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Coronavirus Pandemic

Clonal dispersion of *Acinetobacter baumannii* in an intensive care unit designed to patients COVID-19

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Abstract

Introduction: SARS-CoV2 pandemic marks the need to pay attention to bacterial pathogens that can complicate the hospital stay of patients in the intensive care unit (ICU). ESKAPE bacteria which includes *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter cloacae* are considered the most important, because of their close relationship with the development of ventilator-associated pneumonia (VAP). The aim of this work was to identify and characterize ESKAPE bacteria and to detect their possible clonal spread in medical devices, patients, and medical personnel of the ICU for COVID-19 patients of the Hospital Juarez de Mexico.

Methodology: Genetic identification of ESKAPE bacteria was performed by analyzing the 16S rRNA gene. Resistance assays were performed according to the CLSI guidelines. Assembly of *AdeABCRS* operon and inhibition assays of pumps efflux in *Acinetobacter baumannii* isolates were performed. Associated gene involved in biofilm formation (*icaA*) was performed in isolates belonging to the *Staphylococcus* genus. Finally, typing by ERIC-PCR and characterization of mobile genetic element *SCCmec* were done.

Results: Heterogeneous distribution of ESKAPE and non-ESKAPE bacteria was detected in various medical devices, patients, and medical personnel. *Acinetobacter baumannii* and *Staphylococcus aureus* were the predominant ESKAPE members. The analysis of intergenic regions revealed an important clonal distribution of *A. baumannii* (*AdeABCRS*+). Genotyping of *SCCmec* mobile genetic elements and the *icaA* gene showed that there is no clonal distribution of *S. aureus.*

Conclusions: Clonal spread of *A. baumannii* (*AdeABCRS*+) highlights the importance of adopting good practices for equipment disinfection, surfaces and management of COVID-19 patients.

Key words: ESKAPE bacteria; clonal dispersion; *Acinetobacter baumannii*; intensive care unit; COVID-19.

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Introduction

The current worldwide contingency by SARS-CoV-2, the causative agent of COVID-19 disease highlights the need to pay greater attention to those bacterial pathogens that could complicate the hospital stay of patients in the intensive care unit (ICU). Colonization by microorganisms in hospital environments is made up of hundreds of bacterial genera and species [1]. The spread of bacteria in these environments is often caused by cross-contamination events between nearby areas, mediated by health personnel. Previous studies have reported that the spread of pathogens occurs through direct contact between health personnel and patients [2]. Also, immediate areas where the patients are, have an important role as sources of contamination and spread of bacteria. A particular example of the negative effect of bacterial contamination in the hospital environment is the ICU, due to the severe clinical condition of the patients, including patients with COVID-19 [3-5]. Bacterial infections acquired in the ICU are the cause of high morbidity and mortality rates worldwide, with ventilator-associated pneumonia (VAP) being the most frequent [6-7]. Bacteria that have been described as potential hospital pathogens are grouped with the acronym ESKAPE, which includes *Enterococcus*

faecium, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter cloacae* [8]. Molecular evidence has shown that the ESKAPE group has acquired various resistance mechanisms to evade most of the available therapeutic options. Resistance cassettes, antibiotic efflux pumps, plasmids, transposons, and integrons have been recognized as the genetic elements responsible for multi-resistance to antibiotics in ESKAPE bacteria [9].

With the emergence of infections caused by multi-resistant bacteria to antibiotics, the health problem is greater and a challenge for health personnel, especially if there are critically ill patients, and currently patients with SARS-CoV-2 infection [3,10,11]. Under this context, surface contamination in ICUs has already been identified, as well as pathogen contamination among patients and medical personnel [12-14]. Contamination events occur after contact with surfaces or after the use of equipment in constant use, for example, stethoscopes, monitors, ventilators, telephones, and even medical records [15,16]. It has been shown that 20 to 40% of pathogens that cause hospital infections come from contamination of the hands of health personnel [17,18]. The microbiological analysis of hospital environments is done with the aim of proposing strategies that reduce the spread of bacteria that could be potentially pathogenic. However, these analyses are limited by the lack of molecular evidence to demonstrate the spread of clones, one of the main causes of the emergence of hospital outbreaks [19]. In recent years, the ICU of the *Hospital Juarez de Mexico* has shown an increase in the incidence of health-care associated infections (HAI) by Gram-negative bacilli resistant to antibiotics, with VAP being the most frequent disease caused by ESKAPE bacteria [7]. Therefore, it is necessary to have molecular evidence to evaluate the patient/personnel correlation in the transmission of pathogens in order to know the possible implications of the hospital environment in COVID-19 patients with secondary infections by ESKAPE bacteria. Implications for complications of COVID-19 patients by secondary infections, such as VAP of bacterial origin are discussed.

Table 1. Microbiological sampling sites selected of in Adult Intensive Care Unit of Hospital Juárez de México for COVID-19 patients.

Microbiological sampling sites	Sites (n)
Bed railing	7
Infusion pump	7
Oxygen source	7
Vital sign monitor	7
Blood pressure monitor	7
Patient (ulnar area)	7
Health personnel (hands)	7
Bureau	7
Hand washing area	1
Medication preparation area	2
Ultrasound equipment	1
Medical records	2
Computer keyboards	2
Clothing containers	1
Access doors	2
Total	**67**

Methodology

Ethical considerations

The institutional Committee of Research, Ethics, and Biosafety from *Hospital Juarez de Mexico* approved the protocol under the registration number HJM 0432/18-I in accordance with the Regulation of the General Health Law on Research for Health [20]. An informed consent was obtained from participants prior to their recruitment into the study, and a self-administered questionnaire was gathered from each participant.

Procedure and sites sampling

Six inanimate surfaces for each patient unit were chosen to determine the bacterial bioburden. The criteria for choosing the sampling points were based on those that were in constant contact by health personnel, such as bureau, bed railing, infusion pump, oxygen source, vital sign monitor, and blood pressure monitor. Similarly, samples were taken from the patient (ulnar area) and health personnel (hands), who were close to the patient. Finally, microbiological samples of common sites in the ICU were taken, such as the hand washing area, medication preparation area, ultrasound equipment, medical records, computer keyboards, access doors, and clothing containers. The sampling method (10 cm^2) was performed by using Sanicult™ sampling swabs (Starplex® Scientific, Etobicoke, Ontario, Canada). Samples were transported at 4 °C to the research laboratory for their microbiological culture. In Table 1, microbiological sampling sites selected in the ICU are shown.

Isolation of ESKAPE bacteria

Samples were cultured on selective MacConkey, Mannitol Salt agar (Becton Dickinson & Co., Franklin Lakes, NJ, USA) and bile-esculin azide agar (Hardy Diagnostics, Santa Maria, CA, United states). The plates were incubated aerobically at 37 °C for 24–48 hours. Subsequently, microbial strains were purified in

the LB agar. All strains were growth in LB-broth, then frozen in glycerol (50%) and stored at −70 °C for future experiments. For molecular Biology assays, total DNA from all strains was extracted and purified as described by using the QIAamp DNA Mini QIAcubeKit (QIAGEN, Venlo, The Netherlands). The integrity of the DNA was verified by electrophoresis in 0.8% agarose gels.

Genetic identification of ESKAPE bacteria

All the amplification reactions were performed in a Touchgene Gradient thermal cycler Gene Amp®PCR System 9700 (Applied Biosystems Forrest City, CA, USA). 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*., (Table 2) [21]. Amplicons were analyzed on horizontals 1.5% agarose gels by using 1×Tris–Borate–EDTA buffer (TBE), purified and sequenced by the Biology Institute of the *Universidad Nacional Autónoma de México* (UNAM) using a DNA Analyzer 3730xL (Applied Biosystems, Forrest City, CA, USA). Nucleotide sequences were compared with the nucleotide sequence database (GenBank) by means of the Blast algorithm (http://blast.ncbi.nlm.nih.gov).

Susceptibility/resistance assays

The antimicrobial resistance to different antibiotics was confirmed by using the disk diffusion method on Mueller–Hinton agar plates according to the guidelines set by ‘‘The Clinical and Laboratory Standards Institute” (CLSI, 2019) [22]. The antimicrobial susceptibility was performed for seventeen antimicrobial agents: amikacin (AN, 30 μg), ampicillin (AM, 10 μg), ciprofloxacin (CIP, 5 μg), gentamicin (GM, 10 μg), trimethoprim/sulfamethoxazole (SXT, 23.75/1.25 μg), ceftriaxone (CRO, 30 μg), cefotaxime (CTX, 30 μg), dicloxacillin (CLOX, 1 μg), cephalotin (CF, 30 μg), chloramphenicol (C, 30 μg), penicillin (10, U), nitrofurantoin (NF, 100 μg), netilmycin (NET, 30 μg), clindamycin (CC, 2 μg), erythromycin (E, 15 μg), vancomycin (VA, 30 μg), and tetracycline (TE, 30 μg) (BD, Brea, CA, USA). *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 according to the criteria specified by the CLSI. The frequency of antibiotic resistance was calculated and represented in percentages (%).

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 isolates identified as *A. baumanni.* 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

Table 2. Primers used in this study.

Primer	Molecular target	Sequence (5´→3´)	Size (bp)	Reference
27F 1492R	*16S rRNA*	AGAGTTTGATCMTGGCTCAG TACGGYTACCTTGTTACGACTT	1495	[20]
mecA plus *mecA* minus	*mecA* gene	TGGCTATCGTGTCACAATCG CTGGAACTTGTTGAGCAGAG	370	[22]
16S plus 16S minus	*16S rRNA*	AGGAGGTGATCCAACCGCA AACTGGAAGAAGGTGGGGAT	310	
ERIC1R ERIC2	Intergenic consensus	ATGTAAGCTCCTGGGGATTCA AAGTAAGTGACTGGGGTGAGC	Variable	[42]
adeA-F adeA-R	*adeA* gene	TCTGCAATATGCAACAGTTC GGCTATTGGTAATACGAACG	236	This work
adeB-F adeB-R	*adeB* gene	TTAACGATAGCGTTGTAACC TGAGCAGACAATGGAATAGT	541	[44]
adeC-F adeC-R	*adeC* gene	AGCCTGCAATTACATCTCAT TGGCACTTCACTATCAATAC	560	[45]
AdeR-F AdeR-F	*adeR* gene	GCGTCAGATTAAGCAAG ACTACGATATTGGCGACATT	447	[46]
AdeS-F AdeS-R	*adeS* gene	AGTGGAAGTTAGGTCAAGTT TTGGTTAGCCACTGTTATCT	544	
icaA-F icaA-R	*icaA* gene	ACACTTGCTGGCGCAGTCAA TCTGGAACCAACATCCAACA	188	[24]
SCCmec	Types I to V	See McClure-Warnier *et al*., 2013	Variable	[23]

second reaction to amplify *adeB* gene (encoding an intermembrane protein) was carried out. Finally, a third reaction was performed to amplify *AdeC* gene (encoding an extramembrane protein). Additionally, *AdeR* and *AdeS* genes encoding a regulator protein and activating protein kinase respectively were amplified. Sequences of primers used for *AdeABCRS* amplification are shown in Table 2. Specificity of the primers, a bioinformatic analysis was performed by using MFEprimer-2.0 server available at http://biocompute.bmi.ac.cn/CZlab/MFEprimer-2.0. Finally, to confirm the identity of the PCR products, the amplicons were subjected to sequencing.

Activity confirmation of AdeABCRS in A. baumannii

Acinetobacter baumannii isolates were tested for resistance before and after exposure to the efflux pump inhibitor with Phe-Arg-b-naphthylamide (PAbN; Sigma, St. Louis, Mo, USA). This inhibitor was supplemented in the culture medium at a final concentration of 20 µg/ml. Significant inhibition was defined as a 4-fold or greater reduction to the halo of inhibition in the presence of PAbN and the antibiotic tested.

Molecular typing of A. baumannii by ERIC-PCR

Isolates belonging to *A. baumannii* were subjected to molecular typing by ERIC-PCR, by using the primers ERIC1R and ERIC2 (Table 1). The total reaction volume was 50 mL and consisted of Molecular Biology grade water, 1× PCR buffer, 20 nM $MgCl_2$, 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 seconds, denaturation at 90 °C for 30 seconds, annealing at 58 °C for 1 minute, and extension at 65 °C for 8 minutes, with a final extension at 68 °C for 16 minutes at the end for 30 cycles. Genetic profiles were run in 1×TBE buffer, pH 8.3, and separated in horizontal electrophoresis in 1.5% agarose gels, visualized, photographed under UV illumination, and analyzed by intra-gel pattern comparison.

Molecular typing by characterization of mobile genetic element SCCmec

In order to identify the possible clonal dispersion of Gram-positive bacteria, typing based in multiplex PCR assay of Staphylococcal cassette chromosome mec *SCCmec* (Types I to V) in strains belonging to *Staphylococcus* genus was performed. In a first phase, a duplex PCR for the *mecA* gene and internal control (16S rRNA gene) was performed according to Acosta-Peréz *et al*., by using *mecA plus* and *mecA minus* primers (Table 2) [23]. Isolates that were positive to *mecA* gene were subjected to a second amplification assay by multiplex PCR using a set of 27 primers in order to identify mobile genetic elements *SCCmec* (type I, II, IIA, IIb, III, IIIA, IVa, IVb, IVc, IVd, IVE, IVF, V, VI, and VIII) according to McClure-Warnier *et al.,*. *Staphylococcus aureus* ATCC 43300 was used as a positive control for *mecA* and *SSmec* gene (type II) [24].

Detection of the icaA gene in Staphylococcus spp. strains

In order to identify associated genes involved in biofilm formation (exopolysaccharide poly-N-acetylglucosamine), Gram-positive isolates were subjected to PCR amplification for the detection of the *icaA* gene according to Mariana *et al*., by using *icaA-F* and *icaA-R* primers (Table 2) [25]. In order to to confirm the identity of the PCR products, the amplicons were subjected to sequencing.

Results

Isolation of ESKAPE in the ICU

Sixty-seven sites (medical devices, inert surfaces, medical personnel, and patients) from the intensive care unit of the *Hospital Juarez de Mexico* were microbiologically analyzed for the search of ESKAPE bacteria (Table 1). Microbiological isolation methods in selective and differential media revealed that 61 analyzed sites presented bacterial development (91%), of which 24 (39.3%) corresponded to isolates of Gram-negative origin and 37 (60.6%) to Gram-positive isolates. Finally, isolation sites that presented multiple contamination (10/6.1%) by Gram-positive and Gram-negative bacteria were detected, respectively.

Genetic identification of ESKAPE bacteria

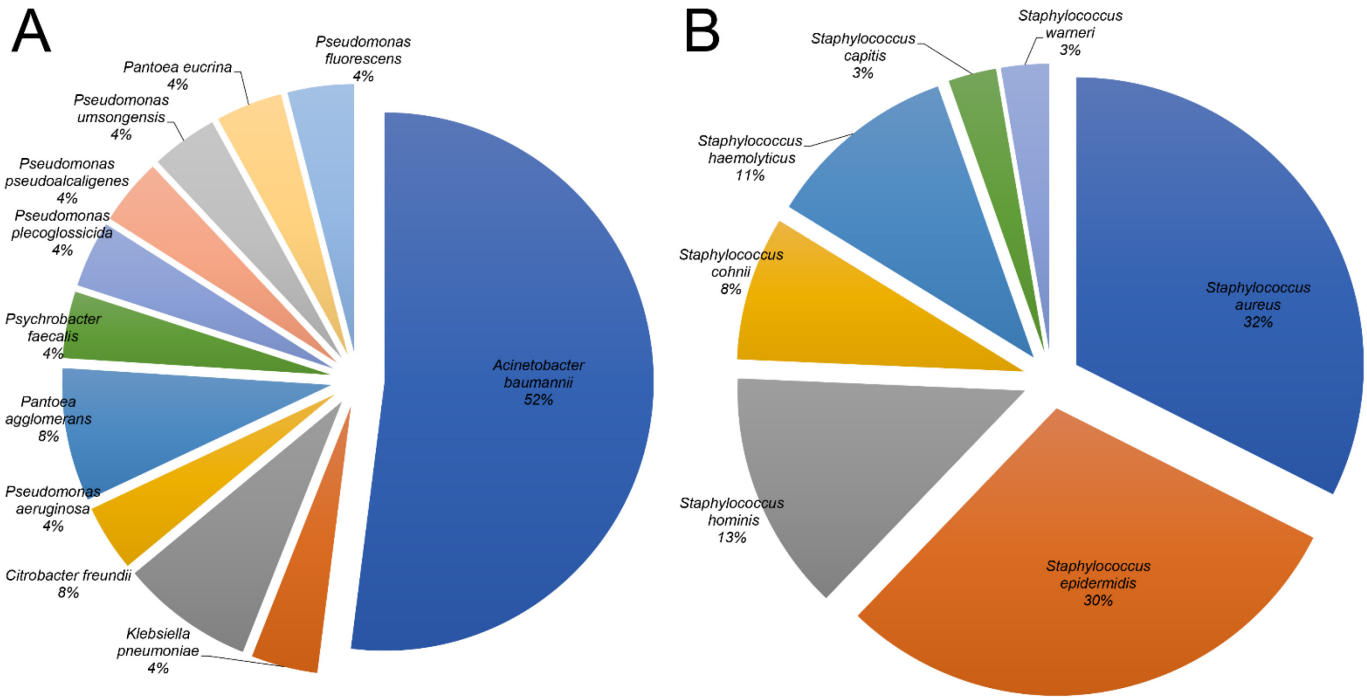
Genetic identification consisted in PCR amplification assays of V1–V9 regions (1492 bp) of the 16S rRNA gene. The PCR products were sequenced and compared to those of the GenBank database by using strict filter parameters with more than 99% nucleotide homology and at least 80% query coverage. The sequences revealed a broad diversity of microorganisms phylogenetically different, including important members of ESKAPE bacteria, such as *A. baumannii* (MN326497.1), *K. pneumoniae* (CP044047.1), *C. freundii* (KR996132.1), *P. aeruginosa* (KY819093.1), and *S. aureus* (LC216327.1) (Figure 1).

Table 3. ESKAPE bacteria distribution (including others genera) in medical devices, inert surfaces, medical personnel and patients from the intensive care unit of the Hospital Juárez de México for COVID-19 patients.

Gram negative bacteria*			**Gram positive bacteria***		
Genus and specie	***n***	***Microbiological sampling sites (n)***	***Genus and specie***	***n***	***Microbiological sampling sites***
*Acinetobacter baumanii***	13	Vital sign monitor (1), patient (1), health personnel (1), bed railing (2), bureau (3), blood pressure monitor (1), infusion pump (1), ultrasound equipment (1), medical records (1), computer keyboard (1)	*Staphylococcus aureus***	11	Blood pressure monitor (2), health personnel (1), bureau (2), oxygen source (1), vital sign monitor (2), infusion pump (1), ultrasound equipment (1), clothing container (1)
*Klebsiella pneumoniae***	1	Patient (1)	*Staphylococcus epidermidis*	11	Bed railing (2), blood pressure monitor (2), infusion pump (2), patient (2), vital sign monitor (1), health personnel (1), medical record (1)
*Citrobacter freundii***	2	Bureau (1), vital sign monitor (1)	*Staphylococcus hominis*	5	Infusion pump (1), bed railing (1), patient (1), computer keyboard (1), access door (1)
*Pseudomonas aeruginosa***	1	Patient (1)	*Staphylococcus haemolyticus*	4	Patient (1), bed railing (1), oxygen source (1), access door (1)
Pantoea agglomerans	1	Health personnel (1)	*Staphylococcus cohnii*	3	Bed railing (2), health personnel (1)
Psychrobacter faecalis	1	Vital sign monitor (1)	*Staphylococcus capitis*	1	Blood pressure monitor (1)
Pseudomonas plecoglossicida	1	Hands washing area (1)	*Staphylococcus warneri*	1	Health personnel (1)
Pseudomonas pseudoalcalígenes	1	Hands washing area (1)			
Pseudomonas umsongensis	1	Access door (1)			
Pantoea eucrina	1	Clothing container (1)			
Pseudomonas fluorescens	1	Clothing container (1)			

*Genetically identify by 16s rRNA gene analysis sequence; ** Belonging to ESKAPE bacteria group.

Figure 1. ESKAPE bacteria distribution (including other genera) in medical devices, inert surfaces, medical personnel, and patients from the intensive care unit of the Hospital Juarez de Mexico. Isolation strains bacterial percentage obtained from 67 analyzed points. A) Gram-negative bacteria and B) Gram-positive bacteria. All strains were genetically identify by the 16s rRNA gene analysis sequence.



Other bacterial species were identified, including commensal bacteria. Phylogenetic analysis and maximum-likelihood indicated that *A. baumannii* 01, 07, 09, 12, 27, 28, 44, 45, 51, 53, 60, 61, and 64 strains were genetically related 99.99% of similarity (correlated with the phenotypic resistance assays). ESKAPE bacteria distribution (including other genera) in medical devices, inert surfaces, medical personnel, and patients from the ICU are shown in Figure 1.

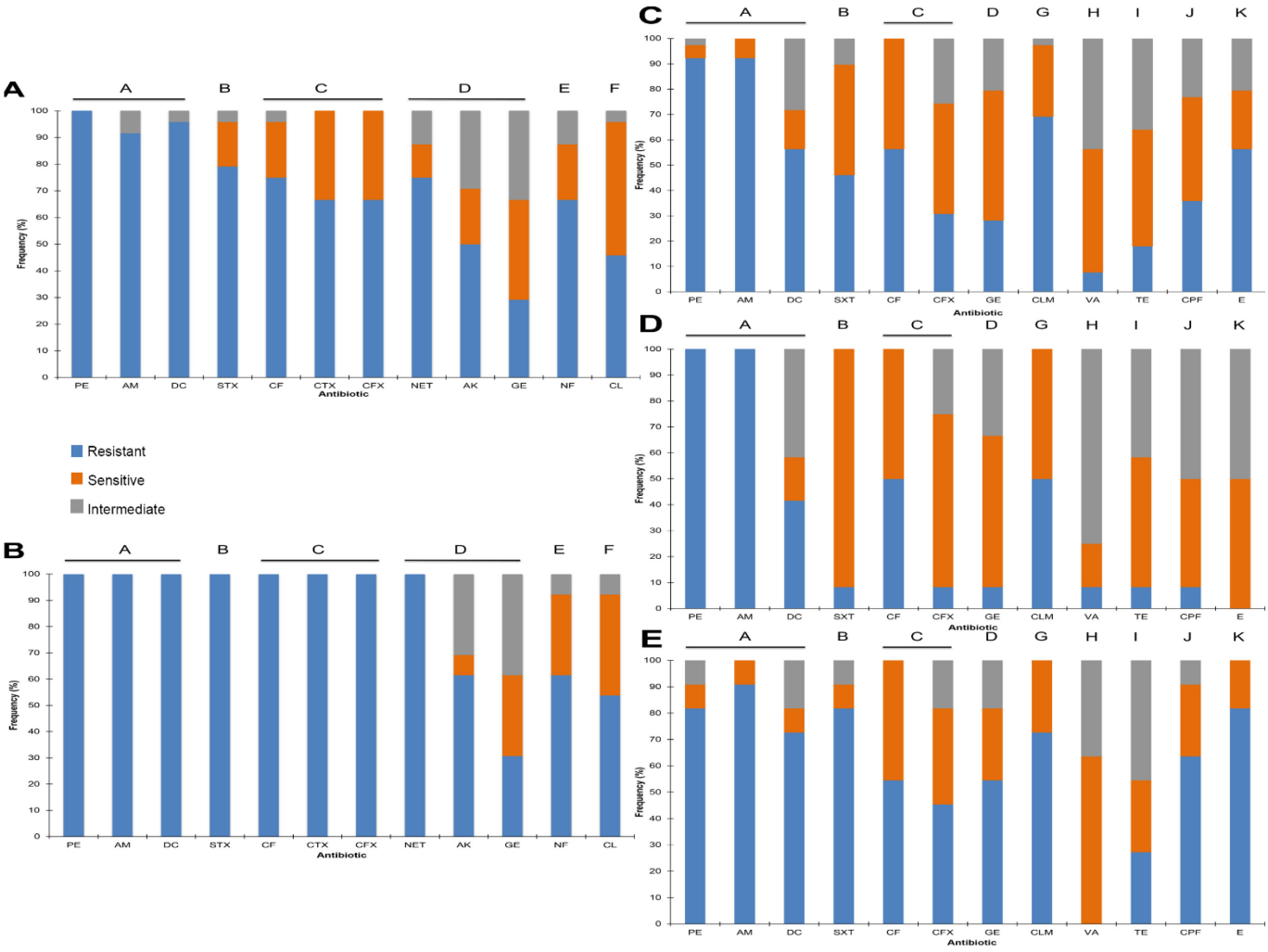
ESKAPE bacteria distribution in medical devices, surfaces, medical personnel, and patients

As shown in Table 3, a heterogeneous distribution of ESKAPE bacteria (and other bacterial species) was identified in medical devices, inert surfaces, medical personnel, and patients. However, bureaus, vital sing monitors, blood pressure monitors, and patients were the most contaminated by ESKAPE bacteria; in contrast, health personnel, bed railings, patients, access doors, blood pressure monitors, and infusions pumps were the most contaminated by other bacterial species, including commensal bacteria.

Susceptibility/resistance assays

All strains (ESKAPE bacteria and others) were subjected to phenotypic resistance assays. The results showed differences in susceptibility and resistance to the eleven antimicrobial families tested. In the first group of strains (Gram-negative population), the results show that cephalosporins, aminoglycosides, and phenicols, were the drugs with the best antimicrobial activity against those strains. On the contrary, penicillins, sulfonamides, and nitrofurans, showed lower inhibitory activity on the tested strains (Figure

Figure 2. Antimicrobial resistance of ESKAPE bacteria (including other genera) in medical devices, inert surfaces, medical personnel, and patients from the ICU of the Hospital Juarez de Mexico. 2A. Total Gram-negative bacteria 2B. *Acinetobacter baumannii* strains, 2C. Total Gram-positive bacteria 2D *Staphylococcus aureus* strains and 2E *Staphylococcus epidermidis* strains. Antimicrobial families tested A) penicillins, B) sulfonamides, C) cephalosporins, D) aminoglycosides, E) nitrofurans, F) phenicols, G) lincosamides, H) glycopeptides, I) tetracyclines, J) fluoroquinolones, and K) macrolides.



2A. An analysis of antimicrobial resistance including only *A. baumannii* strains was performed. The results revealed that all strains were multidrug-resistant with minor differences in their phenotype (aminoglycosides, nitrofurans, and phenicols); therefore, this suggested that all *A. baumannii* are conformed in a clonal group (Figure 2B). In relation to the Gram-positive bacteria group (including *S. aureus*), interestingly the majority of the population presented high antimicrobial susceptibility, where only two antibiotics from the penicillin family (ampicillin and penicillin), showed antimicrobial resistance activity (Figure 2C). In order to know the profile resistance of *S. aureus* strains (ESKAPE member), analysis of sensitivity and resistance were performed. The results showed that frequency of sensitivity was highest (Figure 2D). The above mentioned indicates that although *S. aureus* is a member of the ESKAPE bacteria, it does not present multidrug-resistance. Interestingly, the commensal strains of *S. epidermidis* presented a high frequency of resistance to most of the antimicrobials tested (compared to *S. aureus*). Antibiotics that showed better activity with *S. epidermidis* strains were glycopeptides and tetracyclines (Figure 2E).

Assembly of AdeABC operon and regulator genes AdeRS in MDR A. baumannii

Full operon *AdeABC* and their regulator genes *AdeRS* were amplified in all *A. baumanni* MDR strains (Figure 3). Additionally to genetic detection of the efflux pump, assays of inhibition of pump functionality

Figure 4. Clonal dispersion of *Acinetobacter baumannii* (*AdeABCRS*+) in medical devices, inert surfaces, medical personnel, and patients from the intensive care unit of the Hospital Juarez de Mexico. Clonal dispersion was detected by ERIC-PCR. Lanes: M: molecular size marker 2 log (New England, Bio Labs®), 1-13: *A. baumannii* clones (Ab1-Ab13). *, **, ***, ****: Points analyzed sites for each immediate patient area.

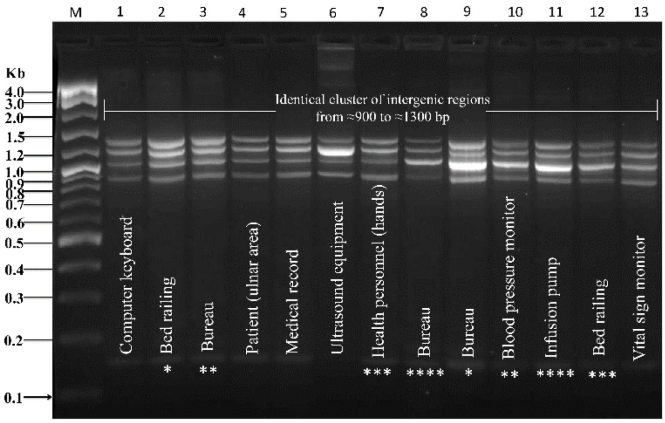
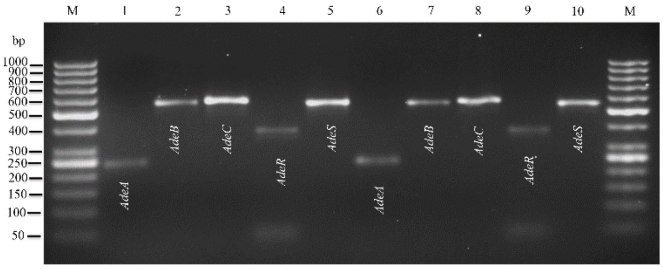


Figure 3. Representative assembly of *AdeABC* operon and regulator genes. *AdeRS* by end-point PCR in MDR *Acinetobacter baumannii* strains isolated from the intensive care unit of the Hospital Juarez de Mexico. Lanes: M: molecular size marker 50 bp (New England, Bio Labs®), 1-5: *A. baumannii* (Ab1). 6-10: *A. baumannii* clones (Ab2).



were performed in order to confirm that the phenotype previously identified, is conferred by the *AdeABCRS* efflux pump. Significant inhibitions (p = 0.05) to the tested antibiotics (except for antibiotics of the penicillin family) were observed after treatment with PAβN compared to the control (no PAβN treatment). This shows that the MDR phenotype is conferred by the presence of the *AdeABCRS* efflux pump.

Molecular typing by ERIC-PCR

Genomic diversity analysis of *A. baumannii* was carried out by using the ERIC-PCR fingerprinting method with ERIC-type primers. Profiles of the intergenic products revealed sizes of amplicons ranged from slightly more than ≈900 bp to about ≈1300 bp (Figure 4). Intergenic region diversity did not allowed the differentiation of twelve isolates; therefore, they were clustered in one genetic group. According to the above, the six strains were indistinguishable from each other (100% similarity).

Molecular typing by characterization of the mobile genetic element SCCmec

In order to identify the possible clonal dispersion of Gram-positive bacteria, characterization of the mobile genetic element *SCCmec* in *Staphylococcus* strains was performed. In the first phase we detected a broad distribution of the *mecA* gene (18/48.6%), where *S. epidemidis* was the predominant species carriyng this gene, followed by *S. hominis, S. aureus, S. haemolyticus,* and finally *S. cohnii* (Table 4). Results of the second phase (typing of *SCCmec*) showed that *SCCmec* types IIb, III, and V were the most prevalent in *S. aureus, S. epidemidis,* and *S. hominis.* Typing assays showed that no clonal relation is present in the five groups of *Staphylococcus* spp.

Detection of the icaA gene in Staphylococcus spp. strains

The *icaA* gene, involved in biofilm formation was detected only in 24% (9/37) of the isolates of *Staphylococcus* spp., where only *S. aureus* was the species carrying the *icaA* gene. Only three isolates of *S. aureus* were carrying both genes [*icaA* and *mecA* (IIb and III)].

Discussion

The current worldwide research on SARS-CoV-2, is focused on the study of its pathogenesis, evolution, treatment, vaccines, and others; however, the study of bacterial pathogens that cause secondary pneumonia associated with mechanical ventilation, which delay and complicate the hospital stay of COVID-19 patients also became relevant. Since studies have shown that pathogens found in ICU patients have also been identified on inanimate surfaces and health personnel, the characterization of VAP-causing pathogens in a contingency situation is of great importance. In the present work it was shown that 91% of the analyzed sites were colonized by bacteria (pathogenic and commensal), where *S. aureus* and *A. baumannii* MDR (*AdeABCRS*+) showed a high incidence, and only *A. baumannii* MDR (*AdeABCRS*+) showed a clonal distribution in surfaces, patients, and health personnel. Therefore, prospective character epidemiological surveillance strategies were implemented to identify potential risk factors in the development of hospital infections in patients admitted to hospital with COVID-19. Inadequate disinfection of surfaces, medical equipment, and mainly of the hands of health professionals was identified. It is worth mentioning that even when there is scrupulous protection in medical personnel in order to avoid the transmission of SARS-CoV-2 from patients to health personnel, the transmission of other pathogens such as ESKAPE bacteria causing VAP is not considered. The possible existence of new variants of SARS-CoV-2 strains could be a potential risk of transmission "in the future" between patients during their direct or indirect manipulation with medical equipment [26]. It has been recommended that inanimate surfaces and immediate equipment to the patient should be disinfected regularly with 70% ethanol, chlorhexidine, among others, due to a high rate of surface recontamination. In a previous study, it was shown that the bacterial recontamination of contact surfaces in the ICU occurred after 4 hours after standard cleaning with detergents with chlorine-releasing agents, isopropyl alcohol, and sodium hypochlorite [27]. The identification of pathogens in medical devices and health personnel clearly indicates the potential risk that patients admitted to the ICU may acquire additional infections to COVID-19. In previous reports, the analysis of the medical records of patients hospitalized with nosocomials in Wuhan, have reported secondary bacterial infections of 5 to 27% in adults infected with SARS-CoV-2, and until February 2020, 50 to 100% of the deceased patients had secondary infections [28-32]. Other reports have identified this type of infection from 13.5% to 44% in ICU patients diagnosed with COVID-19 [30,33,34]. The studies showed that the pathogens most frequently isolated from cases of VAP were of bacterial and fungal origin, such as: MDR *A. baumannii*, KPC, EBLS *K. pneumoniae*, ESBL *P. aeruginosa*, E. *cloacae, Serratia marcescens, Aspergillus fumigatus, Aspergillus flavus, Candida albicans*, and *C. glabrata* [33-36]. This is related to the isolates of the ESKAPE group identified in the present work (*A. baumannii, K. pneumoniae, P. aeruginosa, Citrobacter freundii,* and *S. aureus*). Therefore, the evidence presented clearly shows how the emergence of bacterial infections secondary to

Table 4. Molecular typing by characterization of mobile genetic element SCCmec in Staphylococcus spp. isolated of medical devices, inert surfaces, medical personnel and patients from the intensive care unit of the Hospital Juárez de México for COVID-19 patients.

Specie	*n*	Duplex PCR *mecA* (First phase)		Multiplex PCR *SSCmec* typing (Second phase)		Clonally related?
		Housekeeping 16S rRNA (310 bp)	*mecA gene* (370 bp)	Housekeeping *mecA* (147 bp)	*SSCmec* type (*n*)	
*Staphylococcus aureus****	12	12	3	3	IIb(2) and III(1)	Yes (2/3)
Staphylococcus epidermidis	11	11	8	8	IIb(1), III(2), V(1) and ND(4)	Yes (2/8)
Staphylococcus hominis	5	5	4	4	III(1) and ND(3)**	NO
Staphylococcus haemolyticus	4	4	2	2	ND(2)	NO
Staphylococcus cohnii	3	3	1	1	NA	NA
Staphylococcus capitis	1	1	0	NA*	NA	NA
Staphylococcus warneri	1	1	0	NA	NA	NA

*Non-applicable; **Non-detected; ***ESKAPE bacteria.

COVID-19 could be closely related to poor clinical practices by health personnel. Our findings are consistent with previous reports, where inanimate surfaces and medical equipment of ICU have been shown to be heavily contaminated by bacteria, including potentially pathogenic bacteria resistant to antibiotics from the ESKAPE group [13,15]. Furthermore, antibiotic resistant bacteria have been identified as pathogens contaminating surfaces, medical equipment, and common use equipment (telephones, keyboards, monitors, handles) in the ICU [37]. We consider important to know the resistance profiles to offer a predictive value of the resistance phenotype and, consequently, to provide effective treatments to VAP patients. Interestingly, the antimicrobial resistance results showed that *A. baumannii* had multiple resistance, which was conferred by the expression of an *AdeABCRS* efflux pump, which is considered one of the most important mechanisms in this nosocomial pathogen [38]. Although the microbiological analysis did not include patients with diarrheal episodes, other studies have identified a higher rate of environmental contamination; in addition, some of these bacteria (mainly Gram-positive and sporulating bacteria) are able to survive on inanimate dry surfaces, with greater persistence under humid and low temperature conditions [39,40]. The identification of sites that are frequently contaminated has a fundamental role for the improvement or incorporation of new disinfection protocols to avoid cross contamination events [41]. Using molecular tools in epidemiological studies, the molecular identification and typing of bacterial strains responsible for hospital infections provide valuable information on the spread of bacterial contaminants in this type of environment. In other works, the use of molecular tools has allowed the detection of identical genetic profiles of patient isolates and the immediate environment, confirming the role of vehicles for the transmission of infections to patients [18]. In this work, we were able to detect, by means of intergenic sequence analysis, a genetically identical cluster of isolates of *A. baumannii*, which clearly shows the clonal dispersion of this microorganism in the ICU, with health personnel being the main vehicle of transmission. The heterogeneous presence of isolates of the genus of *Staphylococcus* variant carriers of the mobile genetic element *SCCmec* and the *icaA* gene, showed that there is no clonal dissemination of this pathogen from the ESKAPE group in the analyzed sites. Nevertheless, its role as a causative agent of VAP is not dismissed, since previously methicillin-resistant *S. aureus* have been identified as bacterial agents transmissible by contact between medical personnel and patients [42].

Conclusion

Clonal spread of *A. baumannii* (*AdeABCRS*+) highlights the importance of adopting good practices for equipment disinfection, surfaces and management of COVID-19 patients. Even though there are reports of bacterial contamination in hospital environments together with recommendations aimed at reducing that of infections acquired in the ICU, at this stage of worldwide contingency by SARS-CoV-2, it is necessary to reinforce its importance, through evidence that show the importance of adopting good hygiene practices in the management of COVID-19 patients.

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