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ORIGINAL ARTICLE

Case report of *Curtobacterium* isolated from a catheter tip sample misidentified as *Cronobacter*

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Significance and Impact of the Study: This study reports a rare case of *Curtobacterium* infection isolated from a catheter tip sample, which was misidentified by VITEK® 2 as *Cronobacter* spp. When VITEK® 2 identifies species or genera that are highly unlikely, it is recommended to confirm the outcome by polyphasic analysis.

Keywords
16S rRNA, *Cronobacter*, Curtobacterium, molecular diagnostics, SARS-CoV-2.

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Abstract

The *Curtobacterium* genus is a member of the family Microbacteriaceae, *and Curtobacterium* species are recognized as plant pathogens. The aim of this study was to investigate a dubious result of species identification for an infection located on a catheter tip of a patient with Covid-19. A strain isolated from a catheter tip sample, identified by VITEK® 2 as *Cronobacter* spp., was submitted to polyphasic analysis: Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) using VITEK® MS, real-time polymerase chain reaction targeting *dna*G gene, and 16S rRNA full gene Sanger sequencing analysis for confirmation. The strain presented negative result using qPCR and could not identified by MALDI-TOF MS. 16S rRNA full gene Sanger sequencing analysis identified the strain as *Curtobacterium* spp. The Gram-variable characteristic (Gram-negative instead of Gram-positive) of the isolated strain was the responsible for the misidentification by VITEK® 2 and VITEK® MS did not identify the strain. 16S rRNA full gene sequencing analysis identified the strain as *Curtobacterium* genus, but other complementary techniques are necessary to identify at species level.

Introduction

In August 2021, a 45-year-old male patient, with a history of systemic arterial hypertension, diabetes mellitus and grade I obesity, was admitted with severe respiratory syndrome, requiring non-invasive oxygen support in the emergency room of a hospital in Campo Grande, Mato Grosso do Sul, Brazil. Nasal swab collection was performed and real-time reverse transcriptase-polymerase chain reaction for SARS-CoV-2 was positive. Initial tests showed leukocytosis with neutrophilia and lymphopenia in addition to the presence of higher C-reactive protein (CRP). Chest tomography showed bilateral ground-glass opacities and extensive involvement of the lung parenchyma above 50% and consolidations in bases.

The patient was kept in the intensive care unit (ICU) and received ceftriaxone 1 g intravenously every 12 h and azithromycin 500 mg orally daily, hydrocortisone 100 mg intravenously every 12 h and enoxaparin 40 mg subcutaneously daily. There was stability in the first 5 days, with clinical worsening, being submitted to orotracheal intubation (OTI), mechanical ventilation and elevation of

nitrogenous waste (creatinine: 2·75 mg dl^{-1} and urea: 188·9 mg dl^{-1}), requiring haemodialysis.

The patient progressed to prolonged OTI, and treatment was introduced for ventilator-associated pneumonia and clinical stabilization. After 30 days of hospitalization, there was a new clinical worsening with fever, elevation of CRP, thrombocytopenia and erythema at the site of central venous catheter (CVC) insertion, with the CVC being changed and referred for catheter tip culture, in addition to collection of blood cultures. Blood cultures were negative. The catheter was seeded on horse blood agar using the Maki technique (catheter rolling) and incubated at 37°C for 24 h. One yellow colony (1 colony-forming unit/plate) was observed, that was Gram-negative bacilli and oxidase-negative. After identification with VITEK® 2 (bioMérieux, Craponne, France) using GN identification card the micro-organism was identified as '*Cronobacter sakazakii* group' (*Cronobacter* spp.). The antibiogram was also performed with VITEK® 2 considering the strain as *Cronobacter* spp., but the patient did not receive specific antimicrobial treatment for *Cronobacter* spp.

The Hospital Infection Control Service of the institution was immediately notified, as this agent was not part of the commonly hospital microbiota and was only identified on a single occasion in a late premature newborn in September 2017 (Chaves *et al.* 2018). The presence of this bacterium in CVC with low CFU count was discussed with the ICU visiting physician, and it was considered the only micro-organism colonizing the CVC. To investigate other colonization sites, nasal and anal swabs were collected, but the results were negative.

The patient remained hospitalized for 80 days and presented with several infectious complications, including ventilator-associated pneumonia and pseudomembranous colitis, with the use of various antimicrobial therapies for other micro-organisms, including undergoing exploratory laparotomy due to vascular complications from COVID-19. However, the patient died on 29 October 2021.

The aim of this study was to characterize the isolated strain using molecular biology techniques to elucidate this case of infection.

Results and discussion

The isolate failed to produce results in real-time polymerase chain reaction targeting *dna*G gene. No match was found (<75%) using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS), and the datacount was satisfactory (>100) according to the manufacturing instructions. So that the result of that test was recorded as 'unidentified'.

The complete 16S rRNA full gene was amplified and sequenced. When this sequence was used to query at EzBioCloud Database, it retrieved hits to entries from the following species sequence identity: *Curtobacterium ocenosedimentum* (99·86%), *Curtobacterium citreum* (99·79%), *Curtobacterium luteum* (99·65%), *Curtobacterium albidum* (99·57%), *Curtobacterium pusillum* (99·45%), *Curtobacterium flaccumfaciens* (99·38%) and *Curtobacterium ammoniigenes* (99·17%). These sequences were retrieved and a neighbour-joining tree was constructed (Figure 1). Its Gram-stain result (Gram-negative instead of Gram-positive) was responsible for the misidentification by VITEK®2 and for the failure in MALDI-TOF MS.

The *Curtobacterium* genus is a member of the family Microbacteriaceae, and *Curtobacterium* species are recognized as plant pathogens. The main natural habitats are different plants, although they also occur in soil and other environments (Park *et al.* 1993–1994). *Curtobacteria* are obligate aerobic Gram-positive coryneform bacteria with a group B-type peptidoglycan structure markedly different from that of true corynebacteria and characteristic cell wall lipid composition (Collins and Jones 1983; Funke *et al.* 2005). Cases of human infections caused by *Curtobacterium* members are very rare in the literature (Funke *et al.* 2005; Francis *et al.* 2011).

In the present study, the Gram staining characteristic (Gram-negative instead of Gram-positive) of the isolated strain was responsible for the misidentification by VITEK® 2, since the choice of the identification card will depend on the characteristics of the micro-organism visualized in the Gram-staining. However, even if a Gram-positive staining would have been visualized, the card for gram-positive (GP) or card for Bacillus (BCL) would not have correct identify the isolate because *Curtobacterium genus is not included in list of micro-organisms identified by VITEK® 2*. The misidentification of other micro-organisms as *Cronobacter* spp. have already been reported, but in all the cases they were also Gram-negative bacteria (Townsend *et al.* 2008; Giammanco *et al.* 2011; Warnken *et al.* 2012; Horinouchi *et al.* 2022). Due to the lack of robustness, biochemical kits are no longer recommended for the identification of *Cronobacter* isolates (Jackson and Forsythe 2016; Anonymous 2017; Brandão and Forsythe 2022).

Funke *et al.* (2005) recovered five *Curtobacterium* strains from human clinical specimens. In three cases, a disease association was suggested by heavy growth in pure culture with a moderate leukocyte reaction on direct Gram stains of the clinical material. In two other cases, a disease association was not so definitive and the two strains were isolated from sputum in mixed culture. The most recent case occurred in 2011, when a child with septic arthritis following puncture with a Coxspur Hawthorn plant thorn (Francis *et al.* 2011).

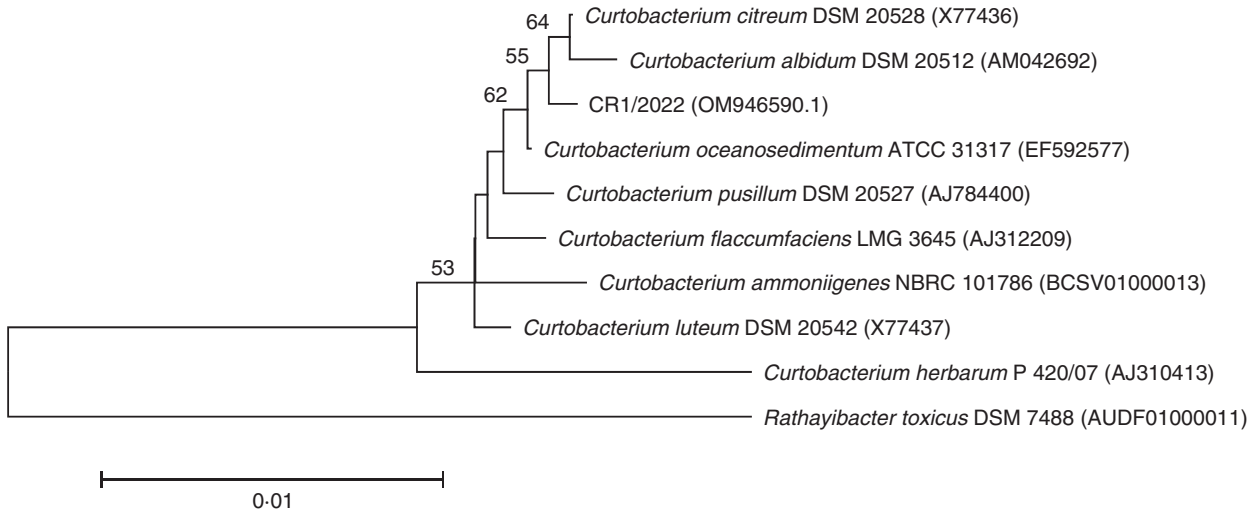

Figure 1 Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic position of the strain CR1/2022 with closely related species from the genus *Curtobacterium*. The numbers at the nodes indicate the percentage of 1000 bootstrap replicates; only values >50% are shown. *Rathayibacter toxicus* DSM 7488 was used as an outgroup. The scale bar represents 0.01 substitutions per nucleotide position. GenBank accession number is given in the parentheses.

Regrettably, even if the identification had been correct (*Curtobacterium* spp. instead of *Cronobacter* spp.), no specific clinical treatment would have been initiated for the patient and probably the outcome would have been the same.

In summary, this is a rare case report of *Curtobacterium* infection isolated from a catheter tip sample of a patient with Covid-19 that was initially misidentified as *Cronobacter* by phenotypical identification.

Materials and methods

The isolate strain CR1/2022 was sent to Nacional Institute of Quality Control in Health from Oswaldo Cruz Foundation (INCQS/Fiocruz), a public laboratory from Brazilian System of Sanitary Surveillance. Upon receival, the Gram staining was repeated, which again resulted in Gram-negative bacilli. Repetition of the VITEK®2 analysis using GN identification card reproduced the identification as *Cronobacter* spp. The isolate was submitted to real-time polymerase chain reaction (qPCR) targeting *dna*G gene (Chen *et al.* 2012). The qPCR reaction was carried out in a total volume of 25 µl containing 12·5 µl of TaqMan® Universal Master Mix (Applied Biosystems®, Alameda, CA), 2·0 µl of DNA template (20–60 ng μl^{-1}), 10·0 pmol of primers CronoF 5′-GGGATATTGTCCCCTGAAACAG-3′ and CronoR 5′-CGAGAATAAGCCGCGCATT-3′ (Invitrogen, Carlsbad, CA) and 0·3 µmol l^{-1} of the probe Crono P^{FAM}-AGA GTAGTAGTTGTAGAGGCCGTGCTTCCGAAAG-^{TAMRA} (Applied Biosystems®). The reaction was carried out on ABI 7500 Real-Time PCR System (Applied Biosystems®) using the cycling: 50°C per 2 min; 95°C per 3 min; 40X (95°C per 15 s, 52°C per 40 s, 72°C per 15 s). The isolate was submitted to MALDI-TOF MS. The strain was seeded on blood agar plate and incubated at 32·5 ± °C for 48 h. A portion of a colony was applied to a slide in quadruplicate together with 1 µl of alpha-cyano-4-hydroxycinnamic acid matrix solution (VITEK MS-CHCA; bioMérieux). *Escherichia coli* ATCC 8739 was used as control, according to the manufacturer's instructions. After matrix crystallization, the slides were introduced in the VITEK® MS RUO equipment (bioMérieux), and the results were analysed by SARAMIS Premium software v. 4·0·0·14 (Costa *et al.* 2022). Lastly, the isolate was identified by 16S rRNA full gene Sanger sequencing analysis using MicroSEQ™ Full Gene 16S rDNA kit (Thermo Fisher Scientific, Waltham, MA), according to the manufacturer's instructions. The sequences were processed using DNA Star LaserGene SeqMan software v. 7·0·0 and deposited at https://www.ncbi.nlm.nih.gov/ with the access number **OM946590**. The identification results were obtained from the website https://www.ezbiocloud.net/, Database Update: 07/07/2021, last access: 03/18/2022, and the species whose identification percentage was ≥98·7% were considered valid (Yoon *et al.* 2017). The sequences of the strains: *C. citreum* DSM 20528 (X77436), *C. albidum* DSM 20512 (access number AM042692), *Curtobacterium oceanosedimentum* ATCC 31317 (access number EF592577), *C. pusillum* DSM 20527 (access number AJ784400), *C. flaccumfaciens* LMG

3645 (access number AJ312209), *C. ammoniigenes* NBRC 101786 (access number BCSV01000013), *C. luteum* DSM 20542 (access number X77437), *Curtobacterium herbarum* P 420/07 (access number AJ310413) and *Rathayibacter toxicus* DSM 7488 (access number AUDF01000011) were included to assess the relationships of these species with the isolate identified in the present study. Phylogenetic tree based on multiple alignments of nearly complete 16S rRNA gene sequences was constructed using the neighbour-joining and the ClustalW algorithm with the software MEGA X (Kumar *et al.* 2018) by employing the Kimura two-parameter model (Kimura 1980) with branch support based on 1000 bootstrap replicates.

Authors contribution

Lygia Maria Paulo da Silva Braga—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Bruna Abdul Ahad Saad—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Caroline Tieppo Flores de Oliveira—the conception and design of the study, acquisition of data, analysis and interpretation of data, revising it critically for important intellectual content, final approval of the version to be submitted. Cláudia Elizabeth Volpe-Chaves—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Mara Luci Gonçalves Galiz Lacerda—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Stephen James Forsythe—revising it critically for important intellectual content, final approval of the version to be submitted. James Venturini—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Sandra Maria do Valle Leone de Oliveira—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Anamaria Mello Miranda Paniago—acquisition of data, analysis and interpretation of data, revising it critically for important intellectual content, final approval of the version to be submitted. Luciana Veloso da Costa—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Rebeca Vitória da Silva Lage—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Cristhiane Moura Falavina dos Reis—the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. Marcelo Luiz Lima Brandão—acquisition of data, analysis and interpretation of data, revising it critically for important intellectual content, final approval of the version to be submitted.

Conflict of Interest

No conflict of interest has been declared.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Anonymous. (2017) *EN ISO 22964:2017 Microbiology of the Food Chain - Horizontal Method for the Detection of Cronobacter* spp. Geneva: International Organization for Standardization.

Brandão, M.L.L., Forsythe, S. (2022) Cronobacter. In *Molecular Typing in Bacterial Infections* ed. de Filippis, I. Volume II. Cham: Springer.

Chaves, C.E.V., Brandão, M.L.L., Lacerda, M.L.G.G., Rocha, C.A.B.C., Leone de Oliveira, S.M.D.V., Parpinelli, T.C., Vasconcellos, L., Forsythe, S.J. *et al.* (2018) Fatal *Cronobacter sakazakii* sequence type 494 meningitis in a newborn. *Brazil Emerg Infect Dis* **24**, 1948–1950.

Chen, Y., Lampel, K., Hammack, T. (2012) Cronobacter. Chapter 29. In *Bacteriological Analytical Manual*. 8ª ed. Revision A, 1998. United States: United States: Food and Drug Administration. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-29-cronobacter

Collins, M.D. and Jones, D. (1983) Reclassification of *Corynebacterium flaccumfaciens*, *Corynebacterium betae*, *Corynebacterium oortii* and *Corynebacterium poinsettiae* in the genus *Curtobacterium*, as *Curtobacterium flaccumfaciens* comb. nov. *J Gen Microbiol* **129**, 3545–3548.

Costa, L.V.D., Miranda, R.V.D.S.L., Fonseca, E.L.D., Gonçalves, N.P., Reis, C.M.F.D., Frazão, A.M., Cruz, F.V., Brandão, M.L.L. *et al.* (2022) Assessment of VITEK® 2, MALDI-TOF MS and full gene 16S rRNA sequencing for aerobic endospore-forming bacteria isolated from a pharmaceutical facility. *J Microbiol Methods* **194**, 106419.

Francis, M.J., Doherty, R.R., Patel, M., Hamblin, J.F., Ojaimi, S. and Korman, T.M. (2011) *Curtobacterium flaccumfaciens* septic arthritis following puncture with a Coxspur hawthorn thorn. *J Clin Microbiol* **49**, 2759–2760.

Funke, G., Aravena-Roman, M. and Frodl, R. (2005) First description of *Curtobacterium* spp. isolated from human clinical specimens. *J Clin Microbiol* **43**, 1032–1036.

Giammanco, G.M., Aleo, A., Guida, I. and Mammina, C. (2011) Molecular epidemiological survey of *Citrobacter freundii* misidentified as *Cronobacter* spp. (*Enterobacter sakazakii*) and *Enterobacter hormaechei* isolated from powdered infant milk formula. *Foodborne Pathog Dis* **8**, 517–525.

Horinouchi, N., Shiota, S., Takakura, T., Yoshida, A., Kikuchi, K., Nishizono, A. and Miyazaki, E. (2022) Bacteremia caused by *Enterobacter asburiae* misidentified biochemically as *Cronobacter sakazakii* and accurately identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: a case report. *J Med Case Reports* **16**, 19.

Jackson, E.E. and Forsythe, S.J. (2016) Comparative study of *Cronobacter* identification according to phenotyping methods. *BMC Microbiol* **16**, 146.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* **35**, 1547–1549.

Park, Y.H., Suzuki, K., Yim, D.G., Lee, K.C., Kim, E., Yoon, J., Kim, S., Kho, Y.H. *et al.* (1993–1994) Suprageneric classification of peptidoglycan group B actinomycetes by nucleotide sequencing of 5S ribosomal RNA. *Antonie Van Leeuwenhoek* **64**, 307–313.

Townsend, S.M., Hurrell, E., Caubilla-Barron, J., Loc-Carrillo, C., Forsythe, S.J. (2008) Characterization of an extended-spectrum beta-lactamase *Enterobacter hormaechei* nosocomial outbreak, and other *Enterobacter hormaechei* misidentified as *Cronobacter* (*Enterobacter*) *sakazakii*. *Microbiology (Reading)* **154**(Pt 12), 3659–3667.

Warnken, M.B., Brandao, M.L.L., Souza, A.E., Romão, C.M.C.P., Nogueira, A.C.M.A. and Destro, M.T. (2012) Phenotypic profiles and detection of target genes by PCR in isolates from different sources and reference strains, identified as *Cronobacter* spp. (*Enterobacter sakazakii*). *Rev Inst Adolfo Lutz* **71**, 21–31.

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H. and Chun, J. (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* **67**, 1613–1617.