



Year: 2024

Novel Organism Verification and Analysis (NOVA) study: identification of 35 clinical isolates representing potentially novel bacterial taxa using a pipeline based on whole genome sequencing

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Abstract: BACKGROUND Reliable species identification of cultured isolates is essential in clinical bacteriology. We established a new study algorithm named NOVA - Novel Organism Verification and Analysis to systematically analyze bacterial isolates that cannot be characterized by conventional identification procedures MALDI-TOF MS and partial 16 S rRNA gene sequencing using Whole Genome Sequencing (WGS). RESULTS We identified a total of 35 bacterial strains that represent potentially novel species. *Corynebacterium* sp. (n = 6) and *Schaalia* sp. (n = 5) were the predominant genera. Two strains each were identified within the genera *Anaerococcus*, *Clostridium*, *Desulfovibrio*, and *Peptoniphilus*, and one new species was detected within *Citrobacter*, *Dermabacter*, *Helcococcus*, *Lancefieldella*, *Neisseria*, *Ochrobactrum* (*Brucella*), *Paenibacillus*, *Pantoea*, *Porphyromonas*, *Pseudoclavibacter*, *Pseudomonas*, *Psychrobacter*, *Pusillimonas*, *Rothia*, *Sneathia*, and *Tessaracoccus*. Twenty-seven of 35 strains were isolated from deep tissue specimens or blood cultures. Seven out of 35 isolated strains identified were clinically relevant. In addition, 26 bacterial strains that could only be identified at the species level using WGS analysis, were mainly organisms that have been identified/classified very recently. CONCLUSION Our new algorithm proved to be a powerful tool for detection and identification of novel bacterial organisms. Publicly available clinical and genomic data may help to better understand their clinical and ecological role. Our identification of 35 novel strains, 7 of which appear to be clinically relevant, shows the wide range of undescribed pathogens yet to define.

DOI: <https://doi.org/10.1186/s12866-023-03163-7>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-254832>

Journal Article

Published Version



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Originally published at:

Muigg, Veronika; Seth-Smith, Helena M B; Adam, Kai-Manuel; Weisser, Maja; Hinic, Vladimira; Blaich, Annette; Roloff, Tim; Heininger, Ulrich; Schmid, Hanna; Kohler, Maurus; Graf, Lukas; Winterflood, Dylan M; Schlaepfer, Pascal; Goldenberger, Daniel (2024). Novel Organism Verification and Analysis (NOVA) study: identification of 35










clinical isolates representing potentially novel bacterial taxa using a pipeline based on whole genome sequencing.
BMC Microbiology, 24(1):14.
DOI: <https://doi.org/10.1186/s12866-023-03163-7>

RESEARCH

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Novel Organism Verification and Analysis (NOVA) study: identification of 35 clinical isolates representing potentially novel bacterial taxa using a pipeline based on whole genome sequencing

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Abstract

Background Reliable species identification of cultured isolates is essential in clinical bacteriology. We established a new study algorithm named **NOVA – Novel Organism Verification and Analysis** to systematically analyze bacterial isolates that cannot be characterized by conventional identification procedures MALDI-TOF MS and partial 16 S rRNA gene sequencing using Whole Genome Sequencing (WGS).

Results We identified a total of 35 bacterial strains that represent potentially novel species. *Corynebacterium* sp. (n=6) and *Schaalia* sp. (n=5) were the predominant genera. Two strains each were identified within the genera *Anaerococcus*, *Clostridium*, *Desulfovibrio*, and *Peptoniphilus*, and one new species was detected within *Citrobacter*, *Dermabacter*, *Helcococcus*, *Lancefieldella*, *Neisseria*, *Ochrobactrum* (*Brucella*), *Paenibacillus*, *Pantoea*, *Porphyromonas*, *Pseudoclavibacter*, *Pseudomonas*, *Psychrobacter*, *Pusillimonas*, *Rothia*, *Sneathia*, and *Tessaracoccus*. Twenty-seven of 35 strains were isolated from deep tissue specimens or blood cultures. Seven out of 35 isolated strains identified were clinically relevant. In addition, 26 bacterial strains that could only be identified at the species level using WGS analysis, were mainly organisms that have been identified/classified very recently.

Conclusion Our new algorithm proved to be a powerful tool for detection and identification of novel bacterial organisms. Publicly available clinical and genomic data may help to better understand their clinical and ecological role. Our identification of 35 novel strains, 7 of which appear to be clinically relevant, shows the wide range of undescribed pathogens yet to define.

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Keywords Clinical isolates, Difficult to identify strains, Whole genome sequencing (WGS), Novel bacteria, Clinical significance, Type (strain) genome server (TYGS)

Background

Species identification is the first and crucial step in the workflow of clinical microbiology as it provides essential guidance regarding treatment [1]. While the vast majority of pathogens isolated in clinical microbiology laboratories belong to well characterized species, a small number of bacterial isolates may not be reliably identified using conventional identification methods due to lack of sufficient reference data or to the presence of a previously uncharacterized organisms. In cases where the rapid Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) methods do not provide a clear identification, molecular techniques are often used. The establishment of 16 S rRNA gene sequence analysis has provided a simple and rapid method for species identification in such cases, and has led to the reclassification and renaming of numerous bacterial genera and species [2, 3]. However, in some cases, analysis of the 16 S rRNA gene sequence also fails to distinguish between species. In these cases, whole genome sequencing (WGS) can be used, which offers better resolution at the species level [1, 4].

We have established an algorithm to identify and characterize strains which are not identifiable by standard methods, i.e., MALDI-TOF MS and partial 16 S rRNA gene sequence analysis, using WGS in a systematic approach. The aim of the study is to detect and characterize new bacterial organisms isolated from clinical specimens and to reliably detect difficult to identify strains. In this report, we describe 35 isolates that represent novel bacterial species, 7 of which were clinically relevant, as well as 26 strains (22 species) whose identification in the routine laboratory was problematic. We provide genome sequences of these species to expand the public database for taxonomic and epidemiological purposes, and we additionally present detailed clinical information about the patients and an assessment of the clinical relevance of the isolates to gain clinical and ecological knowledge about the novel bacterial species.

Methods

The Novel Organism Verification and Analysis (NOVA) study is a prospective study with the aim of characterizing bacterial isolates that are not identifiable by routine diagnostic methods using WGS and thereby describing potential new species. The study was conducted at the Department of Clinical Bacteriology and Mycology of the University Hospital Basel, a tertiary care hospital in Switzerland, and was initiated in 2014. Here we present phenotypic and molecular data on bacterial isolates as well

as clinical information on the patients within a time span from December 2014 to January 2022. Isolates that qualified for the NOVA study were identified using a specific algorithm that was integrated into the routine diagnostic process (Fig. 1).

Description of the NOVA algorithm

Microscopy, aerobic and anaerobic cultures from the various clinical specimens were performed according to standard microbiological procedures including enrichment culture using thioglycolate medium. Anaerobic cultures were incubated and manipulated in an anaerobic workstation (Whitley A 95, Don Whitley Scientific Ltd., Bingley, UK). Species identification of bacterial isolates from routine culture procedures was conducted by MALDI-TOF MS (Bruker Daltonics GmbH, Bremen, Germany) using a simple smear technique with a 1- μ l formic acid overlay and cyano-4-hydroxyinnamic acid (CHCA) matrix solution. Measurements were analyzed with the main spectra library Bruker Daltonics database. If no reliable species identification was achieved with MALDI-TOF MS; i.e., score < 2.0, divergent results on the first and second hit, no validly published species, e.g., *Corynebacterium* lipophilic group F1, or no identification on species level, isolates were subsequently analyzed using partial 16 S rRNA gene PCR and sequence analysis of approximately 800 bp of the first part [5]. The resulting sequences were compared to the 16 S rRNA gene sequence nucleotide databases of the National Center for Biotechnology Information (NCBI) network service (<https://blast.ncbi.nlm.nih.gov>). If seven or more mismatches/gaps (corresponding to $\leq 99.0\%$ nucleotide identity) were identified in the analyzed sequence compared to the closest correctly described bacterial species, the isolates were included into the NOVA study (Fig. 1). A species was considered correctly described if it was designated as validly published in the List of Prokaryotic names with Standing in Nomenclature (LPSN) of the German strain collection database (<https://www.bacterio.net>) [6].

The NOVA pipeline

DNA Extraction was executed with EZ1 DNA Tissue Kit using EZ1 Advanced Instrument (Qiagen, Hilden, Germany). WGS was performed using Illumina technology (MiSeq or NextSeq500) following library creation (NexteraXT or Illumina DNA prep). Assemblies were created from trimmed (trimmomatic v 0.38) [7] reads using unicycler v0.3.0b [8] and annotated using Prokka v1.13 [9]. Assemblies were analyzed using rMLST [10] and TYGS

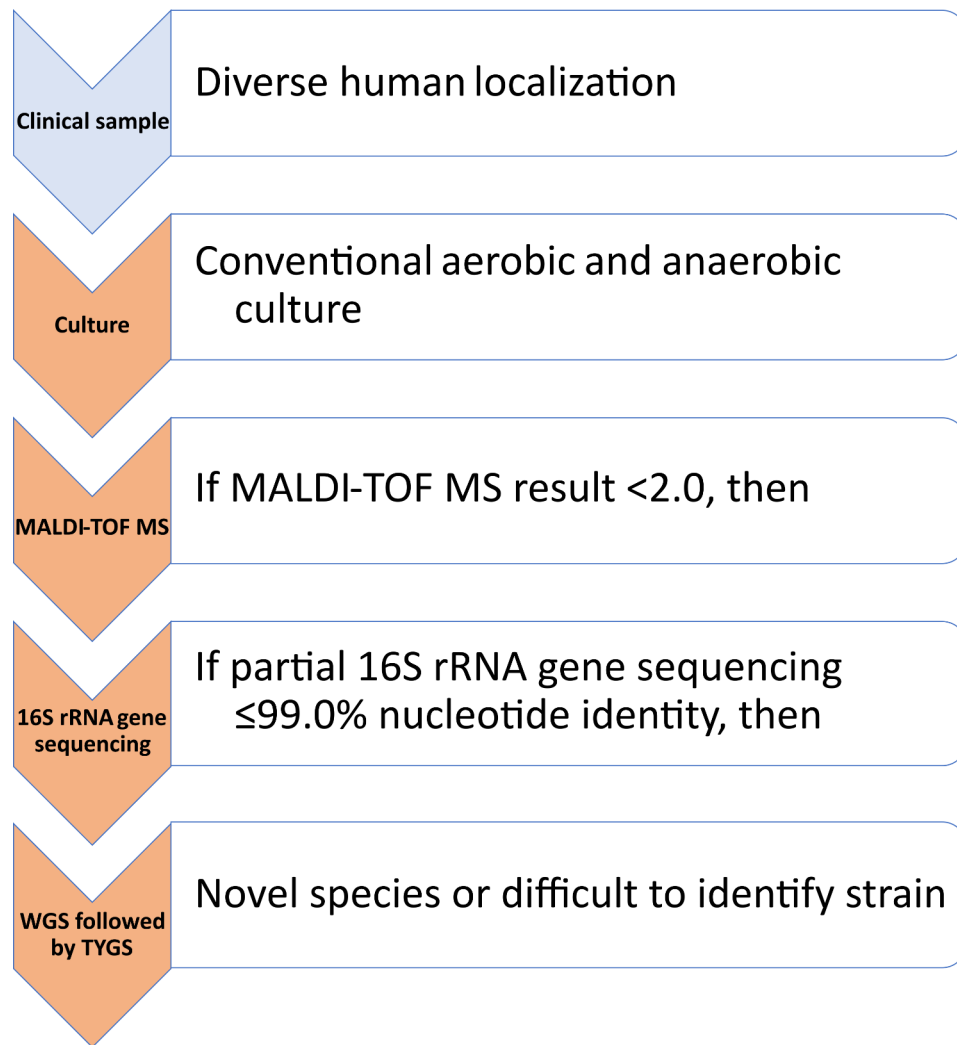


Fig. 1 Algorithm for identification of clinical isolates suitable for the NOVA study

(<https://tygs.dsmz.de> using the 70% digital DNA:DNA hybridization (dDDH) cutoff and method 2 [11]. The date of ultimate TYGS analysis was August 8, 2023.

Average Nucleotide Identity (ANI) values were calculated using the OrthoANIu [12]. Calculations were automated using a windows batch file (GitHub: https://github.com/schlaepferp/win_batch_ani).

Evaluation of clinical relevance by infectious diseases specialists

Patient data were retrospectively extracted from medical records, and the microbiological findings were evaluated individually along with the patient's clinical presentation by an infectious disease specialist. Clinical relevance was estimated on the basis of the following criteria: clinical signs and symptoms, presence of concomitant pathogens, pathogenic potential of the genus of the isolate, and clinical plausibility. The impact on patient care in terms of

antibiotic use or antibiotic switching was not investigated in our study.

Availability of data

Genome data of 56 isolates of this study is accessible at NCBI under BioProject number PRJEB55530. Genome data of *Gulosibacter hominis* strains USB_NOVA_36, USB_NOVA_37, and USB_NOVA_38 are available under CAJGWQ000000000, CAJHCD000000000, and CAJHCF000000000, respectively [13]. The genomes of *Pseudoclavibacter triregionum* (USB_NOVA_49) [14] and *Cutibacterium modestum* (USB_NOVA_51) [15] are accessible under OU365335 and PRJEB41775, respectively. Scripts for calculating ANI-values between two genomes are deposited in GitHub [https://github.com/schlaepferp/win_batch_ani].

Results

A total of 61 isolates, 41 (67%) Gram positive and 20 (33%) Gram negative strains, were not identifiable using routine methods and were included in the NOVA study within the study period. Thirty-five (57%) organisms were identified to be novel bacterial species and 26 (43%) isolates represented difficult to identify organisms.

Predominant genus was *Corynebacterium* with 11 isolates (2 *C. pseudogenitalium*, 1 *C. hindlerae*, 1 “*C. phoceense*”, 1 “*C. provencense*”, and 6 *Corynebacterium* sp. nov. (Fig. 2), followed by *Schaalia* sp. with 5 strains.

The anatomical localization of these 61 clinical samples are indicated in Tables 1 and 2. Predominant specimen was blood culture (n=9). Detailed microbiological results from the 61 cases including type of specimen, microscopy, cultured isolates, MALDI-TOF MS, and partial 16 S rRNA gene sequencing are listed in Table S1.

Overall, medical history and information on clinical relevance were available from 47/61 cases. In 15/47 of cases, the respective bacterial isolate was considered clinically relevant, and in 21 cases as not clinically relevant. In the remaining 11 cases, clinical relevance was unclear. In 3/15 cases classified as clinically relevant, culture growth was monomicrobial. In 2 of these 3 cases, patients had received antibiotics for >3 days at the time of sample collection.

The age range of the 47 patients was from 7 to 94, median age 61 years. Thirty (64%) were males and 17 (36%) females.

Isolates representing novel species, (n = 35)

Twenty-one isolates grew under aerobic and 14 under anaerobic conditions. Twenty-four (69%) strains were Gram positive and 11 (31%) Gram negative. Six isolates belong to novel species within the genus *Corynebacterium* (Fig. 2), followed by *Schaalia* (n=5). There were two strains representative for each of the following genera: *Anaerooccus*, *Clostridium*, *Desulfovibrio*, and *Peptoniphilus*. For each of the following genera one new species was identified: *Citrobacter*, *Dermabacter*, *Helcococcus*, *Lancefieldella*, *Neisseria*, *Ochrobactrum* (*Brucella*), *Pae-nibacillus*, *Pantoea*, *Porphyromonas*, *Pseudoclavibacter*, *Pseudomonas*, *Psychrobacter*, *Pusillimonas*, *Rothia*, *Sneathia*, and *Tessaracoccus* (Table 1).

The following isolates represent the same novel species based on an ANI index ≥ 96.0 : *Corynebacterium* sp. nov.: isolate USB_NOVA_02 and USB_NOVA_03, ANI 99.96 (Fig. 2); *Desulfovibrio* sp. nov. USB_NOVA_16 and USB_NOVA_17, ANI 98.5; *Peptoniphilus* sp. nov. USB_NOVA_18 und USB_NOVA_19, ANI 97.7.

Clinical data were available from 26 cases. Seven/26, 6/26, and 13/26 were classified clinically relevant, unclear, and clinically not relevant, respectively (Table 1).

Difficult to identify isolates, (n = 26)

Twenty-six isolates belong to previously described species which could not be identified by standard identification methods, but only by WGS. These strains represent 19 species already validly published and three species not yet validly published. (Table 2). Seventeen (65.4%) strains were Gram-stain-positive and 9 (34.6%) Gram-stain-negative. Four isolates were identified as *Gulosibacter hominis*, and one isolate as *Pseudoclavibacter triregionum*. Both aerobic Gram-stain-positive bacilli have been described taxonomically from our group in collaboration with the BCCM/LMG Bacteria Collection, Ghent, Belgium in 2021 and 2022, respectively [13, 14]. Two isolates represent *Corynebacterium pseudogenitalium* that has been published validly very recently [16]. In addition, one isolate for each of the following species were identified: *Anaerococcus degeneri*, *Corynebacterium hindlerae*, *Corynebacterium phoceense*, *Corynebacterium provencense*, *Cutibacterium modestum*, *Devosia equisanguinis*, *Enterococcus dongliensis*, *Fenollaria massiliensis*, *Kingella negevensis*, *Kingella pumchi*, *Mogibacter kristianse-nii*, *Pantoea agglomerans*, *Parvimonas parva*, *Prevotella brunnea*, *Pseudomonas yangonensis*, *Pseudoramibacter alactolyticus*, *Saezia sanguinis*, *Slackia exigua*, and *Vandamella animalimorsus*. Of these, “*Corynebacterium phoceense*”, “*Corynebacterium provencense*”, and “*Kingella pumchi*” represent not yet validly published bacterial species. Fifteen (63.6%) from a total of 22 species have been described recently (\geq year 2019) and 14 (53.8%) out of 26 isolates have been isolated before the valid description of the species.

Clinical data were available from 21 cases. Eight/21, 5/21, and 8/21 were classified clinically relevant, unclear, and clinically not relevant, respectively (Table 2).

Discussion

We present an innovative algorithm based on WGS for systematic and reliable identification of bacterial isolates that can not be identified by routine diagnostic methods. Using this algorithm, we collected and analyzed a total of 61 clinical isolates, 35 of them represent potentially novel species and from February 2022 to July 2023 another 21 potentially novel isolates have been collected (not presented in this publication).

The idea of this study arose with the introduction of the WGS technology in our laboratory. Initially, analysis of the genomes was performed in individual time-consuming procedures. A milestone was the availability of the web-based TYGS platform in 2019, which allows genomic data to be analyzed in a standardized manner to determine the correct taxonomic species or define the organism as a novel taxon based on WGS data [11]. Our NOVA tool is now integrated in routine diagnostic procedures and is performed weekly. It represents a relatively

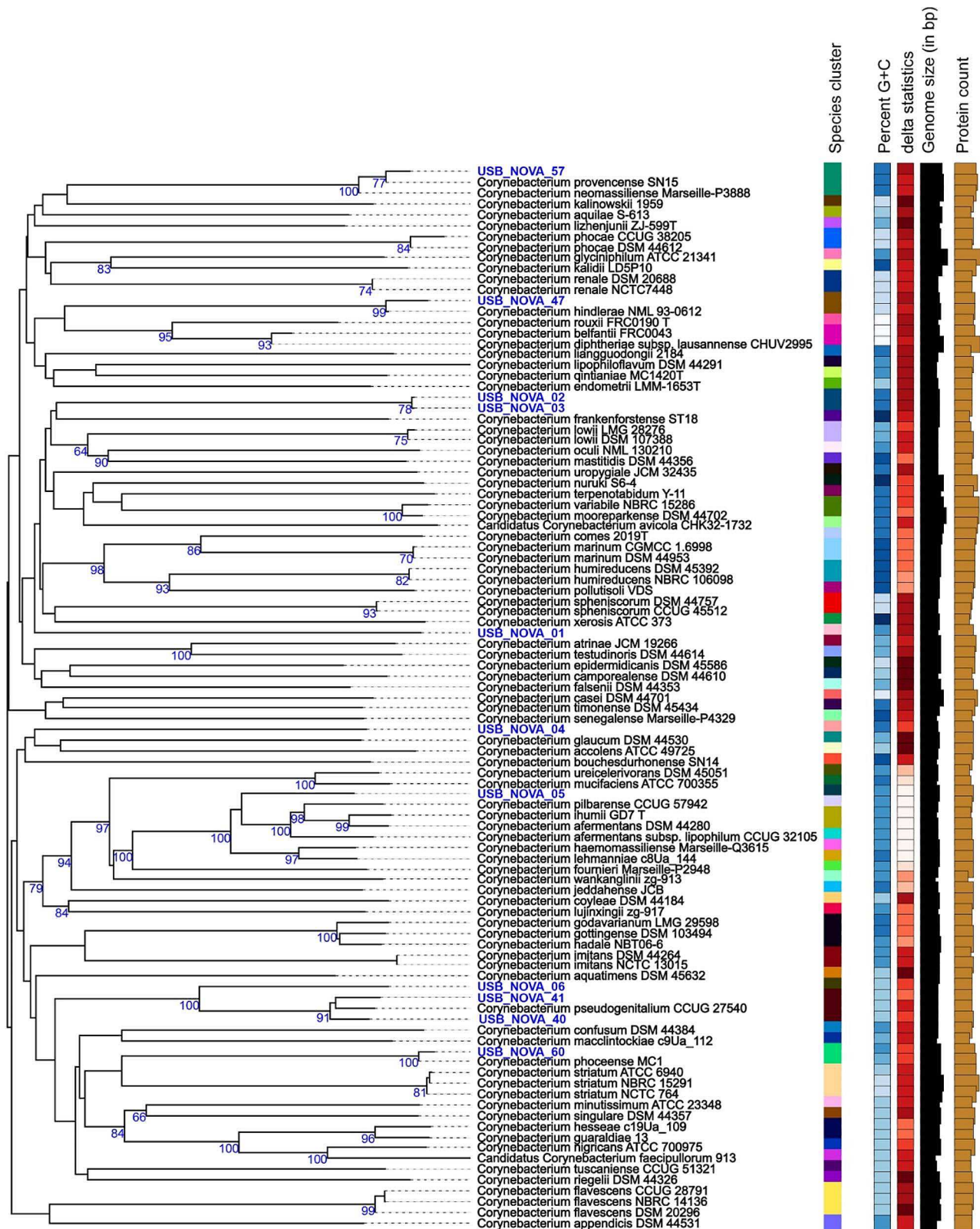


Fig. 2 TYGS GBDP tree showing relationships between *Corynebacterium* genomes and references. Figure was generated by TYGS (<https://doi.org/10.1038/s41467-019-10210-3>), with 11 genomes sequenced for this publication shown in blue colour. Species clusters are defined by dDDH with a 70% cutoff shown in the first metadata column. Isolates USB_NOVA_40 and USB_NOVA_41 represent *C. pseudogenitalium*, isolates USB_NOVA_47, USB_NOVA_57, and USB_NOVA_60 cluster with species *C. hindlerae*, “*C. provencense*”, and “*C. phoceense*”, respectively; the other 6 genomes do not form clusters with any sequenced type strains

Table 1 List of 35 clinical isolates representing novel taxa and corresponding clinical data

| ID Number | Species | Genbank Accession No. (BioSample): SAMEA | Origin of specimen | Clinical data | | | |
|-------------|-------------------------------|--|------------------------------|---------------|---|---|--------------------|
| | | | | Age(y)/sex | Clinical presentation | Relevant underlying disease | Clinical relevance |
| USB_NOVA_01 | <i>Corynebacterium</i> sp.nov | 111,563,050 | Swab toe | 61/m | Abscess formation | None | Not relevant |
| USB_NOVA_02 | <i>Corynebacterium</i> sp.nov | 111,563,052 | Urine | 57/f | Urolithiasis | None | Not relevant |
| USB_NOVA_03 | <i>Corynebacterium</i> sp.nov | 111,563,049 | Swab external auditory canal | 53/m | Acute otitis media due to <i>Pseudomonas aeruginosa</i> | Squamous cell carcinoma floor of mouth | Not relevant |
| USB_NOVA_04 | <i>Corynebacterium</i> sp.nov | 111,563,047 | Blood culture | 55/m | Intoxication with drug of abuse | DM Typ 2 | Not relevant |
| USB_NOVA_05 | <i>Corynebacterium</i> sp.nov | 111,563,048 | Blood culture | 68/m | Cholangitis | Hepatocellular carcinoma | Not relevant |
| USB_NOVA_06 | <i>Corynebacterium</i> sp.nov | 111,563,051 | Urine | 48/m | Urolithiasis | None | Not relevant |
| USB_NOVA_07 | <i>Schaalia</i> sp.nov. | 111,563,087 | Biopsy jaw | 90/f | Chronic osteomyelitis of the jaw/ MRONJ | Metastatic breast cancer | Unclear |
| USB_NOVA_08 | <i>Schaalia</i> sp.nov. | 111,563,089 | Swab mouth | 65/f | SSI of the mouth and jaw | None | Not relevant |
| USB_NOVA_09 | <i>Schaalia</i> sp.nov. | 111,563,086 | Biopsy submandibular | 61/f | Perimandibular abscess | None | Not relevant |
| USB_NOVA_10 | <i>Schaalia</i> sp.nov. | 111,563,085 | Biopsy lung | 68/m | Pleural effusion after pleurodesis | Pleuramesothelioma | Unclear |
| USB_NOVA_11 | <i>Schaalia</i> sp.nov. | 111,563,088 | Swab jaw | 61/f | Abscess fossa canina | None | Relevant |
| USB_NOVA_12 | <i>Anaerococcus</i> sp.nov. | 111,563,038 | Biopsy bone toe | 88/f | Chronic polymicrobial osteomyelitis | Peripheral arterial occlusive disease | Relevant |
| USB_NOVA_13 | <i>Anaerococcus</i> sp.nov. | 111,563,039 | Biopsy bone | 93/f | Implant associated infection of the tibia | None | Not relevant |
| USB_NOVA_14 | <i>Clostridium</i> sp.nov. | 111,563,043 | Biopsy hand | 38/f | Soft tissue infection | None | Relevant |
| USB_NOVA_15 | <i>Clostridium</i> sp.nov. | 111,563,042 | Blood culture | 41/m | Possible pneumococcal pneumonia | IVDU | Unclear |
| USB_NOVA_16 | <i>Desulfovibrio</i> sp.nov. | 111,563,058 | Swab abdomen | 64/f | Tertiary peritonitis | Perforation of the rectum | Relevant |
| USB_NOVA_17 | <i>Desulfovibrio</i> sp.nov. | 111,563,057 | Blood culture | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_18 | <i>Peptoniphilus</i> sp.nov. | 111,563,074 | Biopsy abdomen | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_19 | <i>Peptoniphilus</i> sp.nov | 111,563,073 | Biopsy upper leg | 52/m | Chronic soft tissue inflammation/fistula | DM Typ 2 | Unclear |
| USB_NOVA_20 | <i>Porphyromonas</i> sp.nov | 111,563,075 | Abscess mamma | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_21 | <i>Pusillimonas</i> sp.nov | 111,563,082 | Swab external auditory canal | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_22 | <i>Dermabacter</i> sp.nov | 111,563,056 | Swab toe | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_23 | <i>Helcococcus</i> sp.nov. | 111,563,063 | Blood culture | 58/m | Spondylodiscitis due to <i>Streptococcus dysgalactiae</i> | IVDU | Unclear |
| USB_NOVA_24 | <i>Neisseria</i> sp.nov. | 111,563,067 | Swab leg | 71/m | Soft tissue infection | Postoperative wound healing disorder and DM Typ 2 | Unclear |
| USB_NOVA_25 | <i>Pseudomonas</i> sp.nov | 111,563,078 | Biopsy hand | 67/m | Open fracture | Traumatic amputation of the hand | Relevant |
| USB_NOVA_26 | <i>Pantoea</i> sp.nov | 111,563,071 | Biopsy hand | 67/m | Open fracture | Traumatic amputation of the hand | Relevant |

Table 1 (continued)

| ID Number | Species | Genbank Accession No. (BioSample): SAMEA | Origin of specimen | Clinical data | | | |
|-------------|---------------------------------------|--|------------------------------|---------------|---|-------------------------------|--------------------|
| | | | | Age(y)/sex | Clinical presentation | Relevant underlying disease | Clinical relevance |
| USB_NOVA_27 | <i>Lancefieldella</i> sp.nov | 111,563,065 | Swab maxilla | 67/m | MRONJ | Metastatic prostata carcinoma | Not relevant |
| USB_NOVA_28 | <i>Rothia</i> sp. nov. | 111,563,083 | Swab sacral | 26/m | Pilonidal cyst | None | Not relevant |
| USB_NOVA_29 | <i>Pseudoclavibacter</i> sp.nov. | 111,563,077 | Swab external auditory canal | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_30 | <i>Tessaracoccus</i> sp.nov | 111,563,092 | Biopsy finger | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_31 | <i>Citrobacter</i> sp.nov. | 111,563,041 | Swab rectal | N.a. | ESBL-screening | Leukemia | N.a. |
| USB_NOVA_32 | <i>Paenibacillus</i> sp.nov | 111,563,069 | Aspirate pleura | 42/f | Pulmonary lesion, possible pulmonary fascioliasis | None | Not relevant |
| USB_NOVA_33 | <i>Ochrobactrum (Brucella)</i> sp.nov | 111,563,040 | Bronchial secretion | 69/m | Multifactorial respiratory failure, aspiration | Lung cancer, COPD Gold III | Not relevant |
| USB_NOVA_34 | <i>Sneathia</i> sp.nov | 111,563,091 | Swab pharynx | 23/m | Retropharyngeal abscess | None | Relevant |
| USB_NOVA_35 | <i>Psychrobacter</i> sp.nov. | 111,563,081 | Blood culture | N.a. | N.a. | N.a. | N.a. |

Abbreviations. ID, identification; y, year; f, female; m, male; DM, diabetes mellitus; MRONJ, medication-related osteonecrosis of the jaw;

SSI, surgical site infection; IVDU, intravenous drug use; N.a. not applicable; ESBL, extended spectrum beta-lactamases COPD; chronic obstructive pulmonary disease.

fast and reliable tool to identify difficult to identify bacterial strains and allows to discuss the clinical relevance with our infectious disease specialists in a timely manner.

The predominant genus among our 61 NOVA isolates was *Corynebacterium* with 11 isolates. Five of them were difficult to identify and six represent novel species (Fig. 2). Non-diphtheria corynebacteria are part of the normal microbiota of human skin and mucosa and are therefore very common isolates in clinical samples [17]. This may explain our finding, as well as the fact that none of the 11 corynebacteria isolates were considered clinically relevant. However, the growing number of immunocompromised patients and the use of invasive devices are accompanied by an increase in infections with opportunistic pathogens [17, 18]. For this reason and due to the different antibiotic resistance patterns of the different *Corynebacterium* sp., the identification of this bacterial group on species level is of great importance [19]. For this purpose, in addition to MALDI-TOF-MS analysis, various molecular methods such as PCR-based assays or sequencing of the *rpoB* and 16 S rRNA gene have been described [17, 20–23]. However, a recent review by Church and colleagues states that approximately 35% of *Corynebacterium* sp. cannot be distinguished using 16 S rRNA gene sequencing [24]. In these cases, sequencing of the *rpoB* target may provide additional diversity to distinguish some closely related species [21]. WGS, with its higher resolution, ultimately offers another means of species identification as well as the advantage of being

able to describe the entire genome of a potentially new species.

We assume, that *Vandammella animalimorsus* represents a novel and emerging pathogen. Our isolate USB_NOVA_58 originated from a biopsy of a thumb after a dog bite with the clinical diagnosis of septic arthritis and tenosynovitis in 2021. It was identified at that time as a potentially novel organism classified as *Corticibacter* sp. After reanalysis using the TYGS tool in 2023, the isolate was now identified as *V. animalimorsus*. This novel genus and species was described by Bernard et al. in 2022 using strains provisionally named “CDC group NO-1” recovered from human wound infections following animal bites [25]. Another potential new pathogen is *Kingella pumchi*. Our strain USB_NOVA_42 was isolated in 2018 from a patient with paronychia and assessed as clinically relevant. At that time it was identified as novel organism tentatively named “unidentified *Neisseria* sp.”. It was described as “*Kingella pumchi*” in February 2023 by a Chinese group using a strain, that had been isolated from a human vertebral biopsy [26]. A novel *Cutibacterium*, *C. modestum*, was identified from a prosthetic hip fluid. We identified this strain (USB_NOVA_51) in 2020 as “*Propionibacterium humerusii*”, a tentatively named species published in 2011. Some weeks afterwards, *C. modestum* was described by Dekio I. et al. from an isolate obtained from the meibomian gland [27] showing similar genome data to our strain USB_NOVA_51. We then summarized multiple published data on this organism and showed

Table 2 List of 26 clinical isolates which were identified by using WGS and corresponding clinical data

| ID Number | Species / Reference | Genbank Accession No. (BioSample): SAMEA | Origin of specimen | Clinical data | | | |
|-------------|---|--|--------------------------------|---------------|--|---|--------------------|
| | | | | Age(y)/sex | Clinical presentation | Relevant underlying disease | Clinical relevance |
| USB_NOVA_36 | <i>Gulosibacter hominis</i> | See ref (13) | Swab external auditory canal | 77/m | Otitis media and tympanic drainage | None | Unclear |
| USB_NOVA_37 | <i>Gulosibacter hominis</i> | See ref (13) | Swab external auditory canal | 53/m | Acute otitis media | None | Unclear |
| USB_NOVA_38 | <i>Gulosibacter hominis</i> | See ref (13) | Swab external auditory canal | 90/m | Chronic otitis media, tympanic membrane perforation | Squamous cell carcinoma of the concha auris, leukemia | Unclear |
| USB_NOVA_39 | <i>Gulosibacter hominis</i> | 111,563,062 | Biopsy foot | 80/f | Implant associated infection due to <i>Staphylococcus epidermidis</i> and <i>Enterococcus faecalis</i> | None | Not relevant |
| USB_NOVA_40 | <i>Corynebacterium pseudogenitalium</i> | 111,563,053 | Biopsy placenta fetal site | 27/f | Premature vaginal birth | None | Not relevant |
| USB_NOVA_41 | <i>Corynebacterium pseudogenitalium</i> | 111,563,055 | Urine | 60/f | Asymptomatic bacteriuria | Nephrostoma | Not relevant |
| USB_NOVA_42 | " <i>Kingella pumchi</i> " | 111,563,068 | Swab finger nail | 14/m | Paronychia | None | Relevant |
| USB_NOVA_43 | <i>Fenollaria massiliensis</i> | 111,563,061 | Biopsy bone symphysis | 52/m | Possible SSI with fistula of the symphysis pubis | Open-book-fracture of the pelvis four months before | Unclear |
| USB_NOVA_44 | <i>Mogibacterium kristiansenii</i> | 111,563,066 | Biopsy scrotum | 76/m | Fournier gangrene, sepsis due to colo-scrotal fistula | Radiotherapy and resection of the rectum | Relevant |
| USB_NOVA_45 | <i>Anaerococcus degeneri</i> | 111,563,037 | Biopsy abscess plantar | 90/m | Wet gangrene with osteomyelitis calcaneus | Chronic ulcers | Relevant |
| USB_NOVA_46 | <i>Slackia exigua</i> | 111,563,090 | Biopsy abscess plantar | 90/m | Wet gangrene with osteomyelitis calcaneus | Chronic ulcers | Relevant |
| USB_NOVA_47 | <i>Corynebacterium hindlerae</i> | 111,563,054 | Biopsy bone heel | 64/m | Chronic ulcer | None | Not relevant |
| USB_NOVA_48 | <i>Devosia equisanguinis</i> | 111,563,059 | Blood culture | 35/m | Septic thrombophlebitis | IVDU | Relevant |
| USB_NOVA_49 | <i>Pseudoclavibacter triregionum</i> | See ref (14) | Blood culture | 7/f | Bacteraemia and fever | Pelvic osteotomy, cerebral palsy, | Unclear |
| USB_NOVA_50 | <i>Pseudomonas yangonensis</i> | 111,563,079 | Swab lower leg | 54/f | Wound healing disorder after compartment syndrome | Hemorrhagic diatheses, liver cirrhosis | Not relevant |
| USB_NOVA_51 | <i>Cutibacterium modestum</i> | See ref (15) | Sonicated fluid prosthetic hip | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_52 | <i>Pseudoramibacter alactolyticus</i> | 111,563,080 | Blood culture | 86/m | SARS-CoV-2 infection | None | Not relevant |
| USB_NOVA_53 | <i>Enterococcus dongliensis</i> | 111,563,060 | Aspirate bile | 70/f | Cholangitis | Cholangiocellular carcinoma | Relevant |
| USB_NOVA_54 | <i>Prevotella brunnea</i> | 111,563,076 | Biopsy abdomen | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_55 | <i>Parvimonas parva</i> | 111,563,072 | Biopsy tibia | 15/m | Osteomyelitis and chronic cutaneous fistula | Osteosarcoma tibia | Relevant |
| USB_NOVA_56 | <i>Kingella negevensis</i> | 111,563,064 | Swab vaginal | N.a. | N.a. | N.a. | N.a. |
| USB_NOVA_57 | " <i>Corynebacterium provencense</i> " | 111,563,046 | Urine | 87/m | Asymptomatic bacteriuria | Urolithiasis and uretral stent | Not relevant |
| USB_NOVA_58 | <i>Vandammella animalimorsus</i> | 111,563,044 | Biopsy thumb | 38/f | Septic arthritis and tenosynovitis after dog bite | None | Relevant |
| USB_NOVA_59 | <i>Saezia sanguinis</i> | 111,563,084 | Swab rectal | N.a. | ESBL-screening | None | N.a. |

Table 2 (continued)

| ID Number | Species / Reference | Genbank Accession No. (BioSample): SAMEA | Origin of specimen | Clinical data | | | |
|-------------|----------------------------------|---|-----------------------|---------------|---|----------------------------------|------------------------------|
| | | | | Age(y)/sex | Clinical presentation | Relevant under- lying disease | Clini- cal rel- evance |
| USB_NOVA_60 | <i>Corynebacterium phoceense</i> | 111,563,045 | Biopsy back | 94/m | Postoperative hema- toma/seroma after spine surgery | None | Not relevant |
| USB_NOVA_61 | <i>Pantoea agglomerans</i> | 111,563,070 | Swab forehead | N.a. | N.a. | N.a. | N.a. |

Abbreviations. ID, identification; y, year; f, female; m, male; SSI, surgical site infection; IVDU, intravenous drug use; N.a. not applicable; ESBL, extended spectrum beta-lactamases

that “*P. humerusii*” and *C. modestum* represent the same species and that this bacterium often is misidentified as *Cutibacterium acnes* [15]. The recently described *Gulosibacter hominis* (4 isolates) and *Pseudoclavibacter tri-regionum* (1 isolate) may represent commensals that are part of the human skin microbiome [13, 14].

As a strength of our study, we identified and described novel species from clinical samples, while also providing clinical information and evaluating the clinical relevance of the respective bacterial isolate. In approximately one-third (15/47) of all cases where clinical data were available, the bacterial isolate was considered clinically relevant. However, in 12/15 cases, other concomitant pathogens could be identified as possible cause of the infection, so determination of their clinical relevance was difficult. Moreover, we did not evaluate antibiotic efficacy or change in antibiotic administration based on strain identification. This is a limitation of this study because the impact on patient care is difficult to assess without this information. In 11/47 cases the clinical relevance of the isolate was unclear. Six of these 11 isolates belong to novel species. This demonstrates the importance of identifying bacterial species and collecting clinical data on patients to gain insight into the role of these species as a human pathogen and to better assess their clinical significance in the future.

In our findings, 26 of 61 isolates were difficult to identify at the time point of study inclusion, when combining MALDI-TOF MS testing with partial 16 S rRNA gene sequencing. However, the long collection time limits this classification. Technical advances occurring within the timeframe of study inclusion and reporting may or may not allow for identification with one or both of the methods. Yet our NOVA algorithm was implemented to detect novel species which led to 35 of 61 strains being classified as such at timepoint of reporting (August 8 2023).

Overall, the majority (33/61, 54%) of our isolates were Gram positive rods, which are generally difficult to identify biochemically. This is consistent with observations from other laboratories. Church and colleagues found that the largest group of organisms to be sequenced were

Gram-positive bacilli, which accounted for 48.5% of all isolates sequenced over a six-year period [24].

The implementation of WGS in clinical microbiology for pan-bacterial identification seems to be more challenging and this method is currently performed mainly at large reference and public health laboratories [28, 29]. Difficulties arise from the lack of guidelines and standards, as well as financial and technical obstacles [28]. Price and colleagues conducted a study using WGS to identify bacteria in a clinical laboratory, evaluated their clinical relevance, and thereby provided a model for validating and implementing WGS in such a setting. They used a diverse set of 125 bacterial isolates, and were able to identify 100% (89/89) and 89% (79/89) of isolates to genus and species levels, respectively. WGS also provided better results for isolates (71% (25/35) originally reported at the genus level or descriptively only. In addition, review of patient records showed that improved identification at the genus or species level through WGS may have had a positive impact on patient care. For example, unnecessary use or use of ineffective antibiotics could be identified, as well as the results provide assistance with outbreak investigations [28, 29]. These benefits of WGS is weighed against the question of clinical practicability with regard to the long turnaround time (1–2 weeks in Price’s study). Faster WGS methods, such as nanopore sequencing, could help overcome this problem [30].

Current literature indicates that the MALDI-TOF MS method identifies approximately 98% of routine clinical isolates to the genus level and >90% to the species level, with <1% misidentified [24, 31]. This is in line with observations in our laboratory. The vast majority of samples coming through our clinical microbiology laboratory are easily resolvable as species using standard methods. Nevertheless, the identification here of 35 potentially novel taxa, seven of which appear to have had a clinically relevant role, shows that there is still a wide range of undescribed bacterial organisms from clinical samples. Clinical microbiologists and infectious disease specialists should be aware of this spectrum and we encourage other laboratories to apply or to adapt our algorithm to improve the identification of difficult to identify isolates.

A next important step within our NOVA study will be the correct taxonomical description of these isolates.

Conclusions

To conclude, we developed an algorithm to characterize strains which are not identifiable by standard methods using WGS that allowed the identification of multiple, potentially novel taxa as well as difficult to identify strains. Public availability of corresponding genome sequences and detailed clinical information may help to expand the clinical and ecological understanding regarding these novel bacterial organisms.

Abbreviations

WGS Whole Genome Sequencing

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-023-03163-7>.

Supplementary Material 1: Supplemental material is available online only. Supplemental Table S1.

Acknowledgements

We thank the whole team of technicians from the Clinical Bacteriology / Mycology Unit from the University Hospital Basel for excellent technical assistance. Bioinformatic analyses were partially performed at sciCORE (<http://scicore.unibas.ch>), the scientific computing center at the University of Basel.

Author contributions

DG: Conceptualization NOVA study, supervision, writing original draft. VM: Data collection and analysis, writing original draft, HSS: Conceptualization WGS-based pipeline, data collection and analysis. KMA, MW, UH, HS, MK, LG: clinical data analysis, assessment clinical significance. DG, VM, VH, AB: microbiological data collection and analysis. TR, DMW, PS, HSS: genome data collection and analysis. All authors reviewed the original draft.

Funding

Open access funding provided by University of Basel. No specific funding was used for this study.

Open access funding provided by University of Basel

Data availability

Genome data of 56 isolates of this study is accessible at NCBI under BioProject number PRJEB55530. Genome data of *Gulosibacter hominis* strains USB_NOVA_36, USB_NOVA_37, and USB_NOVA_38 are available under CAJGWQ000000000, CAJHCD000000000, and CAJHCF000000000, respectively [13]. The genomes of *Pseudoclavibacter tireregionum* (USB_NOVA_49) [14] and *Cutibacterium modestum* (USB_NOVA_51) [15] are accessible under OU365335 and PRJEB41775, respectively. Scripts for calculating ANI-values between two genomes are deposited in github [https://github.com/schlaepfer/wbin_batch_anl].

Declarations

Ethics approval and consent to participate

The study was approved by the local ethics committee named Ethikkommission Nordwest- und Zentralschweiz (EKNZ), BASEC Project ID No. 2023-00058, according to the standards of the Swiss Human Research Act. Informed consent to participate was obtained from 47 patients. All experiments were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 9 October 2023 / Accepted: 15 December 2023

Published online: 05 January 2024

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