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Case report

Osteomyelitis infection caused by *Arcanobacterium haemolyticum* in a diabetic patient: A first case report

Amr T.M. Saeb[c], Khalid A. Al-Rubeaan[c], Balavenkatesh Mani[d], Hamsa T. Tayeb[a,b,*]

[a] Genetics Department, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
[b] Saudi Human Genome Project, King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia
[c] Strategic Center for Diabetes Research, College of Medicine, King Saud University, Saudi Arabia
[d] Integrated Gulf Biosystems, Riyadh, Saudi Arabia

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ABSTRACT

Arcanobacterium haemolyticum can cause deep infections, including osteomyelitis. In this study, an automated system misidentified this causal agent as *Cellulomonas* species but 16 s rRNA sequencing correctly identified it as *A. haemolyticum*. Recognizing the capability of *A. haemolyticum* to establish the disease is of great importance to enable accurate diagnosis and begin the suitable antibiotic therapy. Here we present the first case of successfully treated *A. haemolyticum* infective osteomyelitis in a 64-year-old Saudi patient with diabetes mellitus type 2 and review the characteristics of this seldom pathogenic agent.

Introduction

Arcanobacterium haemolyticum is a facultative anaerobic gram-positive bacillus. This genus was previously classified as Corynebacterium, and later in 1982, it was re-classified as *Arcanobacterium* genus [1]. The nomenclature *Arcanobacterium* translates to 'mysterious bacterium,' which is a sobriquet rather appropriate for an organism that is rarely recognized as a clinical pathogen [2]. Though, it can be isolated from the pharynx and skin of healthy people, it is a well-established causal agent of pharyngitis and skin and soft-tissue infections [3]. Fewer incidences, *A. haemolyticum* can cause deep infections, including osteomyelitis [4]. For example, a case of chronic osteomyelitis confirmed by the clinical, radiological, and histopathological perspectives. However, the investigators isolated a rare bacterium, *A. haemolyticum*, and the patient recovered via clindamycin treatment, prescribed in agreement with the sensitivity tests [5].

Another study reported three rural Indian patients aged 60–65 years with polymicrobial wound infections associated with *A. haemolyticum* as the causal agent. Two patients that had *A. haemolyticum* isolated repeatedly from their wounds along with β-hemolytic streptococci, one with cellulitis and the other with postoperative wound infection succeeding a limb amputation. The third case, was a diabetic patient with chronic osteomyelitis, had *A. haemolyticum* collected from his wound in the presence of *Proteus vulgaris* [6]. Furthermore, a Korean study reported a case of osteomyelitis and bacteremia in a diabetic patient caused by *A. haemolyticum*.

Case report

Clinical information

The reported patient is 64 years old, Saudi gentleman known to have Type 2 Diabetes for 32 years. His Diabetes was poorly controlled with HbA1c level of 10.6 % when he presented with chronic wound infection with evidence of Cellulitis and Osteomyelitis involving the distal phalange proven radiologically using plain X-ray and Bone scan. He's known also to have severe Bilateral Neuropathy involving both lower extremities in addition to Bilateral Background Retinopathy. He was managed with Glibenclamide 10 mg BID and Metformin 1 g BID in addition to Aspirin 81 mg daily. Deep wound swab from the ulcer side grow

Abbreviations: EUCAST, The European Committee on Antimicrobial Susceptibility Testing; CARD, the comprehensive antibiotic resistance database; NDARO, national database of antibiotic resistant organisms; PATRIC, the Pathosystems Resource Integration Center; ISPs, ion sphere particles; AMR, anti microbial resistance; Ion PGM, ion personal genome machine; MUSCLE, multiple sequence comparison by log- expectation; RAxML, randomized axelerated maximum likelihood; CDS, coding sequence; NCBI, National Center for Biotechnology Information; RASTtk, rapid annotation using subsystem technology tool kit; Q20, base call quality; AQ20, alignment quality.

* Corresponding author at: Genetics Department, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.
E-mail address: hamsa3000@hotmail.com (H.T. Tayeb).

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Staphylococcus aureus sensitive to Clindamycin and Cefradine. He was covered with the selected Antibiotics for 6 weeks and had complete healing and wound close-up and resolution of his Osteomyelitis (Fig. 1).

Sample collection and sequencing

We collected a deep wound swab using BD Culture Swab MaxV swabs and immediately cultured them in sheep blood agar. We performed the initial characterization using the VITEK-2 identification system (Biomerieux, USA). We performed DNA isolation for the colonies with narrow zones and 0.5 mm size on an automated Maxwell® 16 System (Promega Corporation, USA) according to the manufacturer's instructions. We amplified the isolated DNA using 16 s rRNA universal primers and sequenced using 3730xl Genetic analyzer (Thermo Fisher Scientific, USA). The sequence was submitted to the NCBI and obtained the accession number KJ769249.1. Further we subjected the genomic DNA (gDNA;100–500 ng) to whole genome fragment library preparation and sequenced on an Ion PGM (Thermo Fisher Scientific, USA) using Ion PGM™ Hi-Q™ View sequencing Kit (Thermo Fisher Scientific, USA).

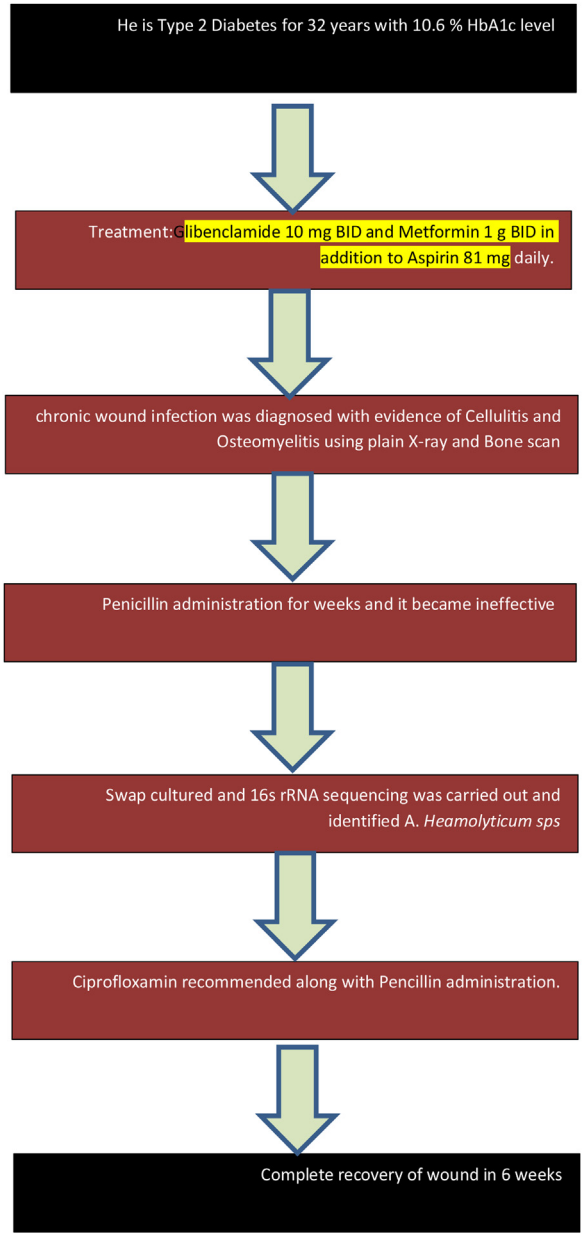


Fig. 1. Patient information starting with the case report to the clinic until the complete recovery.

Data analysis

Sanger sequencing data of 16 s rRNA was quality checked and analyzed using Codon Code Aligner v 6.0 software and we further performed a nucleotide BLAST search to identify the species. The primary bioinformatics analysis involved in removing duplicated reads, Q20 (base call quality), and AQ20 (alignment quality) using Torrent Suite Software v 5.2 (Thermo Fisher Scientific, USA). Q20 is the Phred scale score calculated based on the error probability ($-10\times\log10$) and it corresponds to a predicted error rate of 1%. AQ20 is the read length quality at which the error rate is 1% or less. We also performed a whole-genome assembly using the NCBI Prokaryotic genome annotation pipeline and then submitted the final assembled genome to the NCBI GenBank with accession number CP034038.1.

Discussion

16s rRNA sequencing and treatment

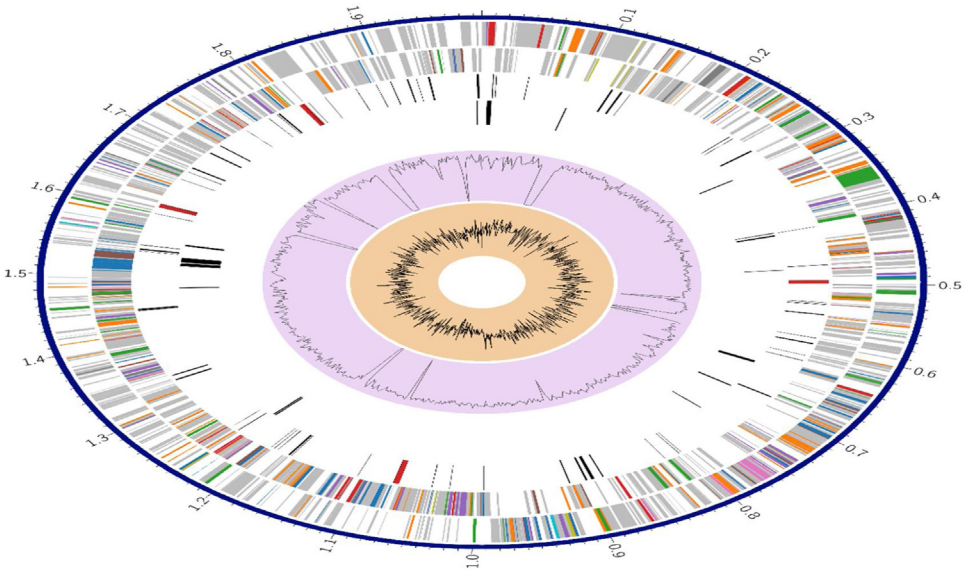
A. *haemolyticum* is a potential pathogen in several clinical reports, and this pathogen may be underreported; therefore, its diagnostic evaluation and characterization are emphasized [5,7]. To address the differentiation by automated identification systems, we used high-resolution sequencing techniques to achieve the definitive identification and characterization of *A. haemolyticum*. In this case, we showed that the VITEK-2 system identification method was not able to provide conclusive results and gave low differentiation results among the pathogenic bacteria, *Erysipelothrix rhusiopathiae, Gardnerella vaginalis,* and the possible participant in diabetic foot ulcers *Helcococcus kunzii* [8]. The VITEK-2 system first identified the gram-negative organism as a *Cellulomonas* species, and then later 16 s rRNA sequence analysis identified it as *A. haemolyticum*. Bae et al. (2010) obtained similar results [9]. Blasting results of UDC1 showed 100 % similarity to *A. haemolyticum* with the GenBank sequences MH569545.1, MH569544.1, LS483427.1, HG003663.1, and NR_074602.1.

Based on the results of the 16 s rRNA hits, we suggested antibiotic courses for the patient. It is worth mentioning that there are no antibiotic standards for antimicrobial susceptibility testing available for *A. haemolyticum* to determine the drugs for treatment. Thus, we followed the treatment as per EUCAST clinical breakpoint 2014 for the phylogenetically close relative *Corynebacterium* spp. Ciprofloxacin and penicillin treatment showed good results against the infection. A similar study used a combination of ciprofloxacin and penicillin successfully against *A. haemolyticum* foot ulcer wound infection [6]. In addition to the antibiotic treatment, dressing the wound of the foot ulcer paved the way for successful wound healing of the patient after 6 weeks. Initial treatment with penicillin was not effective in eradicating the infection. In previous studies, penicillin treatment alone was ineffective in curing the infection [10]. Despite the suggested penicillin-tolerance of *A. haemolyticum* clinical isolates, we can suggest that continuous ciprofloxacin administration would be very effective against the pathogen *A. haemolyticum.* Thus, 16 s rRNA sequencing in routine testing would add significant value in improving exact identification and treatment.

Whole-genome annotations and features

We carried out complete genome annotations of *A. haemolyticum* owing to its rare prevalence and insufficient clinical information.

Hence, we decoded the genome features of *A. haemolyticum* via comprehensive genome annotation using PATRIC services such as genome composition, subsystem analysis, and specialty genes, including AMR genes, and phylogenetic classification using whole-genome annotations. The genome assembly of SCDR1 contains 100 contigs with a total length of 1986154 bp and an average GC content of 53.2 %. We annotated the *A. haemolyticum* SCDR1 denovo genome using the RAST kit (RASTtk). SCDR1 is in the Super Kingdom, and the taxonomy of this genome is *Actinobacteria* > *Actinomycetacea* > *Arcanobacterium* > *Arcanobactrium haemolyticum*. SCDR1 genome contains 1897 CDS, 48 tRNA, and 2 rRNA genes. Protein annotation included 664 hypothetical and 1233 functional proteins, and two protein families. The distribution of the SCDR1 genome indicates a circular graphical display that includes CDS on the forward and reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes (AMR), and virulence factors (Fig. 2a). Many homology CDS or specialty genes are antibiotic resistance and drug targets based on the open-source databases CARD, NDARO, PATRIC, and DrugBank (Table 2). We analyzed the AMR gene function and the corresponding



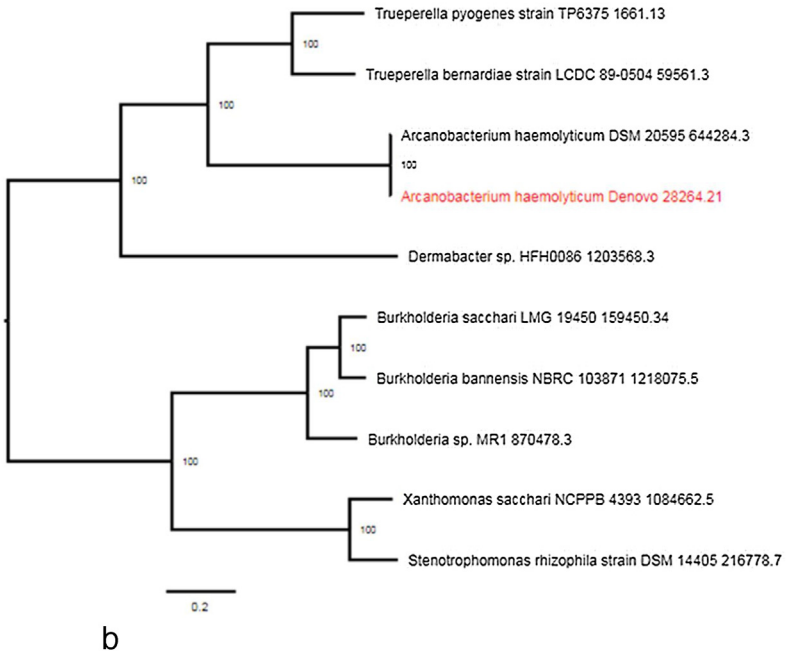


Fig. 2. (a) The distribution of the SCDR1 genome indicates a circular graphical display that includes CDS on the forward and reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes (AMR), and virulence factors.
(b) phylogenetic analysis by selecting the closest reference and representative genomes of the PATRIC global protein families. Finally, we concatenated multiple alignments and phylogenic tree construction using MUSCLE and RaxML with fast bootstrapping.

Table 1a
Potential novel virulence factors *Arcanobacterium haemolyticum* strain SCDR 1 genome.

Property	Source Organism	Gene	Product	Classification	Identity	E-value
Virulence Factor	Escherichia coli O157:H7 str. EDL933	hrpA	ATP-dependent helicase HrpA	Virulence	82	2e-14
Virulence Factor	Mycobacterium tuberculosis H37Rv	sigA	RNA polymerase sigma factor RpoD	Regulation of gene expression	81	4e-80
Virulence Factor	Listeria monocytogenes EGD-e	clpC	ATP-dependent Clp protease, ATP-binding subunit ClpC / Negative regulator of genetic competence clcC/ mecB		80	7e-12
Virulence Factor	Mycobacterium tuberculosis CDC1551		Bacterial proteasome-activating AAA-ATPase (PAN)	Virulence	84	4e-29
Virulence Factor	Mycobacterium tuberculosis H37Rv	uvrA	Excinuclease ABC subunit A	Intracellular survival and replication	94	1e-29
Virulence Factor	Bacillus anthracis str. CDC 684	clpX	ATP-dependent Clp protease ATP-binding subunit ClpX		83	3e-22
Virulence Factor	Escherichia coli O157:H7 str. EDL933	hrpA	ATP-dependent helicase HrpA		82	2e-14
Virulence Factor	Listeria monocytogenes EGD-e	clpC	ATP-dependent Clp protease, ATP-binding subunit ClpC / Negative regulator of genetic competence clcC/ mecB	Stress protein	80	7e-12
Virulence Factor	Listeria monocytogenes 10403S		Protein translocase subunit SecA	Defense against host immune response, Secretion	87	4e-12
Virulence Factor	Mycobacterium tuberculosis H37Rv	sigA	RNA polymerase sigma factor RpoD	Regulation of gene expression	90	3e-08

Table 1b
Potential novel drug targets *Arcanobacterium haemolyticum* strain SCDR 1 genome.

DRUGBANK ID	NAME	DRUG GROUP	PHARMACOLOGICAL ACTION
DB02930	Adenosine 5′-[γ-thio] triphosphate	experimental	A nucleoside triphosphate analogue that is ATP in which one of the oxygens attached to 3-phosphate group is replaced by sulfur.
DB03222	dATP	experimental	RecA
DB04444	Tetrafluoroaluminate Ion	experimental	RecA

mechanisms of SDR1 using the K-Mer-based AMR gene detection method. We discovered potential novel virulence factors and drug targets using deep sequencing and comparative genomics analysis (Table 1a and 1b). We performed the phylogenetic analysis by selecting the closest reference and representative genomes of the PATRIC global protein families. Finally, we concatenated multiple alignments and phylogenic tree construction using MUSCLE and RaxML with fast bootstrapping (Fig. 2b).

Table 2
Specialty genes of *Arcanobacterium haemolyticum* strain SCDR 1 genom.

	Source	No of genes
Antibiotic resistance	CARD	3
Antibiotic resistance	NDARO	2
Antibiotic resistance	PATRIC	24
Drug target	DrugBank	1

Conclusion

In this study, we showed that even using automated identification systems for aerobic or facultative gram-positive rods can be difficult and can lead to misleading information. We also emphasized the use of partial 16 s rRNA sequencing annotation to reach the ultimate identification and characterization of the pathogen to solve the dispute of bacterial identification and its treatment. Thus, the exact identification of *A. haemolyticum* helped us in predicting successful antibiotic combinations for the treatment. We also sequenced and annotated the whole genome of the rare infectious pathogen *A. haemolyticum* (SDR1) and deposited it in NCBI for open access.

Importance of the study

An automated system misidentified the causal agent *A. haemolyticum* as *Cellulomonas* species but the 16 s rRNA sequencing correctly identified it (Soo et al., 2010). Recognizing the ability of *A. haemolyticum* to establish the disease is of great importance to enable accurate diagnosis and begin the suitable antibiotic therapy. Here we describe the first case of a successfully managed *A. haemolyticum* infective osteomyelitis in a 64-year-old Saudi patient with diabetes mellitus type 2 and review the characteristics of this infrequently detected pathogen.

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Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Ethics approval

This study was approved by the institutional review board of King Saud University, College of Medicine Riyadh, Kingdom of

Saudi Arabia. The subject provided written informed consent for participating in this study. No. E-19-3967 IRB Approval.

Authors' contributions

ATMS: Involved in study conception and design, data analysis, and interpretation and involved in drafting the manuscript or revising it critically for important intellectual content.

BM: Performed the DNA sequencing and Involved in drafting the manuscript. Involved in data and statistical analysis.

KA: Involved in study conception and design. Preparing the final approval of the version to be published.

HTT: Involved in study conception and design and drafted the manuscript or revised it critically for important intellectual content. Preparing the final approval of the version to be published. Admistratioanal supervision of the research and preparing the final approval of the version to be published. Providing research fund for the research and final approval of the version to be published.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

The authors report no declarations of interest.

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