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REVIEW

Application and Limitations of 16S rRNA Gene Sequencing for Identifying WHO Priority Pathogenic Gram-Negative Bacilli

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Abstract: Antimicrobial resistance (AMR) poses one of the greatest global health challenges, particularly in healthcare-associated infections caused by multidrug-resistant Gram-negative bacilli. Rapid and reliable identification of these pathogens is critical to guide therapy, improve patient outcomes, and support infection control measures. This review explores the application of 16S ribosomal RNA (rRNA) gene sequencing for the identification of pathogenic Gram-negative bacilli included in the World Health Organization (WHO) antimicrobial resistance priority list. The 16S rRNA gene, with its conserved and hypervariable regions, provides a robust molecular marker widely used in bacterial taxonomy and clinical diagnostics. The analysis covers conventional Sanger sequencing, next-generation sequencing (NGS), and third-generation approaches, outlining their advantages, limitations, and clinical applicability. Results indicate that while 16S rRNA sequencing is a valuable tool for genus-level identification, comparative analysis reveals its resolution is often insufficient for distinguishing closely related species such as *Escherichia coli* and *Shigella* spp. or for taxa with low interspecies variability. In these cases, complementary strategies – such as multilocus sequence analysis, whole genome sequencing, or advanced mass spectrometry-based methods – are required to achieve accurate identification. Furthermore, the reliability of 16S-based identification depends heavily on the quality of reference databases, as demonstrated by in silico analysis of type strains, and adherence to interpretative guidelines. In conclusion, 16S rRNA sequencing remains a cornerstone of molecular diagnostics and epidemiological surveillance of multidrug-resistant Gram-negative pathogens, but its integration with additional molecular and proteomic tools is essential to overcome its limitations and strengthen infection management strategies.

Keywords: infectious diseases, antimicrobial resistance, multidrug resistance, gram-negative bacilli, one health, molecular characterization

Introduction

Since their discovery in the 20th century, antibiotics have revolutionized medicine, saving millions of lives over the decades[1]. However, the widespread and often indiscriminate use of these drugs has driven the emergence of antibiotic-resistant bacterial strains.[1,2] Thus, antimicrobial resistance (AMR) is recognized as the most urgent global public health challenges of the 21st century, especially after the COVID-19 pandemic, when the misuse of antibiotics was even more intense.[3,4] This challenge is particularly critical in healthcare settings, where multidrug resistant pathogens are the primary cause of healthcare-associated infections, representing one of the greatest threats to the patient's safety.[5,6]

Healthcare-associated infections (HAIs) are a significant global burden, increasing hospital stay length, treatment costs, and, critically, patient morbidity and mortality.[7,8] This issue was further exacerbated during the COVID-19 pandemic, when hospitalization rates and antimicrobial use increased significantly.[9] Langlete et al[9] performed

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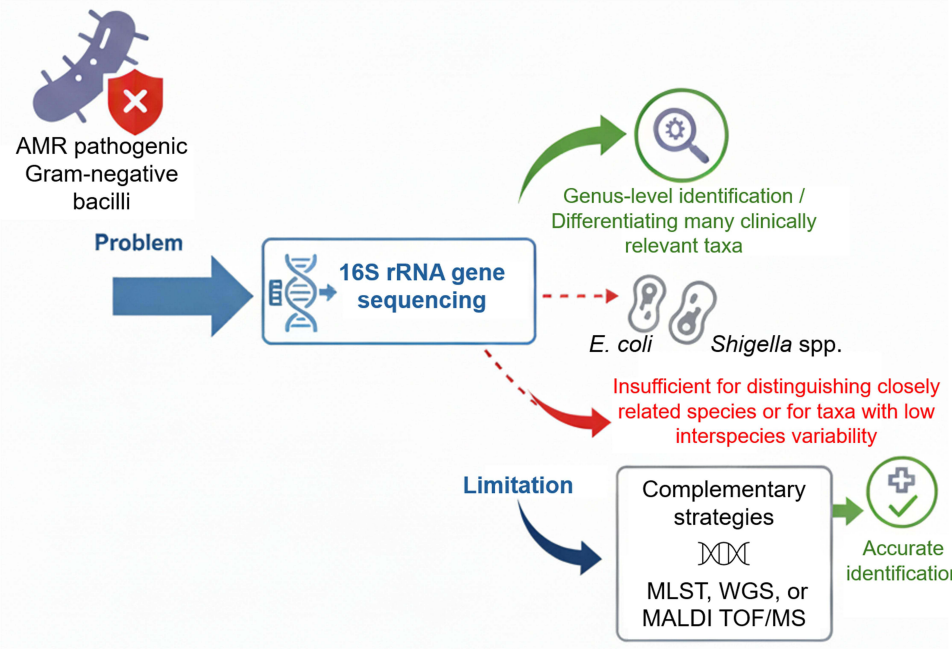
Graphical Abstract



a retrospective registry-based study comparing HAIs and community-associated COVID-19 infections in 54,885 COVID-19 cases identified in patients hospitalized between January 1st, 2019, and January 1st, 2023. The authors reported that mortality rates were consistently higher among HAIs patients compared to community-associated COVID-19 infections patients, the difference being highest shortly after infection. The rise of AMR complicates this landscape, as it limits therapeutic options for infections that are already difficult to treat, leading to poorer clinical outcomes.[6,10] Gram-negative bacteria are already intrinsically resistant to a wide range of antibiotics, including β-lactams, quinolones and polymyxin, due to their outer membrane structure.[11] Among them, *Acinetobacter baumannii, Pseudomonas aeruginosa* and order Enterobacterales stand out as relevant infectious agents.[6,12,13] Along with their intrinsic resistance, these species also exhibit a remarkable capacity to acquire new resistance determinants, having rapid adaptation to selective pressure and high genetic plasticity that allows the accumulation of multiple factors, ultimately leading to multidrug resistance.[6,14,15]

The World Health Organization (WHO) has included *A. baumannii, P. aeruginosa*, carbapenem-resistant Enterobacterales and third-generation cephalosporin-resistant Enterobacterales in its list of priority bacterial pathogens, highlighting the urgent need to prioritize research and studies focusing on these microorganisms.[6] Actually, the order Enterobacterales possesses eight valid families: *Budviciaceae, Enterobacteriaceae, Erwiniaceae, Gallaecimonadaceae, Hafniaceae, Morganellaceae, Pectobacteriaceae*, and *Yersiniaceae*.[16] Among all the genus and species from the order, members of *Enterobacteriaceae* are the most associated with human infections associated to AMR.[17] *Klebsiella pneumoniae, Enterobacter* spp. and *Escherichia coli* belong to ESKAPEE (*Enterococcus faecium, Staphylococcus aureus, K. pneumoniae, A. baumannii, P. aeruginosa, Enterobacter* spp. and *E. coli*), a clinical relevant group of bacteria that can "escape" from the effects of standard antibiotics, thus making them fatal adversaries, especially in clinical settings.[18] Due to the clinical relevance of these species, bacterial diagnostic testing is critical for the identification and characterization of bacterial infections, the prescription of targeted treatments, and the prevention of the spread of disease.[2,19]

Although diagnostic technologies have advanced significantly, most individuals with infectious diseases continue to receive empiric treatment, which also leads to the excessive use of antibiotics.[2,10,20] Moreover, different species present different resistance profiles, and the incorrect identification may deprive the patient of receiving the most appropriate antibiotic choice.[21] Conventional diagnostic methods for bacterial identification, including culture-based biochemical testing, are still widely used in clinical microbiology; however, these approaches are inherently time-consuming and often lack precision for closely related or fastidious species.[19] The process typically requires 24 to 72 h for growth and phenotypic characterization, delaying appropriate therapeutic or containment measures.[10] Furthermore, phenotypic variability and overlapping biochemical profiles among certain genera can lead to misidentification or inconclusive results.[19] Therefore, the use of fast and accurate identification techniques is essential, as it would speed up the procedures of contamination control, the traceability of microorganisms, reduce the misuse of antibiotics and serve as a crucial tool for the control and prevention of healthcare-associated infections.[22]

One of the techniques that can be applied for bacteria identification is the 16S ribosomal RNA (rRNA) gene sequencing.[23] This is one of the most consolidated and widely used molecular approaches for studying bacterial phylogeny and taxonomy.[24] The 16S rRNA gene is present in all bacteria, is approximately 1,500 base pairs (bp), and contains conserved and hypervariable regions (V1 to V9), which makes it ideal for the identification and classification of microorganisms.[25] The conserved regions allow the use of universal primers for amplification. The hypervariable regions (V1 to V9) allow differentiation between genera and, in some cases, species.[26]

The 16S rRNA gene is encoded within the rRNA operon (*rrn*), which contains the genes responsible for the synthesis of ribosomal RNA, essential for the assembly of bacterial ribosomes (Figure 1). The 16S rRNA gene is not translated into proteins. It is transcribed into RNA and becomes part of the small subunit (30S) of the bacterial ribosome. The number of copies of the *rrn* operon can vary between bacterial species (from 1 to more than 15 copies).

Although the presence of multiple rrn operons enhances ribosomal synthesis and cellular adaptability, it also introduces complexity in molecular diagnostics. They are generally very similar to each other, but microvariations can exist. For example, *Escherichia coli* has seven copies of the rrn operon distributed along the chromosome.[27] The existence of several 16S rRNA gene copies within a single genome, often showing slight sequence heterogeneity, can complicate consensus sequence assembly and lead to ambiguous taxonomic assignments.[24] This intra-genomic variation may generate conflicting signals during sequence alignment or database comparison, occasionally resulting in misidentification at the species level. Recognizing this limitation is crucial for accurate interpretation of 16S rRNA-based analyses, particularly in clinical diagnostics where precision and reliability are essential.[25,26]

Among the diverse molecular and proteomic techniques currently employed for bacterial identification, 16S rRNA gene sequencing remains one of the most consolidated and widely adopted approaches. While methods such as Matrix-Assisted Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) have gained prominence for their rapid and cost-effectiveness, 16S sequencing continues to provide more taxonomy resolution.[22,25]

While 16S rRNA gene sequencing has revolutionized microbial ecology, there are recognized limitations for precise species-level identification, particularly within closely related taxa like the genus *Bacillus* and related genus.[25] These limitations arise from insufficient sequence diversity within the highly conserved 16S gene region, often leading to ambiguous speciation. This review, however, is specifically focused on the application of 16S rRNA gene sequencing to Gram-negative organisms associated with antibiotic resistance in clinical settings. In this context, this article aimed to review the use of 16S rRNA gene sequencing for identification of pathogenic Gram-negative bacilli included in WHO antimicrobial resistance priority list,[6] spanning Sanger classical methods to innovative techniques such as next-generation

Figure 1 Organization of the bacterial rrn operon.

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sequencing (NGS). This data tries to elucidate the advantages and limitations of each approach, underscoring the urgent need for methodological standardization to enhance clinical diagnostics, epidemiological surveillance, and infection management caused by these emerging pathogens. By integrating data from standardized databases and international diagnostic guidelines, this work highlights the strengths and limitations of each approach in terms of resolution, reliability, and applicability in clinical microbiology. This comparative perspective aims to delineate the practical diagnostic value of each technique, offering a framework for evidence-based selection of identification strategies.

Methods

This study was conducted as an integrative review of the scientific literature. The following databases were searched: Embase, Web of Science, LILACS, MEDLINE, PubMed, and Scopus. Keywords used included: 16S rRNA, Gram-negative, sequencing, antimicrobial resistance, Enterobacterales, *Pseudomonas aeruginosa, Acinetobacter baumannii*, whole genome sequencing, MLST, MALDI TOF, carbapenem and cephalosporin. Inclusion criteria included articles that reported diagnostic methods used for identification of Gram-negative bacilli from clinical samples. Articles that were not related to Gram-negative bacilli identification were excluded. This methodological approach was chosen to comprehensively evaluate a broad range of scientific data and provide a broad understanding of 16S rRNA gene sequencing techniques. The primary objective of this review was to identify, analyze, and discuss the principal methodologies available for 16S rRNA gene sequencing, including their respective advantages, disadvantages, and applications in the identification of Gram-negative bacilli pathogenic identification included in WHO antimicrobial resistance priority list,[6] including keywords, inclusion and exclusion criteria.

Articles unrelated to human infections or identified as duplicates were excluded. Studies meeting inclusion criteria were fully reviewed, Articles not meeting eligibility after full-text review were excluded with documented reasons.

Results and Discussion

16S rRNA Sequencing Methods

Over the years, 16S rRNA gene sequencing methodologies have evolved considerably, differing in terms of read length, cost, accuracy, and application. The 16S rRNA gene can be sequenced using Sanger, NGS or third-generation methodologies.[28]

Sanger Method

Sanger sequencing, also known as chain termination sequencing, is ideal for the complete sequencing of the 16S rRNA gene, approximately 1,500 base pairs (bp), of a single isolate and has high accuracy.[29] Before sequencing, the DNA must first be amplified by PCR and then purified. The choice of primers in the amplification stage depends on the objective: to amplify the complete gene or only specific hypervariable regions (~250–500 bp). The pair of primers 27F (=PA) and 1492R is widely used to amplify the complete gene.[30] Partial amplification of the 16S rRNA gene consists of sequencing the hypervariable regions (V1-V9), and is widely used in microbiome studies and NGS sequencing.[23] Some examples of primers used for partial or complete amplification and sequencing of the 16S rRNA gene are shown in Table 1.

Table 1 Examples of Primers Used in the Partial or Complete Amplification and Sequencing of the 16S rRNA gene[31–34]

Application	Primers	Sequence (5′-3′)	References	Expected Size (bp)
Complete gene	27F	AGAGTTTGATCMTGGCTCAG	[31]	1500
	1492R	TACGGYTACCTTGTTACGACTT	[32]	
Region V1-V3	27F	AGAGTTTGATCMTGGCTCAG	[31]	~500
	519R	GWATTACCGCGGCKGCTG	[33]	
Region V3-V4	341F	CCTACGGGNGGCWGCAG	[34]	~460
	805R	GACTACHVGGGTATCTAATCC		

Abbreviation: bp, base pairs.

Despite its higher cost, full gene sequencing has more advantages over partial sequencing, as it covers all the hypervariable regions of the gene and consequently has a higher taxonomic resolution. Partial gene sequencing may not distinguish phylogenetically close species, such as *Escherichia coli* vs *Shigella* spp.[28] The complete gene is more reliable for robust phylogenetic analysis.[35,36] The phylogenetic tree can reveal evolutionary relationships between bacterial isolates and helps to discover new species, but the resolution of the 16S rRNA gene may not be enough to separate closely related species, requiring the use of additional genes, such as the housekeeping genes *rpoB, gyrB, recA*, among others, or whole genome sequencing.[25]

There are currently standardized and validated commercial kits for partial or complete sequencing of the 16S rRNA gene using the Sanger method. These kits are widely used in clinical, microbiology laboratories, and include the identification of Gram-negative bacilli.[37–39]

Thermo Fisher Scientific commercializes the MicroSEQ™ 500 16S rDNA and MicroSEQ™ Full Gene 16S rDNA kits (Thermo Fisher Scientific, USA), which are designed for bacterial identification and have their own database for automated identification. The difference between the two kits is the size of the 16S rRNA gene region sequenced and, consequently, the level of taxonomic resolution that each can achieve. According to the manufacturer, the first kit amplifies the V1-V2 region of the bacterial 16S rRNA gene, generating a fragment of approximately 500 bp. This region is widely used for bacterial identification due to its high conservation and variability.

For comparing the 16S gene sequences, the most common public databases used are: 1) the Genbank from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/nucleotide/),[40] and 2) EzBioCloud Database (https://www.ezbiocloud.net/).[41,42] The "Guideline for Interpretive Criteria for Identification of Bacteria by DNA Target Sequencing" published by Clinical & Laboratory Standards Institute (CLSI)[43] establishes standardized interpretive criteria for the identification of bacteria from clinical cultures, using target regions such as the 16S rRNA. The criteria suggested by the Guideline are shown in Table 2.

For interpretation, when using EzBioCloud, a similarity of ≥98.7% identity and >90% coverage against a reference 16S sequence is considered sufficient for identification at the species level.[42] An analysis in EzBioCloud Database Update 2025.04.21 (https://www.ezbiocloud.net/), selecting valid names only, and using the first 500 bp and the full gene sequence (~1,500 bp) of the type strain sequence of each bacilli Gram-negative species and genus (for Enterobacterales order) in WHO Priority List[6] is shown in Table 3. Although the EzBioCloud database criterion for species identification considers a similarity percentage of ≥98.7%, Table 3 also include the CLSI parameters according to the genus/species analyzed.

Table 2 Evaluation of 16S rRNA Gene Sequencing Analysis for Pathogenic Gram-Negative Bacilli Identification Using BLAST Analysis in NCBI Database According to Clinical & Laboratory Standards Institute Guideline[43]

Classification	Priority Taxonomic Group According to WHO (2024)	Interpretation
Enterobacteriaceae[a] (except *Klebsiella, Enterobacter, Citrobacter,* and *Pantoea*)	Enterobacterales	≥99.0% identity for species identification (with greater than 0.8% separation between different species); report "[Genus and species]" ≥97.0% identity for genus identification; consider reporting "[Genus], most closely related to [species]" ≥95.0% identity cannot be definitively identified by 16S rRNA gene sequencing; consider reporting "Unable to identify by 16S rRNA gene sequencing, most closely relate to [Genus]"
Klebsiella, Enterobacter, Citrobacter, and *Pantoea*	Enterobacterales	≥99.5% identity for species identification (with greater than 0.5% separation between different species); report "[Genus and species]" ≥97.0% identity for genus identification with good separation between genera; consider reporting "[Genus], most closely related to [species]" ≥97.0% identity for genus identification with poor separation between genera; consider reporting all closely related genera ≥95.0% identity cannot be definitively identified by 16S rRNA gene sequencing; consider reporting "Unable to identify by 16S rRNA gene sequencing, most closely relate to [Genus]"

(*Continued*)

Table 2 (Continued).

Classification	Priority Taxonomic Group According to WHO (2024)	Interpretation
Non- *Enterobacteriaceae*	Enterobacterales	≥99.0% identity for type strain (percent identity with greater than 0.8% separation from other species); report "[Genus and species]" ≥99.0% identity for not a type strain, but published in peer-reviewed literature (percent identity with greater than 0.8% separation from other species); report "[Genus and multiple species]" ≥99.0% identity for type strain (another species has less than 0.8% sequence divergence); report "[Genus and species]" ≥99.0%; >99.5% to published reference strain, but only 99% to the type strain (percent identity with greater than 0.8% separation from other species); report "[Genus and species]" ≥99.0%; >99.5% to published reference strain, but only 97% to the type strain (percent identity with greater than 0.8% separation from other species); report "[Genus and species]" 97.0–98.9% identity for type strain or other validly named strain (good separation from other genera); report "[Genus]" 97.0–98.9% identity for type strain or other validly named strain (poor separation from other genera); report "[Most closely related to 'genus' but other genera 'state which ones' cannot be excluded]"
Glucose Nonfermenting Gram-Negative Bacilli	*Acinetobacter baumannii; Pseudomonas aeruginosa*	≥99.0% identity for species identification (with greater than 0.8% separation between different species); report "[Genus and species]" ≥97.0% identity for genus identification; consider reporting "[Genus], most closely related to [species]" ≥95.0% identity cannot be definitively identified by 16S rRNA gene sequencing; consider reporting "Unable to identify by 16S rRNA gene sequencing, most closely relate to [Genus]"

Notes: [a]*Escherichia coli* and *Shigella* spp. are indispensable. *Enterobacter* spp. and *Pantoae* spp. have poor resolution for genus and species. *Klebsiella* spp. and *Citrobacter* spp. far resolution for genus and species identification. *Salmonella* spp. resolution to genus with limited resolution to species identification.
Abbreviation: WHO, World Health Organization.

Alternative Methodologies to 16S rRNA Sequencing

MALDI-TOF MS can be a helpful and faster alternative to 16S rRNA sequencing in bacteria identification. It has already been a reliable tool for clinical microbiology laboratories due to its fast, reliable and effective results. It has reduced in 24 h the time to obtain a microbiological diagnosis in comparison to conventional biochemical automatic systems,[44] which can make all the difference in patients' treatment, especially in cases of life-threatening infections or in cases of slow-growing strains.[45] In the last few years, MALDI-TOF MS has been also used to the rapid detection of antibiotic resistance[46–48] which represents a promising solution for nosocomial infections improvements. However, the effectiveness of this methodology depends directly on the robustness of its database, which is related to the presence of a given pathogen spectra and the ability to differentiate closely related species.

The main MALDI-TOF MS instruments used worldwide are the Vitek® MS Prime (bio-Mérieux, Marcy l'Etoile, France) and the MALDI Biotyper® Sirius (Bruker Daltonics GmbH, Bremen, Germany). Despite their database and algorithms differing considerably,[44,49] many studies show that there are no significant differences in the identification of clinically important pathogens when using the two devices.[49,50]

The databases of Vitek MS Prime (v. 3.3, 2022) and MALDI Biotyper Sirius (Revision G, 2023) were consulted to assess their robustness in relation to the type species of the genera presented in the WHO priority list (Table 3). When the species is listed in the database, MALDI-TOF MS analysis is a faster and cheaper alternative than 16S for identifying these bacterial species. However, among the 87 type species of the genera analyzed, 24 (27.6%) (*Acerihabitans arboris, Apirhabdus apintestini, Biostraticola tofi, Buchnera aphidicola, Chania multitudinisentens, Chimaeribacter arupi, Dryocola boscaweniae, Duffyella gerundensis, Enterobacillus tribolii, Gallaecimonas pentaromativorans, Gibbsiella quercinecans, Huaxiibacter chinensis, Intestinirhabdus alba, Limnobaculum parvum, Mangrovibacter plantisponsor, Phaseolibacter flectens, Prodigiosinella aquatilis, Rosenbergiella nectarea, Saccharobacter fermentatus, Shigella dysenteriae, Silvania hatchlandensis, Symbiopectobacterium purcellii, Tenebrionibacter intestinalis*, and *Tenebrionicola larvae*) are not included in the database of either device (Table 4), showing that its continuous improvement is essential

Table 3 Evaluation of 16S rRNA Gene Sequencing Analysis for Identification of Type Species of Pathogenic Gram-Negative Bacilli Described in World Health Organization Priority List[6] Using EzBioCloud Database Update 2025.04.21 and Clinical & Laboratory Standards Institute Guideline[43]

Species	Type Strain	Accession Number (Sequence Length, Base Pairs)	Result of Identification (% of Similarity) Using			
			EzBioCloud		Clinical & Laboratory Standards Institute Guideline	
			500 bp	Full Gene (~1,500 bp)	500 bp	Full Gene (~1,500 bp)
Critical group[a]						
Acinetobacter baumannii	ATCC 19606; CCUG 19096; CIP 70.34; DSM 30007; DSM 6974; JCM 6841; LMG 1041; NCCB 85021; NCTC 12156	ACQB01000091 (1,459)	*Acinetobacter baumannii* (100), *A. nosocomialis* (99.00), *A. seifertii* (99.00)	*Acinetobacter baumannii* (100)	*Acinetobacter baumannii*	*Acinetobacter baumannii*
Acerihabitans arboris	DSM 104038; KCTC 52622; SAP-6	MN737198 (1,461)	*Acerihabitans arboris* (100)	*Acerihabitans arboris* (100)	*Acerihabitans arboris*	*Acerihabitans arboris*
Apirhabdus apintestini	ATCC TSD-396; CA-0114; DSM 116385	OR030829 (1,540)	*Mixta intestinalis* (96.37)	*Brenneria bubanii* (96.41)	Not identified	Not identified
Arsenophonus nasoniae	ATCC 49151; DSM 15247; LMG 12584; SK14	AY264674 (1,460)	*Arsenophonus nasoniae* (100), *A. apicola* (98.80), *A. arthropodicus* (98.80)	*Arsenophonus nasoniae* (100), *A. apicola* (98.70)	*Arsenophonus nasoniae*	*Arsenophonus nasoniae*
Biostraticola tofi	BF36; CIP 109699; DSM 19580	SMCR01000029 (1,465)	*Biostraticola tofi* (100)	*Biostraticola tofi* (100)	*Biostraticola tofi*	*Biostraticola tofi*
Brenneria salicis	ATCC 15712; CCUG 48855; CFBP 802; CIP 105204; DSM 30166; ICMP 1587; LMG 2698; NCPPB 447	MJMA01000033 (1,465)	*Brenneria salicis* (100)	*Brenneria salicis* (100)	*Brenneria salicis*	*Brenneria salicis*
Buchnera aphidicola	Primary endosymbiont of Schizaphis graminum; (Schizaphis graminum); the primary endosymbiont found in the mycetocytes of Schizaphis graminum is designated the type strain	CP001161 (1,470)	*Buchnera aphidicola* (100)	*Buchnera aphidicola* (100)	*Buchnera aphidicola*	*Buchnera aphidicola*
Budvicia aquatica	20186HG01; ATCC 35567; CIP 103240; CNCTC 20186; CNCTC 350; DSM 5075; Eb 13/82; LMG 8813; Strain 20186	AJ233407 (1,455)	*Budvicia aquatica* (100)	*Budvicia aquatica* (100)	*Budvicia aquatica*	*Budvicia aquatica*
Buttiauxella agrestis	ATCC 33320; CDC 1176–81; CIP 80.31; CUETM 77–167; DSM 4586; Gavini F-44; JCM 1090; LMG 7861; NCTC 12119	JMPI01000079 (1,462)	[b]*Buttiauxella agrestis* (100), *B. noackiae* (100), *B. ferragutiae* (100), *B. gaviniae* (100), *B. massiliensis* (100) … *Scandinavium hiltneri* (99.00)	*Buttiauxella agrestis* (100), *B. ferragutiae* (100), *B. massiliensis* (100), *B. brennerae* (99.59), *B. noackiae* (99.45), *Scandinavium hiltneri* (99.00)	*Buttiauxella* spp.	*Buttiauxella* spp.
Cedecea davisae	005; ATCC 33431; CCUG 12370; CDC 3278–77; CIP 80.34; DSM 4568; JCM 1685; LMG 7862; NCTC 13724	ATDT01000040 (1,464)	*Cedecea davisae* (100)	*Cedecea davisae* (100), *C. lapagei* (98.98), *C. neteri* (98.91)	*Cedecea davisae*	*Cedecea davisae/lapagei/neteri*

(*Continued*)

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Table 3 (Continued).

Species	Type Strain	Accession Number (Sequence Length, Base Pairs)	Result of Identification (% of Similarity) Using			
			EzBioCloud		Clinical & Laboratory Standards Institute Guideline	
			500 bp	Full Gene (~1,500 bp)	500 bp	Full Gene (~1,500 bp)
Chania multitudinisentens	DSM 28811; LMG 28304; RB 25	CP007044 (1,464)	*Chania multitudinisentens* (100)	*Chania multitudinisentens* (100)	*Chania multitudinisentens*	*Chania multitudinisentens*
Chimaeribacter arupi	2016-Iso3; ATCC TSD-180; DSM 110101	MK530421 (1,457)	*Chimaeribacter arupi* (100), *C. californicus* (99.40), *C. coloradensis* (98.99)	*Chimaeribacter arupi* (100), *C. californicus* (99.72), *C. coloradensis* (99.59)	*Chimaeribacter arupi/ californicus*	*Chimaeribacter arupi/ californicus/coloradensis*
Citrobacter freundii	ATCC 8090; CCUG 418; CIP 57.32; DSM 30039; HAMBI 1695; IFO 12681; JCM 1657; LMG 3246; NBIMCC 3731; NBRC 12681; NCAIM B.01468; NCTC 9750; NRRL B-2643	AJ233408 (1,464)	[b]*Citrobacter freundii* (100), *C. pasteurii* (100), *C. meridianamericanus* (99.80), *C. braakii* (99.60), *C. portucalensis* (99.60), *Kluyvera georgiana* (98.80)	[b]*Citrobacter freundii* (100), *C. braakii* (99.80), *C. portucalensis* (99.80), *C. meridianamericanus* (99.73), *C. cronae* (99.73), *Klebsiella oxytoca* (98.68)	*Citrobacter* spp.	*Citrobacter* spp.
Cronobacter sakazakii	ATCC 29544; CCUG 14558; CDC 4562–70; CIP 103183; DSM 4485; JCM 1233; LMG 5740; NBIMCC 8667; NBRC 102416; NCTC 11467	BAWU01000071 (1,464)	*Cronobacter sakazakii* (100), *C. malonaticus* (99.20)	*Cronobacter sakazakii* (100), *C. malonaticus* (99.73)	*Cronobacter sakazakii*	*Cronobacter sakazakii/ malonaticus*
Dickeya chrysanthemi	ATCC 11663; CCUG 38766; CFBP 2048; CIP 82.99; DSM 4610; ICMP 5703; LMG 2804; NCAIM B.01392; NCPPB 402	Z96093 (1,464)	*Dickeya chrysanthemi* (100), *D. dieffenbachiae* (98.78)	*Dickeya chrysanthemi* (100), *D. lacustris* (99.11), *D. dieffenbachiae* (98.95), *D. zeae* (98.76), *D. parazeae* (98.70)	*Dickeya chrysanthemi*	*Dickeya chrysanthemi/ lacustris*
Dryocola boscaweniae	CCUG 76177; H6W4; LMG 32610	OM971056 (1,344)	*Dryocola boscaweniae* (100), *Trabulsiella odontotermitis* (98.80)	*Dryocola boscaweniae* (100)	*Dryocola boscaweniae/ Trabulsiella odontotermitis*	*Dryocola boscaweniae*
Duffyella gerundensis	CCOS 903; E_g_EM595; EM595; LMG 28990	FJ611848 (1,348)	*Duffyella gerundensis* (100)	*Duffyella gerundensis* (100)	*Duffyella gerundensis*	*Duffyella gerundensis*
Edwardsiella tarda	ATCC 15947; CCUG 1638; CIP 78.61; DSM 13696; DSM 30052; JCM 1656; LMG 2793; NCCB 73021; NCTC 10396	BANW01000030 (1,466)	*Edwardsiella tarda* (100), *E. hoshinae* (99.40)	*Edwardsiella tarda* (100), *E. hoshinae* (99.73), *E. anguillarum* (99.45), *E. piscicida* (99.39), *E. ictaluri* (99.32)	*Edwardsiella tarda/ hoshinae*	*Edwardsiella* spp.
Enterobacillus tribolii	IG-V01; KCTC 42159; MCC 2532	QRAP01000026 (1,464)	*Enterobacillus tribolii* (100)	*Enterobacillus tribolii* (100)	*Enterobacillus tribolii*	*Enterobacillus tribolii*
Enterobacter cloacae subsp. *cloacae*	ATCC 13047; CCUG 28448; CCUG 29301; CCUG 6323; CIP 60.85; DSM 30054; HAMBI 1295; HAMBI 96; IFO 13535; JCM 1232; LMG 2783; NBIMCC 8570; NBRC 13535; NCTC 10005	CP001918 (1,464)	[b]*Enterobacter cloacae* subsp. *cloacae* (100), *E. kobei* (99.80), *E. ludwigii* (99.80), *E. cloacae* subsp. *dissolvens* (99.80), *E. siamensis* (99,79), *Klebsiella oxytoca* (98.79)	[b]*Enterobacter cloacae* subsp. *cloacae* (100), *E. cloacae* subsp. *dissolvens* (99.73), *Kosakonia oryzendophytica* (99.14), *E. sichuanensis* (99.04), *E. pseudoroggenkampii* (98.99) … *E. roggenkampii* (98.70)	*Enterobacter* spp.	*Enterobacter* spp.

Erwinia amylovora	ATCC 15580; CFBP 1232; CIP 82.82; DSM 30165; ICMP 1540; IFO 12687; LMG 2024; NBRC 12687; NCAIM B.01108; NCPPB 683	BAYW01000035 (1,462)	*Erwinia amylovora* (100)	*Erwinia amylovora* (100), *E. pyrifoliae* (99,38), *E. piriflorinigrans* (98.81), *E. uzenensis* (98.70)	*Erwinia amylovora*	*Erwinia amylovora/pyrifoliae*
Escherichia coli	ATCC 11775; CCUG 24; CCUG 29300; CIP 54.8; DSM 30083; JCM 1649; LMG 2092; NBIMCC 3398; NBRC 102203; NCCB 54008; NCTC 9001	X80725 (1,432)	*Escherichia coli* (100), *E. fergusonii* (99.00), *Shigella dysenteriae* (99.00), *S. flexneri* (98.80)	[b]*Escherichia coli* (100), *Shigella flexneri* (99.58), *S. sonnei* (99.44), *S. boydii* (99.09), *E. albertii* (98.95). *S. dysenteriae* (98.81)	*Escherichia coli*	*Escherichia coli/Shigella flexneri/sonnei*
Ewingella americana	ATCC 33852; CCUG 14506; CDC 1468–78; CIP 81.94; DSM 4580; JCM 5911; LMG 7869; NCTC 12157	JMPJ01000013 (1,466)	*Ewingella americana* (100)	*Ewingella americana* (100), *Rahnella contaminans* (98.70)	*Ewingella americana*	*Ewingella americana*
Franconibacter helveticus	513/05; DSM 18396; JCM 16470; LMG 23732	MK184301 (1,418)	*Franconibacter helveticus* (100)	*Franconibacter helveticus* (100), *F. daqui* (99,42), *F. pulveris* (98,95)	*Franconibacter helveticus*	*Franconibacter helveticus/ daqui*
Gallaecimonas pentaromativorans	CECT 7479; CEE_131; DSM 21945	RJUL01000020 (1,459)	*Gallaecimonas pentaromativorans* (100)	*Gallaecimonas pentaromativorans* (100)	*Gallaecimonas pentaromativorans*	*Gallaecimonas pentaromativorans*
Gibbsiella quercinecans	DSM 25889; FRB 97; LMG 25500; NCPPB 4470	CP014136 (1,464)	*Gibbsiella quercinecans* (100), *G. greigii* (91.81), *G. acetica* (98.76)	*Gibbsiella quercinecans* (100), *G. acetica* (99.58), *G. dentisursi* (99.32), *G. greigii* (99.03)	*Gibbsiella quercinecans/greigii*	*Gibbsiella quercinecans/ acética/greigii*
Hafnia alvei	ATCC 13337; CCUG 41547; CIP 57.31; DSM 30163; HAMBI 1279; HAMBI 1876; JCM 1666; LMG 10392; NBIMCC 1229; NCTC 8105; NRRL B-4260	JMPK01000127 (1,465)	*Hafnia alvei* (100), *Obesumbacterium proteus* (99.60), *H. paralvei* (99.20), *Buttiauxella izardii* (98.80)	*Hafnia alvei* (100), *Obesumbacterium proteus* (99.66), *H. paralvei* (98.77)	*Hafnia alvei/ Obesumbacterium proteus*	*Hafnia alvei/ Obesumbacterium proteus*
Huaxiibacter chinensis	155047; GDMCC 1.2980; JCM 35262	OL712205 (1,462)	[b]*Huaxiibacter chinensis* (100), *Leclercia pneumoniae* (99.77), *Klebsiella spallanzanii* (99.40), *Raoultella ornithinolytica* (98.99), *Lelliottia jeotgali* (98.88). *K. michiganensis* (98.79)	[b]*Huaxiibacter chinensis* (100), *Leclercia pneumoniae* (99.56), *Klebsiella spallanzanii* (99.45), *K. aerogenes* (99.32), *K. michiganensis* (99.27). *Raoultella electrica* (98.69)	*Huaxiibacter chinensis/ Leclercia pneumoniae/ Klebsiella spallanzanii*	*Huaxiibacter* spp.
Intestinirhabdus alba	BIT- B35; CGMCC 1.17042; KCTC 72448	MK734184 (1,411)	*Intestinirhabdus alba* (100)	*Intestinirhabdus alba* (100)	*Intestinirhabdus alba*	*Intestinirhabdus alba*
Klebsiella pneumoniae	ATCC 13883; CCUG 225; CIP 82.91; DSM 30104; HAMBI 450; IFO 14940; JCM 1662; LMG 2095; NBIMCC 3670; NBRC 14940; NCTC 9633	AJJI01000018 (1,462)	[b]*Klebsiella pneumoniae* subsp. *pneumoniae* (100), *K. variicola* subsp. *tropica* (99.66), *K. pneumoniae* subsp. *rhinoscleromatis* (99.20), *K. quasipneumoniae* subsp. *similipneumoniae* (99.20), *K. variicola* subsp. *variicola* (99.20), *K. africana* (98.80)	[b]*Klebsiella pneumoniae* subsp. *pneumoniae* (100), *K. variicola* subsp. *variicola* (99.66), *K. quasipneumoniae* subsp. *quasipneumoniae* (99.59), *K. variicola* subsp. *tropica* (99.52), *K. pneumoniae* subsp. *ozaenae* (99.52), *Enterobacter cloacae* subsp. *cloacae* (98.70)	*Klebsiella pneumoniae* subsp. *pneumoniae/ K. variicola* subsp. *tropica*	*Klebsiella* spp.

(*Continued*)

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Table 3 (Continued).

Species	Type Strain	Accession Number (Sequence Length, Base Pairs)	Result of Identification (% of Similarity) Using			
			EzBioCloud		Clinical & Laboratory Standards Institute Guideline	
			500 bp	Full Gene (~1,500 bp)	500 bp	Full Gene (~1,500 bp)
Kluyvera ascorbata	ATCC 33433; CCUG 15716; CDC 648–74; CIP 82.95; DSM 4611; JCM 21070; LMG 7871; NBRC 102466	JMPL01000225 (1,462)	*Kluyvera ascorbata* (100), *K. intermedia* (99.00), *K. georgiana* (98.80), *K. sichuanensis* (98.74)	[b]*Kluyvera ascorbata* (100), *K. sichuanensis* (99.22), *K. georgiana* (98.97), *K. intermedia* (98.97), *Silvania hatchlandensis* (98.88), *Citrobacter tructae* (98.77)	*Kluyvera ascorbata*	*Kluyvera ascorbata/ sichuanensis*
Kosakonia cowanii	888-76; CCUG 45998; CCUG 45998 A; CCUG 45998 B; CIP 107300; DSM 18146; JCM 10956	BBEU01000098 (1,463)	[b]*Kosakonia cowanii* (100), *Atlantibacter hermannii* (98.80), *E. hormaechei* subsp. *oharae* (98.80), *E. hormaechei* subsp. *steigerwaltii* (98.80), *Enterobacter hormaechei* subsp. *hoffmannii* (98.80). *E. quasihormaechei* (98.80)	*Kosakonia cowanii* (100), *Atlantibacter hermannii* (98.82)	*Kosakonia* spp.	*Kosakonia cowanii*
Leclercia adecarboxylata	ATCC 23216; CIP 82.92; HAMBI 1696; JCM 1667; LMG 2803; NBRC 102595; NCTC 13032	BCNP01000062 (1,462)	[b]*Leclercia adecarboxylata* (100), *Enterobacter kobei* (99.80), *E. ludwigii* (99.80), *E. cloacae* subsp. *dissolvens* (99.80), *Kosakonia oryzendophytica* (99.79). *Klebsiella oxytoca* (98.79)	[b]*Leclercia adecarboxylata* (100), *Enterobacter kobei* (99.79), *E. ludwigii* (99.59), *Silvania hatchlandensis* (99.48), *E. chuandaensis* (99.45), *Lelliottia jeotgal* (98.66)	*Leclercia* spp.	*Leclercia* spp.
Lelliottia nimipressuralis	ATCC 9912; CIP 104980; ICMP 1577; JCM 6050; NCPPB 2045	Z96077 (1,465)	*Lelliottia nimipressuralis* (100)	[b]*Lelliottia nimipressuralis* (100), *L. steviae* (99.37), *L. jeotgali* (99.25), *Silvania hatchlandensis* (99.18), *Leclercia pneumoniae* (99.12), *Huaxiibacter chinensis* (98.70)	*Lelliottia nimipressuralis*	*Lelliottia nimipressuralis/ steviae/jeotgali*
Leminorella grimontii	ATCC 33999; CDC 1944–81; CIP 103359; DSM 5078; JCM 5902; LMG 7912; NCTC 12152	AJ233421 (1,454)	*Leminorella grimontii* (100)	*Leminorella grimontii* (100)	*Leminorella grimontii*	*Leminorella grimontii*
Limnobaculum parvum	HYN0051; KACC 19186; NBRC 112742	CP029185 (1,465)	*Limnobaculum parvum* (100)	*Limnobaculum parvum* (100)	*Limnobaculum parvum*	*Limnobaculum parvum*
Lonsdalea quercina	ATCC 29281; CCUG 48867; CFBP 3617; CFCC 10717; CIP 105201; DSM 4561; ICMP 1845; LMG 2724; NCPPB 1852	JIBO01000012 (1,464)	*Lonsdalea quercina* (100), *L. iberica* (99.80), *L. populi* (99.00)	*Lonsdalea quercina* (100), *L. iberica* (99.32), *L. populi* (99.04)	*Lonsdalea quercina/ iberica*	*Lonsdalea quercina/iberica*
Mangrovibacter plantisponsor	DSM 19579; LMG 24236; MSSRF40	EF643377 (1,368)	*Mangrovibacter plantisponsor* (100), *M. phragmitis* (99.60), *M. yixingensis* (99.00)	*Mangrovibacter plantisponsor* (100), *M. phragmitis* (99.71), *M. yixingensis* (99.56)	*Mangrovibacter plantisponsor/ phragmitis*	*Mangrovibacter plantisponsor/phragmitis/ yixingensis*
Mixta calida	1400/07; DSM 22759; LMG 25383	MLFO01000205 (1,464)	*Mixta calida* (100), *M. gaviniae* (99.40), *M. intestinalis* (99.20)	*Mixta calida* (100), *M. gaviniae* (99.32)	*Mixta calida/gaviniae*	*Mixta cálida/gaviniae*

Moellerella wisconsensis	2896-78; ATCC 35017; CIP 103034; DSM 5076; JCM 5895; LMG 10145; NCTC 12132	JN175344 (1,465)	*Moellerella wisconsensis* (100)	*Moellerella wisconsensis* (100)	*Moellerella wisconsensis*	*Moellerella wisconsensis*
Obesumbacterium proteus	ATCC 12841; CCUG 2078; CIP 82.93; DSM 2777; LMG 3054; NCIB 8771; NCIMB 8771; VKM B-964	CP014608 (1,465)	*Obesumbacterium proteus* (100), *Hafnia alvei* (99.60), *H. paralvei* (99.80)	*Obesumbacterium proteus* (100), *Hafnia alvei* (99.45), *H. paralvei* (99.04)	*Obesumbacterium proteus/Hafnia alvei/ paralvei*	*Obesumbacterium proteus/ Hafnia alvei*
Pantoea agglomerans	ATCC 27155; CCUG 539; CDC 1461–67; CFBP 3845; CIP 57.51; DSM 3493; ICMP 12534; ICPB 3435; JCM 1236; LMG 1286; NBRC 102470; NCTC 9381	AJ233423 (1,447)	[b]*Pantoea agglomerans* (100), *P. eucalypti* (99.54), *P. anthophila* (99.34), *P. vagans* (99.34), *P. brenneri* (99.20). *P. ananatis* (98.80)	[b]*Pantoea agglomerans* (100), *P. pleuroti* (99.65), *P. vagans* (99.57), *Flavobacterium acidificum* (99.21), *P. anthophila* (99.14). *P. coffeiphila* (97.58)	*Pantoea agglomerans/ eucalypti*	*Pantoea agglomerans/ pleuroti/vagans*
Pectobacterium carotovorum	ATCC 15713; CFBP 2046; CIP 82.83; DSM 30168; HAMBI 1429; ICMP 5702; LMG 2404; NCAIM B.01109; NCPPB 312; VKM B-1247	JQHJ01000001 (1,464)	*Pectobacterium carotovorum* (100), *P. aroidearum* (99.00), *P. brasiliense* (98.80), *P. jejuense* (98.72)	[b]*Pectobacterium carotovorum* (100), *P. jejuense* (99.50), *P. brasiliense* (99.39), *P. polaris* (99.11), *P. aroidearum* (99.11), *P. versatile* (98.75)	*Pectobacterium carotovorum*	*Pectobacterium carotovorum/jejuense / brasiliense*
Phaseolibacter flectens	ATCC 12775; CFBP 3281; DSM 27043; ICMP 745; LMG 2187; NCPPB 539	AB021400 (1,464)	*Phaseolibacter flectens* (100)	*Phaseolibacter flectens* (100)	*Phaseolibacter flectens*	*Phaseolibacter flectens*
Photorhabdus luminescens	ATCC 29999; CIP 106429; DSM 3368; Hb	JXSK01000003 (1,467)	*Photorhabdus luminescens* subsp. *luminescens* (100), *Photorhabdus luminescens* subsp. *venezuelensis* (99.76), *Photorhabdus luminescens* subsp. *mexicana* (99.29), *Photorhabdus aballayi* (99.29)	*Photorhabdus luminescens* subsp. *luminescens* (100), *Photorhabdus luminescens* subsp. *venezuelensis* (99.70), *Photorhabdus luminescens* subsp. *mexicana* (99.56)	*Photorhabdus* spp.	*Photorhabdus luminescens* subsp. *luminescens* /subsp. *venezuelensis*/subsp. *mexicana*
Phytobacter diazotrophicus	CGMCC 1.5339; CGMCC 1.5539; DSM 17806; LMG 23328; LS 8	DQ821583 (1,435)	*Phytobacter diazotrophicus* (100)	*Phytobacter diazotrophicus* (100), *P. ursingii* (98.74)	*Phytobacter diazotrophicus*	*Phytobacter diazotrophicus*
Pluralibacter gergoviae	ATCC 33028; CCUG 14557; CDC 604–77; CIP 76.01; DSM 9245; JCM 1234; LMG 5739; NCTC 11434	AB004748 (1,450)	*Pluralibacter gergoviae* (100)	*Pluralibacter gergoviae* (100)	*Pluralibacter gergoviae*	*Pluralibacter gergoviae*
Pragia fontium	ATCC 49100; CCUG 18073; CDC 963–84; CIP 103791; CNCTC Eb 11/ 82; DRL 20125; DSM 5563; HG16; IP 20125; LMG 7875	AJ233424 (1,460)	*Pragia fontium* (100)	*Pragia fontium* (100)	*Pragia fontium*	*Pragia fontium*
Prodigiosinella aquatilis	CFBP 8826; LMG 32072; LS101	CP128857 (1,465)	*Prodigiosinella aquatilis* (100), *Brenneria uluponensis* (99.60)	*Prodigiosinella aquatilis* (100), *Brenneria uluponensis* (99.80)	*Prodigiosinella aquatilis/Brenneria uluponensis*	*Prodigiosinella aquatilis/ Brenneria uluponensis*
Proteus vulgaris	ATCC 29905; CCUG 35382; CCUG 39507; CDC PR1; CIP 104989; DSM 13387; LMG 16708; NCTC 13145; PR 1	DQ885257 (1,464)	*Proteus vulgaris* (100), *P. cibi* (99.37), *P. penneri* (99.26), *P. faecis* (99.14), *P. alimentorum* (99.14), *P. columbae* (98.93)	[b]*Proteus vulgaris* (100), *P. cibi* (99.72), *P. alimentorum* (99.64), *P. faecis* (99.49), *P. columbae* (99.49). *P. terrae* subsp. *terrae* (99.10)	*Proteus vulgaris/cibi / penneri*	*Proteus* spp.

(*Continued*)

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Table 3 (Continued).

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			EzBioCloud		Clinical & Laboratory Standards Institute Guideline	
			500 bp	Full Gene (~1,500 bp)	500 bp	Full Gene (~1,500 bp)
Providencia alcalifaciens	ATCC 9886; CCUG 6325; CIP 82.90; DSM 30120; JCM 1673; NCTC 10286	ABXW01000071 (1,463)	*Providencia alcalifaciens* (100), *P. rustigianii* (99.40), *P. burhodogranariea* (99.00), *P. huaxiensis* (99.00), *P. rettgeri* (98.80)	[b]*Providencia alcalifaciens* (100), *P. rustigianii* (99.66), *P. burhodogranariea* (99.38), *P. huaxiensis* (98.97), *P. rettgeri* (98.84). *P. sneebia* (98.70)	*Providencia alcalifaciens/rustigianii*	[d]*Providencia alcalifaciens/ rustigianii/burhodogranariea*
Pseudescherichia vulneris	ATCC 33821; CCUG 15715; CDC 875; CDC 875–72; CIP 103177; DSM 4564; HAMBI 1694; JCM 1688; LMG 7868; NBRC 102420; NCTC 12130	BBMZ01000044 (1,462)	[b]*Pseudescherichia vulneris* (100), *Kosakonia pseudosacchari* (100), *Yokenella regensburgei* (99.60), *Enterobacter cancerogenus* (99.60), *E. bugandensis* (99.60), *E. timonensis* (98.80)	[b]*Pseudescherichia vulneris* (100), *Enterobacter bugandensis* (99.52), *E. cancerogenus* (99.38), *E. nematophilus* (99.36), *E. chuandaensis* (99.04), *Klebsiella oxytoca* (98.68)	*Pseudescherichia* spp.	*Pseudescherichia* spp.
Pseudocitrobacter faecalis	25 CIT; CCM 8479; DSM 27453; LMG 27751	QNRL01000033 (1,464)	*Pseudocitrobacter faecalis* (100), *P. vendiensis* (99,60), *P. corydidari* (99,58), *Silvania confinis* (99,05), *S. hatchlandensis* (99,05)	*Pseudocitrobacter faecalis* (100), *P. vendiensis* (99,45), *P. corydidari* (99,22)	*Pseudocitrobacter faecalis/vendiensis/ corydidari*	*Pseudocitrobacter faecalis/ vendiensis/corydidari*
Rahnella aquatilis	133; ATCC 33071; CCUG 14185; CIP 78.65; DSM 4594; HAMBI 1280; JCM 1683; LMG 2794	CP003244 (1,465)	*Rahnella aquatilis* (100), *R. perminowiae* (100), *R. aceris* (99.60)	*Rahnella aquatilis* (100), *R. aceris* (99.59), *R. perminowiae* (99.03)	*Rahnella aquatilis/. perminowiae/aceris*	*Rahnella aquatilis/aceris*
Rosenbergiella nectarea	8N4; DSM 24150; LMG 26121	jgi.1084674 (1,465)	*Rosenbergiella nectarea subsp. nectarea* (100), *R. epipactidis subsp. epipactidis* (99,80), *R. epipactidis subsp. japonicus* (99,80), *R. nectarea subsp. apis* (99,80), *R. epipactidis subsp. californiensis* (99,80). *R. australiborealis* (99.00)	*Rosenbergiella nectarea subsp. nectárea* (100), *R. epipactidis subsp. epipactidis* (99,93), *R. nectarea subsp. apis* (99,93), *R. epipactidis subsp. californiensis* (99,93) *R, metrosideri* (99,86). *R. australiborealis (*99,52)	*Rosenbergiella* spp.	*Rosenbergiella* spp.
Rouxiella chamberiensis	130333; CIP 110714; DSM 28324	JRWU01000013 (1,465)	*Rouxiella chamberiensis* (100), *R. badensis* (99.60), *R. silvae* (99.40), *Rahnella inusitata* (98.80)	*Rouxiella chamberiensis* (100), *R. badensis* (99.39), *R. silvae* (98.91), *Rahnella inusitata* (98.70)	*Rouxiella chamberiensis/ badensis/silvae*	*Rouxiella chamberiensis/ badensis*
Saccharobacter fermentatus	WVB 8512	Not available	Not performed	Not performed	Not performed	Not performed
Salmonella enterica	ATCC 43971; CCUG 42060; CIP 60.62; DSM 17058; DSM 27656; IFO 13245; LT2; NBRC 13245; NCIB 11450; NCIMB 11450; NCTC 12416	KX863495 (1,491)	*Salmonella enterica* subsp. *arizonae* (100)	*Salmonella enterica* subsp. *arizonae* (100)	*Salmonella enterica* subsp. *arizonae*	*Salmonella enterica* subsp. *arizonae*
Samsonia erythrinae	CFBP 5236; DSM 16730; ICMP 13937	AF273037 (1,503)	*Samsonia erythrinae* (100)	*Samsonia erythrinae* (100)	*Samsonia erythrinae*	*Samsonia erythrinae*

Scandinavium goeteborgense	CCUG 66741; CECT 9823; NCTC 14286	MK558235 (1,552)	*Scandinavium goeteborgense* (100), *S. tedordense* (99.58), *S. hiltneri* (99.58), *S. manionii* (99.37)	[b]*Scandinavium goeteborgense* (100), *S. hiltneri* (99.86), *S. manionii* (99.80), *Silvania hatchlandensis* (99.18), *Enterobacter wuhouensis* (99.11), *Leclercia pneumoniae* (98.68)	*Scandinavium* spp.	*Scandinavium goeteborgense/hiltneri*
Serratia marcescens	ATCC 13880; CCUG 1647; CFBP 4226; CIP 103235; DSM 30121; HAMBI 1286; JCM 1239; LMG 2792; NBRC 102204; NCTC 10211; NRRL B-2544; VKM B-1248	JMPQ01000005 (1,464)	*Serratia marcescens* (100), *S. bockelmannii* (99.80), *S. nematodiphila* (99.60), *S. nevei* (99.60), *S. ureilytica* (99.59)	*Serratia marcescens* (100), *S. nevei* (99.86), *S. nematodiphila* (99.73), *S. bockelmannii* (99.66)	*Serratia* spp.	*Serratia* spp.
Shigella dysenteriae	ATCC 13313; CIP 57.28; DSM 4781; NCTC 4837	X96966 (1,463)	[b]*Shigella dysenteriae* (100), *S. flexneri* (99.80), *S. sonnei* (99.60), *Escherichia fergusonii* (99.60), *E. ruysiae* (99.40) … *E. coli* (99.00)	[b]*Shigella dysenteriae* (100), *S. flexneri* (99.11), *Escherichia fergusonii* (99.04), *E. whittamii* (99.04), *S. sonnei* (98.97), *E. coli* (98.81)	*Shigella* spp.	*Shigella dysenteriae*
Shimwellia pseudoproteus	521; DSM 3038; LMG 24835; NCIMB 14534	FJ267523 (1,422)	*Shimwellia pseudoproteus* (100)	*Shimwellia pseudoproteus* (100)	*Shimwellia pseudoproteus*	*Shimwellia pseudoproteus*
Siccibacter turicensis	508/05; DSM 18397; JCM 16472; LMG 23730	AVPP01000089 (1,464)	*Siccibacter turicensis* (100)	*Siccibacter turicensis* (100), *S. colletis* (99.02)	*Siccibacter turicensis*	*Siccibacter turicensis*
Silvania hatchlandensis	CCUG 76185; H 19S6; LMG 32608	OM987253 (1,344)	[b]*Silvania hatchlandensis* (100), *Lelliottia steviae* (99,60), *Citrobacter freundii* (99,40), *Lelliottia amnigena* (99,40), *Citrobacter pasteurii* (99,40), *Pseudocitrobacter vendiensis* (98,80)	[b]*Silvania hatchlandensis* (100), *Leclercia adecarboxylata* (99,48), *Lelliottia amnigena* (99,40), *Lelliottia steviae* (99,40), *Leclercia tamurae* (99,40), *Enterobacter quasimori* (98,81)	*Silvania* spp.	*Silvania* spp.
Sodalis glossinidius	DSM 16929; M1; MIT; NCIMB 13495	AP008232 (1,464)	*Sodalis glossinidius* (100)	*Sodalis glossinidius* (100), *S. praecaptivus* (99,04), *S. endolongispinus* (98,77)	*Sodalis glossinidius*	*Sodalis glossinidius*
Symbiopectobacterium purcellii	CECT 30436; LMG 32449; SyEd1	OK044380 (1,464)	*Symbiopectobacterium purcellii* (100)	*Symbiopectobacterium purcellii* (100), *S. endolongispinus* (99,04)	*Symbiopectobacterium purcellii*	*Symbiopectobacterium purcellii*
Tatumella ptyseos	ATCC 33301; CCUG 14188; CDC 9591–78; CDC D6168; CIP 81.97; DSM 5000; H36; LMG 7888; NCTC 11468	JMPR01000058 (1,463)	*Tatumella ptyseos* (100), *T. terrea* (99,00),	*Tatumella ptyseos* (100), *T. terrea* (99,52), *T. saanichensis* (98,90), *T. punctata* (98,70)	*Tatumella ptyseos*	*Tatumella ptyseos/terrea*
Tenebrionibacter intestinalis	BIT-L3; CCTCC AB 2020371; LMG 32222; TBRC 14825	MW411192 (1,431)	*Tenebrionibacter intestinalis* (100), *Tenebrionicola larvae* (98,80)	*Tenebrionibacter intestinalis* (100), *Tenebrionicola larvae* (99,51)	*Tenebrionibacter intestinalis/larvae*	*Tenebrionibacter intestinalis/larvae*
Tenebrionicola larvae	CCM 9152; KCTC 82597; YMB-R21	MW680835 (1,464)	*Tenebrionicola larvae* (100), *Tenebrionibacter intestinalis* (98,77)	*Tenebrionicola larvae* (100), *Tenebrionibacter intestinalis* (99,51)	*Tenebrionicola larvae*	*Tenebrionicola larvae/ intestinalis*
Trabulsiella guamensis	ATCC 49490; CDC 370–85; CIP 103637; DSM 16940; JCM 21227; NBRC 103172	JMTB01000142 (1,464)	*Trabulsiella guamensis* (100), *T. odontotermitis* (99,07)	*Trabulsiella guamensis* (100), *T. odontotermitis* (98,71)	*Trabulsiella guamensis*	*Trabulsiella guamensis*

(*Continued*)

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Table 3 (Continued).

Species	Type Strain	Accession Number (Sequence Length, Base Pairs)	Result of Identification (% of Similarity) Using			
			EzBioCloud		Clinical & Laboratory Standards Institute Guideline	
			500 bp	Full Gene (~1,500 bp)	500 bp	Full Gene (~1,500 bp)
Xenorhabdus nematophila	ATCC 19061; CCUG 14189; DSM 3370; LMG 1036	FN667742 (1,465)	*Xenorhabdus nematophila* (100)	*Xenorhabdus nematophila* (100)	*Xenorhabdus nematophila*	*Xenorhabdus nematophila*
Yersinia pestis	ATCC 19428; CIP 80.26; NCTC 5923	AF366383 (1,461)	*Yersinia pestis* (100), *Y. pseudotuberculosis* (99,80), *Y. similis* (99,80), *Y. wautersii* (99,60), *Y. frederiksenii* (99,00)	*Yersinia pestis* (100), *Y. pseudotuberculosis* (99,93), *Y. wautersii* (99,79), *Y. similis* (99,66), *Y. frederiksenii* (99,18), *Y. alsatica* (98,90)	*Yersinia* spp.	*Yersinia* spp.
Yokenella regensburgei	ATCC 49455; BCRC 12225; CCRC 12225; CIP 105435; JCM 2403; NBRC 102600; NCTC 11966; NIH 725-83	JMPS01000045 (1,462)	*Yokenella regensburgei* (100), *Pseudescherichia vulneris* (99,60), *Kosakonia pseudosacchari* (99,40), *Enterobacter cancerogenus* (99,20), *E. bugandensis* (99,20). *Huaxiibacter chinensis* (98,80)	*Yokenella regensburgei* (100), *Klebsiella michiganensis* (99,27), *K. grimontii* (99,11), *Citrobacter pasteurii* (99,04), *Enterobacter bugandensis* (99,04). *Silvania hatchlandensis* (98,66)	*Yokenella regensburgei/ Pseudescherichia vulneris/Kosakonia pseudosacchari*	*Yokenella regensburgei/ Klebsiella michiganensis*
Wigglesworthia glossinidia	Description; mycetome; primary endosymbiont of Glossina morsitans morsitans	BA000021 (1,473)	*Wigglesworthia glossinidia* (100)	*Wigglesworthia glossinidia* (100)	*Wigglesworthia glossinidia*	*Wigglesworthia glossinidia*
High group						
Pseudomonas aeruginosa	ATCC 10145; ATCC 10145-U; CCEB 481; CCUG 28447; CCUG 29297; CCUG 551; CFBP 2466; CIP 100.720; DSM 50071; IBCS 277; IFO 12689; JCM 5962; LMG 1242; NBIMCC 3732; NBRC 12689; NCCB 76039; NCIB 8295; NCIMB 8; NCIMB 8295; NCTC 10332; NRRL B-771; RH 815; VKM B-588	BAMA01000316 (1,458)	*Pseudomonas aeruginosa* (100), *P. paraeruginosa* (100)	*Pseudomonas aeruginosa* (100), *P. paraeruginosa* (100)	*Pseudomonas aeruginosa/ paraeruginosa*	*Pseudomonas aeruginosa/ paraeruginosa*

Notes: [a]Including Enterobacterales carbapenem and third-generation cephalosporin-resistant; [b]Presented more than five possible species, the last species with >98.7% of similarity is shown after "…".

Abbreviations: ATCC, American Type Culture Collection; CCUG, Culture Collection, University of Gothenburg; CIP, Collection of the Institute Pasteur; DSM, Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH; JCM, Japan Collection of Microorganisms; LMG, Laboratory of Microbiology at Ghent University in Belgium; NCCB, Netherlands Culture Collection of Bacteria; NCTC, National Collection of Type Cultures; KCTC, Korean Collection for Type Cultures; ICMP, International Collection of Microorganisms; NCPPB, National Collection of Plant Pathogenic Bacteria; CNCTC, Czech National Collection of Type Cultures; CDC, Centers for Disease Control and Prevention; HAMBI, Microbial Domain Biological Resource Centre; IFO, Institute for Fermentation, Osaka; NBIMCC, National Bank for Industrial Microorganisms and Cell Cultures; NRRL, Northern Regional Research Laboratory - ARS Culture Collection; CFBP, French Collection for Plant Associated Bacteria; MCC, Microbial Culture Collection; CECT, Colección Española de Cultivos Tipo; NRRL, Northern Regional Research Laboratory; GDMCC, Guangdong Microbial Culture Collection Center; KACC, Korean Agricultural Culture Collection; MSSRF, M.S. Swaminathan Research Foundation; VKM, All-Russian Collection of Microorganisms; NCAIM, National Collection of Agricultural and Industrial Micro-organisms; CCRC, Culture Collection and Research Center; NIH, National Institutes of Health; CCEB, Culture Collection of Beijing Agricultural University, Beijing, China.

for the identification of clinically important pathogens. In any case, all bacterial species in the ESKAPEE group[18] are included in both databases. However, from the 27 species validity species described in *Enterobacter* genera, only 8 (29,6%) and 9 (33,3%) are listed in the MALDI Biotyper and VITEK MS Prime databases, respectively, showing that their continuous improvement is essential for the identification of clinically important pathogens. For *Cronobacter*, an emerging bacterial pathogen associated with infections such as necrotizing enterocolitis, sepsis, and meningitis in neonates and infants,[51] MALDI Biotyper database only identifies the species at genus level, whereas Vitek MS database possesses six of the seven valid species described.[16]

Next-Generation Sequencing Methods

Next-Generation Sequencing (NGS) methods allow millions of DNA fragments to be sequenced simultaneously and have revolutionized microbiome studies by enabling large-scale analysis of bacterial communities using the 16S rRNA gene. Platforms such as Illumina (Illumina Inc, CA, USA) and Ion Torrent (Thermo Fisher Scientific, USA) use short paired-end reads, usually covering partial regions of the gene, such as V3-V4 or V4, ensuring a high depth of sequencing and a reduced cost per sample. These technologies allow the simultaneous detection of thousands of taxa, making them an excellent choice for studies of microbial diversity, ecology and clinical biomarkers, which can be applied to mixed cultures or faecal material. However, because they are limited to 200–500 bp fragments, they can have limited resolution at the species level, as well as being subject to primer bias and the loss of phylogenetic information. Kits such as the "16S Metagenomic Sequencing Library Preparation" (Illumina Inc, CA, USA), which amplify and sequence the V3-V4 regions, are used in amplicon metagenomics for studies of the microbiome of various environments, whether clinical, environmental or industrial. For data processing, bioinformatic tools such as QIIME (Quantitative Insights into Microbial Ecology), a pioneering program for analyzing amplicon sequencing data, mainly the 16S rRNA gene, it was developed to process raw data for microbial diversity analysis, taxonomic classification, and visualization of results. QIIME2 is the successor to QIIME and was developed as a modular platform that analyzes amplicon, metagenome, and transcriptome sequences.[52] Both are integrated with databases such as SILVA, with cured ribosomal RNA sequences, widely used for taxonomic classification of ASVs/OTUs (amplicon sequence variants/operational taxonomic units) of microorganisms in amplicon-based metagenomics studies.[53] Even so, the robustness, scalability, and well-established protocols consolidate NGS methods as the gold standard for large-scale analysis in microbiology. Recent reviews highlight that, despite the growth of long-read technologies, second-generation platforms continue to be widely used for their reliability and cost-effectiveness.[23,54]

In recent years, advances in sequencing technologies have revolutionized genomic and metagenomic research, significantly expanding the accuracy and scope of microbial analyses. Currently, the Illumina platform is one of the most widely used, producing short, high-quality reads that are ideal for amplicon-based microbial community profiling and whole genome sequencing, combining high throughput and low cost. More recently, third-generation sequencing technologies, such as PacBio SMRT (Pacific Biosciences Single Molecule Real-Time) and Oxford Nanopore Technologies (ONT), have made it possible to obtain long reads, allowing the assembly of complete genomes, plasmids, and operons without extensive fragmentation of the assembly, overcoming several limitations of NGS, especially in the resolution of repetitive genomic regions and the recovery of complete genomes, better described in the following topic.[55,56]

Third-Generation Sequencing Methods

Third-generation sequencing technologies are DNA reading methods that allow individual molecules to be analyzed in real time, without the need for prior large-scale amplification. Among the main platforms are PacBio SMRT and Oxford Nanopore Technologies. In general, both platforms produce long reads, improving taxonomic resolution and offering significant advances in 16S rRNA gene analysis, allowing long reads to be obtained that cover the entire gene region (V1-V9). These technologies provide more accurate taxonomic resolution, especially at the species level, compared to second-generation approaches. Recent studies have shown that in a comparative analysis between Illumina, PacBio and Oxford Nanopore, the latter two platforms offered better resolution at the species level, although they still present challenges related to taxonomic annotation due to limitations in reference databases.[55–57] One of the most important

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Table 4 Genera and Species Listed MALDI Biotyper and Vitek MS Databases

Genera (No. of Species Described)[a]	Species in MALDI Biotyper Database (n).	Species in Vitek MS Database (n).
Acinetobacter (87)	*A. albensis, A. apis, A. baumannii, A. baylyi, A. beijerinckii, A. bereziniae, A. bohemicus, A. boissieri, A. bouvetii, A. brisouii, A. calcoaceticus, A. celticus, A. chinensis, A. colistiniresistens, A. courvalinii, A. cumulans, A. defluvii, A. dispersus, A. equi, A. gandensis, A. gerneri, A. guillouiae, A. gyllenbergii, A. haemolyticus, A. halotolerans, A. harbinensis, A. indicus, A. johnsonii, A. junii, A. kookii, A. lactucae, A. larvae, A. lwoffii, A. modestus, A. nectaris, A. nosocomialis, A. parvus, A. piscicola, A. pittii, A. populi, A. pragensis, A. proteolyticus, A. pseudolwoffii, A. puyangensis, A. qingfengensis, A. radioresistens, A. rudis, A. schindleri, A. seifertii, A. sichuanensis, A. soli, Acinetobacter* sp., *A. tandoii, A. tjernbergiae, A. towneri, A. ursingii, A. variabilis, A. venetianus, A. vivianii, A. wuhouensis* (59)	*A. baumannii, A. beijerinckii, A. bereziniae, A. calcoaceticus, A. courvalinii, A. guillouiae, A. gyllenbergii, A. haemolyticus, A. johnsonii, A. junii, A. lwoffii, A. nosocomialis, A. pittii, A. radioresistens, A. schindleri, A. seifertii, A. ursingii* (17)
Acerihabitans arboris (1)	Absent	Absent
Apirhabdus apintestini (1)	Absent	Absent
Arsenophonus (2)	*Arsenophonus nasoniae* (1)	Absent
Biostraticola tofi (1)	Absent	Absent
Brenneria (12)	*B. alni, B. nigrifluens, B. rubrifaciens, B. salicis* (4)	Absent
Buchnera aphidicola (1)	Absent	Absent
Budvicia (2)	*B. aquatica, B. diplopodorum* (2)	*B. aquatica* (1)
Buttiauxella (8)	*B. agrestis, B. brennerae, B. ferragutiae, B. gaviniae, B. izardii, B. noackiae, B. warmboldiae* (7)	*B. agrestis* (1)
Cedecea (5)	*C. davisae, C. lapagei, C. neteri (3)*	*C. davisae, C. lapagei, C. neteri (3)*
Chania multitudinisentens (1)	Absent	Absent
Chimaeribacter (3)	Absent	Absent
Citrobacter (17)	C. *amalonaticus, C. braakii, C. farmeri, C. freundii, C. gillenii, C. koseri, C. murliniae, C. rodentium, C. sedlakii, C. youngae* (10)	C. *amalonaticus, C. braakii, C. farmeri, C. freundii, C. gillenii, C. koseri, C. murliniae, C. rodentium, C. sedlakii, C. werkmanii, C. youngae* (11)
Cronobacter (7)	*Cronobacter* spp. (0)	*C. dublinensis* subsp. *dublinensis, C. dublinensis* subsp. *lactaridi, C. dublinensis* subsp. *lausannensis, C. malonaticus, C. muytjensii, C. sakazakii, C. turicensis, C. universalis* (6)
Dickeya (14)	*D. chrysanthemi, D. dadantii, D. dianthicola, D. zeae* (4)	*D. chrysanthemi, D. dianthicola, D. paradisiaca, D. zeae* (4)
Dryocola (2)	Absent	Absent
Duffyella gerundensis (1)	Absent	Absent
Edwardsiella (5)	*E. hoshinae, E. ictaluri, E. tarda* (3)	*E. hoshinae, E. tarda* (2)
Enterobacillus tribolii (1)	Absent	Absent

Enterobacter (27)	*E. asburiae, E. bugandensis, E. cancerogenus, E. cloacae, E. hormaechei, E. kobei, E. ludwigii, E. roggenkampii* (8)	*E. asburiae, E. bugandensis, E. cancerogenus, E. cloacae* subsp. *cloacae, E. cloacae* subsp. *dissolvens, E. hormaechei* subsp. *hoffmannii, E. hormaechei* subsp. *hormaechei, E. hormaechei* subsp. *oharae, E. hormaechei* subsp. *xiangfangensis, E. hormaechei* subsp. *steigerwaltii, E. kobei, E. ludwigii, E. roggenkampii, E. soli* (9)
Erwinia (20)	*E. amylovora, E. aphidicola, E. billingiae, E. mallotivora, E. oleae, E. papayae, E. persicina, E. piriflorinigrans, E. psidii, E. pyrifoliae, E. rhapontici, E. tasmaniensis, E. teleogrylli, E. toletana, E. tracheiphila, E. typographi* (16)	*E. billingiae, E. mallotivora, E. rhapontici, E. tasmaniensis, E. tracheiphila* (5)
Escherichia (7)	*E. albertii, E. coli, E. fergusonii, E. hermannii, E. marmotae* (5)	*E. albertii, E. coli* (2)
Ewingella americana (1)	*E. americana* (1)	*E. americana* (1)
Franconibacter (3)	*F. helveticus, F. pulveris* (2)	*F. helveticus, F. pulveris* (2)
Gallaecimonas (3)	Absent	Absent
Gibbsiella (3)	Absent	Absent
Hafnia (3)	*H. alvei* (1)	*H. paralvei* (1)
Huaxiibacter chinensis (1)	Absent	Absent
Intestinirhabdus alba (1)	Absent	Absent
Klebsiella (17)	*K. aerogenes, K. oxytoca, K. pneumoniae, K. variicola* (4)	*K. aerogenes, K. oxytoca, K. pneumoniae* subsp. *ozaenae, K. pneumoniae* subsp. *pneumoniae, K. pneumoniae* subsp. *rhinoscleromatis, K. variicola* (4)
Kluyvera (5)	*K. ascorbata, K. cryocrescens, K. georgiana, K. intermedia* (4)	*K. ascorbata, K. cryocrescens, K. intermedia* (3)
Kosakonia (10)	*K. cowanii, K. radicincitans* (2)	Absent
Leclercia (3)	*L. adecarboxylata* (1)	*L. adecarboxylata* (1)
Lelliottia (4)	*L. amnigena, L. nimipressuralis* (2)	*L. amnigena* (1)
Leminorella (2)	*L. grimontii, L. richardii* (2)	Absent
Limnobaculum (5)	Absent	Absent
Lonsdalea (4)	*L. quercina* (1)	Absent
Mangrovibacter (3)	Absent	Absent
Mixta (5)	*M. calida, M. gaviniae, M. intestinalis, M. theicola* (4)	*M. calida* (1)
Moellerella wisconsensis (1)	*M. wisconsensis* (1)	*M. wisconsensis* (1)
Obesumbacterium proteus	Absent	*O. proteus* (1)

(*Continued*)

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Table 4 (Continued).

Genera (No. of Species Described)[a]	Species in MALDI Biotyper Database (n).	Species in Vitek MS Database (n).
Pantoea (21)	*P. agglomerans, P. ananatis, P. anthophila, P. cypripedii, P. dispersa, P. eucrina, P. piersonii, P. septica, P. stewartii, P. vagans* (10)	*P. agglomerans, P. ananatis, P. dispersa* (3)
Pectobacterium (22)	*P. atrosepticum, P. betavasculorum, P. cacticida, P. carotovorum, P. odoriferum, P. wasabiae* (6)	*P. carotovorum, P. odoriferum* (2)
Phaseolibacter flectens (1)	Absent	Absent
Photorhabdus (25)	*P. akhurstii, P. asymbiotica, P. australis, P. bodei, P. caribbeanensis, P. cinerea, P. heterorhabditis, P. kayaii, P. kleinii, P. laumondii, P. luminescens, P. namnaonensis, P. temperata, P. thracensis* (14)	*P. asymbiotica* subsp. *asymbiotica, P. australis, P. akhursti, P. caribbeanensis, P. hainanensis, P. kayaii, P. kleinii, P. noenieputensis, P. laumondii* subsp. *laumondii* (9)
Phytobacter (4)	*P. massiliensis, P. ursingii* (2)	Absent
Pluralibacter (2)	*P. gergoviae, P. pyrinus* (2)	*P. gergoviae* (1)
Pragia fontium (1)	*Pragia fontium* (1)	Absent
Prodigiosinella aquatilis (1)	Absent	Absent
Proteus (10)	*P. hauseri, P. mirabilis, P. penneri, P. vulgaris* (4)	*P. hauseri, P. mirabilis, P. penneri, P. vulgaris* (4)
Providencia (17)	*P. alcalifaciens, P. heimbachae, P. rettgeri, P. rustigianii, P. stuartii, P. vermicola* (6)	*P. alcalifaciens, P. rettgeri, P. rustigianii, P. stuartii* (4)
Pseudescherichia vulneris (1)	*P. vulneris* (1)	*P. vulneris* (1)
Pseudocitrobacter (3)	Absent	*P. faecalis* (1)
Pseudomonas (356)	*P. abietaniphila, P. aeruginosa, P. agarici, P. alcaligenes, P. alcaliphila, P. anguilliseptica, P. antarctica, P. asplenii, P. avellanae, P. avenae, P. azotifigens, P. azotoformans, P. balearica, P. borbori, P. boreopolis, P. brassicacearum, P. brenneri, P. caricapapayae, P. cedrina, P. chlororaphis, P. cichorii, P. citronellolis, P. composti, P. congelans, P. corrugata, P. cuatrocienegasensis, P. extremorientalis, P. flavescens, P. fluorescens, P. fragi, P. frederiksbergensis, P. fulva, P. gessardii, P. graminis, P. grimontii, P. guariconensis, P. indica, P. japonica, P. jessenii, P. jinjuensis, P. kilonensis, P. koreensis, P. kuykendallii, P. libanensis, P. lundensis, P. lutea, P. luteola, P. mandelii, P. marginalis, P. massiliensis, P. mendocina, P. migulae, P. monteilii, P. mosselii, P. mucidolens, P. nitroreducens, P. nosocomialis, P. oleovorans, P. orientalis, P. oryzihabitans, P. otitidis, P. panipatensis, P. plecoglossicida, P. poae, P. pohangensis, P. protegens, P. proteolytica, P. putida, P. resinovorans, P. rhizosphaerae, P. rhodesiae, Pseudomonas* sp., *P. savastanoi, P. segetis, P. straminea, P. stutzeri, P. synxantha, P. syringae, P. taetrolens, P. taiwanensis, P. thermotolerans, P. thivervalensis, P. tolaasii, P. trivialis, P. umsongensis, P. vancouverensis, P. veronii, P. viridiflava, P. xanthomarina* (91)	*P. aeruginosa, P. alcaligenes, P. anguilliseptica, P. asplenii, P. chlororaphis* subsp. *aureofaciens, P. chlororaphis* subsp. *chlororaphis, P. citronellolis, P. cuatrocienegasensis, P. fluorescens, P. fragi, P. luteola, P. mendocina, P. migulae, P. monteilii, P. mosselii, P. oleovorans, P. oryzihabitans, P. otitidis, P. putida, P. rhodesiae, P. straminea, P. stutzeri, P. syringae, P. syringae pv. delphinii, P. veronii, P. viridiflava* (24)
Rahnella (14)	*R. aquatilis, R. bruchi, R. inusitata, R. woolbedingensis* (4)	*R. aquatilis* (1)
Rosenbergiella (6)	Absent	Absent
Rouxiella (4)	*R. badensis, R. chamberiensis* (2)	Absent

Saccharobacter fermentatus (1)	Absent	Absent
Salmonella (3)	*Salmonella* spp. (0)	*S. enterica* subsp. *arizonae, S. enterica* subsp. *diarizonae, S. enterica* subsp. *enterica,* S. ser. Enteritidis, S. ser. Gallinarum, S. ser. Paratyphi A, S. ser. Paratyphi B, S. ser. Paratyphi C, S. ser. Typhi, S. ser. Typhimurium, *S. enterica* subsp. Houtenae (1)
Samsonia erythrinae (1)	*Samsonia erythrinae* (1)	Absent
Scandinavium (5)	*S. goeteborgense* (1)	Absent
Serratia (24)	*S. entomophila, S. ficaria, S. fonticola, S. grimesii, S. liquefaciens, S. marcescens, S. nematodiphila, S. odorifera, S. plymuthica, S. proteamaculans, S. quinivorans, S. rubidaea, S. ureilytica* (13)	*S. ficaria, S. fonticola, S. grimesii, S. liquefaciens, S. marcescens, S. odorifera, S. plymuthica, S. proteamaculans, S. quinivorans, S. rubidaea* (10)
Shigella (4)	Absent	Absent[b]
Shimwellia (2)	*S. blattae* (1)	Absent
Siccibacter (2)	*S. colletis, S. turicensis* (2)	*S. turicensis* (1)
Silvania (2)	Absent	Absent
Sodalis (3)	*S. glossinidius* (1)	Absent
Symbiopectobacterium purcellii (1)	Absent	Absent
Tatumella (6)	*T. citrea, T. ptyseos, T. punctata, T. terrea* (4)	*T. ptyseos* (1)
Tenebrionibacter intestinalis (1)	Absent	Absent
Tenebrionicola larvae (1)	Absent	Absent
Trabulsiella (2)	*T. guamensis* (1)	Absent
Xenorhabdus (29)	*X. beddingii, X. bovienii, X. budapestensis, X. eapokensis, X. ehlersii, X. innexi, X. japonica, X. nematophila, X. poinarii, X. szentirmaii* (10)	*X. nematophila* (1)
Yersinia (26)	*Y. aldovae, Y. aleksiciae, Y. bercovieri, Y. enterocolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. massiliensis, Y. mollaretii, Y. pseudotuberculosis, Y. rohdei, Y. ruckeri* (12)	*Y. aldovae, Y. bercovieri, Y. enterocolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. massiliensis, Y. mollaretii, Y. pseudotuberculosis, Y. rohdei, Y. ruckeri, Y. similis* (12)
Yokenella regensburgei (1)	*Y. regensburgei* (1)	*Y. regensburgei* (1)

Notes: [a]According to the List of Prokaryotic names with Standing in Nomenclature (Accessed on 08/24/2025). [b]*Shigella* species are identified as *Escherichia coli*. Confirmatory tests are necessary to distinguish *E. coli* species from *Shigella*.

advances in third-generation sequencing is PacBio HIFI (High-Fidelity Reads), which combines long reads (>25 kb) with high accuracy (99.9%), allowing for the complete analysis of complex regions, circumventing the biases intrinsic to amplification-based approaches. This occurs due to the repeated sequencing of the same molecule by DNA polymerase, and multiple reads of the same molecule are combined to generate circular consensus sequences (CCS). Similarly, continuous improvements in ONT's chemistry and basecalling algorithms have increased accuracy and throughput, as well as making ONT's long reads more reliable for assembling complete genomes.[58]

In addition to the partial or complete sequencing of the 16S rRNA gene by NGS and third-generation methodologies, respectively, it is fully possible to extract 16S rRNA gene sequences directly from genomes to carry out taxonomic identification and phylogenetic inference analyses. This approach circumvents the limitations associated with sequencing partial regions per amplicon, taking advantage of the complete 16S gene (~1.5 kb) for greater resolution at the species level. Bioinformatics tools such as Contest16S[59] can automatically locate and extract 16S genes from genome FASTA files, which can then be aligned, compared with reference database, and used to build robust phylogenetic trees, inferring evolutionary relationships between bacteria. This procedure is particularly useful in microbial taxonomy studies, characterization of clinical or environmental isolates, as well as allowing cross-validation with amplicon sequencing data, consolidating bacterial identification in a more complete and reliable way.

The choice of sequencing technology for bacterial identification based on the 16S rRNA gene depends on factors such as accuracy, read length, and data transfer speed. Sanger sequencing offers high-quality reads, ideal for low-yield studies, while next-generation sequencing produces higher yields but generates shorter reads, which can decrease taxonomic resolution. Third-generation platforms, such as Oxford Nanopore and PacBio, produce long reads that increase resolution across the entire 16S gene. Therefore, the selection of a platform should consider the balance between accuracy, detail, and the actual limitations of each sequencing method.[29,56]

A recent study compared three sequencing platforms – Illumina MiSeq, PacBio HIFI, and Oxford Nanopore Technologies - to characterize the gut microbiome of rabbits based on the 16S rRNA gene. On the Illumina platform, short reads from the V3–V4 regions of the 16S gene were sequenced, producing many reads but lower taxonomic resolution (48% identification at the species level). PacBio and ONT, which generate long reads, achieved 63% and 76% taxonomic resolution at the species level, respectively. Despite this, the three platforms showed significant differences in microbial composition and diversity, and many species-level classifications were ambiguous ("uncultured_bacterium").[55] In another study, a comparative evaluation of 16S rRNA gene sequencing in soil microbiomes was performed using the Illumina (V4 and V3-V4 regions), PacBio (full sequences and truncated V3-V4/V4 regions), and ONT (full sequences) platforms. There was significant variation in detection sensitivity between platforms. PacBio showed a slight advantage in identifying low-abundance taxa, while ONT provided results very similar to those of PacBio, indicating that ONT's typical sequencing errors have limited impact on the interpretation of well-represented taxa. These results highlight the importance of choosing the appropriate sequencing platform to achieve the desired taxonomic resolution and meet specific research objectives.[56]

Conclusion

The analysis presented in this review underscores the importance of 16S rRNA gene sequencing as a valuable tool for the identification of pathogenic Gram-negative bacilli listed in the WHO antimicrobial resistance priority list. Its conserved and hypervariable regions provide a solid framework for phylogenetic studies and for distinguishing a wide range of clinically relevant taxa. Nevertheless, this method also presents intrinsic limitations, particularly in differentiating closely related species, or in genera with low taxonomic resolution. Furthermore, the accuracy of identification is highly dependent on the choice of primers, sequencing approach, and, critically, the quality and comprehensiveness of reference databases. Thus, while 16S rRNA sequencing remains a cornerstone for bacterial taxonomy and clinical diagnostics, it should be complemented by additional molecular targets or whole genome sequencing to achieve reliable species-level resolution. The integration of these strategies will enhance diagnostic accuracy, infection control, AMR identification, support epidemiological surveillance, and strengthen infection control practices against multidrug-resistant pathogens.

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