The Pan-Genome of the Emerging Multidrug-Resistant Pathogen Corynebacterium striatum

Hendor N. R. Jesus
Federal University of Bahia
Juliana N. Ramos
Rio de Janeiro State University — LDCIC/FCM/UERJ
Danilo J. P. G. Rocha
Federal University of Bahia
Daniele A. Alves
Federal University of Bahia
Carolina S. Silva
Federal University of Bahia
João V. O. Cruz
Federal University of Bahia
Verônica V. Vieira
Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz
Cassius Souza
Rio de Janeiro State University — LDCIC/FCM/UERJ
Louisy S. Santos
Rio de Janeiro State University — LDCIC/FCM/UERJ
Jesus Navas
Cantabria University
Rommel T. J. Ramos
Federal University of Para
Vasco Azevedo
Federal University of Minas Gerais
Eric R. G. R. Aguiar
State University of Santa Cruz
Ana L. Mattos-Guaraldi
Rio de Janeiro State University — LDCIC/FCM/UERJ
Luis G. C. Pacheco (luis.pacheco@ufba.br)
Federal University of Bahia

Research Article

Keywords: Corynebacterium striatum, pan-genome, multidrug resistance, emerging pathogen

Posted Date: May 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1666801/v1

Abstract

Corynebacterium striatum, a common constituent of the human skin microbiome, is now considered an emerging multidrug-resistant pathogen of immunocompromised and chronically ill patients. However, little is known about the molecular mechanisms in the transition from colonization to the multidrug-resistant (MDR) invasive phenotype in clinical isolates. This study performed a comprehensive pan-genomic analysis of *C. striatum*, including isolates from 'normal skin microbiome' and from MDR infections, to gain insights into genetic factors contributing to pathogenicity and multidrug resistance in this species. For this, three novel genome sequences were obtained from clinical isolates of *C. striatum* of patients from Brazil, and other 24 complete or draft *C. striatum* genomes were retrieved from GenBank, including the ATCC6940 isolate from the Human Microbiome Project. Analysis of *C. striatum* strains demonstrated the presence of an open pan-genome (α = 0.852803) containing 3,816 gene families, including 15 antimicrobial resistance (AMR) genes and 32 putative virulence factors. The core and accessory genomes included 1,297 and 1,307 genes, respectively. The identified AMR genes are primarily associated with resistance to aminoglycosides and tetracyclines. Of these, 66.6% are present in genomic islands, and four AMR genes, including *aac(6')-ib7*, are located in a class 1-integron. In conclusion, our data indicated that *C. striatum* possesses genomic characteristics favorable to the invasive phenotype, with high genomic plasticity, a robust genetic arsenal for iron acquisition, and important virulence determinants and AMR genes present in mobile genetic elements.

1. Introduction

Corynebacterium striatum is generally a common inhabitant of the human skin and upper respiratory tract. Still, it is also considered an emerging nosocomial pathogen in humans, affecting patients in opportunistic circumstances due to chronic diseases, immunosuppression, invasive medical procedures, previous antibiotic therapies and use of prosthetic devices (Khan et al. 2021). A growing number of reports have demonstrated the relevance of *C. striatum* in the etiology of a variety of infectious processes, in both immunocompromised and immunocompetent patients: endocarditis (Mansour et al. 2020; Rasmussen et al. 2020; Chang and Chen 2020; Biscarini et al. 2021; Bläckberg et al. 2021), meningitis (Zhang et al. 2020), and chronic septic arthritis (Hollnagel et al. 2020).

Hospital outbreaks by multidrug resistant (MDR) *C. striatum* isolates have now been reported in many countries worldwide, including Brazil (Otsuka et al. 2006; Renom et al. 2007; Verroken et al. 2014; Wang et al. 2016; Alibi et al. 2017; Ramos et al. 2018; Suh et al. 2019; Asgin and Otlu 2020). In these previous studies, different *C. striatum* strains presented resistance to antimicrobial agents from varied classes: penicillin, ciprofloxacin, moxifloxacin, gentamicin, clindamycin, erythromycin, rifampicin, imipenem, chloramphenicol, levofloxacin, tetracycline, tobramycin, as well as for daptomycin, one of the newest therapeutic alternatives (Galimand et al. 2015; Hahn et al. 2016; Nudel et al. 2018; Ramos et al. 2019; Garcia et al. 2020; Mitchell et al. 2021; Souza 2021; Abe et al. 2021).

Only a few studies have investigated the virulence mechanisms of *C. striatum*. Some demonstrated the relevance of biofilm formation during bloodstream infections, contributing to host invasive diseases. *C. striatum* strains were reported as etiologic agents of serious nosocomial invasive bloodstream infections of inpatients submitted to invasive medical procedures including endotracheal intubation and catheterization (Souza et al. 2015; Kang et al. 2018; Ramos et al. 2018); adherence properties to several medical devices made of different abiotic and biotic (hydrophilic and hydrophobic) materials (Alibi et al. 2021; Souza 2021); and, increased resistance of sessile and planktonic forms to some biocides, antiseptics and varied antibiotic classes (Souza et al. 2020). Furthermore, other virulence factors have also been investigated in the *Caenorhabditis elegans*-based virulence assay (Souza et al. 2019).

Recent studies have investigated the genetic composition of the species *C. striatum* and demonstrated that clonal multidrug-resistant isolates can be identified as the causative agents of nosocomial outbreaks (Pereira Baio et al. 2013; Nudel et al. 2018; Wang et al. 2019, 2021) Genetically diverse isolates carrying similar mobile genetic elements conferring multiple resistance to antimicrobials have already been described, then confirming the role of horizontal gene transfer in the rapid acquisition of the MDR phenotype in the species (Navas et al. 2016; Ramos et al. 2018).

In this study, we performed a pan-genomic analysis of the *C. striatum* species, including comparative analyzes of the genomes of three clinical isolates newly sequenced by our research consortium associated to additional twenty-four genomes retrieved from public databases. We observed that *C. striatum* has an open pan-genome, with extensive horizontal gene transfer activity probably being the primary driver of the rapid acquisition of resistance to multiple antimicrobial agents in the species.

2. Materials And Methods

2.1. Whole-genome sequencing and analysis

The QIAamp DNA mini kit (Qiagen) was used to extract total genomic DNA from three *C. striatum* clinical isolates (2023, 2230, 2247) (Ramos et al. 2019) obtained from the Hospital Universitário Pedro Ernesto, State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil. Complete genome sequencing was performed with the Ion Torrent Personal Genome Machine System, using a 318 chip and a fragments library, exactly as previously described by our research team (Mattos-Guaraldi et al. 2015). The FastQC tool was used for quality assessment of the generated sequences (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), trimming was performed to keep only reads with Phred values > 20. *de novo* genome assembly was achieved by SPAdes 3.0 (Bankevich et al. 2012) and MIRA 4.0 (Chevreux et al. 1999).

2.2. Data retrieval from public databases and through literature mining

Twenty-four additional genomic sequences from *C. striatum* isolates were retrieved from NCBI's GenBank (see supplementary material Table S1). Clinical data, isolation sites, and phenotypic profiles of susceptibility to antimicrobial agents were obtained through literature searches. All genomic sequences

were re-annotated using the Pathosystems Resource Integration Center (PATRIC) platform (Wattam et al. 2017) for standardization of genomic annotation with the RASTtk pipeline (Brettin et al. 2015).

2.3. Phylogenetic and phylogenomic analyses

A Multilocus Sequence Analysis (MLSA) and phylogenetic network analysis were performed with the following genes, retrieved from the standardized genomic sequences:
atpA, *dnaE*, *dnaK*, *fusA*, *leuA*, *odhA* and *rpoB* (Mattos-Guaraldi et al. 2015). A public genomic sequence for the PES1 strain of *Corynebacterium simulans* was used as an external group. The seven gene sequences for each genome were recovered from the standardized annotations, concatenated, and then aligned using MAFFT (Katoh et al. 2017). GBlocks were used to obtain regions with improved syntenies between the concatenated sequences (Castresana 2000). The MLSA tree was built using PhyML 3.0 (Guindon et al. 2010), with a substitution model GTR +G +I. A phylogenetic network was constructed with SplitsTree 4 (Huson and Bryant 2006). In addition, a minimum spanning tree based on Core Genome Multilocus Sequence Typing (cgMLST) was built with BacWGSTdb (Ruan and Feng 2015).

2.4. Pan-genomic analysis of *C. striatum*

The *C. striatum* pan-genome was inferred for the 27 standardized genomic sequences using the Bacterial Pan Genome Analysis (BPGA 1.3) pipeline (Chaudhari et al. 2016), using USEARCH v11 (Edgar 2010) for gene grouping and an identity cutoff value of 50%. The Power Law regression model ($n = k \, . \, N^{\alpha}$) was used to determine whether the pan-genome is open ($\alpha \leq 1$) or closed ($\alpha > 1$) (Tettelin et al. 2005, 2008). The subgroups of the pan-genome were submitted for functional annotation of the Cluster of Orthologous Groups (COG) categories using the eggNOG-Mapper (Huerta-Cepas et al. 2017).

2.5. Predictions of antimicrobial resistance genes (AMRs) and virulence factors

The prediction of antimicrobial resistance (AMR) genes was performed in the PATRIC platform, using annotations of the Database of Antibiotic Resistant Organisms (NDARO) (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/) and the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al. 2017). Predictions of genes coding for potential virulence factors were made with VFanalyzer (Liu et al. 2018), using pattern searches by Hidden Markov Models with GLIMMER3 (Delcher et al. 2007); the Virulence Factor Database (VFDB) includes domain profiles generated by hmmbuild and searched by hmmsearch, both from the HMMER3 package (Mistry et al. 2013).

2.6. Predictions of genomic islands, prophages, plasmids and CRISPR arrays

The prediction of genomic islands (GIs) was performed with IslandViewer 4 (Bertelli et al. 2017), which is a web server that integrates four different prediction methods: IslandPick (Langille et al. 2008), SIGI-HMM (Waack et al. 2006), Islander (Hudson et al. 2015) and IslandPath-DIMOB (Hsiao et al. 2003). The system automatically displays additional predictions of virulence factors and antimicrobial resistance

genes. Prophage sequences were predicted by Phage Search Tool Enhanced Release (PHASTER) (Arndt et al. 2016). PlasmidFinder was used to identify plasmid-derived sequences in the genomes (Carattoli et al. 2014). Insertion sequences (ISs) were searched using both the IS Finder tool (https://www-is.biotoul.fr/blast.php), for individual sequences, and the Mobile Element Finder, for complete genomes (https://cge.cbs.dtu.dk/services/MobileElementFinder/). We searched for Integrons using Integron Finder 2.0 (Néron et al. 2022) and compared integrases from the INTEGRALL database (Moura et al. 2009).

The CRISPR-CasFinder tool (Couvin et al. 2018) was used to identify CRISPR arrays and spacers sequences in the *C. striatum* genomes, using a 100 bp flanking distance an evidence level higher than 2.

2.7. Circular plot diagrams and genomic context analysis of antimicrobial resistance genes

Circular plot charts of the *C. striatum* genomes were generated with the Blast Ring Image Generator (BRIG 0.95) (Alikhan et al. 2011) by comparing all genomic sequences against a reference genome using the Basic Local Alignment Search Tool (BLAST 2.10.1) (Camacho et al. 2009). Predicted coordinates for genomic islands, prophages, virulence factors and antimicrobial resistance genes, including Illustrator for Biological Sequences (IBS 1.0.3) (Liu et al. 2015).

3. Results And Discussion

3.1. General features of the *C. striatum* genomes

C. striatum genomes presented a predicted size ranging from 2.61 Mb to 3.13 Mb, with a slight variation in G+C percentage between genomes (range: 59.2% - 59.8%). The total number of predicted coding sequences (CDSs) varied between 2,089 and 2,924. A phylogenetic network analysis based on seven housekeeping genes indicates the existence of two well-defined groups of *C. striatum* strains separated by geographical origin (Fig. 1a). The presence of different MDR *C. striatum* clones, identified by the cgMLST analysis (Fig. 1b), corroborates with findings from previous studies based on pulsed-field gel electrophoresis (PFGE) (Ramos et al. 2019). Interestingly, while strains with very similar phenotypic antimicrobial susceptibility profiles could be found forming clonal complexes (strains 2308 and 2023) (Fig. 1b), we also found strains carrying significantly different contents of AMR genes forming a clonal group (strains LK37 and 797_CAUR) (Fig. 1b). This finding underscores the potential role of horizontal gene transfer as a significant force driving the acquisition of antimicrobial resistance genes in the hospital environment by the species *C. striatum*.

The single strain from a non-human host, Kc-Na-01, presented the most significant genetic divergence from all studied isolates (Fig. 1a). Then, we analyzed this genome using average nucleotide identity by BLAST (ANIb) and found that this genomic sequence shares a higher than 94.5% identity with all genomic sequences included in the study (Supplementary Fig. S1). Even though it is generally regarded that a standard cutoff for species circumscription would be at ≥ 95% ANIb, some studies have shown that an ANIb value of *ca.* 94% equals to *ca.* 70% DNA-DNA hybridization (DDH) (Konstantinidis and Tiedje

2005; Rodriguez-R and Konstantinidis 2014; Qin et al. 2014). This assumption would permit classifying the strain Kc-Na-01 as belonging to the species *C. striatum*.

3.2. Pan-genomic analysis of *C. striatum*

The species *C. striatum* possesses an open pan-genome (α = 0.852803) (Fig. 2a), with 3,816 gene families, of which 33.99% (1,297) are present in the core genome and 34.25% (1,307) in the accessory genome. The unique genes represent 31.76% of the predicted gene families (1,212) (Fig. 2b). The strains KC-Na-01, from a non-human host, and BM4687, a clinical isolate from France, concentrate 42.8% of the species' total number of unique genes (260 and 326 individual genes, respectively). For strain KC-Na-01, the high number of unique genes may be partially explained by the existence of sequences derived from two different plasmids that contribute to the complete gene set of this isolate. Noteworthy, a new aminoglycoside 3-N-acetyltransferase (AAC(3)-XI) from chromosomal origin was recently discovered from the analysis of the genome of isolate BM4678 (Galimand et al. 2015).

The gene families in the *C. striatum* pan-genome are mainly distributed in the following COG functional categories: Metabolism (36.9%); Information storage and processing (35.3%); Cellular processes and signaling (8.2%); and Poorly characterized (19.4%) (Fig. 3a). Regarding the core genome, the most prevalent functional categories included: Translation, ribosomal structure and biogenesis (9.6%); Amino acid transport and metabolism (9.2%); and Transcription (9.1%). The accessory genes are mainly classified into the categories of Amino acid transport and metabolism (10.1%); Replication, recombination and repair (8.6%); and Transcription (8%). Finally, unique genes predominate in the categories of Replication, recombination and repair (19.7%); Transcription (10.9%); and Defense mechanisms (10.4%) (Fig. 3b). A high number of genes related to transcription regulation was an essential feature of the three pan-genome subsets.

3.3. Prediction of antimicrobial resistance genes (AMRs)

Through automated prediction, we identified 15 antimicrobial resistance genes (AMRs) in the C. striatum genomes (Fig. 4a). The single gene identified in all strains was *rpsL*, which codes for the 30S subunit-S12 ribosomal protein, presenting mutations similar to those described in streptomycin-resistant *M. tuberculosis* (Sreevatsan et al. 1996). However, the ATCC6940 and 1961 strains have a streptomycin susceptibility phenotype (Fig. 4b), whereas all the other strains isolated in Brazil have a streptomycin-resistant phenotype (Fig. 4b).

The AMR genes *aph(3')-Ia*, *aph(3')-Ib* and *aph(6)-Id* code for aminoglycoside phosphotransferases (Wright and Thompson 1999) that were found in the *C. striatum* genomes in the following proportions: 40.7%, 37.0% and 37.0%, respectively (Fig. 4a). These genes encode enzymes that catalyze the phosphorylation of various aminoglycosides. The *aac(3)-XI* gene, found in 22.0% of the strains, encodes the enzyme aminoglycoside 3-N-acetyltransferase type XI, initially described in *C. striatum* (Galimand et al. 2015).

The *cmx* gene, part of the MFS transporters family and promotes chloramphenicol efflux (Tauch et al. 1998), was present in 44.0% of the strains (Fig. 4a). As expected from previous studies, all strains that possessed this gene presented phenotypic non-susceptibility to chloramphenicol (Fig. 4b).

The *ermX* gene was found in 74.0% of the strains and codes for the rRNA methyltransferase enzyme responsible for the ineffective binding of macrolides, lincosamides, and streptogramins to the 23S ribosomal binding site (Roberts et al. 1999).

The *sul1* gene was identified in 18.5% of the strains and is part of a gene family that codes for alternatives to the dihydropteroate synthase enzymes, which have less affinity for sulfonamides (Changkaew et al. 2014).

Although the *tetA* and *tetW* genes were found in 44.4% and 40.7% of the *C. striatum* strains, respectively (Fig. 4a), resistance to tetracycline did not correlate well with the identification of these genes in the genomic sequences (Fig. 4b). These genes confer resistance to tetracycline through different mechanisms: *tetA* encodes transport proteins of the MFS family, whereas *tetW* encodes ribosomal protection protein (Roberts 2005).

Four AMR genes in the *C. striatum* pan-genome were identified as unique genes: *tetB*, *aac(6')-Ib7*, *aadA* and *qacE*. The *tetB* gene was found in the 2023 strain, but this strain is phenotypically susceptible to tetracycline, despite carrying the *tetA* and *tetB* genes, encoding efflux pumps for tetracyclines (Fig. 4b). Additionally, mutations in the *rpoB* gene similar to those found in rifampicin-resistant strains of *Mycobacterium tuberculosis* were found in the 2023 strain, which is phenotypically resistant to rifampicin (Ramos et al. 2019).

The other three unique genes (*aac(6')-Ib7*, *aadA* and *qacE*) were only found in the LK37 lineage (Fig. 4a). The *aac(6')-Ib7* gene encodes the aminoglycoside acetyltransferase enzyme (Roberts et al. 1999) and the *aadA* the aminoglycoside nucleotidyltransferase enzyme (Clark et al. 1999) responsible for aminoglycoside resistance, while the *qacE* gene codes for a proton-dependent efflux pump for monovalent cationic antiseptics such as ammonium quaternary (Paulsen et al. 1996).

3.4. Prediction of virulence factors

We identified 32 genes potentially related to virulence in *C. striatum*, with 19 (59.3%) genes present in all strains, 11 (34.3%) genes appearing in at least two strains, but not in all, and 2 (6.3%) genes uniquely present in one strain (Fig. 5). These virulence factors are distributed in 10 functional categories (Fig. 5). In the ‘iron uptake category’, three operons were identified as present in all *C. striatum* strains, namely the *fagABCD* operon, also present in *Corynebacterium pseudotuberculosis* strains (Billington et al. 2002; Dorella et al. 2006) the *hmuTUV* and the *irp6ABC* operons, also found in *C. diphtheriae* (Allen and Schmitt 2009; Schmitt 2014). Additionally, the *itrAB* operon is present in 17 *C. striatum* lineages (63%), and the *mbtH* and *fxbA* genes in 12 lineages (44.4%); these genes have been widely described in bacteria of the genus *Mycobacterium* (Dussurget et al. 1999; Timms et al. 2015).

Four genes coding for transcriptional regulators of potential virulence genes were also found in all *C. striatum* genomes (Fig. 5). The iron-activated *dtxR* gene is involved in regulations of genes related to iron homeostase, such as the genes of the *fagABC*, *hmuTUV* and *ipr6ABC* operons in *Corynebacteria spp.* (Qian et al. 2002; Trost et al. 2010). This gene may also be involved in regulating of *irtA*, *irtB*, *mbtH* and *fxbA*, as demonstrated by (Manabe et al. 2005). The *senx3*, *sigA* and *sigD* genes have already been shown to play essential roles in the virulence and persistence of *Mycobacteria spp.* (Gomez et al. 1998; Raman et al. 2004; Singh and Kumar 2015).

The *pafA* and *mpA* genes, present in all strains, are part of the Pup proteasome System in Actinobacteria and have relevance in the persistence of *M. tuberculosis* in the host (Darwin 2009). The *SpaFED* pili are present in 21 strains, together with the genes of the sortases *srtB* and *strC* necessary for the pili's assembly (Gaspar and Ton-That 2006). These protein structures are displayed on the cell surface and participate in biofilm formation, DNA translocation, and interactions with other bacteria, besides working as phage receptors, contributing to pathogenesis (Mandlik et al. 2008; Proft and Baker 2009; Kline et al. 2010).

The *secA2* secretion system was found in all *C. striatum* strains (Fig. 5). This system has been demonstrated to be responsible for the exportation of multiple effectors that interfere with phagosome maturation and promote intracellular replication in *M. tuberculosis* (Zulauf et al. 2018).

Orthologs of the genes *wecB* and *wecC* code for the enzymes UDP-N-acetilglucosamine-2-epimerase and UDP-N-acetil-d-manosamine desidrogenase, uniquely found in the strain Kc-Na-01 (Fig. 5). Although these enzymes are expected to be found in Gram-negative bacteria, for the synthesis of lipopolysaccharide (Rai and Mitchell 2020), the orthologs *mnaA* and *mnaB* have been described in *Staphylococcus* spp. and are involved in the biosynthesis of the cell envelope.

3.5. Genomic islands (GI), prophages, insertion sequences (IS), and CRISPR loci

Eigthy-four out of 129 AMRs distributed throughout all strains were found within genomic islands (Fig.6). Sixteen strains presented the *ermX* adjacent to *gcrA* and *gcrB*, as shown in the genomic context for strain 2023 (Fig. 7a). Seven AMRs were found within the GI18, including *ermX*, *tetA,* and *tetB* (Fig. 6; Fig. 7b). The genes *aph(3")Ib*, *aph(6)-Id*, and *cmx* are all presented in GI8 (Fig. 6). These together with other genes that are found in the flanking regions of AMR genes were identified in the pTP10 plasmid, which is part of the *C. striatum* M82B genome (Tauch et al. 2000), as well as of the genome of *C. striatum* strain 2308 (Ramos et al. 2018).

Seventy-four bacteriophage signatures were detected in the studied genomes (Supplementary Table S2). Nevertheless, only 16 phage sequences were found intact in the genomes, of which the most prevalent were the PHAGE_Rhodoc_REQ3_NC_016654 and the PHAGE_Staphy_SPbeta_like_NC_029119. Notably, four AMR genes were found within a phage context in strain LK37 (Fig. 7c), present in GI5 (Fig.6).

Integrons are versatile genetic elements, characterized by the ability to insert, excise, rearrange and express genes through a site-specific recombination system and can act as vehicles for intra- and inter-specific transmission of genetic material (El Sayed Zaki et al. 2022). Integrons are characterized into classes based on the type of integrase gene. Class 1 integron is the most frequently observed in clinical isolates, mainly in Gram-negative bacteria (Racewicz et al. 2022). In *Pseudomonas aeruginosa*, the presence of class 1 integron is associated with the emergence of the MDR phenotype (El Sayed Zaki et al. 2022). However, studies on the presence of integrons in Gram-positive bacteria especially in *Corynebacterium* species are scarce. Class 1 integrons have been found in some *Corynebacterium* clinical isolates, such as *Corynebacterium diphtheriae* biovar mitis (Barraud et al. 2011), *Corynebacterium resistens* (Schröder et al. 2012), and *Corynebacterium urealyticum* (Rocha et al. 2020). To date, there are no studies describing the presence of class 1 integrons in *C. striatum* (Leyton et al. 2021). In our study, we found the class 1 integron in LK37 carrying the genes *sul1*, *qacE*, *aadA*, and *aac(6')-Ib7* (Fig. 7c), in strains 2130, 2296, 2425, and 3012STDY7069329 carry only the *sul1* gene (Supplementary Table S3).

We also evaluated the insertion sequences (IS) that appear in the same genomic context as the AMR genes. The main IS families found in these regions were IS3, IS481, IS256, ISL3 and IS6. Interestingly, the ISCre1, belonging to the IS256 family, is associated with the *aac(3)-XI* gene in 7 *C. striatum* isolates. Additionally, the ISCx1 insertion sequence, belonging to the ISL3 family, was found in association with *erm(X)*, *tet(W)* and *aac(3)-XI*. The IS1249 was also found in the genomic context of *erm(X)* and the IS5564 was located near the genes *aph(3")-Ib* and *aph(6')-Id*. Both IS1249 and ISCx1 are part of a transposon Tn5432, which has been identified in the genomic sequences of *C. striatum* by recent studies (Wang et al. 2019; Leyton et al. 2021; Leyton-Carcaman and Abanto 2022) (Fig. 7c).

We also evaluated the genomic context of virulence factors and found that the operons *spaDEF*, *fagABCD*, *hmuTUV*, *irtAB,* and the gene *fxbA* can also be located in GIs. Twenty-one *C. striatum* isolates have mobile genetic elements in the same context of genes coding for SpaD-like pili (Fig. 8). This region is also within a predicted genomic island, GI17 (Fig. 6). Noticeably, five strains present frameshifts in at least one of the pili encoding genes (Fig. 8b), suggesting an incapacity to pili assembly or dependence on sortases StrB and StrC (Gaspar and Ton-That 2006). Some strains did not present the pili assembly machinery within the same genomic context: KC-Na-01, 1961, 962_CAUR, 963_CAUR, 1329_CAUR and LK37 (Fig. 8c).

Great variability in CRISPR-associated loci was identified in the species *C. striatum* (Supplementary Table S4). The studied genomes presented an average number of 52 detected spacer sequences, but with a wide range between 5 and 117 sequences. The identified repeat sequences were more consistently distributed throughout the genomes (Supplementary Table S3). Some genomes presented two clusters of genes coding for CRISPR-associated proteins (*Cas*), but the most prevalent organization found was the type IE CRISPR system (Supplementary Table S3). A recent study suggests the existence of an alternative, as-yet-unidentified CRISPR system organization in this species, termed Type I-E' (Ramos et al. 2022).

4. Conclusions

We found that the *C. striatum* species has an open pan-genome, presenting widespread acquisition of novel genes by horizontal gene transfer. This is particularly demonstrated by the high number of identified genes related to resistance to aminoglycosides and tetracycline. The *C. striatum* genomic sequences illustrate the existence of a robust iron acquisition machinery in the species, suggesting an improved ability to adapt and survive within the host environment. However, the absence of pili or frameshifts in at least one of the five genes in some isolates can lead to a lowered ability to infect and persist in the host, considering that it was the only pili identified in the species. This study highlights the importance of correctly identifying this microorganism in the clinical microbiology laboratory, besides performing molecular surveillance of antimicrobial resistance genes within the hospital environment, due to the widespread presence of clinically relevant AMRs in the species and the apparent facility to share mobile genetic elements.

Declarations

Ethical Approval and Consent to participate

Not applicable.

Human and Animal Ethics

Not applicable.

Consent for publication

All authors read and approved the final version of the manuscript submitted for publication.

Availability of supporting data

The genome sequences generated in this study are deposited in NCBI's GenBank and can be accessed through the following IDs: GCA_002865925.1; GCA_002775055.1; GCA_002775105.1.

Competing interests

The authors declare no conflicts of interest related to this study.

Funding

This study was partially supported by grants from FAPESB, CNPq, CAPES, and FINEP in Brazil, through the following funding schemes: Fundação de Amparo à Pesquisa do Estado da Bahia - BOL0505/2018 and JCB17/2013; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES-PROCAD 071/2013; Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq Nº 09/2018; and Financiadora de Estudos e Projetos, MCT/FINEP/CT-INFRA01/2013.

Authors' contributions

H.N.R.J., J.N.R., D.J.P.G.R., D.A.A., C.S.S., and J.V.O.C.: Investigation, Formal analysis, Methodology, Software, Data Curation, Visualization, Writing - Original Draft; H.N.R.J., C.S., and L.S.S: Investigation, Formal analysis, Methodology, Visualization, Writing - Review & Editing; V.V.V., J.N., R.T.J.R, V.A., E.R.G.R.A., A.L.M-G., and L.G.C.P: Conceptualization, Funding, Resources, Project administration, Supervision, Writing - Review & Editing.

Acknowledgements

HNRJ was the recipient of a Ph.D scholarship from FAPESB. LGCP was the recipient of a research fellowship from CNPq.

Authors' information

Affiliations

Multicenter Post-Graduate Program in Biochemistry and Molecular Biology (PMBqBM), Institute of Health Sciences, Federal University of Bahia, Salvador-BA, Brazil.

Hendor N. R. Jesus and Luis G. C. Pacheco.

Laboratory of Diphtheria and Corinebacteria of Clinical Relevance, School of Medical Sciences, Rio de Janeiro State University — LDCIC/FCM/UERJ, Rio de Janeiro-RJ, Brazil.

Juliana N. Ramos, Cassius Souza, Louisy S. Santos, and Ana L. Mattos-Guaraldi.

Institute of Health Sciences, Federal University of Bahia, Salvador-BA, Brazil.

Danilo J. P. G. Rocha, Daniele A. Alves, Carolina S. Silva, João V. O. Cruz, and Luis G. C. Pacheco.

Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte-MG, Brazil.

Daniele A. Alves and Vasco Azevedo.

Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz, Laboratório Interdisciplinar de Pesquisas Médicas, Rio de Janeiro, RJ, Brazil.

Verônica V. Vieira

Faculty of Medicine. Cantabria University, Santander, Spain.

Jesus Navas

Institute of Biological Sciences, Federal University of Para, Belem-PA, Brazil; and Biological Engineering Laboratory, Science and Technology Park Guama, Belem-PA, Brazil.

Rommel T. J. Ramos

Department of Biological Sciences, State University of Santa Cruz, Ilhéus-BA, Brazil.

Eric R. G. R. Aguiar

References

1. Abe M, Kimura M, Maruyama H, et al (2021) Clinical characteristics and drug susceptibility patterns of Corynebacterium species in bacteremic patients with hematological disorders. Eur J Clin Microbiol Infect Dis 15–17. https://doi.org/10.1007/s10096-021-04257-8
2. Alibi S, Ferjani A, Boukadida J, et al (2017) Occurrence of Corynebacterium striatum as an emerging antibiotic-resistant nosocomial pathogen in a Tunisian hospital. Sci Rep 7:9704. https://doi.org/10.1038/s41598-017-10081-y
3. Alibi S, Ramos-Vivas J, Ben Selma W, et al (2021) Virulence of clinically relevant multidrug resistant Corynebacterium striatum strains and their ability to adhere to human epithelial cells and inert surfaces. Microb Pathog 155:104887. https://doi.org/https://doi.org/10.1016/j.micpath.2021.104887
4. Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA (2011) BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402
5. Allen CE, Schmitt MP (2009) HtaA is an iron-regulated hemin binding protein involved in the utilization of heme iron in Corynebacterium diphtheriae. J Bacteriol 191:2638–2648
6. Arndt D, Grant JR, Marcu A, et al (2016) PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16-21. https://doi.org/10.1093/nar/gkw387
7. Asgin N, Otlu B (2020) Antimicrobial Resistance and Molecular Epidemiology of Corynebacterium striatum Isolated in a Tertiary Hospital in Turkey. Pathogens 9:136. https://doi.org/10.3390/pathogens9020136
8. Bankevich A, Nurk S, Antipov D, et al (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477
9. Barraud O, Badell E, Denis F, et al (2011) Antimicrobial drug resistance in Corynebacterium diphtheriae mitis. Emerg Infect Dis 17:2078–2080. https://doi.org/10.3201/eid1711.110282
10. Bertelli C, Laird MR, Williams KP, et al (2017) IslandViewer 4: Expanded prediction of genomic islands for larger-scale datasets. Nucleic Acids Res 45:W30–W35. https://doi.org/10.1093/nar/gkx343
11. Billington SJ, Esmay PA, Songer JG, Jost BH (2002) Identification and role in virulence of putative iron acquisition genes from Corynebacterium pseudotuberculosis. FEMS Microbiol Lett 208:41–45
12. Biscarini S, Colaneri M, Mariani B, et al (2021) A case of Corynebacterium striatum endocarditis successfully treated with an early switch to oral antimicrobial therapy. Infez Med 29:138–144

13. Bläckberg A, Falk L, Oldberg K, et al (2021) Infective Endocarditis Due to Corynebacterium Species: Clinical Features and Antibiotic Resistance. Open Forum Infect Dis 8:1–6. https://doi.org/10.1093/ofid/ofab055
14. Brettin T, Davis JJ, Disz T, et al (2015) RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365
15. Camacho C, Coulouris G, Avagyan V, et al (2009) BLAST+: architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421
16. Carattoli A, Zankari E, García-Fernández A, et al (2014) In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14
17. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552
18. Chang W-T, Chen Z-C (2020) Pulsatile Strangulation of the Aorta — A Rare Presentation and Etiology of Infective Endocarditis —. Circ J 84:1607. https://doi.org/10.1253/circj.CJ-20-0495
19. Changkaew K, Utrarachkij F, Siripanichgon K, et al (2014) Characterization of Antibiotic Resistance in Escherichia coli Isolated from Shrimps and Their Environment. J Food Prot 77:1394–1401. https://doi.org/10.4315/0362-028X.JFP-13-510
20. Chaudhari NM, Gupta VK, Dutta C (2016) BPGA- an ultra-fast pan-genome analysis pipeline. Sci Rep 6:24373. https://doi.org/10.1038/srep24373
21. Chevreux B, Wetter T, Suhai S (1999) Genome sequence assembly using trace signals and additional sequence information. In: German conference on bioinformatics. Citeseer, pp 45–56
22. Clark NC, Olsvik Ø, Swenson JM, et al (1999) Detection of a Streptomycin/Spectinomycin Adenylyltransferase Gene (aadA) in Enterococcus faecalis Antimicrob Agents Chemother 43:157 LP – 160. https://doi.org/10.1128/AAC.43.1.157
23. Couvin D, Bernheim A, Toffano-Nioche C, et al (2018) CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. Nucleic Acids Res 46:W246–W251
24. Darwin KH (2009) Prokaryotic ubiquitin-like protein (Pup), proteasomes and pathogenesis. Nat Rev Microbiol 7:485–491. https://doi.org/10.1038/nrmicro2148
25. Delcher AL, Bratke KA, Powers EC, Salzberg SL (2007) Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679. https://doi.org/10.1093/bioinformatics/btm009
26. Dorella FA, Pacheco LGC, Oliveira SC, et al (2006) Corynebacterium pseudotuberculosis: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. Vet Res 37:201–218. https://doi.org/10.1051/vetres:2005056
27. Dussurget O, Timm J, Gomez M, et al (1999) Transcriptional control of the iron-responsive fxbA gene by the mycobacterial regulator IdeR. J Bacteriol 181:3402–3408. https://doi.org/10.1128/JB.181.11.3402-3408.1999

28. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. https://doi.org/10.1093/bioinformatics/btq461
29. El Sayed Zaki M, Mostafa Mahmoud N, Anies Rizk M (2022) Molecular Study of Integrase Gene I and Integrase Gene II in Clinical Isolates of Pseudomonas aeruginosa. Infect Disord - Drug Targets 22:925. https://doi.org/10.2174/1871526522666220408111454
30. Galimand M, Fishovitz J, Lambert T, et al (2015) AAC(3)-XI, a New Aminoglycoside 3- N -Acetyltransferase from Corynebacterium striatum. Antimicrob Agents Chemother 59:5647–5653. https://doi.org/10.1128/AAC.01203-15
31. Garcia CM, McKenna J, Fan L, Shah A (2020) Corynebacterium Striatum Bacteremia in End-Stage Renal Disease: A Case Series and Review of Literature. R I Med J (2013) 103:46–49
32. Gaspar AH, Ton-That H (2006) Assembly of distinct pilus structures on the surface of Corynebacterium diphtheriae. J Bacteriol 188:1526–1533. https://doi.org/10.1128/JB.188.4.1526-1533.2006
33. Gomez M, Doukhan L, Nair G, Smith I (1998) sigA is an essential gene in Mycobacterium smegmatis. Mol Microbiol 29:617–628
34. Guindon S, Dufayard J-F, Lefort V, et al (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321
35. Hahn WO, Werth BJ, Butler-Wu SM, Rakita RM (2016) Multidrug-Resistant Corynebacterium striatum Associated with Increased Use of Parenteral Antimicrobial Drugs. Emerg Infect Dis 22:1908–1914. https://doi.org/10.3201/eid2211.160141
36. Hollnagel K, Willen J, Ellis M, et al (2020) Chronic Corynebacterium striatum Septic Arthritis in a Patient Referred for Total Knee Arthroplasty. Case Rep Orthop 2020:1–5. https://doi.org/10.1155/2020/1392182
37. Hsiao W, Wan I, Jones SJ, Brinkman FSL (2003) IslandPath: aiding detection of genomic islands in prokaryotes. Bioinformatics 19:418–420. https://doi.org/10.1093/bioinformatics/btg004
38. Hudson CM, Lau BY, Williams KP (2015) Islander: a database of precisely mapped genomic islands in tRNA and tmRNA genes. Nucleic Acids Res 43:D48–D53. https://doi.org/10.1093/nar/gku1072
39. Huerta-Cepas J, Forslund K, Coelho LP, et al (2017) Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. Mol Biol Evol 34:2115–2122. https://doi.org/10.1093/molbev/msx148
40. Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23:254–267
41. Jia B, Raphenya AR, Alcock B, et al (2017) CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 45:D566–D573. https://doi.org/10.1093/nar/gkw1004
42. Kang SJ, Choi SM, Choi JA, et al (2018) Factors affecting the clinical relevance of Corynebacterium striatum isolated from blood cultures. PLoS One 13:1–12. https://doi.org/10.1371/journal.pone.0199454

43. Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20:1160–1166. https://doi.org/10.1093/bib/bbx108
44. Khan D, Shadi M, Mustafa A, et al (2021) A Wolf in Sheep's clothing; Case reports and literature review of Corynebacterium striatum endocarditis. IDCases 24:e01070. https://doi.org/10.1016/j.idcr.2021.e01070
45. Kline KA, Dodson KW, Caparon MG, Hultgren SJ (2010) A tale of two pili: assembly and function of pili in bacteria. Trends Microbiol 18:224–232
46. Konstantinidis KT, Tiedje JM (2005) Genomic insights that advance the species definition for prokaryotes. Proc Natl Acad Sci U S A 102:2567–2572. https://doi.org/10.1073/pnas.0409727102
47. Langille MGI, Hsiao WWL, Brinkman FSL (2008) Evaluation of genomic island predictors using a comparative genomics approach. BMC Bioinformatics 9:329. https://doi.org/10.1186/1471-2105-9-329
48. Leyton-Carcaman B, Abanto M (2022) Beyond to the Stable: Role of the Insertion Sequences as Epidemiological Descriptors in Corynebacterium striatum. Front Microbiol
49. Leyton B, Ramos JN, Baio PVP, et al (2021) Treat Me Well or Will Resist: Uptake of Mobile Genetic Elements Determine the Resistome of Corynebacterium striatum. Int J Mol Sci 22:7499
50. Liu B, Zheng D, Jin Q, et al (2018) VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res 47:D687–D692. https://doi.org/10.1093/nar/gky1080
51. Liu W, Xie Y, Ma J, et al (2015) IBS: an illustrator for the presentation and visualization of biological sequences. Bioinformatics 31:3359–3361. https://doi.org/10.1093/bioinformatics/btv362
52. Manabe YC, Hatem CL, Kesavan AK, et al (2005) Both Corynebacterium diphtheriae DtxR(E175K) and Mycobacterium tuberculosis IdeR(D177K) are dominant positive repressors of IdeR-regulated genes in M. tuberculosis. Infect Immun 73:5988–5994. https://doi.org/10.1128/IAI.73.9.5988-5994.2005
53. Mandlik A, Swierczynski A, Das A, Ton-That H (2008) Pili in Gram-positive bacteria: assembly, involvement in colonization and biofilm development. Trends Microbiol 16:33–40. https://doi.org/10.1016/j.tim.2007.10.010
54. Mansour MK, Al-Messabi AH, Ahmed SA, et al (2020) Corynebacterium striatum prosthetic valve endocarditis. A case report and literature review. Clin Infect Pract 7–8:100055. https://doi.org/10.1016/j.clinpr.2020.100055
55. Mattos-Guaraldi AL, Guimarães LC, Santos CS, et al (2015) Draft Genome Sequence of Corynebacterium striatum 1961 BR-RJ/09, a Multidrug-Susceptible Strain Isolated from the Urine of a Hospitalized 37-Year-Old Female Patient. Genome Announc 3:e00869-15. https://doi.org/10.1128/genomeA.00869-15
56. Mistry J, Finn RD, Eddy SR, et al (2013) Challenges in homology search: HMMER3 and convergent evolution of coiled-coil regions. Nucleic Acids Res 41:e121–e121. https://doi.org/10.1093/nar/gkt263

57. Mitchell KF, McElvania E, Wallace MA, et al (2021) Evaluating the Rapid Emergence of Daptomycin Resistance in Corynebacterium : a Multicenter Study. J Clin Microbiol 59:1–12. https://doi.org/10.1128/JCM.02052-20
58. Moura A, Soares M, Pereira C, et al (2009) INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. Bioinformatics 25:1096–1098. https://doi.org/10.1093/bioinformatics/btp105
59. Navas J, Fernández-Martínez M, Salas C, et al (2016) Susceptibility to Aminoglycosides and Distribution of aph and aac(3)-XI Genes among Corynebacterium striatum Clinical Isolates. PLoS One 11:e0167856. https://doi.org/10.1371/journal.pone.0167856
60. Néron B, Littner E, Haudiquet M, et al (2022) IntegronFinder 2.0: Identification and Analysis of Integrons across Bacteria, with a Focus on Antibiotic Resistance in Klebsiella. Microorg. 10
61. Nudel K, Zhao X, Basu S, et al (2018) Genomics of Corynebacterium striatum, an emerging multidrug-resistant pathogen of immunocompromised patients. Clin Microbiol Infect 24:1016.e7-1016.e13. https://doi.org/10.1016/j.cmi.2017.12.024
62. Otsuka Y, Ohkusu K, Kawamura Y, et al (2006) Emergence of multidrug-resistant Corynebacterium striatum as a nosocomial pathogen in long-term hospitalized patients with underlying diseases. Diagn Microbiol Infect Dis 54:109–114. https://doi.org/https://doi.org/10.1016/j.diagmicrobio.2005.08.005
63. Paulsen IT, Brown MH, Skurray RA (1996) Proton-dependent multidrug efflux systems. Microbiol Rev 60:575 LP – 608
64. Pereira Baio PV, Franceschi Mota H, D'avila Freitas A, et al (2013) Clonal multidrug-resistant Corynebacterium striatum within a nosocomial environment, Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 108:23–29. https://doi.org/10.1590/S0074-02762013000100004
65. Proft T, Baker EN (2009) Pili in Gram-negative and Gram-positive bacteria—structure, assembly and their role in disease. Cell Mol life Sci 66:613
66. Qian Y, Lee JH, Holmes RK (2002) Identification of a DtxR-Regulated Operon That Is Essential for Siderophore-Dependent Iron Uptake in Corynebacterium diphtheriae. J Bacteriol 184:4846–4856. https://doi.org/10.1128/JB.184.17.4846-4856.2002
67. Qin Q-L, Xie B-B, Zhang X-Y, et al (2014) A proposed genus boundary for the prokaryotes based on genomic insights. J Bacteriol 196:2210–2215. https://doi.org/10.1128/JB.01688-14
68. Racewicz P, Majewski M, Biesiada H, et al (2022) Prevalence and characterisation of antimicrobial resistance genes and class 1 and 2 integrons in multiresistant Escherichia coli isolated from poultry production. Sci Rep 12:1–13
69. Rai AK, Mitchell AM (2020) Enterobacterial Common Antigen: Synthesis and Function of an Enigmatic Molecule. MBio 11:e01914-20. https://doi.org/10.1128/mBio.01914-20
70. Raman S, Hazra R, Dascher CC, Husson RN (2004) Transcription Regulation by the Mycobacterium tuberculosis Alternative Sigma Factor SigD and Its Role in Virulence. J Bacteriol 186:6605 LP – 6616. https://doi.org/10.1128/JB.186.19.6605-6616.2004

71. Ramos JN, Baio PVP, Veras JFC, et al (2022) Novel configurations of type IE CRISPR-Cas system in Corynebacterium striatum clinical isolates
72. Ramos JN, Rodrigues IDS, Baio PVP, et al (2018) Genome sequence of a multidrug-resistant Corynebacterium striatum isolated from bloodstream infection from a nosocomial outbreak in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 113:e180051–e180051. https://doi.org/10.1590/0074-02760180051
73. Ramos JN, Souza C, Faria YV, et al (2019) Bloodstream and catheter-related infections due to different clones of multidrug-resistant and biofilm producer Corynebacterium striatum. BMC Infect Dis 19:672. https://doi.org/10.1186/s12879-019-4294-7
74. Rasmussen M, Mohlin AW, Nilson B (2020) From contamination to infective endocarditis—a population-based retrospective study of Corynebacterium isolated from blood cultures. Eur J Clin Microbiol Infect Dis 39:113–119. https://doi.org/10.1007/s10096-019-03698-6
75. Renom F, Garau M, Rubí M, et al (2007) Nosocomial outbreak of Corynebacterium striatum infection in patients with chronic obstructive pulmonary disease. J Clin Microbiol 45:2064–2067. https://doi.org/10.1128/JCM.00152-07
76. Roberts MC (2005) Update on acquired tetracycline resistance genes. FEMS Microbiol Lett 245:195–203. https://doi.org/10.1016/j.femsle.2005.02.034
77. Roberts MC, Sutcliffe J, Courvalin P, et al (1999) Nomenclature for Macrolide and Macrolide-Lincosamide-Streptogramin B Resistance Determinants. Antimicrob Agents Chemother 43:2823–2830. https://doi.org/10.1128/AAC.43.12.2823
78. Rocha DJP, Azevedo V, Brenig B, et al (2020) Whole-genome sequencing reveals misidentification of a multidrug-resistant urine clinical isolate as Corynebacterium urealyticum. J Glob Antimicrob Resist 23:16–19. https://doi.org/https://doi.org/10.1016/j.jgar.2020.07.020
79. Rodriguez-R LM, Konstantinidis KT (2014) Bypassing Cultivation To Identify Bacterial Species: Culture-independent genomic approaches identify credibly distinct clusters, avoid cultivation bias, and provide true insights into microbial species. Microbe Mag 9:111–118
80. Ruan Z, Feng Y (2015) BacWGSTdb, a database for genotyping and source tracking bacterial pathogens. Nucleic Acids Res 44:D682–D687. https://doi.org/10.1093/nar/gkv1004
81. Schmitt MP (2014) Iron acquisition and iron-dependent gene expression in Corynebacterium diphtheriae. In: Corynebacterium diphtheriae and Related Toxigenic Species. Springer, pp 95–121
82. Schröder J, Maus I, Meyer K, et al (2012) Complete genome sequence, lifestyle, and multi-drug resistance of the human pathogen Corynebacterium resistens DSM 45100 isolated from blood samples of a leukemia patient. BMC Genomics 13:141. https://doi.org/10.1186/1471-2164-13-141
83. Singh N, Kumar A (2015) Virulence factor SenX3 is the oxygen-controlled replication switch of Mycobacterium tuberculosis. Antioxid Redox Signal 22:603–613. https://doi.org/10.1089/ars.2014.6020
84. Souza C (2021) Biofilm Production on Different Abiotic Surface, Positive and Negative Charge by Multi-Drug Resistance Corynebacterium striatum Strains. Open Access J Surg 12:114–128.

https://doi.org/10.19080/OAJS.2021.12.555843

85. Souza C de, Faria YV, Sant'Anna L de O, et al (2015) Biofilm production by multiresistant Corynebacterium striatum associated with nosocomial outbreak. Mem Inst Oswaldo Cruz 110:242–248. https://doi.org/10.1590/0074-02760140373
86. Souza C de, Mota HF, Faria YV, et al (2020) Resistance to antiseptics and disinfectants of planktonic and biofilm-associated forms of Corynebacterium striatum. Microb Drug Resist 26:1546–1558
87. Souza C de, Simpson-Louredo L, Mota HF, et al (2019) Virulence potential of Corynebacterium striatum towards Caenorhabditis elegans. Antonie Van Leeuwenhoek 112:1331–1340. https://doi.org/10.1007/s10482-019-01265-9
88. Sreevatsan S, Pan X, Stockbauer KE, et al (1996) Characterization of rpsL and rrs mutations in streptomycin-resistant Mycobacterium tuberculosis isolates from diverse geographic localities. Antimicrob Agents Chemother 40:1024–1026. https://doi.org/10.1128/AAC.40.4.1024
89. Suh JW, Ju Y, Lee CK, et al (2019) Molecular epidemiology and clinical significance of Corynebacterium striatum isolated from clinical specimens. Infect Drug Resist 12:161–171. https://doi.org/10.2147/IDR.S184518
90. Tauch A, Krieft S, Kalinowski J, Pühler A (2000) The 51,409-bp R-plasmid pTP10 from the multiresistant clinical isolate Corynebacterium striatum M82B is composed of DNA segments initially identified in soil bacteria and in plant, animal, and human pathogens. Mol Gen Genet MGG 263:1–11. https://doi.org/10.1007/PL00008668
91. Tauch A, Zheng Z, Pühler A, Kalinowski J (1998) Corynebacterium striatumChloramphenicol Resistance Transposon Tn5564:Genetic Organization and Transposition inCorynebacterium glutamicum. Plasmid 40:126–139. https://doi.org/10.1006/plas.1998.1362
92. Tettelin H, Masignani V, Cieslewicz MJ, et al (2005) Genome analysis of multiple pathogenic isolates of Streptococcus agalactiae: implications for the microbial "pan-genome." Proc Natl Acad Sci 102:13950–13955
93. Tettelin H, Riley D, Cattuto C, Medini D (2008) Comparative genomics: the bacterial pan-genome. Curr Opin Microbiol 11:472–477. https://doi.org/10.1016/j.mib.2008.09.006
94. Timms VJ, Hassan KA, Mitchell HM, Neilan BA (2015) Comparative genomics between human and animal associated subspecies of the Mycobacterium avium complex: a basis for pathogenicity. BMC Genomics 16:695. https://doi.org/10.1186/s12864-015-1889-2
95. Trost E, Ott L, Schneider J, et al (2010) The complete genome sequence of Corynebacterium pseudotuberculosis FRC41 isolated from a 12-year-old girl with necrotizing lymphadenitis reveals insights into gene-regulatory networks contributing to virulence. BMC Genomics 11:728. https://doi.org/10.1186/1471-2164-11-728
96. Verroken A, Bauraing C, Deplano A, et al (2014) Epidemiological investigation of a nosocomial outbreak of multidrug-resistant Corynebacterium striatum at one Belgian university hospital. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 20:44–50. https://doi.org/10.1111/1469-0691.12197

97. Waack S, Keller O, Asper R, et al (2006) Score-based prediction of genomic islands in prokaryotic genomes using hidden Markov models. BMC Bioinformatics 7:142. https://doi.org/10.1186/1471-2105-7-142
98. Wang J, Wang Y, Du X, et al (2016) Rapid transmission of multidrug-resistant Corynebacterium striatum among susceptible patients in a tertiary hospital in China. J Infect Dev Ctries 10:1299–1305. https://doi.org/10.3855/jidc.7577
99. Wang X, Zhou H, Chen D, et al (2019) Whole-Genome Sequencing Reveals a Prolonged and Persistent Intrahospital Transmission of Corynebacterium striatum, an Emerging Multidrug-Resistant Pathogen. J Clin Microbiol 57:e00683-19. https://doi.org/10.1128/JCM.00683-19
100. Wang X, Zhou H, Du P, et al (2021) Genomic epidemiology of Corynebacterium striatum from three regions of China: an emerging national nosocomial epidemic. J Hosp Infect 110:67–75. https://doi.org/10.1016/j.jhin.2020.10.005
101. Wattam AR, Davis JJ, Assaf R, et al (2017) Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res 45:D535–D542. https://doi.org/10.1093/nar/gkw1017
102. Wright GD, Thompson PR (1999) Aminoglycoside phosphotransferases: proteins, structure, and mechanism. Front Biosci 4:D9-21
103. Zhang M-J, Cao X-J, Fan J, et al (2020) Corynebacterium striatum meningitis combined with suspected brain and lung abscesses: a case report and review. BMC Infect Dis 20:389. https://doi.org/10.1186/s12879-020-05114-3
104. Zulauf KE, Sullivan JT, Braunstein M (2018) The SecA2 pathway of Mycobacterium tuberculosis exports effectors that work in concert to arrest phagosome and autophagosome maturation. PLoS Pathog 14:e1007011–e1007011. https://doi.org/10.1371/journal.ppat.1007011

Figures

Figure 1

Phylogenetic and phylogenomic analyses of *C. striatum. C. striatum* isolates: in orange, environmental isolate (South Korea); green, isolates from Hospital Universitário Pedro Ernesto (Rio de Janeiro – Brazil); yellow, reference strains (ATCC, NCTC and NBRC); black, strains with unknown isolation location; red, isolates from University of Washington Medical Center (Seattle – USA); pink, strain isolated at the University of Pennsylvania (Philadelphia – USA); brown, isolate from the University of Washington (Saint Louis – USA); blue, isolates from the Brigham and Women's Hospital (Boston – USA); purple, isolate from Hôpital Paris Saint-Joseph (Paris – France). **a** SplitTree of the MLSA alignment; **b** Minimum spanning tree based on cgMLST.

Figure 2

Pan-genome development analysis of *Corynebacterium striatum*. **a** There are number of gene families in the pan-genome (orange) and in the core genome (green). **b** The flower diagram demonstrates the dimensions of the core genome and accessory genes in gene numbers, and the contribution of genes per isolate to accessory genes and single genes.

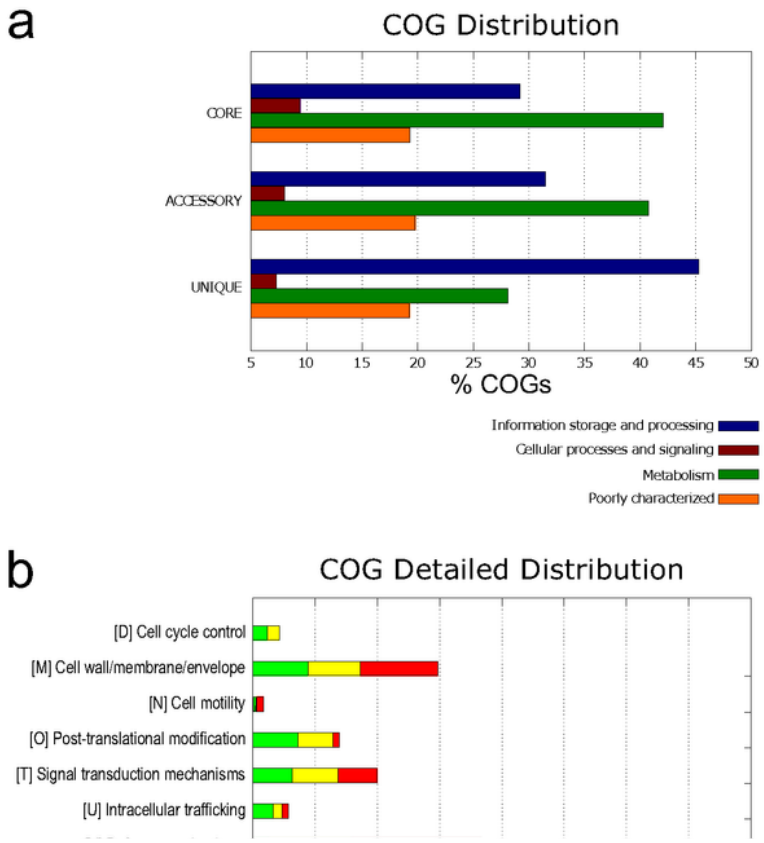

Figure 3

Classifications of the pan-genome's gene set based on COG categories. **a** Type of gene groups by general COG categories. **b** Type of gene groups by detailed COG categories.

Figure 4

Antimicrobial resistance genes. **a** Left panel: MLSA tree; right panel: prediction of antimicrobial resistance genes. **b** Antimicrobial susceptibility phenotype retrieved from the literature. The same color scheme is used for geographic coordinates, as in Fig. 1.

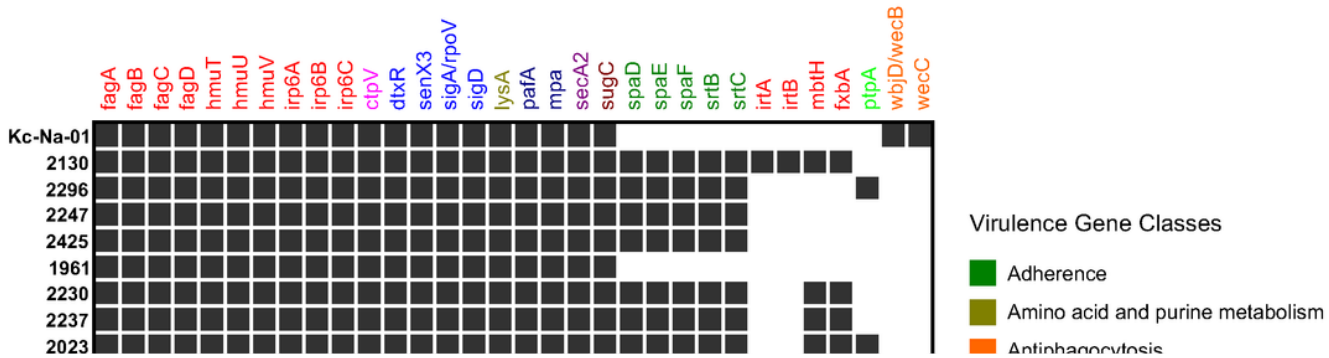

Figure 5

Predictions of virulence factors in *C. striatum*. The colored legend shows the different functional categories of the identified genes.

Figure 6

Circular plot view of the *C. striatum* genomic sequences. All genomes were aligned to a reference genome, and in the outermost circle were represented the Genomic Islands and Prophage sequences identified from the reference genome. Reference genomes: **a** ATCC6940; **b** 2023; **c** LK37.

Figure 7

Genomic context of AMR genes and mobile genetic elements. Shown in blue, are miscellaneous genes; in yellow are mobile genetic elements; in red are antimicrobial resistance genes; in gray are, hypothetical genes. **a** Genomic context of AMR genes from the *C. striatum* 2023 strain. **b** Genomic context of antimicrobial resistance genes from the *C. striatum* LK37 strain. **c** Prediction of prophage sequences in the genomic context of AMR genes in LK37.

Figure 8

Genomic context of virulence factors in *C. striatum* genomes. Shown in blue, miscellaneous genes; in yellow, mobile genetic elements; in green, virulence factor genes; in orange, virulence factor genes with frameshift mutations; in gray, hypothetical proteins. **a** Genomic context of SpaD-type pili in ATCC6940. **b** Demonstrates frameshift in spaD-like pili genes. **c** Genomic context of the KC-Na-01, 1961, CAUR_962, CAUR_963, CAUR_1329 and LK37 strains at the same locus of the ATCC6940 strain.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- HNRJesusetalApr2022FIGSupplementaryMaterial.docx