups admitted to Hospital Juárez de México

Patient classification	Gender (%/n)	
	Male	Female
Pediatric*	0/0	2.9/1
Young adults <sup>†</sup>	3.2/2	5.9/2
Adults <sup>‡</sup>	69.3/43	55.9/19
Elderly <sup>§</sup>	27.5/17	35.3/12
<b>Total</b>	<b>100/62</b>	<b>100/34</b>

\*0-18 years old.  
<sup>†</sup>18-35 years old.  
<sup>‡</sup>36-64 years old.  
<sup>§</sup>>64 years old.

Genomic similarity were confirmed with a bootstrap test of 1,000 repetitions using the Past4 program (Version 4.09).

**RESULTS**

*Description of population of COVID-19 patients with VAP*

During the period from April to June 2020 (first wave), patients with suspected COVID-19 were admitted to HJM. According to the CO-RADS system, these patients were classified according to their degree of severity as reported by Prokop et al., (2020).<sup>37</sup> The study population was categorised as critical and therefore underwent mechanical endotracheal intubation. COVID-19/VAP patients were

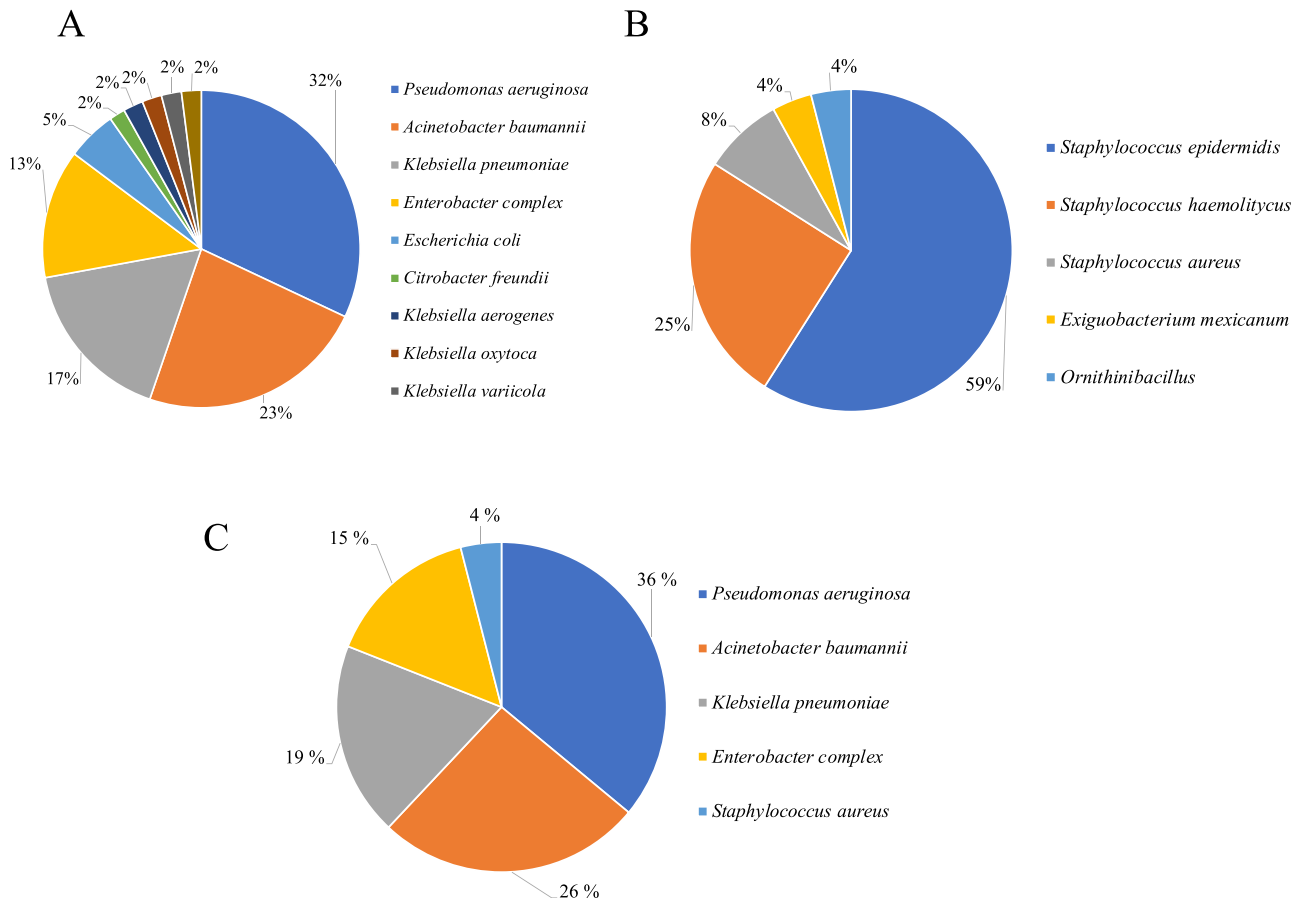
cared for in 6 different areas named COVID-19 AREAS (A, B, C, D, E, F, G). Table 2 shows the distribution of COVID-19/VAP patients by sex and age.

*ESKAPE and non-ESKAPE bacteria isolated from COVID-19/VAP patients*

Bacteriological analysis by classical microbiology revealed that only 56.3% (n = 54) of the tracheal aspirates showed bacterial growth with presumptive clinical value. Polymicrobial and monomicrobial cultures were identified in 42.6% (n = 23) and 57.4% (n = 31), respectively. Patients in the adult (36-64 years old) and elderly (>64 years old) age groups were those who developed polymicrobial infections. Monomicrobial infections were heterogeneously identified in all four age groups. A total of 60 and 23 isolates taxonomically classified as Gram-negative and Gram-positive were identified, respectively. Sequence analysis showed that 63.85% (n = 53) were isolates belonging to the ESKAPE group (Fig 1). Finally, other genera and species implicated in HAI, as well as bacteria considered commensal and environmental, were identified.

*High frequency of MDR and XDR ESKAPE bacteria*

The results showed that *A. baumannii* (n = 14/26.4%), *P. aeruginosa* (n = 5/9.4%) and *K. pneumoniae* (n = 5/9.4%) strains were classified as MDR. XDR phenotype was identified in *P. aeruginosa* in 3 isolates (5.6%). Resistant phenotypes for Enterobacterales group (except *K. pneumoniae*) were classified according to Magiorakos et al., (2012)<sup>38</sup> (Table 3).



**Fig 1.** Frequency of ESKAPE and non-ESKAPE bacteria isolated. A. Gram negative population, B. Gram positive population and C. ESKAPE bacteria population.

**Table 3**  
Frequency of antimicrobial resistant obtained by CLSI in ESKAPE bacteria isolated of COVID-19/VAP patients from Hospital Juárez de México

Antibiotic	Antimicrobial resistant frequency in ESKAPE and non-ESKAPE bacteria n (%)								
	<i>Acinetobacter baumannii</i> (n = 14)			<i>Pseudomonas aeruginosa</i> (n = 19)			Enterobacterales (n = 20)		
	S <sup>*</sup>	I <sup>†</sup>	R <sup>‡</sup>	S	I	R	S	I	R
AMC	ND <sup>*</sup>	ND	ND	ND	ND	ND	8 (40)	8 (40)	4 (20)
AN	0 (0)	0 (0)	14 (100)	13 (68.4)	1 (5.3)	5 (26.3)	17 (8)	2 (20)	1 (5)
CAZ	0 (0)	0 (0)	14 (100)	12 (63.1)	0 (0)	7 (36.8)	9 (45)	0 (0)	11 (55)
COL	14 (100)	0 (0)	0 (0)	14 (100)	0 (0)	0 (0)	14 (100)	0 (0)	0 (0)
CRO	0 (0)	0 (0)	14 (100)	ND	ND	ND	5 (25)	0 (0)	15 (75)
CTX	0 (0)	0 (0)	14 (100)	ND	ND	ND	2 (20)	1 (5)	15 (75)
CZA	ND	ND	ND	12 (63.1)	0 (0)	7 (36.8)	18 (9)	0 (0)	1 (5)
ETP	ND	ND	ND	ND	ND	ND	18 (90)	0 (0)	2 (10)
FEP	0 (0)	3 (21.4)	11 (78.6)	12 (63.2)	2 (10.53)	5 (26.3)	13 (65)	3 (15)	4 (20)
GM	0 (0)	0 (0)	14 (100)	14 (73.7)	0 (0)	5 (26.3)	13 (65)	0 (0)	7 (35)
IPM	1 (7.1)	0 (0)	13 (92.9)	9 (47.4)	0 (0)	10 (52.6)	85 (90)	1 (5)	2 (10)
LVX	0 (0)	0 (0)	14 (100)	9 (47.37)	2 (10.53)	8 (42.1)	ND	ND	ND
MEM	1 (7.2)	0 (0)	13 (92.9)	10 (52.6)	0 (0)	9 (47.4)	85 (90)	1 (5)	2 (10)
SAM	3 (21.4)	4 (28.6)	7 (50)	ND	ND	ND	7 (35)	3 (15)	10 (50)
STX	0 (0)	0 (0)	14 (100)	ND	ND	ND	11 (55)	0 (0)	9 (45)
TET	3 (21.4)	0 (0)	11 (78.6)	ND	ND	ND	ND	ND	ND
TGC	14 (100)	0 (0)	0 (0)	ND	ND	ND	ND	ND	ND
TZP	0 (0)	0 (0)	14 (100)	(47.4)	0 (0)	10 (52.6)	12 (0)	7 (35)	1 (5)

\*Sensitive.

†Intermediate.

‡Resistant.

<sup>\*</sup>Nondetermined. Abbreviations: AMC, Amoxicillin/Ac. Clavulanic; AN, Amikacin; CAZ, Ceftazidime; COL, Colistin; CRO, Ceftriaxone; CTX, Cefotaxime; CZA, Ceftazidime/Avibactam; ETP, Ertapenem; FEP, Cefepime; GM, Gentamicin; IPM, Imipenem; LVX, Levofloxacin; MEM, Meropenem; SAM, Ampicillin/Sulbactam; STX, Trimethopim/Sulfamethoxazole; TET, Tetracycline; TGC, Tigecycline; TZP, Piperacillin/Tazobactam.

#### MDR/XDR phenotype/genotype and their relationship in carbapenemases production

ESKAPE bacteria (30.2%/n = 16) and non-ESKAPE bacteria (1.8%/n = 1) were capable to hydrolyse carbapenems. *Acinetobacter*

*baumannii* (n = 9) was the most prevalent carbapenemase-producer and 6 strains of *P. aeruginosa* were also carbapenemase producers. Finally, *Escherichia coli* (n = 1) and *E. cloacae* (n = 1), were capable to hydrolyse meropenem. The results of antimicrobial resistance genotype are shown in Table 4.

**Table 4**  
Genetic background of antimicrobial resistance in ESKAPE and non-ESKAPE bacteria isolated from COVID-19/VAP patients from Hospital Juárez de México

Strain	Carbapenem resistance <sup>†</sup>	mCIM <sup>§</sup>	Metallo $\beta$ -lactamases** NDM,IMP,VIM	Serine $\beta$ -lactamases		Colistin resistance** mcr-1/2
				KPC**/OXA-48**	OXA-23 <sup>††</sup>	
AB*-10	+	-	-	-	+	-
AB-20	+	-	-	-	+	-
AB-28	+	-	-	-	-	-
AB-66	+	-	-	-	+	-
AB-70	+	+	-	-	-	-
AB-73	+	+	-	-	+	-
AB-84	+	+	*** <i>bla</i> <sub>VIM</sub> <sup>††</sup>	-	-	-
AB-86	+	+	-	-	-	-
AB-97	+	+	-	-	-	-
AB-102	+	+	-	-	-	-
AB-104	+	+	+ <i>bla</i> <sub>VIM</sub>	-	+	-
AB-106	+	+	-	-	-	-
AB-108	+	+	-	-	-	-
AB-112	+	-	-	-	+	-
PA <sup>†</sup> -5(2)	+	+	+ <i>bla</i> <sub>VIM</sub>	-	ND <sup>††</sup>	-
PA-9	+	+	+ <i>bla</i> <sub>VIM</sub>	-	ND	-
PA-11	+	+	+ <i>bla</i> <sub>VIM</sub>	-	ND	-
PA-27	+	+	+ <i>bla</i> <sub>VIM</sub>	-	ND	-
PA-78	+	+	+ <i>bla</i> <sub>VIM</sub>	-	ND	-
PA-81(2)	+	+	+ <i>bla</i> <sub>VIM</sub>	-	-	-
ECL <sup>‡</sup> -77(2)	+	+	+ <i>bla</i> <sub>VIM</sub>	-	ND	-
EC <sup>‡</sup> -23(2)	+	+	+ <i>bla</i> <sub>NDM</sub>	-	ND	-

\**Acinetobacter baumannii*.

†*Pseudomonas aeruginosa*.

‡*Enterobacter cloacae*.

§*Escherichia coli*.

† According to CLSI (2021).

§ Modified carbapenem inactivation method.

\*\*Real time-PCR.

†† End point-PCR.

‡‡ Non-determined.



### *Acinetobacter baumannii* of COVID-19/VAP patients carrying full *AdeABC* operon

Full operon *AdeABC* and their regulator genes *AdeRS* were amplified in all *A. baumannii* MDR strains. Additionally, to genetic detection of the efflux pump, assays of inhibition of this pump were performed to confirm their functionality, through MDR phenotype reversion. The results showed there was no association of efflux pump inhibition and antibiotic resistance.

### Molecular typing of ESKAPE members by ERIC-PCR

Profiles of the intergenic products revealed sizes of amplicons from ESKAPE ranged from slightly more than  $\approx 100$  bp to about  $\approx 3000$  bp. Intergenic region diversity of *A. baumannii* showed that the 14 isolates were grouped in seven unique clones, where 11 of them identified 4 clonal groups. A broad genomic diversity of *P. aeruginosa* strains was detected. This is due to the fact that out of the total of 19 strains, 17 were not grouped, and one clonal group was detected (Fig 2). Finally, *Klebsiella pneumoniae* and *E. cloacae* isolates, a broad genomic diversity was observed (Fig 3).

### *Acinetobacter baumannii* clonal dispersion investigation

Four clonal clusters of *A. baumannii* were detected in the last month of the quarter analysed, according to the chronology of their appearance, the origin of these clonal clusters started in the COVID-19 “A and B” areas. No patients survived. Figure 4 shows the timeline for *A. baumannii* clonal detection events by date and COVID-19 area (A, B, and C). The Table 5 summarises the most relevant characteristics of the COVID-19/VAP patients involved in the detection of the four *A. baumannii* clonal groups.

## DISCUSSION

Research on the new SARS-CoV-2 virus has focused on the study of its pathogenesis, evolution, treatment, vaccines, among others.<sup>39–41</sup> However, the study of the bacterial agents that complicate the clinical status of COVID-19 patients, as well as the genetic basis of antimicrobial resistance and its clonal dispersion in critical areas has been poor explored. It is known that VAP-causing pathogens prolong and complicate their hospital stay, so their identification and containment through prospective epidemiological surveillance should be a priority. This study showed that the pathogens that cause VAP in COVID-19 patients form part of the nosocomial reservoir in non-COVID-19 and pandemic periods.<sup>12,16,22</sup> This is because pathogens found in ICU patients (ESKAPE and non-ESKAPE group) have also been shown to be identified on inanimate surfaces, medical personnel and devices used in respiratory therapy, such as mechanical ventilators. Statistics from this work showed that the male patient population had the highest prevalence of COVID-19 (Table 2). It has been shown that ACE2 receptor levels are significantly higher in this sex, a factor of susceptibility to SARS-CoV-2 infection and consequently fatal outcomes.<sup>42</sup> Conversely, 88.54% of the population studied was over 41 years, where age has been recognised as a predisposing factor, mainly in those who presented the disease during the first wave of the pandemic (Table 2).<sup>43–46</sup> Other susceptibility factors include impaired immune response, which leads to impaired control of viral replication and prolonged inflammatory response.<sup>45,47</sup> All these complications together trigger acute respiratory distress syndrome (ARDS), where mechanical ventilation (MV) plays an important role in supporting respiratory support for the maintenance of these patients.<sup>48</sup> ARDS is a well-known risk factor for VAP and is related to the incidence in patients with MV.<sup>8,48</sup> The percentage of bacterial co-infections in our study was 56.25%, frequencies of 17.2% to 89% have been reported elsewhere in the

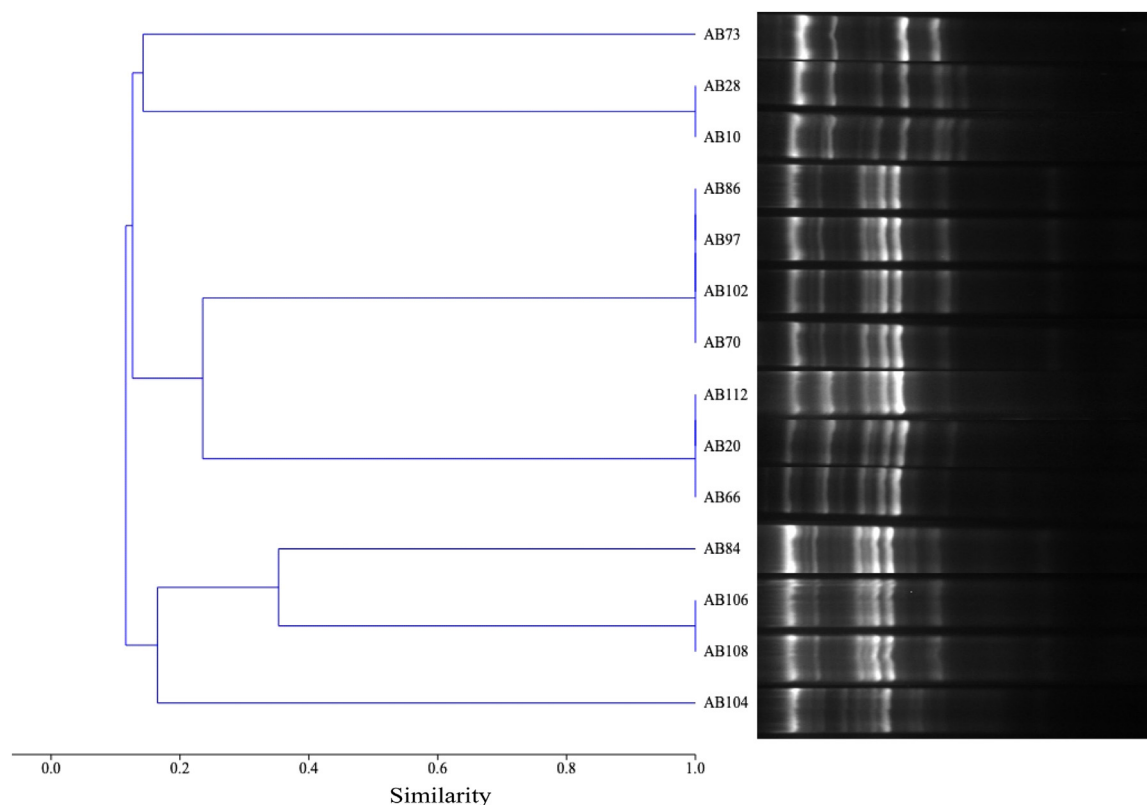
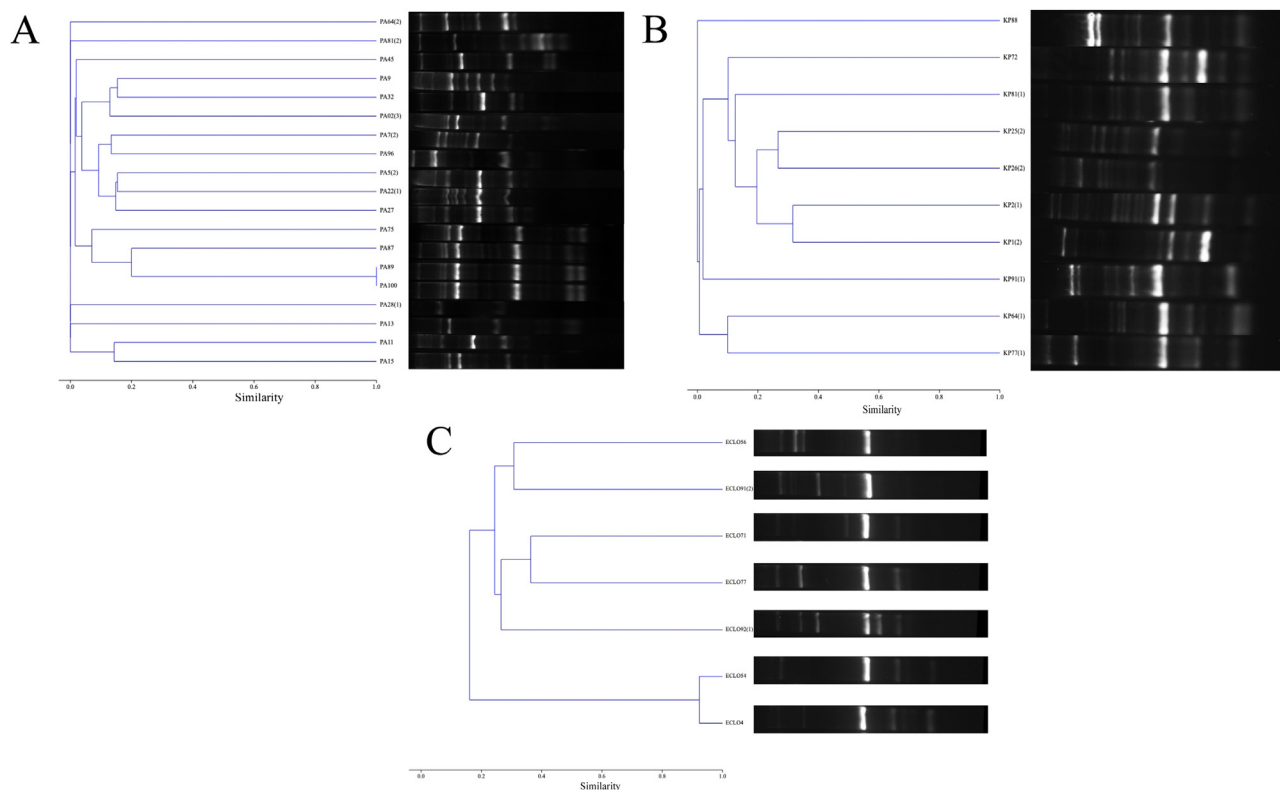


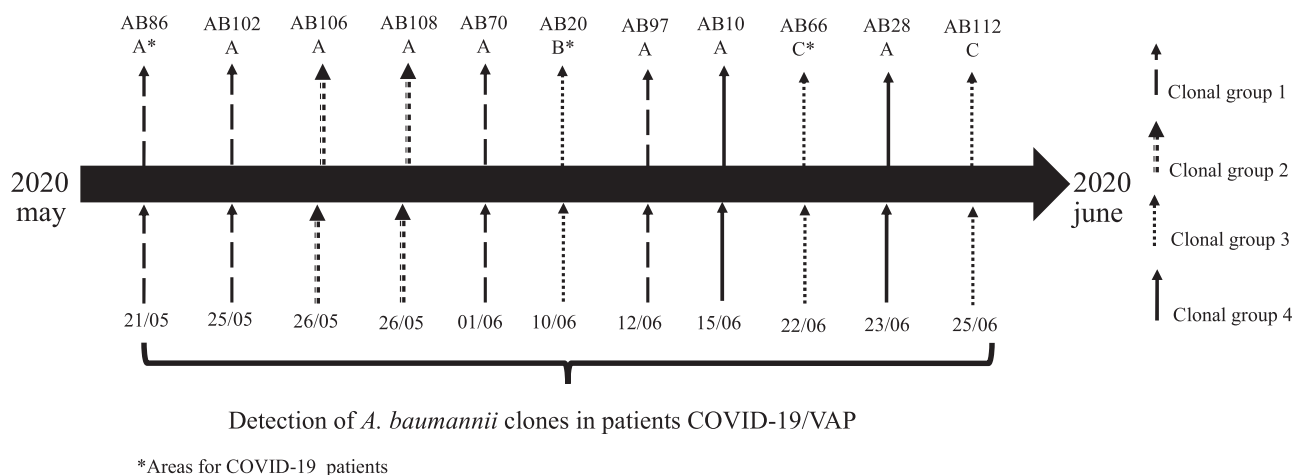
Fig 2. Genomic diversity by dendrogram construction of *Acinetobacter baumannii* isolated from COVID-19/VAP patients of the Hospital Juárez de México by ERIC-PCR.



**Fig 3.** Genomic diversity by dendrogram construction of ESKAPE bacteria isolated from COVID-19/VAP patients of the Hospital Juárez de México by ERIC-PCR.

world.<sup>8,9,13,49,50</sup> We speculate that these frequencies may vary depending on factors, such as adherence to correct clinical practices in patient management, hand washing technique, and adequate disinfection methods.<sup>22</sup> As a result of these problems, the need and interest in epidemiological surveillance for the containment of both SARS-CoV-2 and HAI-causing bacteria arose at the *HJM*. The ESKAPE group was the microorganisms are mainly responsible for HAI, including VAP.<sup>10,48,51</sup> These bacteria have been previously reported in patients with VAP and SARS-CoV-2 infection.<sup>7,9,13</sup> In contrast, 44% of the study population had clinical features suggestive of VAP but failed to isolate the causative agent. We speculate that the microbiological culture bias is due to the fact that only ESKAPE bacteria were

targeted. This may have influenced the detection of other microorganisms related to this nosocomial infection. Previous studies report the presence of pathogens that can cause VAP in COVID-19 patients that could be undetected by microbiological analysis. Co-infections of COVID-19 patients with yeast *Candida* spp., and other bacteria not investigated in this study have been reported.<sup>9,52,53</sup> The COVID-19 pandemic has had several consequences, including a possible increase in antimicrobial resistance (AMR), where the irrational use of antibiotics has been described as the main cause.<sup>54</sup> Antibiotic resistance and its persistence are associated with the risk of therapeutic failure and resilient infections.<sup>55</sup> We detected isolates with resistance to tested antimicrobials, mainly  $\beta$ -lactams and other agents used in



**Fig 4.** Timeline of detection of clonal groups of MDR *Acinetobacter baumannii* isolated from COVID-19/VAP patients of the Hospital Juárez de Mexico.



**Table 5**  
Characteristics of COVID-19/VAP patients involved in clonal dispersion groups of MDR *Acinetobacter baumannii* strains from Hospital Juárez de México

Sex	Age	ICU admission reason	Clonal group	Date of first CR-AB isolate collection	CR-AB <sup>1</sup> isolate source	Hospital Stay (days)	Patient outcomes
M	43	SDRA*	1	21/05/20	Tracheal aspirate	25	Deceased
M	55	SDRA		25/05/20	Tracheal aspirate	12	Deceased
M	57	SDRA		01/06/20	Tracheal aspirate	22	Deceased
F	55	SDRA		12/06/20	Tracheal aspirate	19	Deceased
F	33	SDRA	2	26/05/20	Tracheal aspirate	28	Deceased
M	38	SDRA		26/05/20	Tracheal aspirate	20	Deceased
M	35	SDRA	3	10/06/20	Tracheal aspirate	23	Deceased
M	74	SDRA		22/06/20	Tracheal aspirate	14	Deceased
F	65	SDRA		25/06/20	Tracheal aspirate	9	Deceased
F	63	SDRA	4	15/06/20	Tracheal aspirate	21	Deceased
F	46	SDRA		23/06/20	Tracheal aspirate	26	Deceased

\*Acute respiratory distress syndrome.

<sup>1</sup>Carbapenem-resistant *Acinetobacter baumannii*.

VAP patients, such as aminoglycosides and fluoroquinolones<sup>56</sup> (Table 3). Resistance profiles similar to those of this work have been observed in other studies.<sup>51,57,58</sup> The background of the presence of one of the main mechanisms of antimicrobial resistance in *A. baumannii*,<sup>59,60</sup> a genetic and phenotypic search for efflux pumps in this pathogen was performed, although PCR tests demonstrated the presence of the complete *AdeABC* operon, pump inhibition tests did not demonstrate its activity. We speculate that the multidrug resistance of the *A. baumannii* isolates in this work is not conferred by the *AdeABC* efflux pump. Future works of resistome analysis of *A. baumannii* will be aimed for searching possible mutations in the *AdeABC* operon and antimicrobial resistance mechanisms in order to relate phenotypes and genotypes in the isolates found. The high multidrug resistance in *A. baumannii* isolates, compared to the other members of the ESKAPE group, shows that this pathogen continues to be a problem in our hospital and the main microorganism implicated in cases of VAP caused by MDR bacteria. As reported by Sosa-Hernandez et al., (2019), the incidence of *A. baumannii* as a causative agent of VAP was higher compared to the other pathogens of the ESKAPE group.<sup>12</sup> Derived from this finding, the positive impact of prospective epidemiological surveillance through implementation of a comprehensive quality improvement plan (CQIP) during the following year was demonstrated.<sup>51</sup> The classical epidemiological approach of case association limits the identification of outbreaks that might go unnoticed. To our knowledge, the available literature is only limited to the reporting of isolates without an analysis of whether these isolates have a common ancestor. Therefore, classical Epidemiology must resort to alternative molecular tools to understand the origin of adverse events in critical areas. Through the analysis of intergenic regions, clonal dispersion of *A. baumannii* and *P. aeruginosa* was demonstrated (Fig. 2 and 3). The timeline analysis of VAP cases in COVID-19 patients showed a direct association between the dispersion of clones detected and the area where patients were hospitalized (Fig 4 and Table 5). We supposed that healthcare workers may be the main vehicle of transmission of these pathogens. Several reports support that, poor clinical practices lead to the spread of microorganisms in hospitals, and this has an impact on the incidence of HAI and in turn on mortality, especially in critically ill patients.<sup>17–20,62,63</sup> It is not known exactly how the pandemic has affected antimicrobial resistance as outbreak detection and control measures were suspended due to the health emergency.<sup>54</sup> However, some studies suggest that there was an increase in AMR during the pandemic.<sup>64,65</sup> This paper demonstrates that molecular tools go beyond the traditional scheme of analysis for enriching the causal chain by detecting hidden outbreaks and revealing associations that are not so obvious to classical Epidemiology. The current epidemiological transition positions molecular Epidemiology as an indispensable tool in the pandemic and post-pandemic era in the bacteriological field.

## Acknowledgments

M.A.C.D., G.I.C., G.C.E., and J.M.B.L. received support from “Sistema Nacional de Investigadores (SNI)” from “Consejo Nacional de Ciencia y Tecnología” (CONACyT). G.C.E. received support from “Estímulos al Desempeño en Investigación, Comisión de Operación y Fomento de Actividades Académicas del IPN”. M.A.L.C. received grant-aided support from CONACyT and “Beca de Estímulos Institucional de Formación de Investigadores” (BEIFI) and actually is PhD student of Biomedicine and molecular Biotechnology program of IPN.

## References

- World Health Organization. (2022). COVID-19 weekly epidemiological update, edition 76, 25 January 2022. Accessed February 28, 2022. <https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—25-january-2022>.
- Maes M, Higginso E, Pereira-Dias J, et al. Ventilator-associated pneumonia in critically ill patients with COVID-19. *Crit Care*. 2021;25:25.
- Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395:1054–1062.
- Giacobbe D, Battaglini D, Enrile E, et al. Incidence and prognosis of ventilator-associated pneumonia in critically ill patients with COVID-19: a multicenter study. *J Clin Med*. 2021;10:555.
- Nseir S, Martin-Loeches I, Povoia P, et al. Relationship between ventilator-associated pneumonia and mortality in COVID-19 patients: a planned ancillary analysis of the coVAPid cohort. *Crit Care*. 2021;25:284.
- Ściśło L, Walewska E, Bodys-Cupak I, Gniadek A, Kózka M. Nutritional status disorders and selected risk factors of Ventilator-Associated Pneumonia (VAP) in Patients Treated in the Intensive Care Ward—a retrospective study. *Int J Environ Res*. 2022;19:602.
- Meawed T, Ahmed S, Mowafy S, Samir G M, Anis R. Bacterial and fungal ventilator associated pneumonia in critically ill COVID-19 patients during the second wave. *J Infect Public Health*. 2021;14:375–1380.
- Rouzé A, Martin-Loeches I, Povoia P, Makris D, Artigas A, Bouchereau M. Relationship between SARS-CoV-2 infection and the incidence of ventilator-associated lower respiratory tract infections: a European multicenter cohort study. *Intensive Care Med*. 2021;47:188–198.
- Hughes S, Troise O, Donaldson H, Mughal N, Moore L. Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. *Clin Microbiol Infect*. 2020;26:1395–1399.
- Mulani M, Kamble E, Kumkar S, Tawre M, Pardesi. Emerging strategies to combat ESKAPE pathogens in the Era of antimicrobial resistance: a review. *Front Microbiol*. 2019;10:539.
- Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. *Biomed Res. Int.*. 2016 2475067.
- Sosa-Hernández O, Matías-Téllez B, Estrada-Hernández A, Cureño-Díaz M, Bello-López J. Incidence and costs of ventilator-associated pneumonia in the adult intensive care unit of a tertiary referral hospital in Mexico. *Am. J infect. Control.*. 2019;47:e21–e25.
- Fattorini L, Creti R, Palma C, Pantosti A. Unit of Antibiotic Resistance and Special Pathogens, & Unit of Antibiotic Resistance and Special Pathogens of the Department of Infectious Diseases, Istituto Superiore di Sanità, Rome. Bacterial coinfections in COVID-19: an underestimated adversary. *Ann Ist Super Sanita*. 2020;56:359–364.
- Dettori M, Piana A, Deriu M, et al. Outbreak of multidrug-resistant *Acinetobacter baumannii* in an intensive care unit. *New Microbiol*. 2014;37:185–191.

15. Ababneh Q, Abulaila S, Jaradat Z. Isolation of extensively drug resistant *Acinetobacter baumannii* from environmental surfaces inside intensive care units. *Am. J. Infect. Control.* 2021;S0196-6553. 00589-7.
16. Durán-Manuel E, Cruz-Cruz C, Ibáñez-Cervantes G, et al. Clonal dispersion of *Acinetobacter baumannii* in an intensive care unit designed to patients COVID-19. *J Infect Dev Ctries.* 2021;15:58–68.
17. Morgan D, Liang S, Smith C, et al. Frequent multidrug-resistant *Acinetobacter baumannii* contamination of gloves, gowns, and hands of healthcare workers. *Infect Control Hosp Epidemiol.* 2010;31:716–721.
18. Tajeddin E, Rashidan M, Razaghi M, et al. The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections. *J Infect Public Health.* 2016;9:13–23.
19. Agodi A, Barchitta M, Cipresso R, Giaquinta L, Romeo M, Denaro C. *Pseudomonas aeruginosa* carriage, colonization, and infection in ICU patients. *Intensive Care Med.* 2007;33:1155–1161.
20. Weber D, Rutala W, Miller M, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium difficile*, and *Acinetobacter* species. *Am J Infect Control.* 2010;38:S25–S33.
21. Kalantar M, Jarinsh H, Mehrvarz S, Danial Z, Rasouli H, Rashidi A. Bacterial contamination of ventilators in the Intensive Care Unit. *Trauma Mon.* 2018;23:5.
22. Cureño-Díaz M, Durán-Manuel E, Cruz-Cruz C, et al. Impact of the modification of a cleaning and disinfection method of mechanical ventilators of COVID-19 patients and ventilator-associated pneumonia: one year of experience. *Am J Infect Control.* 2021;49:1474–1480.
23. Johanson W, Pierce A, Sanford J, Thomas G. Nosocomial respiratory infection with gram negative bacilli: the significance of colonization of respiratory tract. *Ann Intern Med.* 1972;77:701–706.
24. Gupta R, Sharma S, Parwez Saxena S. Changing panorama for surveillance of device-associated healthcare infections: challenges faced in implementation of current guidelines. *Indian J Med Microbiol.* 2018;36:18–25.
25. Mehta A, Bhagat R. Preventing ventilator-associated infections. *Clin Chest Med.* 2016;37:683–692.
26. DeSantis T, Brodie E, Moberg J, et al. Highdensity universal16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. *Microb Ecol.* 2007;53:371–383.
27. Jiménez Pearson M, Galas M, Corso A, et al. Consenso latinoamericano para definir, categorizar y notificar patógenos multirresistentes, con resistencia extendida o panresistentes. *Am J Public Health.* 2019;43:e65.
28. Clinical and Laboratory standard institute (CLSI). Performance standards for antimicrobial susceptibility testing, 31th ed. CLSI Supplement M100. (ISBN: 978-1-68440-105-5). 2021.
29. Pierce VM, Simner PJ, Lonsway DR, et al. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among enterobacteriaceae. *J Clin Microbiol.* 2017;55:2321–2333.
30. Cortés-Ortiz IA, Juárez-Gómez JC, Cu-Quijano C, et al. *Klebsiella pneumoniae* blaNDM-1-carrying a class 1 integron causing a hospital outbreak in a Mexican attention center. *J Inf Dev Ctries.* 2021;15:657–664.
31. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. *J Antimicrob Chemother.* 2010;65:490–495.
32. Nordmann P, Poirel L, Carrère A, Toleman MA, Walsh TR. How to detect NDM-1 producers. *J Clin Microbiol.* 2011;49:718–721.
33. Girlich D, Bernabeu S, Gersperrin V, et al. Evaluation of the amplidiag CarbaR+MCR kit for accurate detection of carbapenemase-producing and colistin-resistant bacteria. *J Clin Microbiol.* 2019;57:e01800–e01818.
34. Alsehlawi ZS, Alshara JM, Had ZJ, Almohana AM. First report of the blaOxa-23 gene in a clinical isolates of *Acinetobacter baumannii* in Najaf hospitals-Iraq. *Int J Recent Sci Res.* 2014;5:1407–1411.
35. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and. Application to fingerprinting of bacterial genomes. *Nucleic Acids Res.* 1991;19:6823–6831.
36. 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;33:2233–2239.
37. Prokop M, van Everdingen W, van Rees Vellinga T, Quarles van Ufford H, Stöger L, et al. CO-RADS: a categorical CT assessment scheme for patients suspected of having COVID-19-Definition and evaluation. *Radiology.* 2020;296:E97–E104.
38. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012;18:268–281.
39. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 transmission and pathogenesis. *Trends Immunol.* 2020;41:1100–1115.
40. Awadasseid A, Wu Y, Tanaka Y, Zhang W. Current advances in the development of SARS-CoV-2 vaccines. *Int J Biol Sci.* 2021;17:8–19.
41. Raman R, Patel K J, Ranjan K. COVID-19: unmasking emerging SARS-CoV-2 variants, vaccines and therapeutic strategies. *Biomolecules.* 2021;11:993.
42. Mukherjee S, Pahan K. Is COVID-19 Gender-sensitive? *J Neuroimmune Pharmacol.* 2021;16:38–47.
43. Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G. Risk factors associated with mortality among patients with COVID-19 in intensive care units in Lombardy, Italy. *JAMA Intern Med.* 2020;180:1345–1355.
44. Parra-Bracamonte GM, Lopez-Villalobos N, Parra-Bracamonte FE. Clinical characteristics and risk factors for mortality of patients with COVID-19 in a large data set from Mexico. *Ann Epidemiol.* 2020;52:93–98.e2.
45. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395:1054e62.
46. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P. Coronavirus infections and immune responses. *J Med Virol.* 2020;92:424–432.
47. Opal SM, Girard TD, Ely EW. The immunopathogenesis of sepsis in elderly patients. *Clin Infect Dis.* 2005;41:S504e12.
48. Póvoa H, Chianca GC, Iorio N. COVID-19: An Alert to Ventilator-Associated Bacterial Pneumonia. *Infect Dis Ther.* 2020;9:417–420.
49. Perez S, Innes GK, Walters MS, et al. Increase in hospital-acquired carbapenem-resistant *Acinetobacter baumannii* infection and colonization in an acute care hospital during a surge in COVID-19 admissions - New Jersey, February-July 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69:1827–1831.
50. Zangrillo A, Beretta L, Scandroglio AM, Monti G, Fominskiy E, Colombo S. Characteristics, treatment, outcomes and cause of death of invasively ventilated patients with COVID-19 ARDS in Milan, Italy. *Critical Care Resusc.* 2020;22:200–211.
51. Sader HS, Castanheira M, Arends S, Goossens H, Flamm RK. Geographical and temporal variation in the frequency and antimicrobial susceptibility of bacteria isolated from patients hospitalized with bacterial pneumonia: results from 20 years of the SENTRY Antimicrobial Surveillance Program (1997-2016). *J Antimicrob Chemother.* 2019;74:1595–1606.
52. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020;395:507–513.
53. Lai CC, Wang CY, Hsueh PR. Co-infections among patients with COVID-19: the need for combination therapy with non-anti-SARS-CoV-2 agents? *J Microbiol Immunol Infect.* 2020;53:505–512.
54. Rawson TM, Moore L, Castro-Sanchez E, Charani E, Davies F, Satta G. COVID-19 and the potential long-term impact on antimicrobial resistance. *J Antimicrob Chemother.* 2020;75:1681–1684.
55. Huemer M, Mairpady Shambat S, Brugger SD, Zinkernagel AS. Antibiotic resistance and persistence-Implications for human health and treatment perspectives. *EMBO Rep.* 2020;21:e51034.
56. Ceccato A, Di Giannatale P, Nogas S, Torres A. Safety considerations of current drug treatment strategies for nosocomial pneumonia. *Expert Opin Drug Saf.* 2021;20:181–190.
57. Gales AC, Seifert H, Gur D, Castanheira M, Jones RN, Sader HS. Antimicrobial Susceptibility of *Acinetobacter calcoaceticus-Acinetobacter baumannii* Complex and *Stenotrophomonas maltophilia* clinical isolates: results from the SENTRY Antimicrobial Surveillance Program (1997-2016). *Open Forum Infect Dis.* 2019;6(suppl 1):S34–S46.
58. Garza-González E, Bocanegra-Ibarias P, Bobadilla-Del-Valle M, Ponce-de-León-Garduño LA, Esteban-Kenel V. Drug resistance phenotypes and genotypes in Mexico in representative gram-negative species: results from the infivar network. *PLoS One.* 2021;16: e0248614.
59. Xu C, Bilya SR, Xu W. *adeABC* efflux gene in *Acinetobacter baumannii*. *New Microbes New Infections.* 2019;30: 100549.
60. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB. Biology of *Acinetobacter baumannii*: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. *Front Cell Infect Microbiol.* 2017;7:55.
61. Sosa-Hernández O, Matías-Téllez B, Silva-López YE, Alarcón-Hernández V, Bello-López JM, Cureño-Díaz MA. Economic and epidemiological impact of an improvement plan for the decrease of ventilator-associated pneumonia in a tertiary hospital in Mexico. *J Patient Saf.* 2021;17:e1889–e1893.
62. Rangel K, Chagas T, De-Simone SG. *Acinetobacter baumannii* infections in times of COVID-19 pandemic. *Pathogens.* 2021;10:1006.
63. Eckardt P, Canavan K, Guran R, George E, Miller N, Avendano DH. Containment of a carbapenem-resistant *Acinetobacter baumannii* complex outbreak in a COVID-19 intensive care unit. *Am J Infect Control.* 2022;50:477–481.
64. Ruiz-Garbajosa P, Cantón R. COVID-19: Impact on prescribing and antimicrobial resistance. *Revi Esp Quimioter.* 2021;34(suppl 1):63–68.
65. Ukuhor HO. The interrelationships between antimicrobial resistance, COVID-19, past, and future pandemics. *J Infect Public Health.* 2021;14:53–60.