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Original Article

Resistance determinants of emerging pathogens isolated from an intensive care unit as a parameter of population health conditions of the Legal Amazon microregion

Determinantes de resistência a patógenos emergentes isolados de uma unidade de terapia intensiva como parâmetro das condições de saúde da população da microrregião da Amazônia Legal

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Abstract

Bacteria responsible for causing infections are common in hospital environments, water, soil, and food products. The infection risk is intensified by the absence of public sanitation, poor quality of life, and food scarcity. These external factors promote the dissemination of pathogens by direct contamination or biofilm formation. In this work, we identified bacterial isolates obtained from intensive care units in the southern region of Tocantins, Brazil. We compared matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) techniques and 16S ribosomal ribonucleic acid (rRNA) molecular analysis; we also performed phenotypic characterization. Fifty-six isolates characterized using morphotinctorial tests were classified as gram-positive (80.4%; n = 45) and gram-negative (19.6%; n = 11) and were resistant to several antibiotic classes; notably, we identified the bla_{OXA-23} resistance gene in the ILH10 isolate. Microbial identification using MALDI-TOF MS resulted in the identification of *Sphingomonas paucimobilis* and *Bacillus circulans*. 16S rRNA sequencing revealed four isolates belonging to the genera *Bacillus* and *Acinetobacter*. The similarity was superior to 99% for *Acinetobacter schindleri* in the Basic Local Alignment Search Tool (BLAST), grouped in the clade superior to 90%. Several strains isolated from intensive care units (ICU) were resistant to various antibiotic classes. These techniques allowed for the identification of several microorganisms of importance in public health, enabling improvements in human infection control and proving the quality of inputs, food, and water.

Keywords: multi-drug resistant bacteria, hospital environment, MALDI-TOF MS, 16S rRNA.

Resumo

As bactérias responsáveis por causar infecções são comuns em ambientes hospitalares, água, solo e produtos alimentícios. O risco de infecção é intensificado pela ausência de saneamento público, má qualidade de vida e escassez de alimentos. Esses fatores externos promovem a disseminação de patógenos por contaminação direta ou formação de biofilme. Neste trabalho, identificamos isolados bacterianos obtidos de unidades de terapia intensiva na região sul do Tocantins, Brasil. Comparamos técnicas de espectrometria de massa de tempo de voo com ionização por dessorção a laser assistida por matriz (MALDI-TOF MS) e análise molecular de ácido ribonucleico ribossômico 16S (rRNA); também realizamos caracterização fenotípica. Cinquenta e seis isolados caracterizados por testes morfotintoriais foram classificados como gram-positivos (80,3%; n = 45) e gram-negativos (19,6%; n = 11) e foram resistentes a várias classes de antibióticos; notavelmente, identificamos o gene de resistência bla_{OXA-23} no isolado de ILH10. A identificação microbiana usando MALDI-TOF MS resultou na identificação de *Sphingomonas paucimobilis* e *Bacillus circulans*. O sequenciamento do 16S rRNA revelou quatro isolados pertencentes aos gêneros *Bacillus* e *Acinetobacter*. A similaridade foi superior a 99% para *Acinetobacter schindleri* no BLAST, agrupado no clado superior

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a 90%. Várias cepas isoladas de ICU foram resistentes a várias classes de antibióticos. Essas técnicas permitiram a identificação de diversos microrganismos de importância em saúde pública, possibilitando melhorias no controle de infecções humanas e comprovando a qualidade dos insumos, alimentos e água.

Palavras-chave: bactéria multirresistente, ambiente hospitalar, MALDI-TOF MS, 16S rRNA.

1. Introduction

Bacterial infections inside and outside hospital environments are the primary causes of illness. The excessive use of antimicrobial agents in humans and domestic animals has generated high rates of bacterial resistance and has become a severe problem worldwide (Bonelli et al., 2014; Moretto et al., 2021). Community and environmental factors contribute to the emergence of multi-drug resistant bacteria, promoted by the lack of adequate public sanitation and the transmission of domestic effluents to water sources. These phenomena contribute to the spread of pathogens, especially in regions with low human development indexes (Gallini et al., 2010; Bartley et al., 2019; Moretto et al., 2021).

The emergence of new resistance mechanisms may arise in vulnerable communities, leading to the dissemination of bacterial genes via conjugative plasmids (Meirelles-Pereira et al., 2002; Cerceo et al., 2016). Among the primary multi-drug resistant bacteria (MDRs), including species in the genera *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Klebsiella*, and enterobacteria that are commonly found in hospital environments, water, soil, and food products (Logan, 2012; Smet et al., 2014; Ferreira et al., 2019; Arbab et al., 2021; Adwan and Omar, 2021). Food is a potential transmission source between the community and clinical settings (Carvalheira et al., 2021).

Another critical factor is the ability of bacteria to form a structural community of multiple bacterial cells (biofilm) characterized by high nutritional and metabolic versatility and adherence to living and non-living surfaces. These surfaces include glass, plastic, X-ray equipment, benches, beds, fans, and air circulation systems (Bernards et al., 2004; Gaddy et al., 2009).

Resistance to antibiotics and chemicals (disinfectants) contributes to greater pathogenicity (Gaddy et al., 2009; Nowak and Paluchowska, 2016). β-lactamases act as one of the primary mechanisms associated with resistance; β-lactam resistance is mediated by β-lactamases that inhibit antibiotics, including carbapenems, penicillins, cephalosporins, and monobactams. β-lactamases can be divided into class A (e.g. *Klebsiella pneumoniae* carbapenemase, class B metallo-β-lactamases [e.g. New Delhi metallo-β-lactamase, Verona integrin-encoded metallo-beta-lactamases, imipenem-resistant *Pseudomonas*]) and class D β-lactamases (e.g., oxacylinases) (Bush and Jacoby, 2010; Logan, 2012; Lee et al., 2016).

Conventional methods for diagnosing pathogens involve isolating the organism in culture with subsequent analysis of its phenotypic characteristics (i.e., microscopic and macroscopic morphologies) and biochemical evaluations on various substrates (Stępień-Pyśniak et al., 2017). Other methods used in clinical microbiology laboratories include molecular methods with gene sequencing of 16S rRNA and 23S rRNA. MALDI-TOF MS is a rapid and accurate identification technique for bacteria isolated from biological specimens and can detect various proteins from microbial species (Angeletti, 2017; Tsuchida et al., 2020).

The analysis of microbial diversity in different environments and the multi-drug resistant bacteria profile have essential implications for hospitals stemming from the indiscriminate use of antibiotics. The present work aimed to characterize microbial sources phenotypically in intensive care units (ICUs) and identify isolates using the MALDI-TOF MS and 16S rRNA molecular methods. These techniques are critical for public health because they provide actions to control human infections and benefit the population.

2. Materials and Methods

2.1. Study location; sample processing; ethical and legal permissions.

The Gurupi Regional Hospital – Tocantins – Brazil (11°43'48" S and 49°04'08" W) is located in the Legal Amazon microregion. It is a referral center for 18 municipalities: Aliança do Tocantins, Araguaçu, Alvorada, Cariri do Tocantins, Crixás do Tocantins, Dueré, Figueirópolis, Formoso do Araguaia, Jau do Tocantins, Lagoa da Confusão, Palmeiropolis, Paranã, Peixe, Sandolândia, São Valério, São Salvador, Sucupira, and Talisman. We used samples from beds of patients hospitalized for more than 48 hours. The collection was performed using sterile swabs passed on the bed surface and transported in Cary Blair medium. Samples were cultured on tryptic soy agar (Kasvi, Italy) and incubated aerobically at 37 °C overnight. Bacterial isolates were phenotypically characterized using manual Gram staining methods and measuring motility properties, growth at 44 °C, citrate utilization, oxidase and urease production, and oxidation/fermentation (Castilho et al., 2017). The Research Ethics Committee of the Federal University of Tocantins approved the work (protocol number 72773417.3.0000.5519). After issuing a substantiated opinion of this study, the protocol was forwarded to the Health Department of the State of Tocantins (SESAU/TO) in compliance with ordinance SES-391/2017.

2.2. Phenotypic analysis and resistance classification

We measured antibiotic sensitivity and resistance using the disk diffusion method, following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). The isolates were cultured in 100 ml of TSB broth (soy peptone 17 g/l, casein 3 g/l, dextrose 5 g/l, and monobasic potassium phosphate 5 g/l), stirring at 120 rpm at 37 °C for 8 h. Growth was adjusted to 10^8 colony-forming units/ml and seeded on Mueller-Hinton agar (Kasvi, Italy) between 18 and 24 hours of incubation at 37 °C for recording halo

formation (Castilho et al., 2017). Antibiotics (Sensifar®-Brasil) included ampicillin/sulbactam (20 µg), amikacin (30 µg), ceftazidime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), gentamicin (10 µg), imipenem (10 µg), levofloxacin (5 µg), linezolid (30 µg), meropenem (10 µg), minocycline (30 µg), norfloxacin (10 µg), piperacillin/tazobactam (110 µg), polymyxin B (330 IU), tetracycline (30 µg), and vancomycin (30 µg). Halo inhibition data were measured and ranked, with resistance to ≥ 1 antimicrobial agent in ≥ 3 antimicrobial categories considered MDR. Resistance to ≥ 1 antimicrobial agent in all but two antimicrobial classes was considered extensively drug-resistant (Magiorakos et al., 2012).

2.3. Molecular analysis and detection of genes

The polymerase chain reaction (PCR) was performed using primers for OXA-51, OXA-23, OXA-40, OXA-58 and the insertion sequence (IS) of the ISAba1 (Sohrabi et al., 2012) (Table 1). DNA extraction was performed using an extraction kit (Invitrogen-PureLink® Genomic DNA Kits. The PCR technique was used to amplify the fragments with a final volume of 20 µL containing 20 ng of DNA, 1.5 mM of $MgCl_2$, 250 µM dNTP, 1U Taq DNA Polymerase (Promega, Madison, WI, USA), and 0.47 µM primer. Reactions were carried out in a Thermocycler (Biocycler MJ96G, Applied Biosystems) under the following conditions: denaturation at 95 °C for 4 min; 35 cycles of denaturation (90 °C, 30 seconds), alignment (50 °C, 40 seconds) and extension (72 °C, 1 min); final extension at 72 °C for 5 min. PCR products were separated by 1.5% agarose gel electrophoresis, stained with 0.5 µg/mL ethidium bromide, and visualized using Gel Doc System XR (Bio-Rad, Laboratories, Hercules, CA, USA).

2.4. Identification of emerging pathogens

2.4.1. DNA extraction and sequencing of the 16S rRNA gene

Bacterial isolates were cultured in brain heart broth (Merck, Germany) and incubated overnight at 37 °C with continuous shaking at 120 rpm. DNA extraction was performed using an extraction kit (Invitrogen-PureLink® Genomic DNA Kits). PCR of the rDNA16S region amplified the DNA. The sequence of primers 16SF and 16SR amplify a region of 400 bp (16SF-ACTGCTATCCACCCTCAAAC and 16SR CTGGGTGAAGTTGTAATCGG). The PCR product was purified using exonuclease I and shrimp alkaline phosphatase. The integrity of the genomic DNA was tested by electrophoresis.

The sequencing of the 16S gene was performed on a 3500 sequencer (Thermo Fisher Scientific). Sequencing reactions were set up with 20 ng of DNA, 0.5 µl Big Dye V3.1 (Thermo Fisher Scientific), 2.0 µl of 5X sequencing buffer, and 3.2 µmol of primer. The continuous sequences were compared with reference and outgroup sequences from the National Center for Biotechnology Information using the BLAST system to identify similarities. Phylogenetic analysis of the isolates was performed using the maximum likelihood method and the Tamura-Nei model (Tamura and Nei, 1993) using a MEGA 11 (Tamura et al., 2021). Branch confidence was tested using 500 bootstrap repetitions.

2.4.2. Identification by MALDI-TOF MS

Isolates were seeded in Brain Heart Infusion (Merck, Germany), incubated at 37 ºC for 18 hours, and sent to Instituto Hermes Pardini (Belo Horizonte, Brazil) for microbial identification by automated mass spectrometry equipped with MALDI-TOF MS (BioMérieux, France). Briefly, a fresh colony was spread on a Vitek MS DS target slide, and the preparations were covered with 1 ml of VITEK MS-CHCA matrix solution (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and trifluoroacetic acid at 2.5%). After drying, the target plate was loaded into the Vitek MS mass spectrometer and air-dried for 1 to 2 minutes at room temperature. Microbial identification was achieved by obtaining spectra using MALDI-TOF and analyzing the spectra using the VITEK MS database. The spectral peaks were compared with the characteristic pattern of a species, genus, or family of microorganisms, resulting in the identification

Table 1. Target genes for PCR amplification, fragment size, and primer sequences.

Beds UTI	No. of bacterial colony	No. bacterial isolate	Fenotypic Characteristic		
			Cell Morphology	Gram positive [%]	Gram negative [%]
1	22	5	Cocci; diplococci; bacilli;	80	20
2	34	5	Cocci; diplococci; bacilli;	100	0
3	10	6	Cocci; diplococci; bacilli;	83.3	16.7
4	89	4	Cocci; diplococci; diplobacilli;	50	50
5	457	8	Cocci; diplococci; bacilli;	62.5	37.5
6	5	4	Cocci; diplococci; bacilli;	60	40
7	23	8	Bacillus; diplococci,	75	25
8	36	11	Cocci; diplococci; bacilli; sarcinas	72	27.2
9	8	5	Cocci; Bacilli	40	60
Total	677	56		80.3	19.6

(Dubois et al., 2012; Moon et al., 2013; Gonçalves et al., 2019). A percentage probability (99.9%) represents the similarity of specific peaks between the generated spectrum and the database spectra. Lower values were designated as inconclusive

3. Results

3.1. Phenotypic characterization, susceptibility testing, and detection of resistance genes

We identified 677 colonies from ICU bed surfaces; 56 bacterial isolates were obtained for characterization (8.2%; n = 677) (Table 2). Of these, 80.3% were gram-positive (n = 45) and 19.6% were gram-negative (n = 11) (Table 2). Sensitivity analysis revealed a resistance rate for cotrimoxazole of 44%, ceftazidime of 32%, ciprofloxacin of 28%, norfloxacin of 25%, and cefepime of 23%. The lowest resistance rate was obtained for minocycline and amikacin (1.7%) (Figure 1). Five isolates (ILH10, ILH14, ILH13, ILH16, and ILH2) were resistant to an antibiotic of various classes (penicillins, cephalosporins, quinolones, polymyxins, tetracycline, and sulfonamides) (Table 3). Another isolate (ILH11) showed resistance only to β-lactam class antibiotics (penicillin, cephalosporins, and carbapenems). There was no evidence for the presence of resistance genes related to bla_{OXA-51}, bla_{OXA-40}, bla_{OXA-58}, or the ISAba1 element. By contrast, we identified bla_{OXA-23} genes in the ILH10 isolate. There was no evidence of bla_{OXA-23}, bla_{OXA-51}, bla_{OXA-40}, bla_{OXA-58}, or ISAba1 in the isolates ILH11, ILH13, ILH14, ILH16, and ILH2; however, these isolates were resistant to at least one penicillin, cephalosporin, or quinolone (Tables 3 and 4).

3.2. Bacterial identification using 16S rRNA and MALDI-TOF MS

The isolates ILH10, ILH11, ILH13, and ILH2 were identified only in the sequencing of the 16S rRNA gene and belonged to the genera *Acinetobacter* and *Bacillus* (Table 3). The ILH10 isolate was subjected to phylogenetic analysis involving 19 nucleotide sequences from the genus *Acinetobacter* (Figure 2). All ambiguous positions were removed for each pair of sequences (pair deletion option). There were 1593 positions in the final dataset of the ILH10 isolate that showed more significant similarity with 99.3% to *Acinetobacter schindleri* in BLAST, grouping 90% in the clade with bootstrap higher than 90% (Table 3; Figure 2). The ILH13 isolate was subjected to phylogenetic analysis involving 18 nucleotide sequences from the genus *Bacillus* (Figure 2). All ambiguous positions were removed for each pair of sequences (pair deletion option). There were 1555 positions in the final dataset of the ILH13 isolate that showed more significant similarity with 100% to *Bacillus cereus* in BLAST, in the clade with bootstrap higher than 90% (Table 3; Figure 2). The ILH2 isolate was subjected to phylogenetic analysis involving 22 nucleotide sequences from the genus *Bacillus* (Figure 2). All ambiguous positions were removed for each pair of sequences (pair deletion option). There were 1561 positions in the final dataset of the ILH2 isolate that showed more significant similarity with 99,7% to *Bacillus Licheniformis* in BLAST, in the clade with bootstrap higher than 80% (Table 3; Figure 2). Only

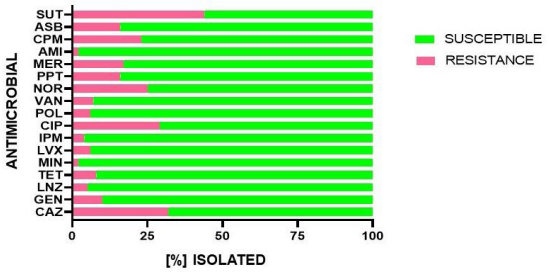

Figure 1. Antimicrobial rates of bacterial resistance isolated from ICU hospital bed surfaces (n = 56). Antibiotics: ceftazidime (CAZ), gentamicin (GEN), linezolid (LNZ), tetracycline (TET), minocycline (MIN), levofloxacin (LVX), imipenem (IPM), ciprofloxacin (CIP), polymyxin B (POL), vancomycin (VAN), norfloxacin (NOR), piperacillin/tazobactam (PPT), meropenem (MER), amikacin (AMI), cefepime (CPM), ampicillin/sulbactam (ASB), cotrimoxazole (sulfatrin) (SUT), imipenem (IPM)

Table 2. Phenotypic characterization of isolates from hospital ICU beds in Tocantins, Brazil.

Group	Gene	Primer sequence (5´-3´)	Temperature melting (°C)	Expected Aplicon size (pb)	Reference
β-Lactamases (Class D)	*Oxa-23*F	GATGTGTCATAGTATTCGTCGT	50	1,057	Sohrabi et al. (2012)
	*Oxa-23*R	TCACAACAACTAAAAGCACTGT			
	*Oxa-51*F	AACAAGCGCTATTTTTATTTCAG	50	641	
	*Oxa-51*R	CCCATCCCCAACCACTTTT			
	Oxa-40F	ATGAAAAAATTTATACTTCCTATATTCAGC	50	825	
	Oxa-40R	TTAAATGATTCCAAGATTTTCTAGC			
	Oxa-58F	AGTATTGGGGCTTGTGCT	49	453	
	*Oxa-58*R	AACTTCCGTGCCTATTTG			
IS element	*ISAba1*F	CATTGGCATTAAACTGAGGAGAAA	50	451	Sohrabi et al. (2012)
	*ISAba1*R	TTGGAAATGGGGAAAACGAA			

Table 3. Antimicrobial resistance of isolates and 16S rRNA gene sequencing.

Isolate Beds UTI (Codes)*	Scientific Name	Number GenBank acession	Query cover (%)	Identity (%)	N. acession GenBank 16S rRNA gene partial sequence	Antimicrobial resistance	Genes					MDR**
							bla_{OXA-23}	bla_{OXA-51}	bla_{OXA-40}	bla_{OXA-58}	*ISAba1*	
ILH10	*A. schindleri*	NR_025412.1	100	99.3	ON853562.1	caz, cpm, lvx, cip, asb, ppt, pol tet, sut,	P	N	N	N	N	P
ILH11	*B. subtilis*	NR_102783.2	98	97.4	ON853563.1	caz, cpm, asb, ppt, mer	N	N	N	N	N	N
ILH13	*B. cereus*	NR_115526.1	100	100	ON853564.1	caz, cpm, nor, lvx, cip asb, pol	N	N	N	N	N	P
ILH2	*B. licheniformis*	NR_118996.1	98	99.7	ON853570.1	cip, asb, ppt, gen, tet lnz,	N	N	N	N	N	P

Antibiotics: ceftazidime (caz), cefepime (cpm), norfloxacin (nor), levofloxacin (lvx), ciprofloxacin (cip), ampicillin/sulbactam (asb), piperacillin/tazobactam (ppt), meropenem (mer), gentamicin (gen), polymyxin B (pol), tetracycline (tet), linezolid (lnz), cotrimoxazole (sulfatrin) (sut). P: Positive; N: Negative. *ILH - Isolated Hospital Beds. **Multidrug resistant bacteria.

Table 4. Antimicrobial resistance of isolates and microbial identification by MALDI-TOF MS.

Isolate Beds UTI (Codes)*	MALDI-TOF MS	Antimicrobial resistance	Genes					MDR**
			bla_{OXA-23}	bla_{OXA-51}	bla_{OXA-40}	bla_{OXA-58}	*ISAba1*	
ILH14	*S. paucimobilis*	cip, ppt, asb, gen, pol, tet lnz,	N	N	N	N	N	P
ILH16	*B. circulans*	caz cpm, nor, lvx, cip ppt, asb, lpm	N	N	N	N	N	P

Antibiotics: ceftazidime (caz), cefepime (cpm),norfloxacin (nor), levofloxacin (lvx), ciprofloxacin (cip), ampicillin/sulbactam (asb), piperacillin/tazobactam (ppt), imipenem (ipm), gentamicin (gen), polymyxin B (pol), tetracycline (tet), linezolid (lnz). P: Positive; N: Negative. *ILH - Isolated Hospital Beds. ** Multidrug resistant bacteria.

ILH14 and ILH 16 were identified using MALDI-TOF MS, revealing *Sphingomonas paucimobilis* and *Bacillus circulans* (Table 4; Figure 3). ILH10, ILH11, ILH13, and ILH2 were not detected using the technique because the system did not include a reference database for identification (Table 4).

4. Discussion

The indiscriminate use of antibiotics inevitably leads to antimicrobial resistance, primarily through natural resistance and antibiotic residues in sewage and soils (Moretto et al., 2021). The Gurupi Regional Hospital, the study site, is a referral center for 18 municipalities in the state of Tocantins, which can favor the dissemination of MDRs via dissemination between communities and environments through domestic effluents and food (Meirelles-Pereira et al., 2002; Cerceo et al., 2016). This factor explains the presence of bacteria in the genera *Bacillus*, *Acinetobacter* and *Sphingomonas*, found in Gurupi Regional Hospital, also commonly found in water, soil, and food products (Logan 2012; Smet et al., 2014; Ferreira et al., 2019; Arbab et al., 2021; Carvalheira et al., 2021).

Infections caused by nosocomial pathogens lead to morbidity and mortality in hospitalized patients with the emergence of MDR bacteria (Weber et al., 2010; Otter et al., 2013; Dancer, 2014; Sood and Perl, 2016). Reports have shown that biofilm formation is favorable to the growth of persistent colonization with methicillin-resistant *Staphylococcus aureus*, *Acinetobacter* spp., *Clostridium difficile*, and *Pseudomonas aeruginosa* (Kramer et al., 2006; Boyce, 2007; Chemaly et al., 2014; Hu et al., 2015). Here, we verified the phenotypic and genotypic characterization of ICU bed surface isolates, showing the presence of MDR bacteria, description of resistance genes, and identification by MALDI-TOF MS and 16S rRNA sequencing.

Limitations regarding the database of protein profiles of isolates make it challenging to use MALDI-TOF MS to identify unconventional bacterial strains of medical interest. Studies have described the failure to identify strains due to the absence of a broad protein spectrum from a reference bank or even the capture of the technique of specific proteins necessary to define the species. (Bizzini et al., 2011; Alizadeh et al., 2021). However, data on its applicability reveal MALDI-TOF MS's importance in quickly typing subspecies of medical importance; the technique contributes to the proper management of antimicrobials and best hospital practices. On the other hand, its limitations highlight the need to increase reference data for reference banks (Croxatto et al., 2012; Angeletti, 2017; Davies et al., 2021).

16S rRNA gene sequencing allowed greater coverage and effectiveness in identifying microorganisms.

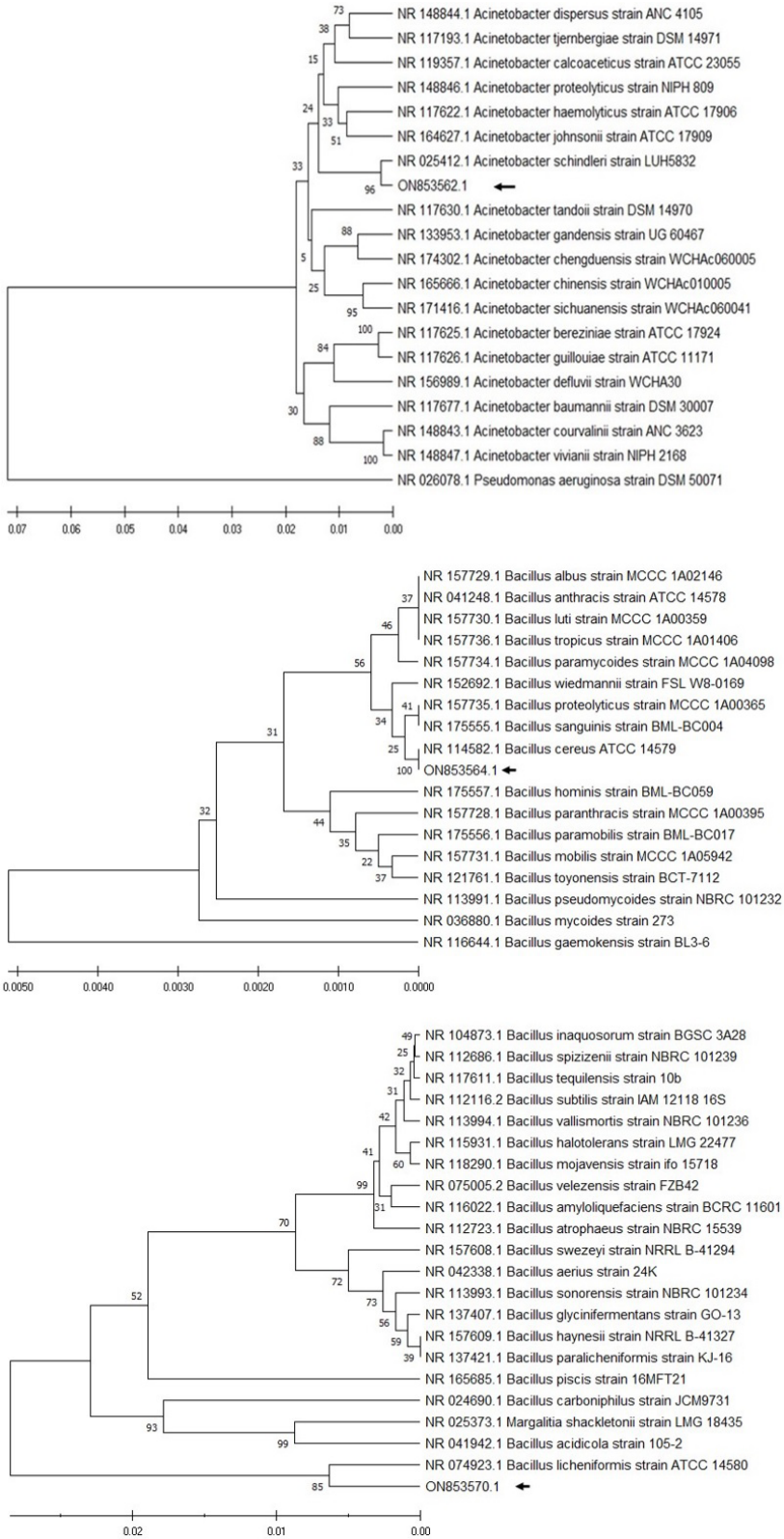

Figure 2. Phylogenetic tree obtained by maximum likelihood of the 16S rRNA gene sequences (400 bp) of the ILH10 isolate with identification of deposit in GenBank ON853562.1 (black arrow). ILH13 isolate with identification of deposit in GenBank ON853564.1 (black arrow). ILH2 isolate with identification of deposit in GenBank ON853570.1 (black arrow). Bootstrap values are indicated at the root of each branch.

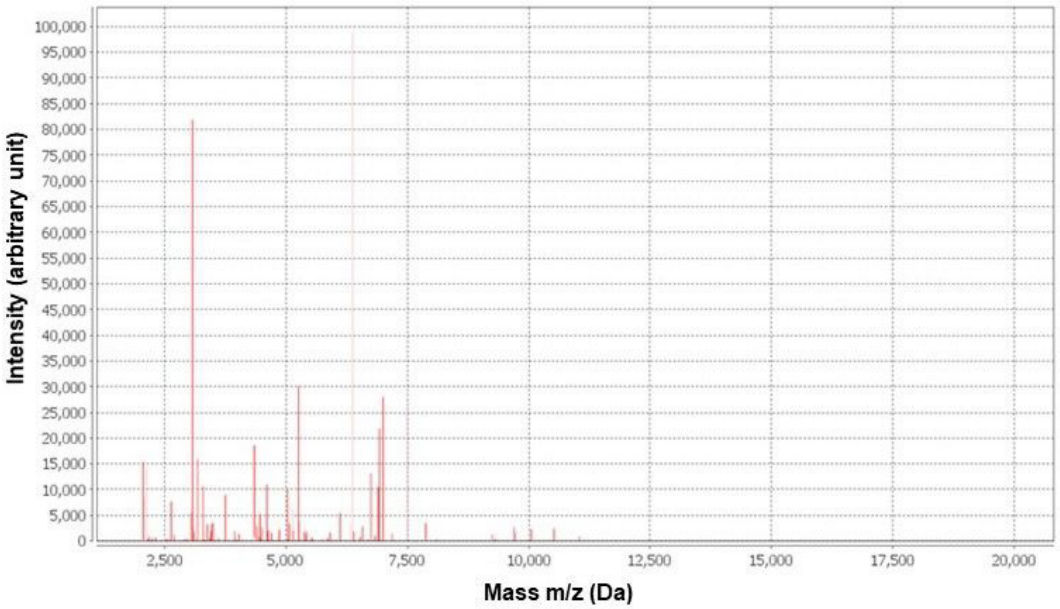

Figure 3. Mass spectrometer with the proteomic profile of isolate ILH16 (*B. circulans*) by MALDI-TOF MS.

The technique is used widely for the identification of most bacterial taxa. However, the technique entails substantial performance time and high processing costs (Clarridge III, 2004; Bizzini et al., 2011; Church et al., 2020; Kosecka-Strojek et al., 2019). Our work using the 16S rRNA technique effectively identified the bacterial isolates classified by similarity and construction of the phylogenetic profile.

For ILH-10 (*A. schindleri*), we observed phenotypes resistant to several antibiotics in different classes. The isolate was positive for OXA-23, which may be associated with a multi-resistance phenotype to beta-lactams. *Acinetobacter* are opportunistic pathogens, and most are clinically significant, typically causing severe nosocomial infections in immunocompromised hosts (Joly-Guillou, 2005; Ibrahim et al., 2021). *Acinetobacter* is often associated with the accumulation of different resistance mechanisms to commercially available antibiotics, including carbapenems (Fournier et al., 2006; Périchon et al., 2014; Zander et al., 2014). Species producing class D β-lactamases of the oxa gene type (e.g., A. *baumannii*, *A. johnsonii*, *A. pittii*, *A. baylyi*, and *A. schindleri*) have been reported worldwide (Boo et al., 2009; Zhou et al., 2011; Zander et al., 2014; Smet et al., 2014). Furthermore, it is essential to note that resistance genes in non-*A. baumannii* (*A. schindleri*) species can cause bacteremia due to carbapenem resistance in immunocompromised patients (Montaña et al., 2017).

S. paucimobilis (ILH14) is an opportunistic pathogen involved in nosocomial infections; it can cause significant problems in clinical settings when resistant to antibiotics. Reports described *S. paucimobilis* in clinical specimens, blood, urine, endotracheal aspirates, bronchoalveolar lavage, and cerebrospinal fluid of patients with septicemia, meningitis, lower respiratory tract infections, and ventilator-associated pneumonia (Rohilla et al., 2021). Cases of early prosthetic valve infective endocarditis caused by MDR *S. paucimobilis* have been reported (Saboe et al., 2021). The *S. paucimobilis* (ILH14) isolated here was identified using the MALDI-TOF MS technique with phenotyping of MDR antibiotics the quinolone, penicillin, aminoglycoside, polymyxin, tetracycline, and oxazolidinone classes.

Bacillus sp. isolates (ILH11, ILH13, ILH2, and ILH16) showed resistance to clinically relevant antibiotics such as cephalosporins, quinolones, and penicillins. This genus is widely distributed in the environment, including soil, dust, air, fomites, water, and hospital environments (Bottone, 2010). The broad diversity of Bacillus allows gene regulation that increases its pathogenicity (Bottone, 2010). Previous studies identified MDR phenotypes in this group (Torkar and Bedenic, 2018; Liu et al., 2018, Mills et al., 2022). Some MDR strains of *Bacillus sp* obtained in the present study cause food poisoning. They can also cause localized wounds and eye infections; in a hospital environment, they can cause nosocomial bacteremia (Kuroki et al., 2009, Ehling-Schulz et al., 2019) and meningitis in immunocompromised patients (Gaur et al., 2001). Opportunistic infections by *Bacillus circulans* were identified in immunocompromised patients (Russo et al., 2021).

In summary, our findings suggest that several strains obtained from ICUs showed resistance to several classes of antimicrobials identified by molecular techniques and MALDI-TOF MS; however, the latter technique may be more relevant when associated with antimicrobial resistance phenotyping to assist antimicrobial management. Our findings reinforce the need to investigate resistance genes of the β-lactamase class. Finally, our findings suggest the need for amplification in detecting specific resistance genes. Our findings also contribute to identifying contamination sources responsible for increasing MDR

organism levels. Monitoring these sources will improve environmental, food, and public health infection controls.

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