Article

First Screening of Beta-Lactam Antibiotic Resistance Genes and Bacterial Diversity in the Public Transport System of Quito, Ecuador

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Abstract: Background: Multidrug-resistant bacteria present resistance mechanisms against β-lactam antibiotics, such as Extended-Spectrum Beta-lactamases (ESBL) and Metallo-β-lactamases enzymes (MBLs) operon encoded in Gram-negative species. Likewise, Gram-positive bacteria have evolved other mechanisms through *mec* genes, which encode modified penicillin-binding proteins (PBP2). This study aimed to determine the presence and spread of β-lactam antibiotic resistance genes and the microbiome circulating in Quito's Public Transport (QTP).
Methods: A total of 29 station turnstiles were swabbed to extract the surface environmental DNA. PCRs were performed to detect the presence of 13 antibiotic resistance genes and to identify 16S rDNA barcoding, followed by clone analysis, Sanger sequencing and BLAST search.
Results: ESBL genes *bla*$_{\text{TEM-1}}$ and *bla*$_{\text{CTX-M-1}}$ and MBL genes *bla*$_{\text{OXA-181}}$ and *mecA* were detected along QPT stations. Two subvariants were found for *bla*$_{\text{TEM-1}}$, *bla*$_{\text{CTX-M-1}}$, and *bla*$_{\text{OXA-181}}$. Almost half of the circulating bacteria found at QPT stations were common human microbiota species including those classified by the WHO as pathogens of critical and high-priority surveillance.
Conclusions: β-lactam antibiotic resistance genes are widely spread throughout QPT. This is the first report of *bla*$_{\text{OXA-181}}$ in environmental samples in Ecuador. Moreover, we detected a new putative variant of this gene. Some commensal coagulase-negative bacteria may have a role as mecA resistance reservoirs.

Keywords: m*ecA; bla*$_{\text{TEM-1}}$; *bla*$_{\text{OXA-181}}$; *bla*$_{\text{CTX-M-1}}$; environmental-DNA; antibiotic-resistance

1. Introduction

Bacterial diseases have an enormous impact on human health and remain a major focus in modern medicine. However, there is ample evidence that multidrug resistance (MDR) in pathogenic microorganisms, defined as showing resistance to three or more classes of antibiotics, has become a serious problem in the mismanagement of infectious diseases [1]. Given that infections are easily transmitted from person to person, microbes in public places, such as transport systems, can be a serious health problem (Yeh et al., 2011.; Romo-Castillo & Pazin-Filho, 2022).

The most widely used class of human antibacterial agents are β-lactams, which target the transpeptidases responsible for cross-linking peptidoglycan in cell walls, thereby

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inhibiting cell wall biosynthesis. There are more than 34 β-lactam antibiotics approved by the US Food and Drug Administration, which together account for approximately 50% of all antibiotic prescriptions worldwide and up to 60% in Ecuador [3–5]. However, to date, bacteria have acquired resistance mechanisms to overcome all major classes of β-lactam antibiotics.

In Gram-negative pathogens, the main clinically determining mechanism of β-lactam resistance is the enzymatic inactivation of the antibiotics by β-lactamases [5]. Extended-spectrum β-lactamase (ESBL) is the classification of β-lactamase enzymes that gives widespread resistance to β-lactam antibiotics, including penicillin, cephalosporins, and monobactam, thus affecting the effectiveness of all beta-lactams but not carbapenems. These enzymes stimulate hydrolysis of the β-lactam ring and thereby inhibit these antibiotics [6]. Among ESBLs, the most widespread and clinically relevant are the class TEM, SHV, and CTX-M types. ESBLs are frequently plasmid-encoded. Plasmids responsible for ESBL production frequently carry operon genes encoding resistance to other drug classes (for example, aminoglycosides or even polymixins) (Paterson & Bonomo, 2005). Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited (Gutiérrez-Gutiérrez & Rodríguez-Baño, 2019). Furthermore, carbapenems evade most β-lactamases but are hydrolyzed by metallo β--lactamases (MBLs) such as New Delhi metallo β-lactamase (NDM), *Klebsiella pneumoniae* carbapenemase (KPC), and OXA [8]. Thus, these enzymes are also named carbapenemases.

Nevertheless, Gram-positive bacteria have evolved other mechanisms to avoid β-lactam inhibition of cell wall biosynthesis. Instead of cleaving the β-lactam ring, these bacteria alter penicillin-binding proteins (PBPs) through successive mutations. As a result, the β-lactams are less effective at disrupting cell wall synthesis. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one of the most widespread types of antibiotic resistance worldwide in Gram-positive pathogens [9]. This notorious spread is possible due to the presence of the staphylococcal chromosomal cassette *mec* (SCCmec) and plasmid-mediated genes, such as *blaZ*. These genetic elements work as main drivers of β-lactam resistance in MRSA. Sharing homologous architecture with the *bla* operon, SCCmec contains the *mecA* gene, which encodes a modified penicillin-binding protein, PBP2a, with reduced affinity for β-lactam antibiotics [10]. Although *mecA* is the predominant variant, a divergent *mecA* homolog, *mecC*, was identified in MRSA strains from human samples in Ireland in 2011 [11]. Later, two new *mec* genes were reported, *mecB* and *mecD*, which are much less frequent and were both detected in *Macrococcus caseolyticus* [12,13].

All these resistance mechanisms have been typically detected from nosocomial environments. However, there is more evidence that the main selection mechanisms of bacterial resistance are increasing in non-hospital settings, having direct influence as reservoirs of resistant pathogens and their spread capacity [14]. In 2015, the World Health Organization (WHO) implemented "the Global Action Plan on Antimicrobial Resistance" to encourage the monitoring and research on bacterial antibiotic resistance by governments and city councils [15]. Thus, the universal collection of data on the microbial communities of horizontal-transfer systems was carried out to contribute to the development of novel health and ecological surveillance programs in cities [16,17].

One of the strategies for this call is the detection of antibiotic resistance genes in pathogenic and non-pathogenic microorganisms by amplifying and sequencing specific genes directly from environmental DNA (eDNA). Among the multitude of urban environments, transportation systems represent a uniquely centralized place. Therefore, a global collection of information on microbial communities of transportation systems has been gathered to contribute to this goal [16,17]. Specifically, Quito's Public Transport (QPT) is used by almost 900,000 people a day and it is where urban and rural dwellers meet and circulate daily. However, records about β-lactam resistance genes circulating in the QTP are absent to date.

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Given the widespread worry but general ignorance of potential bacterial infections in public transportation, the present study aimed to survey the main β-lactam antimicrobial resistance genes associated with QPT by screening the presence of *mecA,* ESBL, and MBL resistance genes, and the circulating bacteria by 16S rDNA assessment from the environmental DNA (eDNA) isolated from QPT surfaces.

2. Materials and Methods

2.1. Study area and sample collection

This was a cross-sectional study using primary data conducted in QPT. Quito is the capital of Ecuador and the second most populous city after Guayaquil with 3.5 million inhabitants [18]. QTP comprises three main routes that cross the city from north to south. These routes are connected by stop stations and terminals. Surface bacteria were collected by rubbering sterilized cotton swabs on the entry and exit turnstiles from 29 stations in March 2022. The swabs were soaked in PBS 1X, pH 7.4, as described by Rawlinson et al. in 2019.

2.2. eDNA extraction and gene amplification

eDNA was directly extracted from collected swabs using the PureLink™ Microbiome DNA Extraction Kit (#A29790 Thermo Fisher, USA) and concentrated with GeneVac™ miVac Centrifugal Concentrator (Fisher Scientific, USA). eDNA concentration and purity were determined in NanoDrop™ at 230, 260, and 280 nm (Thermo Fisher Scientific, USA). The amplification of resistance genes was performed with specific primers for *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTXM-1}$, *bla*$_{CTXM-2}$, *bla*$_{CTXM-9}$, *bla*$_{CTXM-8/25}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{OXA-48/181}$, *mecA*, *mecC*, and *mecD*. The 16S rDNA V1-V3 region was amplified for bacterial identification. Amplification was performed in reactions of 15 µL containing 2X GoTaq® Green Master Mix (Promega), 0.3 µM of each primer, and 0.1 to 0.5 ng/µL of extracted DNA. PCR was performed following the conditions described by the authors in Table 1.

Table 1. List of primers used for molecular identification of β-lactam resistance genes from eDNA of QPT surfaces between March and August 2022.

Gene	Primer's name	Sequence (5′-3′)	PCR product (bp)	Annealing (C°)	Reference
16S rDNA	8F	AGAGTTTGATCCTGGCTCAG	527	57.4	[20]
	534R	ATTACCGCGGCTGCTGG			
***bla*$_{TEM}$**	TEM-410F	GGTCGCCGCATACACTATTCTC	372	60	[21]
	TEM-781R	TTTATCCGCCTCCATCCAGTC			
***bla*$_{SHV}$**	SHV-287F	CCAGCAGGATCTGGTGGACTA	231		
	SHV-517R	CCGGGAAGCGCCTCAT			
***bla*$_{CTXM-1}$**	ctxm1-115F	GAATTAGAGCGGCAGTCGGG	588		
	ctxm1-702R	CACAACCCAGGAAGCAGGC			
***bla*$_{CTXM-2}$**	ctxm2-39F	GATGGCGACGCTACCCC	107		
	ctxm2-145R	CAAGCCGACCTCCCGAAC			
***bla*$_{CTXM-9}$**	ctxm9-16F	GTGCAACGGATGATGTTCGC	475		
	ctxm9-490R	GAAACGTCTCATCGCCGATC			
***bla*$_{CTXM-8/25}$**	ctxm8g25g-533F	GCGACCCGCGCGATAC	186		
	ctxm8g25g-718R	TGCCGGTTTTATCCCCG			
***bla*$_{KPC}$**	KPCfw	CGTCTAGTTCTGCTGTCTTG	798	55	[22]
	KPCrv	CTTGTCATCCTTGTTAGGCG			
***bla*$_{VIM}$**	VIMfw	GATGGTGTTTGGTCGCATA	390		
	VIMrv	CGAATGCGCAGCACCAG			
***bla*$_{NDM}$**	NDMfw	GGTTTGGCGATCTGGTTTTC	621		
	NDMrv	CGGAATGGCTCATCACGATC			
***bla*$_{OXA-48/181}$**	OXA48fw	GCGTGGTTAAGGATGAACAC	438		
	OXA48rv	CATCAAGTTCAACCCAACCG			

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mecA	mecA147-F	GTGAAGATATACCAAGTGATT	147	56	[23]
	mecA147-R	ATGCGCTATA-GATTGAAAGGAT			
mecC	mecALGA251 fw	GAAAAAAAGGCTTAGAA-CGCCTC	138	50	[24]
	mecALGA251 rv	GAAGATCTTTTCCGTTTTCAGC			
mecD	mecDfw	TCCTTTAGCGATAGATGGTGAA	834	54	[25]
	mecDrv	CTCCCATCTTTTCTCCATCCT			

2.3. S rDNA clone library

16S rDNA V1-V3 amplicons were inserted onto a pCRTM4-TOPO® TA vector, following TOPO™ TA Cloning™ Kit for Sequencing protocol (# K4575J10 Thermo Fisher, USA). Chemically competent *E. coli* DH5a cell preparation and transformation protocol were performed as stated by Hanahan in 1983 [26]. X-gal and IPTG were used for the selection of positive colonies. Plasmids were extracted by alkaline lysis [27].

2.4. Sequence analysis

PCR products for β-lactam resistance and 16S rDNA clones were sequenced by the Sanger technique in an ABI 3500xL Genetic Analyzer (Applied Biosystems, USA) by BigDye 3.1® capillary electrophoresis matrix. Sequences were edited with MEGA X software (Kumar et al., 2018) and compared against the GenBank database at the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). Sequences were submitted for the acquisition of accession numbers (see section "Data Availability Statement").

Chimeric sequences were removed with UCHIME2 Algorithm [28]. Kranken2 database analysis with Standard configuration was performed to assign taxonomic labels to sequences and the Krona Pie Chart was used for the visualization of the taxonomic profile [29–31].

3. Results

A total of 29 stations were sampled from the three main lines of QPT, distributed as shown in Figure 1.

3.1. QTP β-lactam resistance genes screening

Four out of thirteen genes for β-lactam antibiotic resistance were detected: *bla*$_{TEM}$, *mecA*, *bla*$_{CTX\text{-}M\text{-}1}$, and *bla*$_{OXA181}$. The most widely spread resistance gene was *bla*$_{TEM}$, detected in 27 stations, followed by *mecA* in 16 stations, *bla*$_{CTX\text{-}M\text{-}1}$ in three, and *bla*$_{OXA181}$ in two. Meanwhile, *bla*$_{SHV}$, *bla*$_{CTX\text{-}M2}$, *bla*$_{CTX\text{-}M8/25}$, *bla*$_{CTX\text{-}M9}$, *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{NDM}$, *mecC*, and *mecD* did not amplify in any sample (Figure 1; Table S1).

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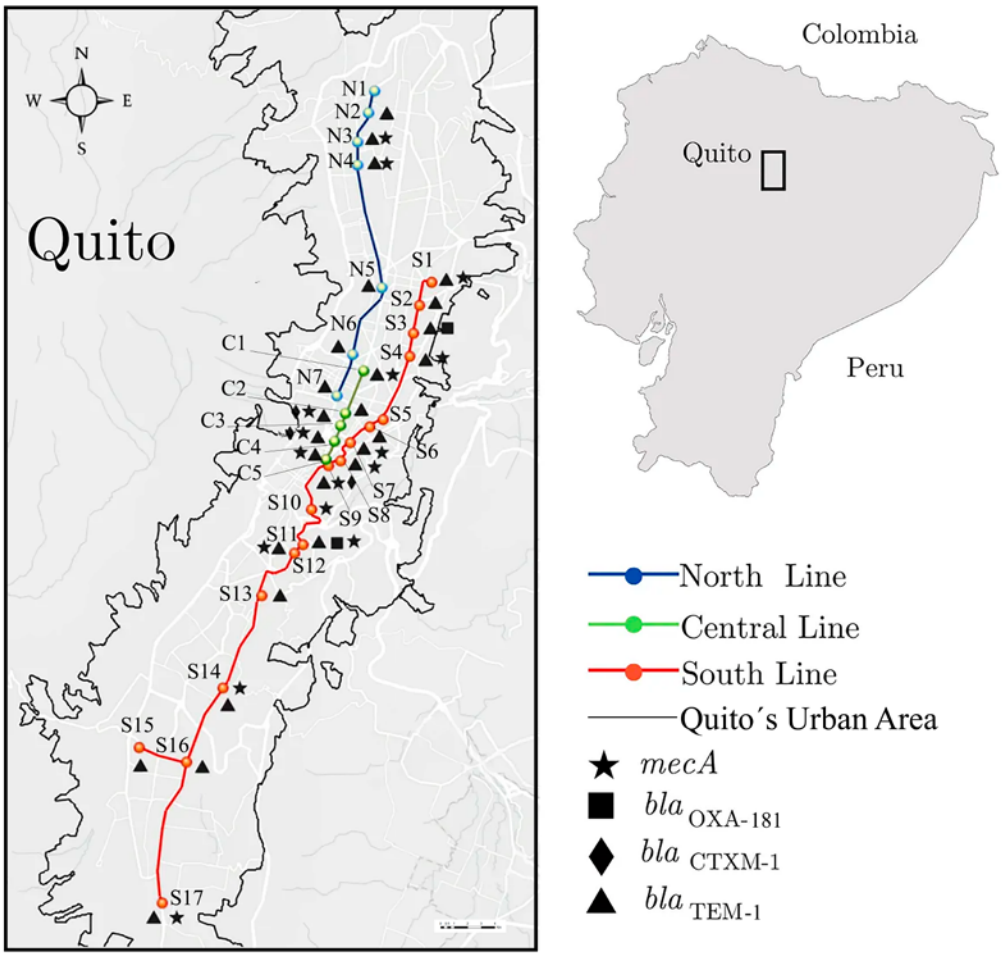

Figure 1. Map of Quito´s Public Transport (QTP) showing North, Central, and South lines. Black icons show the presence of *bla*TEM-1, *bla*CTX-M1, *bla*OXA-48, and *mecA* genes found in each station from environmental DNA (eDNA) in March 2022.

The sequencing of PCR products for *bla*TEM showed two subvariants named TV1 and TV2 with one nucleotide difference that leads to a single amino acid change from Ala116Val. These subvariants were assigned the accession numbers OP846058 and OP846059, respectively. Both variants matched the *bla*TEM-1 variant at the GenBank database. The variant TV1 showed 100% identity with *bla*TEM-1 for synthetic plasmids and species from *Staphylococcus*, *Streptococcus*, and *Pseudomonas* genera. TV2 was 100% identical to *bla*TEM-1 from *Klebsiella pneumoniae*, *E. coli*, and *Enterobacter cloacae* (Figure 1; Figure S1).

Likewise, two different *bla*CTX-M-1 subvariants were sequenced and named CV1 and CV2. The variation in nucleotide sequence leads to a single amino acid change again from Ala56Val in CV1 (accession number OP846056) and CV2 (accession number OP846057) respectively. Both variants were found, with 100% identity, in *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella*, and *Enterobacter* genera at the GenBank. Furthermore, CV2 was found also in *Salmonella enterica* and the *Vibrio* genus (Figure 1; Table S1).

The two *bla*OXA-181 identified subvariants were called OV1 and OV2. The variation in nucleotide sequence leads to a single amino acid change from His174Arg in OV1 (accession number OP846061) and OV2 (accession number OP846062). The OV2 variant had 100% identity matches at the GenBank with the species *E. coli* and *K. pneumoniae* and *Enterobacter*, *Pseudomonas*, and *Shewanella* genera. Just one 100% match was found for OV1, belonging to *E. coli* isolated from urine samples [32]. The other matches were the same as OV2 with 99% of similarity.

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3.2. QTP microbiome.

We examined QPT microbiome through a total of 293 clones of 16S rDNA partial gene amplification from eDNA. After data curation, around 275 sequences were obtained, and 256 were selected by Kraken2 software for taxonomic analysis (Figure 2). All the identified microorganisms belonged to the Bacteria domain, except for two related to chloroplast form *Vicia faba*, one from *Colquhounia coccinea,* and one from *Klebsormidium flaccidum* (Table S2). The results detailed in Figure 2 suggest that species-level identification was achieved for 74% of the sequences, which showed more than 97% of similarity with the sequences identified at the GenBank (Table S2).

Almost half of the 16S amplicons (49%) matched Gram-negative Proteobacteria-phylum species from Alpha and Gammaproteobacteria classes (20% each), followed by the Betaproteobacteria class (8%), and Delta/epsilon subdivision (0.8%). The rest of the Gram-negative species belonged to Bacteroidetes (4%) and Fusobacterium (1%) phylla. Regarding Gram-positive species, Firmicutes with Bacilli class and Actinobacteria with Actinobacteria class were the most abundant phylla with 21% and 20% respectively. The Clostridiales order showed a relative abundance of only 3%.

15% of the Gammaproteobacteria class corresponded to the Enterobacteriaceae family with enteric microbiota species such as *E. coli*, *Salmonella enterica*, *Klebsiella* sp., *Erwinia* sp., and common plant microbiota such as *Pantoea calida*, *P. aglomerans,* and *P. vagans*. Typically, environmental species in the Gammaproteobacteria class were represented by the Pseudomonadales order (4%), including *Pseudomonas psychrotolerans*, *P. putida*, *P. stutzeri*, *P. brassicacearum*, *P. mendocina, Acinetobacter johnsonii,* and *A. radioresistens* in order of abundance (Figure S2D).

The Bacilli class shows the *Staphylococcus* genus as the most abundant, including 9% of the 16S sequences with ten identified species: *S. saprophyticus*, *S. epidermidis, S. simulans*, *S. aureus*, *S. lugdunensis*, *S. capitis*, *S. pettenkoferi*, *S. simiae*, *S. xylosus*, and *S. haemolyticus* in order of abundance. The *Streptococcus* genus also shows a wide diversity with important species such as *S. pneumoniae, S. mitis, S. dentisani, S. thermophilus*, and *S. cristatus* (Figure S2B).

Inside the Alphaproteobacteria class, the most abundant genus was *Mesorhizobium,* with 15% of the sequences (Figure 2C). Finally, the Actinobacteria class was represented by common skin microbiota such as *Cutibacterium acnes* with 7% of the sequences Other typically saprophytic and mammal-skin species, such as *Micrococcus luteus* and 3 species from the *Corynebacterium* genus, were also detected (Figure S2A).

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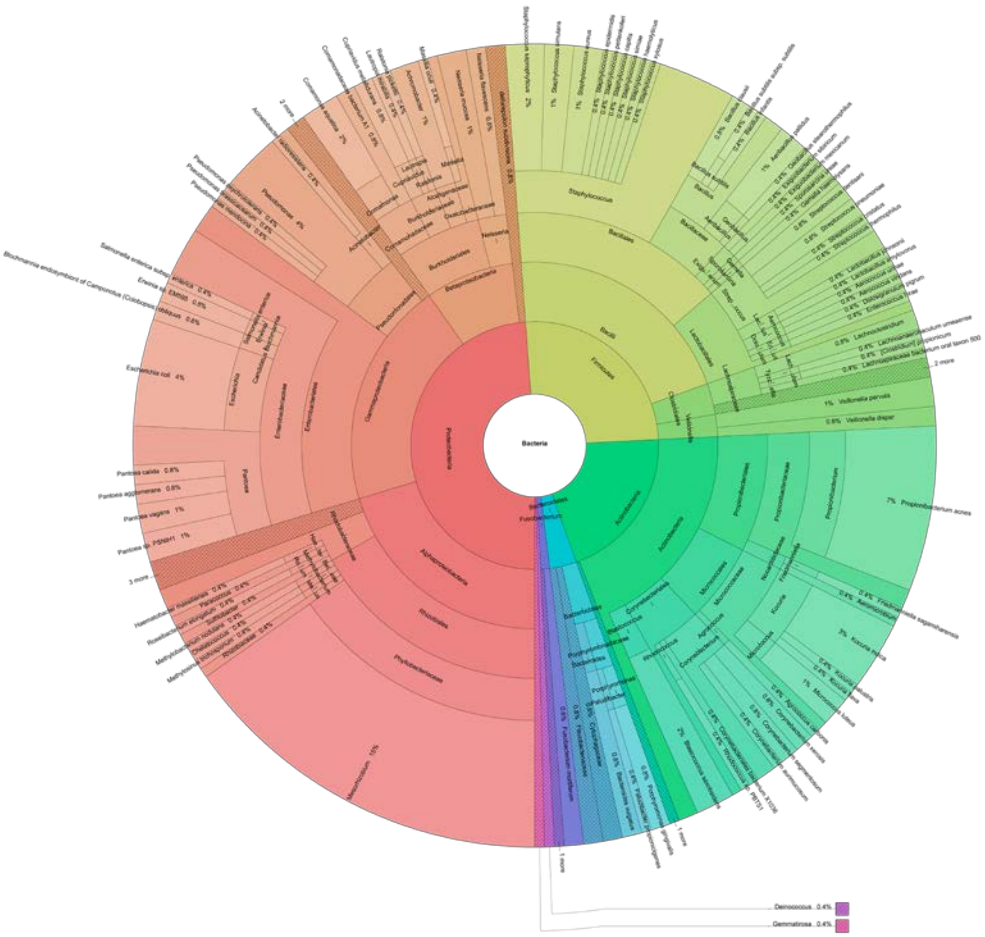

Figure 2. 16S rDNA relative abundance of species detected in eDNA samples from Quito Public Transport (QTP) in Mach 2022. Data set available at: https://usegalaxy.org.au/datasets/a6e389a98c2d1678a9b3a242082abea8/display/?preview=True&dataset=0&node=1&collapse=true&color=false&depth=8&font=10&key=true.

In short, 41.2% of the 16S sequences were putative human microbiota: 29.7% from the skin, 18.7% from the respiratory tract, 9% from the gastrointestinal tract, 14.8% from the oral cavity, and 6.5% from others. The remaining 58.8% belonged to typically environmental bacteria, including one cyanobacterium and several chloroplasts from broad beans, flowers, and green algae (Table S2). 52% of the sequences analyzed were putative pathogens.

4. Discussion

To our knowledge, this is the first study of β-lactam resistance genes and-microbiome in a public transport system in Ecuador. Our results showed the spread of ESBL, MBLs, and *mecA* resistance along QPT. The presence of these genes agrees with previous environmental studies in Ecuador where ESBL pathogens [33] and methicillin resistance in community-acquired infections [34] were reported. Two subvariants were found for *bla*$_{\text{TEM-1}}$, *bla*$_{\text{CTX-M-1}}$, and *bla*$_{\text{OXA-181}}$. The *bla*$_{\text{TEM-1}}$_TV2 subvariant has been widely reported in three leading pathogens for deaths attributed to multidrug resistant (MDR) bacteria, i.e., *Acinetobacter baumanii* [35,36], *E. coli* [37], and *K. pneumoniae* [38,39]. Nevertheless, most of the *bla*$_{\text{TEM-1}}$_TV1 subvariant matches at the GenBank were sequences related to synthetic plasmids. One of these was used for experiments on the evolution of β-lactamase where the antibiotic resistance protein TEM-1 evolved towards resisting the antibiotic cefotaxime in an *E. coli* strain with a high mistranslation rate [40]. Other experiments demon-

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strated that substitutions at residues 69, 130, 165, 244, 275, and 276 are all thought to play an exclusive role in inhibitor resistance [41][42] and most of them are found at a high frequency in the dataset of clinical isolates. Nevertheless, TV1 substitution with respect to TV2 is at residue 116. The *bla*$_{\text{CTX-M-1}}$_CV1 and *bla*$_{\text{CTX-M-1}}$_CV2 subvariants were present in the same species as *bla*$_{\text{TEM-1}}$_TV2. Previous studies found five key substitutions among CTX-M-1 variants: Val80Ala, Asp117Asn, Ser143Ala, Asp242Gly, and Asn289Asp [43] the first one being consistent with the variants found in this study.

This is also the first report for *bla*$_{\text{OXA-181}}$ detection from non-hospital settings in Ecuador. Since broad-spectrum cephalosporins and carbapenems are both weakly hydrolyzed by OXA-48 and OXA-181 carbapenemases, elevated minimum inhibitory concentrations (MICs) to those drugs may not be apparent in traditional phenotypic tests [44]. Therefore, isolates harboring OXA-48 and OXA-181 may go undetected in routine laboratory settings, complicating treatment options (Ying Heng, 2019). In addition, they have a high dissemination rate due to transferable plasmids, making them an important cause of a wide range of infections, both in community and healthcare settings [44]. Thus, molecular detection using eDNA could be a sensitive tool for OXA48/181 surveillance. Since the subvariant OV1 only had a 100% match at the GenBank in 2022, it might be a new circulating variant worth analyzing in future isolates from QPT.

mecA gene was detected in 55.2% of QTP stations. This finding is consistent with the presence of the *Staphylococcus* genus in similar studies on public facilities [45]. Interestingly, the Coagulase-positive staphylococci (CoPS), widely reported as *mecA* carriers including *S. aureus* [45], represented just 1% of the sequences. However, the Coagulase-negative Staphylococci (CoNS) was considerably more abundant with 9 species that comprise 7.4% of QPT bacteria identified (Figure 2; Table S2). Despite being formerly categorized as being less or nonpathogenic, CoNS today comprises a significant group of nosocomial pathogens [46], including *S. epidermidis* and *S. haemolyticus*, both of which were found in QPT (Figure 2; Table S2). The widespread presence of the *mecA* gene in QTP may be a warning sign for the expansion and selection of *mecA* into other commensal and environmental bacteria, including CoNS species [47].

The bacterial diversity in QPT shows the typical classes found in environmental and anthropogenic surfaces, where Alphaproteobacteria, Gammaproteobacteria, Bacilli, and Actinobacteria are the most abundant [48]. Four species found in QPT (S*taphylococcus aureus, E. coli, S. pneumoniae,* and *K. pneumoniae*) along with *P. aeruginosa* were the etiological agent for 54.9% of deaths among 33 investigated bacteria in a systematic analysis for the Global Burden of Disease Study in 2019 [49]. The 16S rDNA barcoding does not allow one to differentiate between *E. coli* strains. Thus, this species might play different roles: from probiotic to pathogen to commensal [50]. However, *E. coli* has been identified as one of the most significant sources of resistance genes that may be to blame for treatment failures in both human and veterinary medicine [44].

Other opportunistic and noncommon pathogens were widely identified in QPT. In general terms, these species are usually significant in hospital settings for infections and immunocompromised patients. The Gammaproteobacteria phylum shows several other species that exemplify this phenomenon, such as *Pantoea aglomerans*, *P. calida* [51], *Pseudomonas mendocina* [52], and *Acinetobacter radioresistens* [53], causing nosocomial bacteremia. Although opportunistic bacteria rarely cause infections, the high abundance described in this study represents a permanent risk of community acquisition and input for nosocomial environments.

The current SARS-CoV-2 pandemic has exacerbated the world's ability to rapidly diagnose infectious disease outbreaks in order to take suitable epidemiological measures to minimize negative impacts [54]. On top of this, the silent pandemic of antimicrobial resistance (AMR) faces several outstanding questions about its evolution and dissemination. In this sense, knowing the resistance genes and bacteria present in public transportation, where thousands of people share reduced spaces, is a necessity in the creation of public health policies for municipalities and governments.

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5. Conclusions

ESBL genes *bla*TEM-1 and *bla*CTX-M-1; MBL gene *bla*OXA-181 and *mecA* were detected along QPT stations. Furthermore, this is the first report of *bla*OXA-181 in an Ecuadorian environmental sample. Our results suggest that CoNS bacteria might have a role as *mecA* resistance reservoirs and other environmental Gram-negative bacteria might act the same for *bla*TEM-1, *bla*CTX-M-1, and *bla*OXA-181. The overlapping presence of these resistances with opportunistic pathogens (classified as critical and high priority by WHO) in QPT should be a warning of the potential hazards to come. Thus, this work is one step forward toward understanding the spread of antibiotic resistance and provides useful information to health practitioners to consider the etiological agents circulating among the inhabitants of Quito´s metropolitan area.

Supplementary Materials:

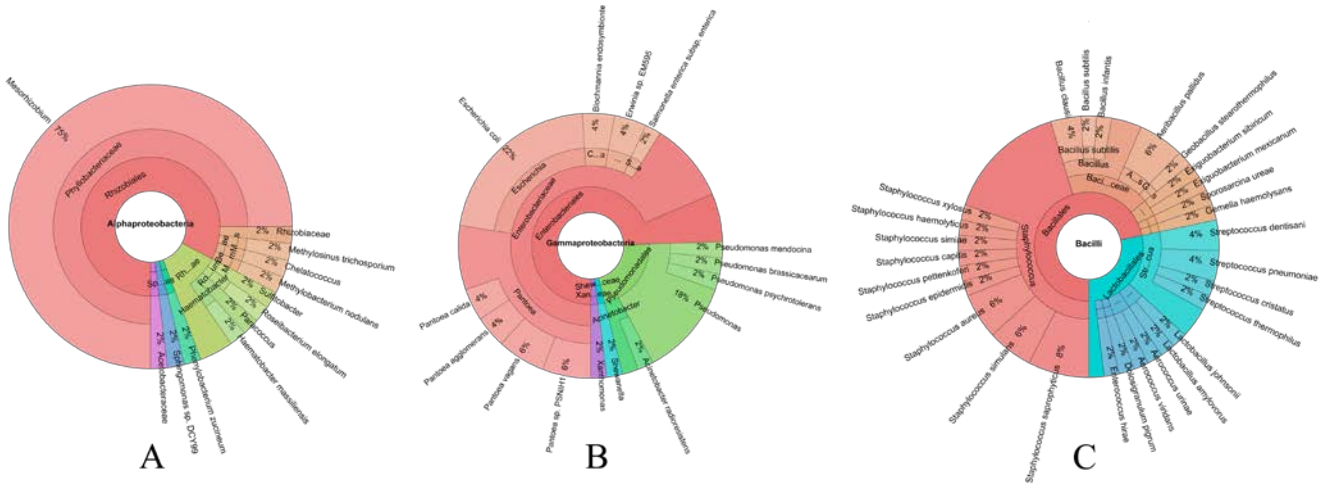

Figure S1. The Alphaproteobacteria (Figure S1A), Gammaproteobacteria (Figure S1B), and Bacilli (Figure S1C) class pie charts show the bacterial diversity of the most abundant species in this study.

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                  *116
TV1   PNDERDTTMPAAMATTLRKLL
TV2   PNDERDTTMPVAMATTLRKLL

                *56
CV1   MCSTSKVMAAAAVLKKSESEP
CV2   MCSTSKVMAVAAVLKKSESEP

                 *174
OV1   ISATQQIAFLHKLYHNKLHVS
OV2   ISATQQIAFLRKLYHNKLHVS
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Figure S2. Protein variants of TEM-1 (TV1-2), CTX-M1 (CV1-2), and OXA-181 (OV1-2) found in QPT (translated from the nucleotide sequence). Asterisks indicate the residue position within the protein.

Table S1. *ß*-lactam resistance genes detected on eDNA from stations of Quito's Public Transport System (March 2022).

Code	Station	*bla*TEM	*bla*CTXM-1	*bla*OXA-48/181	*mecA*
N1	Terminal La Ofelia	-	-	-	-
N2	La Delicia	+	-	-	-
N3	Del Maestro	+	-	-	+

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N4	Vaca de Castro	+	-	-	+
N5	La "Y"	+	-	-	-
N6	San Gabriel	+	-	-	-
N7	Seminario Mayor	+	-	-	-
S1	Terminal Rio Coca	+	-	-	+
S2	Los Sauces	+	-	-	-
S3	Naciones Unidas	+	-	+	-
S4	Eloy Alfaro	+	-	-	+
S5	Baca Ortiz	-	-	-	-
S6	Manuela Cañizares	+	-	-	-
S7	Casa de la Cultura	+	-	-	+
S8	Eugenio Espejo	+	-	-	+
S9	Simon Bolivar	+	+	-	+
S10	Playon La Marin	-	-	-	+
S11	Colegio Montufar	+	-	+	+
S12	Teatro Mexico	+	-	-	+
S13	Terminal El Recreo	+	-	-	-
S14	Ayapamba	+	-	-	+
S15	Terminal Quitumbe	+	-	-	-
S16	Capuli	+	-	-	-
S17	Terminal Sur Guamani	+	-	-	+
C1	Mariana de Jesus	+	-	-	+
C2	Santa Clara	+	-	-	-
C3	La Mariscal	+	+	-	+
C4	El Ejido	+	+	-	+
C5	La Alameda	+	-	-	+

Table S2. Closest match of 16S sequences obtained from QTP and common human microbiota niches obtained from GenBank Database (November 2022). .

Clone	16S Sequences Accession numbers	Closest match	Identity (%)	Common Human Microbiota Niches	Putative pathogen
1	OP965067	*Staphylococcus epidermidis*	99.79	Skin	Yes
2	OP965068	*Staphylococcus epidermidis*	98.99	Skin	Yes
3	OP965069	*Solirubrobacter* sp.	96.79	None	No
4	OP965070	*Cutibacterium acnes*	100	Skin	Yes
5	OP965071	*Staphylococcus* sp.	100	Skin and mucous membranes	Yes
6	OP965072	*Rubellimicrobium* sp.	96.92	None	No
7	OP965073	*Methylobacterium* sp.	99.04	None	No
8	OP965074	*Staphylococcus epidermidis*	99.37	Skin	Yes
9	OP965075	*Staphylococcus epidermidis*	99.79	Skin	Yes
10	OP965076	*Lysobacter* sp.	99.13	None	No

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11	OP965077	Candidatus Saccharibacteria bacterium	97.66	Oral cavity	Yes
12	OP965078	*Streptococcus parasanguinis*	99.59	Respiratory tract	No
13	OP965079	*Exiguobacterium* sp.	99.79	None	No
14	OP965080	*Staphylococcus epidermidis*	98.32	Skin	Yes
15	OP965081	*Staphylococcus epidermidis*	98.57	Skin	Yes
16	OP965082	*Blastococcus* sp.	98.66	None	No
17	OP965083	Acinetobacter lwoffii	99.47	Skin	Yes
18	OP965061	*Pantoea agglomerans*	99.79	None	Yes
19	OP965054	*Pantoea* sp.	99.8	None	Yes
20	OP965066	*Staphylococcus saprophyticus*	100	Gastrointestinal tract	Yes
21	OP965033	*Staphylococcus saprophyticus*	100	Gastrointestinal tract	Yes
22	OP965047	*Staphylococcus simulans*	99.79	Skin	Yes
23	OP965084	*Staphylococcus* sp.	100	Skin and mucous membranes	Yes
24	OP965115	*Neisseria mucosa*	100	Respiratory tract	Yes
25	OP965116	*Parvimonas* sp.	99.78	Abscess	Yes
26	OP965117	*Porphyromonas* sp.	99.16	Oral cavity, gastrointestinal tract, and respiratory tract	Yes
27	OP965148	*Neisseria mucosa*	100	Respiratory tract	Yes
28	OP965118	*Neisseria flavescens*	100	Respiratory tract	Yes
29	OP965131	*Porphyromonas* sp.	99.57	Oral cavity, gastrointestinal tract, and respiratory tract	Yes
30	OP965119	*Streptococcus downii*	99.38	Oral cavity	No
31	OP965130	*Lachnoanaerobaculum* sp.	99.77	Oral cavity and gastrointestinal tract	No
32	OP965120	*Veillonella* sp.	99.79	Oral cavity	Yes
33	OP965129	*Fusobacterium* sp.	99.54	Oral cavity, respiratory, gastrointestinal, and urinary tracts	Yes
34	OP965128	*Gemella* sp.	100	Oral cavity and respiratory tract	Yes
35	OP965127	*Porphyromonas gingivalis*	100	Oral cavity, gastrointestinal tract, respiratory tract, and colon	Yes
36	OP965126	*Neisseria perflava*	100	Respiratory tract	Yes
37	OP965125	*Streptococcus* sp.	99.58	Oral cavity and respiratory tract	Yes
38	OP965124	*Streptococcus* sp.	99.58	Oral cavity and respiratory tract	Yes
39	OP965123	*Porphyromonas* sp.	100	Oral cavity, gastrointestinal tract, respiratory tract, and colon	Yes
40	OP965122	*Granulicatella adiacens*	100	Oral cavity	Yes
41	OP965121	*Streptococcus* sp.	99.79	Oral cavity and respiratory tract	Yes
42	OP965050	*Pantoea dispersa*	100	None	Yes
43	OP965027	*Raoultella terrigena*	100	None	Yes
44	OP965045	*Pantoea agglomerans*	99.57	None	Yes
45	OP965058	*Pantoea calida*	100	None	Yes
46	OP965057	*Pantoea calida*	100	None	Yes

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47	OP965161	*Escherichia coli*	100	Gastrointestinal tract	Yes
48	OP965160	*Escherichia coli*	99.79	Gastrointestinal tract	Yes
49	OP965164	*Escherichia coli*	99.55	Gastrointestinal tract	Yes
50	OP965163	*Escherichia coli*	99.78	Gastrointestinal tract	Yes
51	OP965162	*Escherichia coli*	99.78	Gastrointestinal tract	Yes
52	OP965062	*Pantoea* sp.	99.79	None	Yes
53	OP965063	*Pseudomonas psychrotolerans*	100	None	No
54	OP965026	*Staphylococcus warneri*	99.79	Skin	Yes
55	OP965042	*Staphylococcus aureus*	100	Respiratory tract	Yes
56	OP965023	*Aerococcus viridans*	99.19	Skin and respiratory tract	Yes
57	OP965034	*Staphylococcus warneri*	100	Skin	Yes
58	OP965085	*Paludibacter propionicigenes*	97.89	None	No
59	OP965086	*Acinetobacter johnsonii*	99.35	None	No
60	OP965087	*Dorea longicatena*	99.79	Gastrointestinal tract	No
61	OP965088	*Lawsonella clevelandensis*	98.65	Abscess	Yes
62	OP965089	*Veillonella* sp.	98.96	Oral cavity	Yes
63	OP965090	*Ralstonia pickettii*	99.36	None	Yes
64	OP965091	*Streptococcus mitis*	99.37	Oral cavity	Yes
65	OP965092	*Veillonella* sp.	99.8	Oral cavity	Yes
66	OP965093	*Dorea longicatena*	99.58	Gastrointestinal tract	No
67	OP965094	*Bacillus* sp.	98.94	None	No
68	OP965095	*Cutibacterium acnes*	99.78	Skin	Yes
69	OP965096	*Hymenobacter terrenus*	93.45	None	No
70	OP965097	*Thermomonas* sp.	99.57	None	No
71	OP965098	*Corynebacterium* sp.	99.53	Skin and mucous membranes	No
72	OP965099	*Lawsonella clevelandensis*	99.55	Abscess	Yes
73	OP965100	*Cutibacterium acnes*	100	Skin	Yes
74	OP965101	*Corynebacterium freneyi*	100	Skin	No
75	OP965102	*Streptococcus pseudopneumoniae*	98.95	Respiratory tract	Yes
76	OP965103	*Janthinobacterium* sp.	99.34	None	No
77	OP965104	*Propioniciclava soli*	93.19	None	No
78	OP965112	*Gemmatirosa kalamazoonesis*	91.41	None	No
79	OP965113	*Exiguobacterium undae*	99.8	None	No
80	OP965108	*Propioniciclava soli*	93.17	None	No
81	OP965110	*Veillonella* sp.	99.59	Oral cavity	Yes
82	OP965111	*Massilia* sp.	98.92	None	No
83	OP965109	*Nafulsella* sp.	91.67	None	No
84	OP965107	*Phreatobacter cathodiphilus*	97.38	None	No
85	OP965106	*Fusobacteriaceae bacterium*	95.1	Oral cavity, respiratory, gastrointestinal, and urinary tracts	Yes
86	OP965105	Uncultured organism clone	98.28	None	Unkown

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87	OP965114	*Modestobacter* sp.	97.21	None	No
88	OP965059	*Pseudomonas stutzeri*	100	None	Yes
89	OP965064	*Lelliottia amnigena*	100	None	Yes
90	OP965065	*Pantoea* sp.	100	None	Yes
91	OP965024	*Pantoea eucrina*	100	None	No
92	OP965043	*Staphylococcus simulans*	100	Skin	Yes
93	OP965025	*Staphylococcus aureus*	100	Respiratory tract	Yes
94	OP965044	*Staphylococcus simulans*	99.37	Skin	Yes
95	OP965035	*Staphylococcus saprophyticus*	100	Gastrointestinal tract	Yes
96	OP965036	*Bacillus subtilis*	100	None	No
97	OP965060	*Enterococcus hirae*	99.81	None	Yes
98	OP965046	*Staphylococcus xylosus*	100	Skin	Yes
99	OP965159	*Thioclava* sp	100	None	No
100	OP965158	*Flavobacterium* sp.	97.86	None	No
101	OP965157	*Lactobacillus johnsonii*	99.6	Gastrointestinal tract	No
102	OP965156	*Micrococcus luteus*	100	Skin and respiratory tract	Yes
103	OP965155	*Anaerotignum* sp.	97.67	None	No
104	OP965154	*Comamonas* sp.	99.64	None	No
105	OP965153	*Mesorhizobium loti*	99.76	None	No
106	OP965152	*Rhizobium* sp.	100	None	No
107	OP965151	*Comamonas aquatica*	99.64	None	No
108	OP965150	*Shewanella xiamenensis*	99.86	None	Yes
109	OP965149	*Neisseria mucosa*	100	Respiratory tract	Yes
110	OP965147	*Pseudomonas plecoglossicida*	99.57	None	Yes
111	OP965146	*Pseudomonas putida*	99.79	None	Yes
112	OP965132	*Staphylococcus lugdunensis*	99.79	Skin	Yes
113	OP965133	*Fusobacterium mortiferum*	99.54	Oral cavity	Yes
114	OP965134	*Comamonas aquatica*	100	None	No
115	OP965135	*Micrococcus luteus*	98.97	Skin and respiratory tract	Yes
116	OP965136	*Rubellimicrobium* sp.	98.27	None	No
117	OP965137	Uncultured organism clone	99.78	None	Unkown
118	OP965138	*Methylovirgula* sp.	100	None	No
119	OP965139	*Comamonas aquatica*	99.78	None	No
120	OP965140	*Pseudocitrobacter faecalis*	97.41	Gastrointestinal tract and blood	Yes
121	OP965141	*Haematobacter massiliensis*	100	Respiratory tract	Yes
122	OP965142	*Pseudomonas plecoglossicida*	98.94	None	Yes
123	OP965143	*Donghicola* sp.	97.59	None	No
124	OP965144	*Hymenobacter glacieicola*	99.54	None	No
125	OP965051	*Pantoea agglomerans*	100	None	Yes
126	OP965031	*Klebsiella* sp.	100	Skin and gastrointestinal tract	Yes
127	OP965052	*Enterobacter kobei*	100	Urinary tract	Yes
128	OP965145	*Pseudomonas* sp.	100	None	Yes

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129	OP965037	*Erwinia gerundensis*	99.57	None	No
130	OP965030	*Pantoea* sp.	100	None	Yes
131	OP965053	*Pantoea agglomerans*	99.57	None	Yes
132	OP965038	*Staphylococcus aureus*	100	Respiratory tract	Yes
133	OP965039	*Staphylococcus haemolyticus*	100	Skin	Yes
134	OP965174	*Blastococcus saxobsidens*	98.85	None	No
135	OP965173	*Cutibacterium acnes*	99.11	Skin	Yes
136	OP965172	*Cutibacterium acnes*	99.56	Skin	Yes
137	OP965171	*Cutibacterium acnes*	99.33	Skin	Yes
138	OP965170	*Paracoccus* sp.	99.52	None	Yes
139	OP965169	*Corynebacterium tubercu-lostearicum*	98.87	Skin	Yes
140	OP965168	*Belnapia* sp.	97.58	None	No
141	OP965167	*Paracoccus* sp.	98.82	None	Yes
142	OP965166	*Micrococcus luteus*	99.55	Skin and respiratory tract	Yes
143	OP965165	*Blastococcus aggregatus*	98.86	None	No
144	OP965189	*Cutibacterium acnes*	98.9	Skin	Yes
145	OP965190	*Achromobacter xylosoxidans*	99.36	None	Yes
146	OP965191	*Tepidomonas* sp.	99.79	None	No
147	OP965192	*Aeribacillus* sp.	97.55	None	No
148	OP965193	*Cutibacterium acnes*	99.11	Skin	Yes
149	OP965194	*Tepidomonas* sp.	99.13	None	No
150	OP965195	*Cupriavidus metallidurans*	99.14	None	No
151	OP965196	*Cutibacterium acnes*	99.56	Skin	Yes
152	OP965197	*Aeribacillus pallidus*	99.17	None	No
153	OP965198	*Achromobacter xylosoxidans*	99.78	None	Yes
154	OP965199	*Cupriavidus metallidurans*	99.36	None	No
155	OP965200	*Cutibacterium acnes*	98.92	Skin	Yes
156	OP965201	*Chryseobacterium hispanicum*	100	None	No
157	OP965202	Bacteroidetes bacterium	87.86	None	Yes
158	OP965203	*Chryseolinea soli*	89.33	None	No
159	OP965204	*Caldalkalibacillus uzonensis*	96.5	None	No
160	OP965205	*Escherichia coli*	97.12	Gastrointestinal tract	Yes
161	OP965206	*Mesorhizobium huakuii*	99.02	None	No
162	OP965207	*Mesorhizobium huakuii*	99.02	None	No
163	OP965208	*Mesorhizobium huakuii*	99.01	None	No
164	OP965209	*Mesorhizobium huakuii*	98.77	None	No
165	OP965210	*Streptococcus thermophilus*	99.15	None	No
166	OP965211	*Mesorhizobium terrae*	99.76	None	No
167	OP965212	*Mesorhizobium huakuii*	100	None	No
168	OP965213	*Lautropia mirabilis*	99.06	Oral cavity	No
169	OP965214	*Bacillus* sp.	97.03	None	No

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170	OP965215	*Veillonella* sp.	98.77	Oral cavity	Yes
171	OP965216	*Mesorhizobium huakuii*	99.02	None	No
172	OP965217	*Corynebacterium tuberculostearicum*	99.77	Skin	Yes
173	OP965218	*Brevitalea aridisoli*	86.88	None	No
174	OP965219	*Nocardioides iriomotensis*	99.29	None	No
175	OP965220	*Marmoricola* sp.	96.8	None	No
176	OP965221	*Blastococcus aggregatus*	100	None	No
177	OP965222	*Corynebacterium simulans*	99.08	Skin	Yes
178	OP965223	*Lactobacillus crispatus*	99.59	Gastrointestinal tract	No
179	OP965224	*Aerococcus viridans*	99.57	Skin and respiratory tract	Yes
180	OP965225	*Cutibacterium acnes*	95.58	Skin	Yes
181	OP965226	*Citricoccus* sp.	93.56	None	No
182	OP965227	*Phenylobacterium* sp.	95.26	None	No
183	OP965228	*Arthrobacter agilis*	99.78	None	No
184	OP965229	*Novosphingobium silvae*	100	None	No
185	OP965230	*Mesorhizobium huakuii*	99.04	None	No
186	OP965231	Clostridiaceae bacterium	94.52	None	No
187	OP965232	*Escherichia coli*	98.09	Gastrointestinal tract	Yes
188	OP965233	*Escherichia coli*	98.54	Gastrointestinal tract	Yes
189	OP965234	*Streptococcus* sp.	100	Oral cavity, and respiratory tract	Yes
190	OP965235	*Paracoccus* sp.	100	None	Yes
191	OP965236	*Vagococcus fessus*	98.51	None	Yes
192	OP965237	*Corynebacterium aurimucosum*	98.14	Urinary tract	Yes
193	OP965238	*Paracoccus marcusii*	100	None	Yes
194	OP965239	*Cutibacterium acnes*	99.78	Skin	Yes
195	OP965240	*Cutibacterium acnes*	99.33	Skin	Yes
196	OP965241	*Mesorhizobium huakuii*	99.26	None	No
197	OP965242	*Cutibacterium acnes*	98.88	Skin	Yes
198	OP965243	*Kocuria palustris*	97.8	None	No
199	OP965244	*Methyloligella* sp.	93.4	None	No
200	OP965245	*Pseudomonas* sp.	99.57	None	Yes
201	OP965246	*Mesorhizobium huakuii*	98.3	None	No
202	OP965247	*Streptococcus pseudopneumoniae*	100	Respiratory tract	Yes
203	OP965248	*Mesorhizobium huakuii*	99.51	None	No
204	OP965249	*Pseudomonas* sp.	99.14	None	Yes
205	OP965250	*Mesorhizobium huakuii*	98.77	None	No
206	OP965251	*Mesorhizobium huakuii*	99.03	None	No
207	OP965252	*Mesorhizobium huakuii*	98.53	None	No
208	OP965253	*Aeribacillus pallidus*	99.79	None	No

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209	OP965254	*Mesorhizobium huakuii*	99.26	None	No
210	OP965255	*Mesorhizobium huakuii*	98.07	None	No
211	OP965256	*Mesorhizobium huakuii*	99.01	None	No
212	OP965257	*Mesorhizobium huakuii*	97.62	None	No
213	OP965258	*Mesorhizobium alhagi*	98.598	None	No
214	OP965259	*Paracoccus* sp.	100	None	Yes
215	OP965260	*Mesorhizobium alhagi*	97.9	None	No
216	OP965261	*Kocuria rhizophila*	98.02	None	No
217	OP965262	*Mesorhizobium huakuii*	98.83	None	No
218	OP965263	*Mesorhizobium huakuii*	99.02	None	No
219	OP965264	*Deinococcus budaensis*	94.41	None	No
220	OP965265	*Kocuria rhizophila*	98.25	None	No
221	OP965266	*Mesorhizobium terrae*	99.75	None	No
222	OP965267	*Mesorhizobium loti*	100	None	No
223	OP965268	*Kocuria rhizophila*	98.46	None	No
224	OP965269	*Mesorhizobium loti*	100	None	No
225	OP965270	*Kocuria rhizophila*	98.03	None	No
226	OP965271	*Kocuria rhizophila*	99.33	None	No
227	OP965272	*Kocuria rhizophila*	99.77	None	No
228	OP965273	*Mesorhizobium huakuii*	99.51	None	No
229	OP965274	*Achromobacter xylosoxidans*	97.93	None	Yes
230	OP965275	*Kocuria* sp.	99.14	None	Yes
231	OP965276	*Facklamia* sp.	98.94	None	Yes
232	OP965277	*Labedella gwakjiensis*	98.42	None	No
233	OP965278	*Mesorhizobium huakuii*	98.31	None	No
234	OP965279	*Mesorhizobium terrae*	98.1	None	No
235	OP965280	*Mesorhizobium huakuii*	98.8	None	No
236	OP965281	*Mesorhizobium* sp.	99.26	None	No
237	OP965282	*Cutibacterium acnes*	99.33	Skin	Yes
238	OP965283	*Mesorhizobium* sp.	99.26	None	No
239	OP965284	*Mesorhizobium* sp.	99.27	None	No
240	OP965285	*Mesorhizobium alhagi*	97.88	None	No
241	OP965286	*Mesorhizobium huakuii*	98.8	None	No
242	OP965287	*Lawsonella clevelandensis*	98.85	Abscess	Yes
243	OP965288	*Staphylococcus caprae*	98.3	Blood	Yes
244	OP965289	*Mesorhizobium huakuii*	99.26	None	No
245	OP965290	Uncultured organism clone	98.52	None	Unkown
246	OP965291	*Hymenobacter koreensis*	97.69	None	No
247	OP965292	*Mesorhizobium terrae*	98.57	None	No
248	OP965293	*Cutibacterium acnes*	98.04	Skin	Yes
249	OP965294	*Cutibacterium acnes*	98.26	Skin	Yes
250	OP965295	*Mesorhizobium huakuii*	99.03	None	No

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251	OP965296	*Luteimonas* sp.	94.32	None	No
252	OP965183	*Mesorhizobium huakuii*	99.03	None	No
253	OP965184	*Neobacillus ginsengisoli*	98.09	None	No
254	OP965185	*Agrococcus* sp.	99.1	None	No
255	OP965186	*Friedmanniella* sp.	98.38	Gastrointestinal tract	No
256	OP965187	*Rhodococcus cornyebacterioides*	99.05	None	No
257	OP965188	*Paracoccus* sp.	98.83	None	Yes
258	OP965175	*Escherichia coli*	98.93	Gastrointestinal tract	Yes
259	OP965176	*Escherichia coli*	98.53	Gastrointestinal tract	Yes
260	OP965177	*Escherichia coli*	98.73	Gastrointestinal tract	Yes
261	OP965178	*Escherichia* sp.	99.36	Gastrointestinal tract	Yes
262	OP965179	*Escherichia coli*	99.39	Gastrointestinal tract	Yes
263	OP965180	*Escherichia coli*	98.54	Gastrointestinal tract	Yes
264	OP965181	*Escherichia coli*	98.76	Gastrointestinal tract	Yes
265	OP965182	*Escherichia coli*	98.76	Gastrointestinal tract	Yes
266	OP965048	*Pantoea agglomerans*	100	None	Yes
267	OP965049	*Pantoea agglomerans*	100	None	Yes
268	OP965055	*Pseudomonas* sp.	97.51	None	Yes
269	OP965056	*Pantoea* sp.	99.57	None	Yes
270	OP965028	*Leclercia adecarboxylata*	100	None	Yes
271	OP965029	*Acinetobacter* sp.	99.79	None	No
272	OP965041	*Staphylococcus warneri*	99.79	Skin	Yes
273	OP965040	*Staphylococcus saprophyticus*	100	Gastrointestinal tract	Yes
274	OP965032	*Staphylococcus aureus*	100	Respiratory tract	Yes
275	OP965301	*Chroococcidiopsis* sp.	97.38	None	No
276	OP965300	*Vicia faba chloroplast*	99.53	None	No
277	OP965299	*Vicia faba chloroplast*	99.75	None	No
278	OP965298	*Colquhounia coccinea* chloroplast	99.76	None	No
279	OP965297	*Klebsormidium flaccidum* chloroplast	99.76	None	No

where:

None is used for species that are predominant in environmental niches.

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References

1. Catalano, A.; Iacopetta, D.; Ceramella, J.; Scumaci, D.; Giuzio, F.; Saturnino, C.; Aquaro, S.; Rosano, C.; Sinicropi, M.S. Multidrug Resistance (MDR): A Widespread Phenomenon in Pharmacological Therapies. *Molecules* **2022**, *27*, 1–18, doi:10.3390/molecules27030616.
2. Yeh, P.J.; Simon, D.M.; Millar, J.A.; Alexander, H.F.; Franklin, D. A Diversity of Antibiotic-Resistant Staphylococcus Spp. in a Public Transportation System. *Osong Public Health Res Perspect* **2011**, *2*, 202–209, doi:10.1016/j.phrp.2011.11.047.
3. Romo-Castillo, H.F.; Pazin-Filho, A. Towards Implementing an Antibiotic Stewardship Programme (ASP) in Ecuador: Evaluating Antibiotic Consumption and the Impact of an ASP in a Tertiary Hospital According to World Health Organization (WHO) Recommendations. *J Glob Antimicrob Resist* **2022**, *29*, 462–467, doi:10.1016/j.jgar.2021.11.001.
4. Tahlan, K.; Jensen, S.E. Origins of the β-Lactam Rings in Natural Products. *Journal of Antibiotics* **2013**, *66*, 401–410, doi:10.1038/ja.2013.24.
5. Tooke, C.L.; Hinchliffe, P.; Bragginton, E.C.; Colenso, C.K.; Hirvonen, V.H.A.; Takebayashi, Y.; Spencer, J. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *J Mol Biol* **2019**, *431*, 3472–3500, doi:10.1016/j.jmb.2019.04.002.
6. Castanheira, M.; Simner, P.J.; Bradford, P.A. Extended-Spectrum β-Lactamases: An Update on Their Characteristics, Epidemiology and Detection. *JAC Antimicrob Resist* **2021**, *3*, doi:10.1093/jacamr/dlab092.
7. Gutiérrez-Gutiérrez, B.; Rodríguez-Baño, J. Current Options for the Treatment of Infections Due to Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae in Different Groups of Patients. *Clinical Microbiology and Infection* **2019**, *25*, 932–942, doi:10.1016/j.cmi.2019.03.030.
8. Boyd, S.E.; Livermore, D.M.; Hooper, D.C.; Hope, W.W. Metallo-β-Lactamases: Structure, Function, Epidemiology, Treatment Options, and the Development Pipeline. *Antimicrob Agents Chemother* **2020**, *64*, doi:10.1128/AAC.00397-20.
9. Algammal, A.M.; Hetta, H.F.; Elkelish, A.; Alkhalifah, D.H.H.; Hozzein, W.N.; Batiha, G.E.S.; Nahhas, N. el; Mabrok, M.A. Methicillin-Resistant Staphylococcus Aureus (MRSA): One Health Perspective Approach to the Bacterium Epidemiology, Virulence Factors, Antibiotic-Resistance, and Zoonotic Impact. *Infect Drug Resist* **2020**, *13*, 3255–3265, doi:10.2147/IDR.S272733.
10. Ubukata, K.; Nonoguchi, R.; Matsuhashi, M.; Konno, M. Expression and Inducibility in Staphylococcus Aureus of the MecA Gene, Which Encodes a Methicillin-Resistant S. Aureus-Specific Penicillin-Binding Protein. *J Bacteriol* **1989**, *171*, 2882–2885, doi:10.1128/jb.171.5.2882-2885.1989.
11. Shore, A.C.; Rossney, A.S.; Brennan, O.M.; Kinnevey, P.M.; Humphreys, H.; Sullivan, D.J.; Goering, R. v.; Ehricht, R.; Monecke, S.; Coleman, D.C. Characterization of a Novel Arginine Catabolic Mobile Element (ACME) and Staphylococcal Chromosomal Cassette Mec Composite Island with Significant Homology to Staphylococcus Epidermidis ACME Type II in Methicillin-Resistant Staphylococcus Aureus Genotype ST22-MRSA-IV. *Antimicrob Agents Chemother* **2011**, *55*, 1896–1905, doi:10.1128/AAC.01756-10.
12. Becker, K.; van Alen, S.; Idelevich, E.A.; Schleimer, N.; Seggewiß, J.; Mellmann, A.; Kaspar, U.; Peters, G. Plasmid-Encoded Transferable Mecb-Mediated Methicillin Resistance in Staphylococcus Aureus. *Emerg Infect Dis* **2018**, *24*, 242–248, doi:10.3201/eid2402.171074.
13. Lakhundi, S.; Zhang, K. Methicillin-Resistant Staphylococcus Aureus: Molecular Characterization, Evolution, and Epidemiology. *Clin Microbiol Rev* 2018, *31*.
14. Larsson, D.G.J.; Flach, C.F. Antibiotic Resistance in the Environment. *Nat Rev Microbiol* 2022, *20*, 257–269.
15. World Health Organization *Global Action Plan on Antmicrobial Resistance*; 2018; Vol. 1, pp. 1–28;.
16. Access, O. The Metagenomics and Metadesign of the Subways and Urban Biomes (MetaSUB) International Consortium Inaugural Meeting Report. *Microbiome* **2016**, *4*, 24, doi:10.1186/s40168-016-0168-z.

17. Klimenko, N.S.; Tyakht, A. v.; Toshchakov, S. v.; Shevchenko, M.A.; Korzhenkov, A.A.; Afshinnekoo, E.; Mason, C.E.; Alexeev, D.G. Co-Occurrence Patterns of Bacteria within Microbiome of Moscow Subway. *Comput Struct Biotechnol J* **2020**, *18*, 314–322, doi:10.1016/j.csbj.2020.01.007.
18. INEC INEC Presenta Sus Proyecciones Poblacionales Cantonales | Instituto Nacional de Estadística y Censos. 2022.
19. Rawlinson, S.; Ciric, L.; Cloutman-Green, E. How to Carry out Microbiological Sampling of Healthcare Environment Surfaces? A Review of Current Evidence. *Journal of Hospital Infection* **2019**, *103*, 363–374, doi:10.1016/j.jhin.2019.07.015.
20. Chen, Y.L.; Lee, C.C.; Lin, Y.L.; Yin, K.M.; Ho, C.L.; Liu, T. Obtaining Long 16S RDNA Sequences Using Multiple Primers and Its Application on Dioxin-Containing Samples. *BMC Bioinformatics* **2015**, *16*, doi:10.1186/1471-2105-16-S18-S13.
21. Yamaguchi, T.; Kawahara, R.; Harada, K.; Teruya, S.; Nakayama, T.; Motooka, D.; Nakamura, S.; do Nguyen, P.; Kumeda, Y.; Dang, C. van; et al. The Presence of Colistin Resistance Gene Mcr-1 and -3 in ESBL Producing Escherichia Coli Isolated from Food in Ho Chi Minh City, Vietnam. *FEMS Microbiol Lett* 2018, *365*.
22. Poirel, L.; Walsh, T.R.; Cuvillier, V.; Nordmann, P. Multiplex PCR for Detection of Acquired Carbapenemase Genes. *Diagn Microbiol Infect Dis* **2011**, *70*, 119–123, doi:10.1016/J.DIAGMICROBIO.2010.12.002.
23. Zhang, K.; McClure, J.A.; Elsayed, S.; Louie, T.; Conly, J.M. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome Mec Types I to V in Methicillin-Resistant Staphylococcus Aureus. *J Clin Microbiol* **2005**, *43*, 5026–5033, doi:10.1128/JCM.43.10.5026-5033.2005.
24. Stegger, M.; Andersen, P.S.; Kearns, A.; Pichon, B.; Holmes, M.A.; Edwards, G.; Laurent, F.; Teale, C.; Skov, R.; Larsen, A.R. Rapid Detection, Differentiation and Typing of Methicillin-Resistant Staphylococcus Aureus Harbouring Either MecA or the New MecA Homologue MecALGA251. *Clinical Microbiology and Infection* **2012**, *18*, 395–400, doi:10.1111/j.1469-0691.2011.03715.x.
25. Schwendener, S.; Cotting, K.; Perreten, V. Novel Methicillin Resistance Gene MecD in Clinical Macrococcus Caseolyticus Strains from Bovine and Canine Sources. *Sci Rep* **2017**, *7*, doi:10.1038/srep43797.
26. Hanahan, D. Studies on Transformation of Escherichia Coli with Plasmids,. *J Mol Biol* **1983**, *166*, 557–580, doi:doi.org/10.1016/S0022-2836(83)80284-8.
27. Bimboim, H.C.; Doly, J. A Rapid Alkaline Extraction Procedure for Screening Recombinant Plasmid DNA. *Nucleic Acids Res* **1979**, *7*, 1513–1523, doi:10.1093/nar/7.6.1513.
28. Schloss, P.D.; Westcott, S.L. Assessing and Improving Methods Used in Operational Taxonomic Unit-Based Approaches for 16S RRNA Gene Sequence Analysis. *Appl Environ Microbiol* **2011**, *77*, 3219–3226, doi:10.1128/AEM.02810-10.
29. Wood, D.E.; Salzberg, S.L. Kraken: Ultrafast Metagenomic Sequence Classification Using Exact Alignments; 2014;
30. Ondov, B.D.; Bergman, N.H.; Phillippy, A.M. Interactive Metagenomic Visualization in a Web Browser. *BMC Bioinformatics* **2011**, *12*, doi:10.1186/1471-2105-12-385.
31. Cuccuru, G.; Orsini, M.; Pinna, A.; Sbardellati, A.; Soranzo, N.; Travaglione, A.; Uva, P.; Zanetti, G.; Fotia, G. Orione, a Web-Based Framework for NGS Analysis in Microbiology. *Bioinformatics* **2014**, *30*, 1928–1929, doi:10.1093/bioinformatics/btu135.
32. Mahalingam, N.; Manivannan, B.; Khamari, B.; Siddaramappa, S.; Adak, S.; Bulagonda, E.P. Detection of Antibiotic Resistance Determinants and Their Transmissibility among Clinically Isolated Carbapenem-Resistant Escherichia Coli from South India. *Medical Principles and Practice* **2018**, *27*, 428–435, doi:10.1159/000489885.
33. Bastidas-caldes, C.; Romero-alvarez, D.; Valdez-vélez, V.; Morales, R.D.; Montalvo-hernández, A.; Gomes-dias, C.; Calvopiña, M. Extended-Spectrum Beta-Lactamases Producing Escherichia Coli in South America : A Systematic Review with a One Health Perspective. **2022**, 5759–5779.
34. Bastidas, C.A.; Villacrés-Granda, I.; Navarrete, D.; Monsalve, M.; Coral-Almeida, M.; Cifuentes, S.G. Antibiotic Susceptibility Profile and Prevalence of MecA and LukS-PV/LukF-PV Genes in Staphylococcus Aureus Isolated from Nasal and Pharyngeal Sources of Medical Students in Ecuador. *Infect Drug Resist* **2019**, *12*, 2553–2560, doi:10.2147/IDR.S219358.
35. Brito, B.P.; Koong, J.; Wozniak, A.; Opazo-Capurro, A.; To, J.; Garcia, P.; Hamidian, M. Genomic Analysis of Carbapenem-Resistant Acinetobacter Baumannii Strains Recovered from Chilean Hospitals Reveals Lineages Specific to South America and Multiple Routes for Acquisition of Antibiotic Resistance Genes. *Microbiol Spectr* **2022**, doi:10.1128/spectrum.02463-22.
36. Richards, A.M.; Abu Kwaik, Y.; Lamont, R.J. Code Blue: Acinetobacter Baumannii, a Nosocomial Pathogen with a Role in the Oral Cavity. *Mol Oral Microbiol* **2015**, *30*, 2–15, doi:10.1111/omi.12072.
37. Manishimwe, R.; Moncada, P.M.; Bugarel, M.; Scott, H.M.; Loneragan, G.H. Antibiotic Resistance among Escherichia Coli and Salmonella Isolated from Dairy Cattle Feces in Texas. *PLoS One* **2021**, *16*, doi:10.1371/journal.pone.0242390.
38. Debergh, H.; Maex, M.; Garcia-Graells, C.; Boland, C.; Saulmont, M.; van Hoorde, K.; Saegerman, C. First Belgian Report of Ertapenem Resistance in an ST11 Klebsiella Pneumoniae Strain Isolated from a Dog Carrying BlaSCO-1 and BlaDHA-1 Combined with Permeability Defects. *Antibiotics* **2022**, *11*, doi:10.3390/antibiotics11091253.

39. Wyres, K.L.; Lam, M.M.C.; Holt, K.E. Population Genomics of Klebsiella Pneumoniae. *Nat Rev Microbiol* 2020, *18*, 344–359.
40. Bratulic, S.; Toll-Riera, M.; Wagner, A. Mistranslation Can Enhance Fitness through Purging of Deleterious Mutations. *Nat Commun* **2017**, *8*, 1–9, doi:10.1038/ncomms15410.
41. Chaïbi, E.B.; Sirot, D.; Paul, G.; Labia, R. Inhibitor-Resistant TEM β-Lactamases: Phenotypic, Genetic and Biochemical Characteristics. *Journal of Antimicrobial Chemotherapy* **1999**, *43*, 447–458, doi:10.1093/jac/43.4.447.
42. Yang, J.J.; Cheng, A.; Tai, H.M.; Chang, L.W.; Hsu, M.C.; Sheng, W.H. Selected Mutations by Nemonoxacin and Fluoroquinolone Exposure among Relevant Gram-Positive Bacterial Strains in Taiwan. *Microbial Drug Resistance* **2020**, *26*, 110–117, doi:10.1089/mdr.2019.0048.
43. Mendonça, J.; Guedes, C.; Silva, C.; Sá, S.; Oliveira, M.; Accioly, G.; Baylina, P.; Barata, P.; Pereira, C.; Fernandes, R. New CTX-M Group Conferring B-Lactam Resistance: A Compendium of Phylogenetic Insights from Biochemical, Molecular, and Structural Biology. *Biology (Basel)* **2022**, *11*, doi:10.3390/biology11020256.
44. Poirel, L.; Madec, J.-Y.; Lupo, A.; Schink, A.-K.; Kieffer, N.; Nordmann, P.; Schwarz, S. Antimicrobial Resistance in Escherichia Coli . *Microbiol Spectr* **2018**, *6*, doi:10.1128/microbiolspec.arba-0026-2017.
45. Baroja, I.; Guerra, S.; Coral-Almeida, M.; Ruíz, A.; Galarza, J.M.; de Waard, J.H.; Bastidas-Caldes, C. Methicillin-Resistant Staphylococcus Aureus Nasal Colonization among Health Care Workers of a Tertiary Hospital in Ecuador and Associated Risk Factors. *Infect Drug Resist* **2021**, *14*, 3433–3440, doi:10.2147/IDR.S326148.
46. Nimer, N.A. Nosocomial Infection and Antibiotic-Resistant Threat in the Middle East. *Infect Drug Resist* 2022, *15*, 631–639.
47. Rey, J.; Gil, M.; de Mendoza, J.H.; García, A.; Gaitskell-Phillips, G.; Bastidas-Caldes, C.; Zalama, L. Clonality and Persistence of Multiresistant Methicillin-Resistant Coagulase-Negative Staphylococci Isolated from the Staff of a University Veterinary Hospital. *Antibiotics* **2022**, *11*, doi:10.3390/antibiotics11060811.
48. Gohli, J.; Bøifot, K.O.; Moen, L.V.; Pastuszek, P.; Skogan, G.; Udekwu, K.I.; Dybwad, M. The Subway Microbiome: Seasonal Dynamics and Direct Comparison of Air and Surface Bacterial Communities. *Microbiome* **2019**, *7*, doi:10.1186/s40168-019-0772-9.
49. GBD 2019 Antimicrobial Resistance Collaborators Global Mortality Associated with 33 Bacterial Pathogens in 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *Lancet* **2022**, *6736*, 1–28, doi:10.1016/S0140-6736(22)02185-7.
50. Tyakht, A. v.; Manolov, A.I.; Kanygina, A. v.; Ischenko, D.S.; Kovarsky, B.A.; Popenko, A.S.; Pavlenko, A. v.; Elizarova, A. v.; Rakitina, D. v.; Baikova, J.P.; et al. Genetic Diversity of Escherichia Coli in Gut Microbiota of Patients with Crohn's Disease Discovered Using Metagenomic and Genomic Analyses. *BMC Genomics* **2018**, *19*, doi:10.1186/s12864-018-5306-5.
51. Yamada, K.; Kashiwa, M.; Arai, K.; Satoyoshi, K.; Nishiyama, H. Pantoea Calida Bacteremia in an Adult with End-Stage Stomach Cancer under Inpatient Care. *Journal of Infection and Chemotherapy* **2017**, *23*, 407–409, doi:10.1016/j.jiac.2017.01.001.
52. Ioannou, P.; Vougiouklakis, G. A Systematic Review of Human Infections by Pseudomonas Mendocina. *Trop Med Infect Dis* 2020, *5*.
53. Visca, P.; Petrucca, A.; de Mori, P.; Festa, A.; Boumis, E.; Antinori, A.; Petrosillo, N. Dispatches Emerging Infectious Diseases Community-Acquired Acinetobacter Radioresistens Bacteremia in an HIV-Positive Patient; 2020; Vol. 7;.
54. Oude Munnink, B.B.; Worp, N.; Nieuwenhuijse, D.F.; Sikkema, R.S.; Haagmans, B.; Fouchier, R.A.M.; Koopmans, M. The next Phase of SARS-CoV-2 Surveillance: Real-Time Molecular Epidemiology. *Nat Med* 2021, *27*, 1518–1524.