Actinomyces species are uncommon but important causes of invasive infections. The ability of our regional clinical microbiology laboratory to report species-level identification of Actinomyces relied on molecular identification by partial sequencing of the 16S ribosomal gene prior to the implementation of the Vitek MS (matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-TOF MS]) system. We compared the use of the Vitek MS to that of 16S rRNA gene sequencing for reliable species-level identification of invasive infections caused by Actinomyces spp. because limited data had been published for this important genera. A total of 115 cases of Actinomyces spp., either alone or as part of a polymicrobial infection, were diagnosed between 2011 and 2014. Actinomyces spp. were considered the principal pathogen in bloodstream infections (n = 17, 15%), in skin and soft tissue abscesses (n = 25, 22%), and in pulmonary (n = 26, 23%), bone (n = 27, 23%), intra-abdominal (n = 16, 14%), and central nervous system (n = 4, 3%) infections. Compared to sequencing and identification from the SmartGene Integrated Database Network System (IDNS), Vitek MS identified 47/115 (41%) isolates to the correct species and 10 (9%) isolates to the correct genus. However, the Vitek MS was unable to provide identification for 43 (37%) isolates while 15 (13%) had discordant results. Phylogenetic analyses of the 16S rRNA sequences demonstrate high diversity in recovered Actinomyces spp. and provide additional information to compare/confirm discordant identifications between MALDI-TOF and 16S rRNA gene sequences. This study highlights the diversity of clinically relevant Actinomyces spp. and provides an important typing comparison. Based on our analysis, 16S rRNA gene sequencing should be used to rapidly identify Actinomyces spp. until MALDI-TOF databases are optimized.

Actinomyces species are uncommon human bacterial pathogens that can cause a wide variety of invasive infections (1). The Actinomyces genus is part of the family Actinomycetaceae, of which there are currently 47 published species, but several novel Actinomyces taxa have recently been described by molecular analyses targeting the ribosomal 16S rRNA gene (2). For example, the Human Oral Microbiome Database (www.homd.org) currently reports many species-level Actinomyces taxa that have not yet been named (3). The pathogenic role of known and novel Actinomyces spp. in contributing alone or as part of polymicrobial flora to human invasive infections is underappreciated because many clinical microbiology laboratories continue to rely on phenotypic methods for identification (4–7). Because Actinomyces spp. grow best under anaerobic conditions and are notoriously fastidious, slow-growing organisms, they may be missed as important pathogens by routine aerobic culture techniques and incubation periods (2, 8).

Upon microscopic examination, Actinomyces isolates typically appear as Gram-positive bacilli that may be coryneform-like with palisading or branching structures and have a positive catalase reaction. Commercial biochemical identification panels for aerobic and anaerobic Gram-positive bacilli include the API Coryne (bioMérieux) and the Vitek ANC cards (bioMérieux) and have less than optimal performance for accurately identifying many clinically relevant Actinomyces spp. (9, 10). Although matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is widely being adopted into clinical laboratory practice, there have been limited reports comparing the performance of MALDI-TOF MS to that of molecular methods for the identification of Actinomyces spp. (11–15).

Our laboratory routinely uses fast partial sequencing of the 16S rRNA gene (V1 to V3 region) to identify a wide variety of Gram-positive bacilli that are important primary pathogens or that are clinically relevant as part of polymicrobial invasive infections when isolated from clinical specimens (16). More than one hundred cases of invasive Actinomyces spp. infections have been diagnosed by our laboratory in recent years by 16S rRNA sequencing. We were therefore interested in evaluating the ability of the Vitek MS (MALDI-TOF) to identify the diverse array of Actinomyces spp. recovered and in comparing its performance to the gold standard of 16S rRNA sequencing. Phylogenetic analysis of all available 16S rRNA sequence data was also used to determine if any...
Actinomyces spp. were associated with specific types of infection and to provide additional confirmation/clarity of discordant identifications between the two typing methods.

MATERIALS AND METHODS

Laboratory setting. Calgary Laboratory Services (CLS) is a large centralized regional clinical laboratory performing most diagnostic infectious diseases testing for a health care region that services ~1.5 million people in urban and rural centers.

Samples. Actinomyces spp. isolates described in this study were recovered from clinical specimens tested by CLS over a 4-year period (2011 to 2014). All isolates were anaerobic or aerotolerant Gram-positive bacilli that were catalase positive. Molecular identification was done by fast partial sequencing of the 16S rRNA gene (523 bp) with MicroSeq 500 kits and an ABI Prism 3130 sequencer (Applied Biosystems, Foster City, CA) using standard methods (16). A BLAST search against the SmartGene Integrated Database Network System (IDNS) for bacteria indicated the most closely related species, and the overall identity score for all isolates was ≥99.9% with 0 to 2 mismatches (17, 18). Some isolates had also been tested using the Vitek ANC card (bioMérieux), but few could be definitively identified using phenotypic tests (data not shown). All isolates were also identified using a commercial mass spectrometry system (Vitek MS, ACQ Software R2 version 1.4.2b; bioMérieux) using Myla version 3.2.0-4 in accordance with the manufacturer’s instructions. Ethanol-formic acid extraction was performed on all isolates using the protocol provided by the manufacturer. A quantity of 1 μl of the extracted supernatant was placed on the steel target plate, dried, and overlaid with 1 μl of matrix. The target plate was then loaded into the Vitek MS instrument for analysis. Samples were repeated if the result gave low discrimination or no identification (ID).

Phylogenetic analysis. The clinical sequences (n = 115) and reference sequences (n = 15) from the SILVA database (19, 20) were aligned with MAFFT (version 7.123b) (21) and were visualized in AliView (version 1.18) (22). The multiple-sequence alignment columns containing over 20% gaps were trimmed with trimAL (version 1.2), which resulted in a 432-bp alignment length (23). A neighbor-joining tree was inferred with 100 bootstrap replicates, and evolutionary distances were computed using the Jukes-Cantor method in MEGA5 (24). Branches corresponding to partitions reproduced in <50% of bootstrap replicates were collapsed. The tree was manually rooted on a taxonomic outlier (Trupedella pyogenes; GenBank accession number JN578112) using FigTree (25), with additional color coding of the tree done with the Ape and Picante packages in R (26, 27).

Data analysis. All data pertaining to individual isolate identification, the 16S rRNA sequence, and the site/source data were entered into Microsoft Excel, and the data were analyzed using standard methods. Data were sorted and filtered to determine the distribution of individual Actinomyces spp. recovered from particular types of invasive infections. The performances of MALDI-TOF and 16S rRNA sequencing for identification of Actinomyces spp. to the genus and species levels in addition to PARTITIONs reproduced in <50% of bootstrap replicates were collapsed. The tree was manually rooted on a taxonomic outlier (Trupedella pyogenes; GenBank accession number JN578112) using FigTree (25), with additional color coding of the tree done with the Ape and Picante packages in R (26, 27).

Ethics. The conjoint health ethics board at the University of Calgary approved this study (Ethics ID number 23985).

Nucleotide sequence accession numbers. All sequences were submitted to GenBank under accession numbers KJ937784 to KJ937885 and KP192309 to KP192321.

RESULTS

Patient demographics. A total of 115 individual patients with invasive Actinomyces spp. infections were enrolled during the study period. Most of them were adults (n = 104, 90.5%), and the remainder were children of ≤14 years of age (n = 11, 9.5%). Among the pediatric cases, there was an equal prevalence of disease between girls (n = 5, 46%) and boys (n = 6, 54%), and their ages were similar; girls were aged 7.0 years ± 4.1 standard deviation (SD) (range, 3 to 13 years) while boys were aged 5.6 years ± 4.1 SD (range, 2 to 11 years). Among the adult cases, more disease occurred in males (n = 57, 55%) than in females (n = 47, 45%), but their ages were comparable; females were aged 51.5 years ± 20.3 SD (range, 20 to 96 years), and males were aged 56 years ± 17.2 SD (range, 22 to 91 years).

Epidemiology. The species and source distributions of invasive infections primarily caused by Actinomyces spp. are outlined in Fig. 1A and B, respectively. The anatomical source category and basic clinical information from the reports are displayed as the tip labels in Fig. 2 alongside GenBank accession numbers. Anatomical sources included abdominal (n = 16, 14%), bone (n = 27, 23%), and bloodstream infections (BSIs) (n = 17, 15%) as well as central nervous system (CNS) (n = 4, 3%) and pulmonary sources (n = 26, 23%) and skin and soft tissue abscesses (SSTA) (n = 25, 22%). The SSTA infections were localized to the head or neck (6, 24%), the thoracic region (9, 36%), and the abdomen or lower body (10, 40%). All A. radingae infections (n = 4/4, 100%) occurred as SSTA infections of the thoracic region, and most A. neuii or A. neuii subsp. anitratus (6/9, 67%) presented as SSTA infections. Pulmonary infections included pneumonia, pulmonary infiltrates, pleu-
TABLE 1 Comparison of *Actinomyces* spp. identification using the expanded Vitek MS MALDI-TOF database and using 16S rRNA gene sequence identification

<table>
<thead>
<tr>
<th>16S sequence identification</th>
<th>No. (%) correct to genus</th>
<th>No. (%) correct to species</th>
<th>No. (%) with discordant results</th>
<th>No. (%) with no result/ID</th>
<th>Total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID to a specific sequence*</td>
<td>4 (15)</td>
<td>7 (23)</td>
<td>2 (7)</td>
<td>16 (55)</td>
<td>29 (25)</td>
</tr>
<tr>
<td><em>Actinomyces</em> sp.</td>
<td>1 (12)</td>
<td>5 (63)</td>
<td>2 (25)</td>
<td>8 (7)</td>
<td></td>
</tr>
<tr>
<td><em>Actinomyces</em> europeae</td>
<td>3 (50)</td>
<td></td>
<td>3 (50)</td>
<td>6 (5)</td>
<td></td>
</tr>
<tr>
<td><em>Actinomyces</em> funkei</td>
<td></td>
<td></td>
<td>1 (100)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td><em>Actinomyces</em> georgiae</td>
<td></td>
<td></td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>2 (2)</td>
</tr>
<tr>
<td><em>Actinomyces</em> graevenitii</td>
<td></td>
<td></td>
<td>3 (43)</td>
<td>4 (57)</td>
<td>7 (6)</td>
</tr>
<tr>
<td><em>Actinomyces</em> israelii</td>
<td></td>
<td></td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>3 (2)</td>
</tr>
<tr>
<td><em>Actinomyces</em> meyeri</td>
<td></td>
<td></td>
<td>1 (12)</td>
<td>3 (38)</td>
<td>7 (7)</td>
</tr>
<tr>
<td><em>Actinomyces</em> naeslundii</td>
<td>3 (43)</td>
<td>4 (50)</td>
<td></td>
<td>3 (38)</td>
<td>7 (6)</td>
</tr>
<tr>
<td><em>Actinomyces</em> neuii</td>
<td></td>
<td>1 (14)</td>
<td>1 (12)</td>
<td>3 (38)</td>
<td>7 (6)</td>
</tr>
<tr>
<td><em>Actinomyces</em> odontolyticus</td>
<td></td>
<td>6 (67)</td>
<td></td>
<td>2 (22)</td>
<td>7 (7)</td>
</tr>
<tr>
<td><em>Actinomyces</em> oris</td>
<td>1 (33)</td>
<td>1 (33)</td>
<td>1 (33)</td>
<td></td>
<td>3 (2)</td>
</tr>
<tr>
<td><em>Actinomyces</em> radingae</td>
<td></td>
<td>3 (75)</td>
<td></td>
<td></td>
<td>4 (3)</td>
</tr>
<tr>
<td><em>Actinomyces</em> turicensis</td>
<td>8 (100)</td>
<td></td>
<td></td>
<td></td>
<td>8 (7)</td>
</tr>
<tr>
<td><em>Actinomyces</em> viscosus</td>
<td>1 (100)</td>
<td></td>
<td></td>
<td></td>
<td>1 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>10 (9)</td>
<td>47 (41)</td>
<td>15 (13)</td>
<td>43 (37)</td>
<td>115</td>
</tr>
</tbody>
</table>

* Includes the following species according to 16S sequencing: *Actinomyces* F507T (2), *Actinomyces* ARUP UnID 100, 97, 68, and 50 (4), *Actinomyces* F60622/2008 (1), *Actinomyces* GTC3949 (1), *Actinomyces* oral taxon 169, 170, 171, 172, 175, 180, and A50 (15), *Actinomyces* S8 86-2a (1), *Actinomyces* sp2-iso-aAG2 (3), and *Actinomyces* TeJ5 (2).

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**Phylogenetic analysis of invasive *Actinomyces* spp.** Figure 2 illustrates the phylogenetic tree of all *Actinomyces* spp. isolated from our laboratory during the study period and additional publicly available type strain sequences. Partial 16S rRNA sequences are displayed as circles and are color coded according to the species-level identification given by the SmartGene IDNS database. Sequences only identified to the genus level are shown as black triangles. Phylogenetic tree tips are labeled with GenBank accession number, source of infection, and clinical detail from the CLS database, while the reference strains are labeled in bold with accession number, genus, and species. The 15 discordant Vitek MS results are highlighted with red font and asterisk (*) symbols. Our phylogenetic results confirm the species identification by the IDNS database; the majority of species identifications match well to publicly available type strain sequences. There are some clusters of mixed species, such as those sharing recent common ancestry with *A. oris* and *A. viscosus*, supporting previous reports of heterogeneity within these species (28–30). Interestingly, our data also illustrate how diverse the oral taxa are, as shown by the dark
blue circles dispersed throughout the tree. In addition to the oral
taxon classifications, there are other sequences that are not taxo-
nomically named but that are considered species identifications in
the database (i.e., E507T, GTG3949, SP2-iso-aAG2, and S8 86-2a)
due to the growing number of molecular-based identification pro-
tocols and microbiome studies (31, 32).

DISCUSSION
This is the first study that documents the important role that a wide
range of *Actinomyces* spp. plays in invasive infections on a popula-
tion basis in a large health care region. In all cases, diagnosis of inva-
sive infection was based on clinical and microbiological data from the
sites/source of infection, and the majority of isolates (110/115, 96%)
were identified to the species level using 16S sequencing and identifi-
cation with the IDNS SmartGene database. Although this study de-
scribes the recent clinical experience of a single health care region with
a centralized regional microbiology laboratory, similar rates of inva-
sive infections due to *Actinomyces* spp. would be expected to occur in
other jurisdictions because actinomycosis is an endogenous infec-
tion. These organisms are part of the normal commensal flora on
human mucosal surfaces, and opportunistic invasive infection only
occurs when *Actinomyces* spp., either alone or as part of a polymicro-
bial process, invade into deeper tissues attributed with disruption of
the host’s mucus (1, 2, 8). Usual inciting events for the development
of actinomycosis include mechanical mucosal trauma due to injury,
surgery, or placement of a foreign device.

A recent review on human *Actinomyces* infections by Könönen
and Wade provides a thorough summary of the natural distribu-
tion of *Actinomyces* spp. in or on the body and Actinomyces spp.
that have a predilection for causing invasive infections at specific
body sites (2). Our study confirmed previous reports of the asso-
ciation of oral taxon sequences throughout the phylogenetic tree (Fig.
2). Further work to characterize these oral taxon isolates and their
genomes will no doubt provide valuable information about this
group and will likely lead to the identification of new species.

A growing number of case reports and literature reviews on
actinomycosis have been published, suggesting greater attention is
needed for invasive infections caused by *Actinomyces* spp. (2, 12,
36–38). Furthermore, large clinical and epidemiological studies
using molecular identification methods for recovered *Actinomyces*
spp. are clearly required to delineate the precise role of these com-
mensal organisms as opportunistic human pathogens.

Our study shows that 16S rRNA sequencing remains the cur-
rent gold standard method for definitive species-level identifica-
tion. The Vitek MS system accurately identified approximately
one-half of the isolates to the genus or species level. In a previous
study by Garner et al. (15), *Actinomyces* isolates were correctly
identified to the species level in 20/27 (74%) isolates. However,
this study included only 3 species of *Actinomyces*. In our study,
18/36 (50%) of the same three species were correctly identified to
the species level, but due to the small numbers, it is difficult to
draw statistically significant conclusions. In addition, our study
solely focuses on *Actinomyces*, including a more diverse sample
population with half of the *Actinomyces* spp. that can only be iden-
tified to the genus level by 16S RNA sequencing.

In the other cases, an inaccurate identification or no result was
provided by Myla version 3.1. Although MALDI-TOF MS may be
used initially if validated for the identification of common species
(e.g., *A. europeus, A. meyeri, A. neuii, A. odontolyticus, A. turceni-
sis,* and *A. viscosus*), other Gram-positive branching bacilli isolated
as the sole or predominant pathogen from clinical samples from
invasive tissues, sterile fluids, or blood cultures should be tested
with molecular methods for a definitive identification if required.
As a minimum, *Actinomyces* isolates recovered from suspected
cases of actinomycosis (based on clinical and/or histological re-
results) should be saved so that further microbiological character-
ization can be performed and the databases updated to improve
accuracy for future testing.

In conclusion, this work demonstrates that a diverse number of
*Actinomyces* spp. are recovered from sterile sites, and that half of
these cannot be identified using our current MS platform. Accu-
rate identification of invasive *Actinomyces* spp. may have clinical
importance, as there is clustering of certain species within partic-
ular sites of infection. We also identify that serious infections are
caused by a growing number of new and unusual *Actinomyces* spp.
that have been identified primarily by molecular detection meth-
ods requiring future work to further characterize these species.

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molecular and MALDI-TOF MS testing at Calgary Laboratory Services for
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