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Detection of Bacteria with Potential to Cause Hospital-Associated Infections in a Small-Species Veterinary Hospital in Mexico

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Abstract: Hospital-Associated Infections (HAIs) are caused by microorganisms that are not present before patients are admitted to healthcare facilities, and usually have multidrug resistance profiles. There is ample information and active research in human medicine to create preventive and control measures, but there have been fewer efforts in animal medicine, and studies in only a few countries have been examining how this problem presents in veterinary hospitals. In Mexico, there have been no studies on the presence of multidrug-resistant bacteria associated with HAIs in veterinary medicine. Therefore, the surfaces of inanimate objects and equipment in a university veterinary hospital for small species were sampled to search for bacteria with the potential to cause HAIs. After isolation, molecular identification and multidrug resistance tests were carried out. One bacterial strain was found to be resistant to carbapenems, third-generation cephalosporines, and penicillin/β-lactamase inhibitors. Additionally, other susceptible bacterial genera were identified as potential nosocomial pathogens in humans and animals. The presence of multidrug-resistant bacteria was confirmed. Further studies should be conducted to determine the isolate's origin and its relationship with reported human clinical genotypes. This type of study highlights the importance of epidemiological surveillance and the need to not underestimate the potential risk posed by multidrug-resistant microorganisms.

Keywords: Hospital-Associated Infections; nosocomial infections; veterinary hospital; multidrug resistance; companion animals; small-species veterinary hospital; veterinary microbiology

1. Introduction

Hospital-Associated Infections (HAIs) are caused by microorganisms acquired by patients during hospitalization or treatment in care units. The microorganisms that cause these infections are not present or are in an incubation period before the patient's admission [1]. HAIs are usually transmitted by two routes. The first is direct contact between health personnel and patients through medical devices such as urinary or intravenous

catheters, endotracheal tubes, or medical equipment, such as assisted breathing ventilators and anesthesia systems. The second route is indirect transmission through contaminated surfaces and inanimate objects such as beds, medical tables, doorknobs, light switches, phones, cell phones, and other objects in the hospital environment [2].

In both humans and animals, HAIs increase morbidity and mortality and extend the length of hospitalization. As a result, there is an increase in costs for patients and public health programs [3–5]. Annually, HAIs incur costs of USD 28 to 45 billion for the health system in the United States and EUR 7 billion in Europe [1]. Pathogens associated with HAI include viruses, fungi, and bacteria, although bacteria are of greater importance due to their pathogenicity mechanisms, such as biofilm and endospore development (as non-limiting illustrative examples). These mechanisms allow for them to survive on inanimate surfaces for long periods of time [6]. Additionally, one of the main concerns related to HAIs is that bacteria have an substantially capacity to acquire new sets of genes related to pathogenicity, including antimicrobial resistance [7,8].

Hospitals and healthcare units for humans have reported *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella* spp. as the most frequent bacteria associated with HAIs [9]. However, other commonly reported genera of bacteria are also associated with nosocomial infections in humans, such as non-fermenting bacteria, including *Acinetobacter baumanni* and *Pseudomonas aeruginosa* [10]. All of these bacteria can exhibit multidrug resistance, which has been demonstrated and reviewed extensively [11–15].

Research on the pathogens that cause HAIs in veterinary medicine is limited. Nonetheless, concern about multidrug resistance and HAIs in animal health has been growing due to the close coexistence of humans and animals. In European countries, research has demonstrated the presence of bacterial species and their multidrug resistance profiles in veterinary hospitals, and findings show a prevalence of 82% of pathogens in the ESKAPE group (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp.) [16]. From the "One Health" perspective, this is relevant because other studies show that genotyped *A. baumanii* strains isolated from animals are genetically related to European clones associated with human clinics [17].

In Latin America, studies have also demonstrated the presence of bacteria with resistance to antimicrobials in pets and large animal species, and the bacterial species described are consistent with reports in other countries [18–22]. The spread of HAIs follows a contact pattern related to the social interaction of veterinarians, owners, and animals [23]. This raises concerns about whether there is an exchange of strains between humans and animals and the possibility of zoonosis being underestimated. To our knowledge, there have been no studies in Mexico describing the presence of hospital-associated pathogens in veterinary medicine. It is important to promote research on the epidemiology, genetics, and dispersion patterns of these pathogens related to human–animal relationships, medical practices, and production practices according to the idiosyncrasies of each country.

The aim of this work was to search for bacteria with the potential to be associated with nosocomial infections and to determine their antimicrobial resistance profiles in a university veterinary hospital for small animal species. The results of this study could serve as a starting point to promote awareness of the potential risk of multidrug-resistant bacteria and their unnoticed presence in the daily work of veterinarians. By identifying these pathogens and their resistance patterns, we hope to contribute to improving infection control practices and the development of targeted interventions in veterinary healthcare settings.

2. Materials and Methods

2.1. Ethical Statements

During this study, no animals, owners, or medical staff were sampled. All of the samples were taken from inanimate objects and surfaces. Prior to this study, the protocol was submitted to the Ethics Committee of the Faculty of Natural Sciences at the Autonomous University of Queretaro (FCN-UAQ) due to the potential isolation of bacteria with multidrug resistance profiles, in adherence with national legislation to prevent the propagation

and dissemination of pathogens (approval numbers: 078FCN2023 and 43FCN2022). All of the materials, such as cultures, plastic consumables, etc., were disposed of in accordance with national legislation.

2.2. Sample Collection and Sampling Procedure

Samples were obtained at the Small-Species Specialty Veterinary Hospital of the Autonomous University of Querétaro. Prior to sampling, a map of the facilities was created to determine the flow of patients and medical staff and to identify areas where greater interaction might occur. This was determined by analyst observation. The selected areas of the hospital were the waiting room, reception, preventive medicine clinics, the clinical pathology laboratory, consultation room, radiology area, teaching area, and hospitalization, recovery, and operating rooms. In these areas, samples were obtained from surfaces of furniture specific to the area and intervention equipment (such as ventilators, anesthesia machines, water sources, door handles, and fixed phones).

The samples were collected using a sterile cotton swab which was pre-moistened with buffered peptone water (218105 Difco-BD, Franklin Lakes, NJ, USA). The swab was rolled and rubbed against the surfaces and then placed in a sterile buffered peptone water tube. The tube was labeled with a consecutive number and accompanied by a sample registration form to trace its origin. During sampling, the samples were stored in a cooler with refrigerant gels. At the end of the sampling process, the samples were immediately transported to the Immunology and Vaccines Research Laboratory at FCN-UAQ, where they were processed. This sampling procedure was carried out based on published compilations and recommendations using materials and reagents that were accessible at the time of the study [24–26].

2.3. Sample Processing

The samples were cultured on different media in the following order: blood agar, MacConkey agar, and eosin-methylene blue (EMB) agar (1031-A, 1019-A, and 1011-A, respectively; DIBICO, Mexico State, Mexico). The swab was removed from the transport tube, and the sample was inoculated onto a quarter of a Petri dish by rubbing and rolling the entire surface of the swab over the agar. A quadrant streak was then performed using a nichrome loop. Inoculated Petri dishes were incubated at 37 °C for 18 to 24 h for the first inspection, and the ones that presented growth were separated for further inspection. The Petri dishes with no growth during first inspection were incubated for another 24 h, and a second inspection was performed. Cultures with no growth after 48 h were discarded.

Bacterial colonies were selected for subculture according to their morphology and their Gram-stain patterns. The selected colonies were only those with typical enterobacterial, non-fermenting, and staphylococcal growth on selective agar and blood agar and those with Gram-positive cocci and Gram-negative bacilli. Bacterial morphologies corresponding to common environmental contaminants were excluded. These criteria were used with the aim of searching for bacterial species that are commonly reported as causing HAIs. No anaerobe bacterial species were considered for this study. A subsequent identification was also carried out with greater robustness. Each bacterial isolate was reassigned a new number/letter code to trace its origin.

Bacterial isolates were cultured in Luria–Bertani (LB) broth (110283, Merk, Rahway, NJ, USA) for cryopreservation to avoid the loss of resistance-related mobile genetic elements due to a lack of selective pressure or due to repetitive propagation of subcultures. Bacterial cultures were pelleted at 8000× *g* for 5 min (6767-HS, Corning, New York, NY, USA) and resuspended in half of the original culture volume. Glycerol was added to a final concentration of 50%, and the mixture was aliquoted into 1.5 mL centrifuge tubes (1210-00, SSI Bio, Lodi, CA, USA). Stabilates were stored at −80 °C for subsequent antimicrobial susceptibility testing.

2.4. Molecular Identification of Bacterial Isolates by 16S rRNA Gene Amplification

The molecular identification of each bacterial isolate was carried out according to the protocol described by James [27], with modifications. Briefly, to obtain DNA from each isolated bacterium, 3 mL cultures in LB broth were set up in 50 mL conical tubes (CLS430829, Corning, New York, NY, USA). The cultures were incubated at 37 °C and 200 rpm overnight in an orbital incubator shaker (MaxQ 4450, Thermo, Waltham, MA, USA). On the next day, the culture was collected in 1.5 mL conical tubes, bacterial cells were pelleted using pulse centrifugation at the maximum speed, and the culture media were discarded. Bacterial pellets were resuspended in 500 µL of molecular biology grade water (46-000-CV Corning, New York, NY, USA) and boiled at 90 °C for 10 min to lyse bacterial cells. No further DNA purification of nucleic acids was needed.

16S rRNA gene amplification was performed using the primers U3 (5′-AGT GCC AGC AGC CGC GGT AA-3′) and U4 (5′-AGG CCC GGG AAC GTA TTC AC-3′), which resulted in an amplicon of approximately 1000 bp. The primers are universal and can amplify the 16S rRNA gene of any bacteria for subsequent identification using informatics. Therefore, tubes with negative controls containing a reaction mix and water were placed between each tube containing bacterial DNA to ensure that there was no cross-contamination. This ensured that no sample would be carried over from one reaction to the next.

For amplification, MyTaq Mix (Bio-25041, MeridianBioscience, Cincinnati, OH, USA) was used according to the supplier's instructions. The amplification conditions were 94 °C for 5 min followed by 30 cycles of 96 °C for 15 s, 60 °C for 15 s, and 72 °C for 15 s, with a final extension step of 72 °C for 5 min and 4 °C indefinitely. Polymerase chain reaction (PCR) products and the absence of cross-contamination were visualized using DNA electrophoresis in 1% agarose gel and stained with GelRed (41003-Biotium, CA, USA) in a ChemiDoc Imaging System (12003153, BioRad, Hercules, CA, USA).

Each amplicon was purified using a NucleoSpin PCR Clean-up column kit (740611.50, Macherey-Nagel, Westfalen, Germany) and was sent for sequencing using the dideoxy chain-termination method at the Laboratory of Genomic Services in the Laboratory of Genomics for Biodiversity (LANGEBIO-CINVESTAV) in Irapuato, Mexico. The obtained sequences were curated and compared using nucleotide BLAST (available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 11 October 2023) after setting 16S ribosomal RNA sequences (Bacteria and Archaea) as the search database. The samples with low-quality sequencing results according to electropherograms were discarded.

2.5. Antimicrobial Susceptibility Test

The bacteria isolates' resistance to antimicrobials was determined using a susceptibility test with the Vitek® 2 Compact system (Biomérieux, Marcy-l'Étiole, France) according to the manufacturer's instructions. The antimicrobial susceptibility test was performed at the Research and Teaching Laboratory in Microbiology and Parasitology at the Children's Hospital of Morelia "Eva Samano de López Mateos" in Morelia, Mexico. The bacterial stabilates were reactivated in blood base agar without RBC supplementation. After incubation at 37 °C for 18 h, single isolated colonies from pure cultures were resuspended in 3 mL of physiological saline solution. The optical density of each bacterial suspension was adjusted to match the 0.5 MacFaraland Standard tube, as measured using the Vitek® DensiCheck® (Biomérieux, Marcy-l'Étiole, France).

Next, the adjusted bacterial suspensions were inoculated onto Gram-negative antimicrobial susceptibility testing cards (Vitek® 2 AST-272, 414164, Biomérieux, Marcy-l'Étiole, France). The cards were loaded into the device and a bio-number was assigned to each sample's cards. The results were checked after 18 h. Susceptibility parameters of the minimal inhibitory concentration (MIC) were evaluated according to the CLSI M100 manual [28].

3. Results

In total, 40 swab samples were collected from the waiting room, reception, preventive medicine clinics, clinical pathology laboratory, consultation room, radiology area, teaching

area, and hospitalization, recovery, and operating rooms. The sampled surfaces included tables, chairs, shelves, sink faucets and taps, door handles, computer mice and keyboards, buttons and pipes on anesthesia and ventilator equipment, and other surfaces that had contact with staff's hands. Figure 1 shows a simple distribution map of the hospital and access areas for medical personnel, animal owners, and non-medical personnel.

In accordance with the inclusion and exclusion criteria, 41 different bacteria were isolated. The hospitalization area and radiology room were the areas where most Gram-negative bacilli were found with five isolates. This was followed by preventive medicine rooms, consultation rooms, the recovery area, and dressing room, where four isolates were obtained, as is shown in Table 1. After cryopreserving all 41 samples, 16S rRNA gene amplification was performed, which yielded a 1000 bp amplicon.

Table 1. Hospital areas, sampled objects, and number of bacterial samples were isolated and selected.

Hospital Area	Number of Samples	Sampled Objects and Surfaces
Waiting room	3	Guest chairs
Reception	0	Computer mouse and keyboard, desk surface
Preventive medicine rooms	4	Desks surfaces, examination table surfaces
Clinical laboratory	2	Work bench surfaces
Consulting room 1	2	Desks surfaces, door handles, examination table surfaces
Consulting room 2	4	Desks surfaces, examination table surfaces, ultrasound scanner
Multipurpose room	1	Boardroom table surface
Radiology room	5	Door handles, ultrasound scanner, X-Ray radiography system
Teaching area	0	
Hospitalization area	5	Desk surfaces, door handles, sink faucet and taps
Recovery area	4	Sink faucets and taps
Operating room 1	2	Anesthesia system
Operating room 2	2	Anesthesia system, multi-parameter patient's monitor
Autoclave/washing room	2	Desks and shelves surfaces, sink faucet and taps
Dressing room	4	Door handles, surface bench
Infectious hospitalization	1	Sink faucet and taps
Total samples	41	

There was no contamination between negative control reactions and the bacterial DNA reaction, as expected. Figure 2 shows an example of the first five bacterial isolates amplified and their respective water controls. All of the bacterial DNA isolates were amplified and electrophoresed similarly. After PCR products were purified, 34 samples were sent for sequencing. Five samples were excluded due to a lack of PCR product. These samples were eliminated from further analysis, as other samples from the same area and nearby surfaces had already yielded satisfactory results.

The molecular identification of isolated bacteria was determined with a BLAST analysis using the 16S ribosomal RNA sequence (Bacteria and Archaea) database. As a result, 20 samples (62.5%) were identified as environmental bacteria, while the other 12 samples (37.5%) matched species with potential nosocomial infection risk, including the genera *Enterobacter* and *Klebsiella*. Table 2 shows the results of sequencing identification, as well as a general overview of reported antimicrobial resistance and infections in humans or animals. Two samples were excluded due to very short sequences and low-quality electropherograms, which made their molecular identification inaccurate.

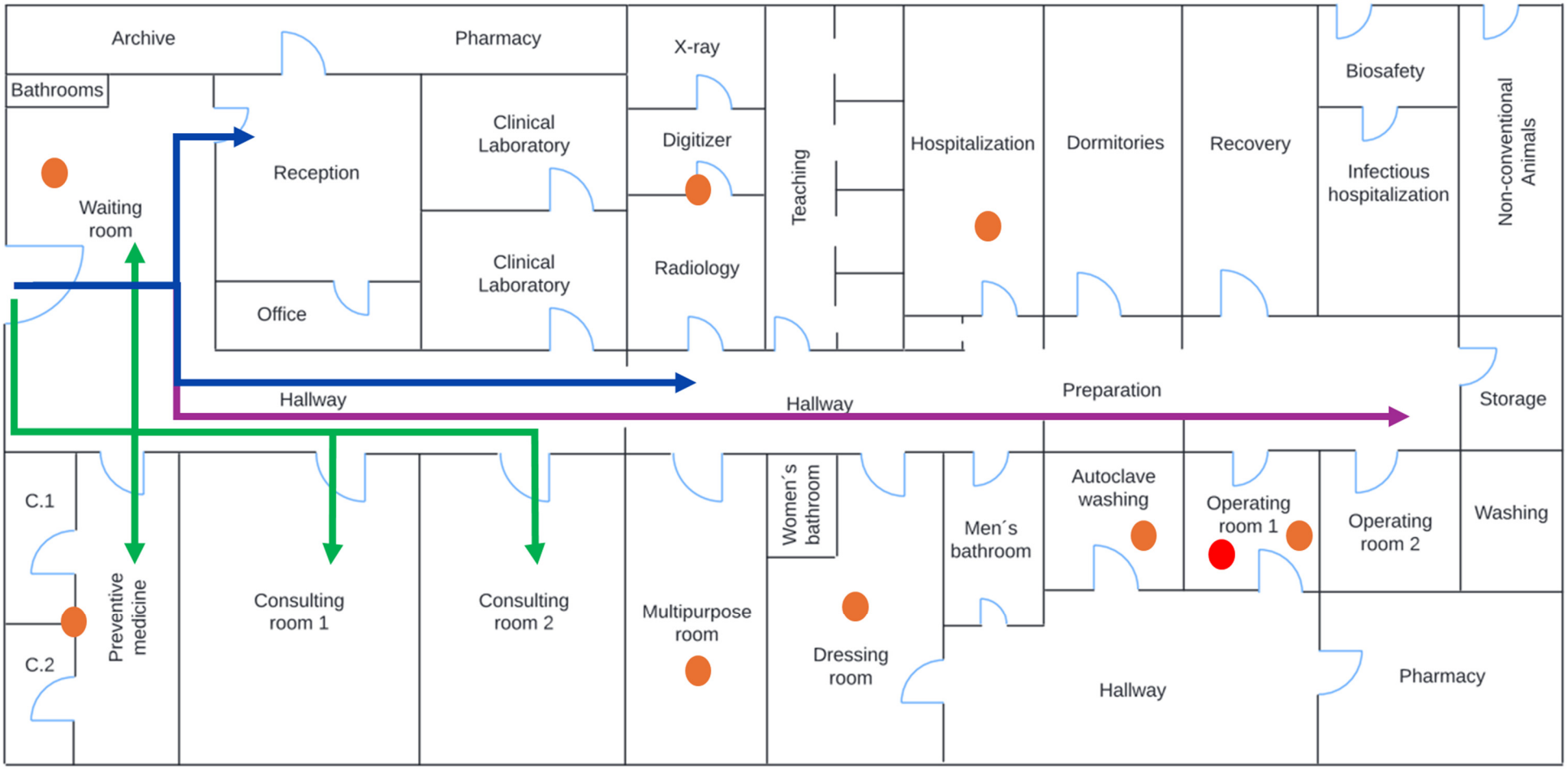

Figure 1. Distribution map of hospital installation. Arrows show the accessibility to the facilities for medical staff (purple), animal owners (green), and administrative staff (blue). Orange dots represent the sampled sites where antimicrobial-susceptible bacteria were isolated, and the red dot indicates where multidrug-resistant bacteria were isolated.

Table 2. Molecular identification of bacteria isolated in the veterinary hospital.

ID	Sample Origin	Identified Bacteria	Query Cover	E-Value	Identity (%)	Accession Number	Pathogenicity Reported	Antimicrobial Resistance Reported	Environmental Or Nosocomial (E or N)	References
1	Waiting room	*Pseudescherichia vulneris*	100	0	99.71	NR_041927.1	Yes	Yes	N	[29,30]
2		*Enterobacter mori*	100	0	100	NR_146667.2	Recently reported in humans	Recently reported	E	[31,32]
3		*Klebsiella aerogenes KCTC*	100	0	100	NR_102493.2	Reported in human and veterinary medicine	Yes	N	[33,34]
4	Preventive medicine room	*Enterobacter mori*	100	0	100	NR_146667.2	Recently reported	Recently reported	E	[32,35]
5		*Enterobacter quasihormaechei*	100%	2.00×10^{-139}	100	NR_180451.1	Recently reported in a human patient	No	N	[36,37]
6		*Yersinia pseudotuberculosis*	100	2.00×10^{-174}	100	NR_118571.1	Yes, described in humans	No	N	[38,39]
7		*Klebsiella aerogenes* KCTC	100	0	100	NR_102493.2	Reported in human and veterinary medicine	Yes	N	[33,34]
8	Consulting room 2	*Citrobacter cronae*	100	3.00×10^{-95}	100	NR_170426.1	Considered opportunistic pathogen	Yes	N	[40,41]
9		*Enterobacter mori*	100	0	100	NR_146667.2	Recently reported in humans	Recently reported	E	[31,32]
10		*Stutzerimonas stutzeri* ATCC	100	1.00×10^{-63}	100	NR_103934.2	No	No	E	[42]
11		*Vibrio ishigakensis*	100	6.00×10^{-39}	100	NR_156028.1	No	No	E	[43,44]
12	Clinical laboratory	*Leclercia pneumoniae*	100	8.00×10^{-65}	100	NR_181872.1	Recently reported	No	E	[45]
13		*Citrobacter bitternis*	100	3.00×10^{-85}	100	NR_178707.1	No	No	E	[46]
14	Multipurpose room	*Enterobacter sichuanensis*	100	0	91.44	NR_179946.1	Recently reported	No	E	[47]
15	Radiology room	*Mixta gaviniae*	99%	0	97.40	NR_117305.1	No	No	E	[48]
16		*Photobacterium leiognathi*	100	1.00×10^{-72}	100	NR_029253.1	No	No	E	[49]
18		*Mixta gaviniae*	100	6.00×10^{-159}	100	NR_117305.1	No	No	E	[48]
19		*Leclercia adecarboxylata*	100	3.00×10^{-162}	100	NR_114154.1	Yes	Recently reported	N	[50,51]
20	Dressing room	*Acinetobacter variabilis*	100	0	100	NR_134685.1	No	No	E	[52,53]
22		*Rosenbergiella australiborealis*	99	0	99.43	NR_126305.1	No	No	E	[54]

Table 2. *Cont.*

ID	Sample Origin	Identified Bacteria	Query Cover	E-Value	Identity (%)	Accession Number	Pathogenicity Reported	Antimicrobial Resistance Reported	Environmental Or Nosocomial (E or N)	References
23	Autoclave washing room	*Citrobacter cronae*	100	0	100	NR_170426.1	Considered opportunistic pathogen	Yes	N	[40,41]
29		*Pseudomonas songnenensis*	100	0	100	NR_148295.1	No	No	E	[55]
24	Operating room 2	*Erwinia uzenensis*	99	0	99.79	NR_113061.1	No	No	E	[56]
25		*Bacillus haynesii*	100	2.00×10^{-60}	100	NR_157609.1	No	No	E	[57]
34	Consulting room 1	Undetermined	–	–	–	–	–	–	–	–
26		*Klebsiella aerogenes* KCTC	100	6.00×10^{-98}	100	NR_102493.2	Reported in human and veterinary medicine	Yes	N	[33,34]
38		*Affinibrenneria salicis*	100%	9.00×10^{-43}	100	NR_173669.1	No	No	E	[58]
30	Hospitalization area	*Stutzerimonas nitritolerans*	100	0	100	NR_169495.1	No	No	E	[59]
31		*Enterobacter ludwigii*	100	0	99.84	NR_042349.1	Yes	Recently reported	N	[60,61]
32		*Enterobacter asburiae*	100%	0	100	NR_024640.1	Yes	No	N	[62]
33		*Dryocola clanedunensis*	100%	4.00×10^{-84}	99.42	NR_189237.1	No	No	E	[63]
39	Recovery area	*Pseudomonas songnenensis*	100%	0	100.00	NR_148295.1	No	No	E	[55]
28		Undetermined	–	–	–	–	–	–	–	–
36	Infectious hospitalization	*Enterobacter asburiae*	100%	0	100.00	NR_024640.1	Yes	No	N	[62]

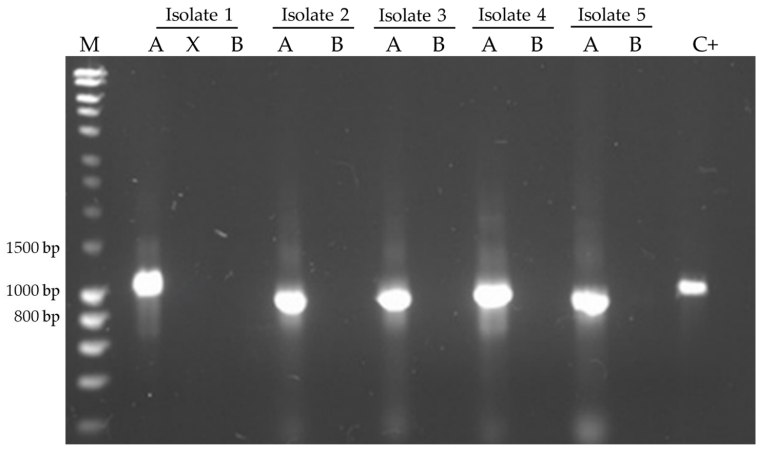

Figure 2. Amplification of 16S rRNA gene from isolated bacteria. The 1% agarose gel shows the first five amplicons from the 16S rRNA gene from isolated bacteria (A). No amplification was observed in water (negative control) between amplicons (B). A PCR positive reaction control was included using *E. coli* (commercial strain One Shot™ top 10 DNA, C404010, Thermo Fisher Scientific, Waltham, MA, USA) (C+). An empty lane was left for isolate 1 (X). M: molecular marker (BIO-33025, MeridianBioscience, Cincinnati, OH, USA).

After molecular identification, a susceptibility test was carried out. Antibiotics included in the test were piperacillin-tazobactam, ceftriaxone, ceftazidime, cefepime, doripenem, imipenem, ertapenem, meropenem, amikacin, gentamicin, and ciprofloxacin. All of the samples except for one were sensitive to antimicrobial agents. Sample 26 was identified as *Klebsiella aerogenes* using molecular identification.

Table 3 compares the results obtained with the CLSI standards for all members included in Enterobacterales. The Vitek results and the CLSI breakpoints are expressed in terms of the MIC in μg/mL. This means that when bacteria can grow in a concentration of a specific antimicrobial agent, the bacteria are resistant; otherwise, they are susceptible. By ensuring a proper preanalytical procedure and correctly labeling the samples, it was possible to trace the samples to determine the origin of the multidrug-resistant bacteria, as shown in Figure 1. The bacteria were isolated from the tubing of the anesthesia system in operating room 1.

Table 3. Antimicrobial susceptibility test of sample 26. The results are expressed as the minimal inhibitory concentration in μg/mL where growth was observed.

Vitek Results			**CLSI MIC Breakpoints for Enterobacterales (μg/mL)**		
Antimicrobial Agent	**MIC (μg/mL)**	**Interpretation [1]**	**≤S**	**I**	**≥R**
Piperacillin-tazobactam	≥128	R	8/4	16/4	32/4
Ceftriaxone	≤1	S	1	2	4
Ceftazidime	≥64	R	4	8	16
Cefepime	≥8	R	2	4–8	16
Doripenem	≥8	R	1	2	4
Imipenem	≥16	R	0.5	1	2
Ertapenem	≥8	R	1	2	4
Meropenem	≥16	R	1	2	4
Amikacin	4	S	4	8	16
Gentamicin	2	S	2	4	8
Ciprofloxacin	≤0.25	S	0.25	0.5	1

[1]: R: Resistant, I: Intermediate, S: Susceptible.

4. Discussion

The results confirm the presence of a multidrug-resistant bacterium in a small-species university veterinary hospital in Mexico. Additionally, we were able to provide a general

overview of the bacterial genera and species found in this hospital environment. According to the literature, 12 isolates have been reported to be pathogenic, opportunistic, or multidrug-resistant, as shown in Table 2. The identification of a multidrug-resistant bacterium is a signal of the importance of continued epidemiological surveillance in the hospital environment.

K. aerogenes was identified as the only multidrug-resistant bacteria. The genus *Klebsiella* has been extensively reported as one of the main multidrug-resistant bacteria in human and veterinary hospitals [33,34,64,65]. Furthermore, the genus has a characteristic of rapid evolution through the acquisition of horizontal gene transfer [64,66]. *K. aerogenes* genomes have been analyzed and have demonstrated the presence of gene islands related to its pathogenicity, multidrug resistance, and strains that are spread across different parts of the world [67].

Two different genotypes of isolates were identified as *K. aerogenes*, but only one of them was resistant to antimicrobial agents. Even though one of them is susceptible to antimicrobial agents, it still represents a significant risk due to its potential for easy acquisition of virulence and resistance genes [66]. Further analysis is suggested to investigate the genotypes of both isolates, determine their origin, and explore the relationship between these and the reported clinical genotypes [64,68,69].

The second most isolated bacterium was *Enterobacter* sp. The genera *Enterobacter* and *Klebsiella* are both types of "ESKAPE bugs", which were coined to refer to multidrug-resistant microorganisms that mainly cause nosocomial infections worldwide [70]. Two species in the *Enterobacter cloacae* complex were isolated: *Enterobacter asburiae* and *Enterobacter ludwigii*. The complex includes *E. cloacae*, *E. asburiae*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, and *E. nimipressuralis.* This complex comprises species for which biochemical phenotypic identification and antimicrobial patterns are insufficient for their differentiation [71,72]. As in humans, the complex has been significantly reported in veterinary medicine and has mainly isolated in dogs and cats [73–76].

Not surprisingly, a study from Japan reported that four out of five colistin-resistant *E. cloacae* complex species isolated from companion animals were identical to those of human origin based on phylogenetic analysis [73]. This finding is supported by a study from the United States of America, where two members of the complex with resistance profiles were isolated from two dogs. Phylogenetic analysis of the dogs' isolates were compared with genome databases, which indicated a close relationship with human clinical isolates [74].

The bacteria isolated in this study were resistant to 7 of the 11 antibiotics tested. They exhibited resistance to third-generation cephalosporins, carbapenems, and a combination of penicillin/β-lactamase inhibitors. Extended-spectrum β-lactamase bacteria have previously been isolated from animals [75]. This examination did not include animal sampling, but doing so would likely increase the number of findings of this type of bacteria.

It is important to consider that the capacity of the veterinary hospital is medium to low, and it is not designed to house many patients (approximately 100 ambulatory consultations and 54 short-term hospitalizations per month). This indirectly limits the presence of nosocomial bacteria, as it has been determined that occupancy and overcrowding can influence the incidence of nosocomial infections [77,78]. Based on our findings, it is crucial to characterize the phylogenetic relationship with human strains and the genetic background responsible for antibiotic resistance in isolated bacteria to understand the origin of this strain. Further clinical surveillance of patients in Mexico must be carried out to increase the number of studies on this topic, including other private veterinary hospitals. This could contribute to highlighting how underestimated the risk of an emerging multidrug-resistant zoonosis scenario is.

5. Conclusions

This study confirmed the presence of multidrug-resistant bacteria in a university veterinary hospital in Mexico. Susceptible bacteria from the "ESKAPE bugs" group were identified. The species of bacteria isolated in this work are major contributors to HAIs

globally and are reported to be genetically related to clinical human isolates. Future research should be conducted to trace the origin of this strain and identify the genetic elements responsible for its resistance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15030117/s1, 16S rRNA gene sequences of the isolates.

Author Contributions: D.J.H.-S. conceived and supervised the project, obtained founds, performed hospital sampling, and wrote the manuscript. A.I.R.-G. performed hospital sampling, bacterial cultures, 16s rRNA amplification and purification, sequence curing, bioinformatics sequence analysis, the antimicrobial susceptibility test, and wrote the manuscript. L.K.A.-B. provided reagents and equipment for antimicrobial susceptibility test and performed the test. M.M.B.-R. contributed to the bacterial cultures, isolation, and molecular identification. C.R.-B. contributed to the bacterial cultures, isolation, and molecular identification. R.M.-G. provided funds and edited the manuscript. L.G.-R. contributed to molecular identification. O.F.C.-M. contributed the hospital facilities access and hospital sampling. G.A.-T. contributed funds and edited manuscript. J.G.G.-S. edited the manuscript. J.M. provided reagents, contributed to the laboratory facilities and equipment, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the current national legislation for temporary storage, safe handling, and disposal of biological-infectious hazardous wastes (NOM-087-SEMARNAT-SSA1-2002). No animals were used in this study. The protocol was approved by the Ethics Committee of the Faculty of Natural Science at the Autonomous University of Queretaro (FCN-UAQ). Approval numbers: 078FCN2023 and 43FCN2022.

Data Availability Statement: No new genomic sequences or bacterial strains were discovered. The sequences obtained are available as Supplementary Materials.

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