Occurrence of *Corynebacterium striatum* as an emerging antibiotic-resistant nosocomial pathogen in a Tunisian hospital

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*Corynebacterium striatum* is a nosocomial opportunistic pathogen increasingly associated with a wide range of human infections and is often resistant to several antibiotics. We investigated the susceptibility of 63 *C. striatum* isolated at the Farhat-Hached hospital, Sousse (Tunisia), during the period 2011–2014, to a panel of 16 compounds belonging to the main clinically relevant classes of antimicrobial agents. All strains were susceptible to vancomycin, linezolid, and daptomycin. Amikacin and gentamicin also showed good activity (MIC90 = 1 and 2 mg/L, respectively). High rates of resistance to penicillin (82.5%), clindamycin (79.4%), cefotaxime (60.3%), erythromycin (47.6%), ciprofloxacin (36.5%), moxifloxacin (34.9%), and rifampicin (25.4%) were observed. Fifty-nine (93.7%) out of the 63 isolates showed resistance to at least one compound and 31 (49.2%) were multidrug-resistant. Twenty-nine resistance profiles were distinguished among the 59 resistant *C. striatum*. Most of the strains resistant to fluoroquinolones showed a double mutation leading to an amino acid change in positions 87 and 91 in the quinolone resistance-determining region of the *gyrA* gene. The 52 strains resistant to penicillin were positive for the gene *bla*, encoding a class A β-lactamase. Twenty-two PFGE patterns were identified among the 63 *C. striatum*, indicating that some clones have spread within the hospital.

*Corynebacterium* species are widely distributed in the environment and in the microbiota of humans and animals. Medically relevant *Corynebacterium* species include *Corynebacterium diphtheriae*, the primary cause of diphtheria, and the non-diphtherial corynebacteria, which are part of the normal flora of the skin and mucous membranes. Nondiphtherial corynebacteria have been frequently dismissed as a contaminant when isolated from clinical materials. However, the role of these bacteria in clinical disease is now more clearly established. *Corynebacterium striatum* is recognized as a true pathogen when isolated in several samples from sterile body sites or from indwelling medical devices¹,². Consideration of whether an isolate represents infection, colonization, or contamination is based upon clinical assessment. A variety of infections have been associated with this bacterium: bacteremia, endocarditis, valvular damage, meningitis, vaginitis and infections of the urinary tract, the respiratory tract, wounds, skin and eye³–¹⁰.

Susceptibility testing of *C. striatum* is necessary to establish a specific therapy. Although initial studies indicated that *C. striatum* clinical isolates were susceptible to a wide range of antibiotics¹¹,¹², recent reports have shown increased multidrug resistance¹³,¹⁴. Patients suffering underlying diseases and receiving multiple antibiotic courses are at high risk for developing serious opportunistic infections by drug-resistant *C. striatum* strains, which can be at the origin of major outbreaks. Thus, several outbreaks of clonal multidrug-resistant *C. striatum* have been recently reported¹⁵–¹⁷.

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and cefotaxime (MICs90 ≤16 mg/L). Only three isolates showed resistance to both penicillin and cefotaxime (MICs90 ≤16 mg/L). Ciprofloxacin and moxifloxacin also showed low activity against our isolates (MICs90 ≤0.5 mg/L).

The compounds of the MLSB group were poorly active against C. striatum, considered as an emerging pathogen in different countries, has been the most frequently isolated Corynebacterium species since 2011 at the Farhat-Hached University (FHU) hospital, Sousse, Tunisia. In this study, we aimed to investigate the susceptibility of the 63 C. striatum isolated at the FHU hospital during the period 2011–2014 to 16 agents, representative of the main groups of antibiotics, including the most used compounds to treat infections caused by Corynebacterium spp. as well as second-line and complementary agents. We describe the high incidence of drug resistance in our C. striatum and characterize the molecular mechanisms related to resistance to aminoglycosides, compounds of the MLSB group, fluoroquinolones and β-lactams. The clonal relationships among C. striatum isolates were analysed by Pulsed-field Gel Electrophoresis (PFGE).

### Results

#### Antimicrobial susceptibility testing and resistance profiles.

Fifty-nine (93.7%) out of the 63 isolates showed resistance to at least one of the 16 tested compounds, whereas the remaining 4 isolates were susceptible. The MIC50 and MIC90 distributions and the percentage of resistance to the different antibiotics for the 63 C. striatum included in this study are presented in Table 1.

| Antimicrobial agent | Range (mg/L) | MIC50 | MIC90 | Breakpoint | S | I | R | Resistant | Intermediate | Total (%) R |
|---------------------|-------------|-------|-------|------------|---|---|---|-----------|--------------|-------------|
| Amikacin            | 0.06–64     | 0.06  | 1     | ≤16        | 32 | ≥64 | 3 | 0         | 3 (4.8%)     |             |
| Gentamicin          | 0.06–64     | 0.06  | 2     | ≤4         | 8  | ≥16 | 3 | 1         | 4 (6.3%)     |             |
| Kanamycin           | 0.016–256   | 0.125 | >64   | ≤16        | 32 | ≥64 | 10| 1         | 11 (17.5%)   |             |
| Tobramycin          | 0.06–64     | 0.06  | 8     | ≤4         | 8  | ≥16 | 3 | 4         | 7 (11.1%)    |             |
| Streptomycin        | 0.125–64    | 2     | >64   | ≤8         | >16| 8   | 2 | 2         | 10 (15.9%)   |             |
| Erythromycin        | 0.06–64     | 0.5   | 8     | ≤0.5      | 1  | ≥2  | 24| 6         | 30 (47.6%)   |             |
| Clindamycin         | 0.06–64     | 1     | >64   | ≤0.5      | 2  | ≥4  | 24| 26        | 50 (79.4%)   |             |
| Doxycycline         | 0.06–16     | 0.06  | 8     | ≤4         | 8  | ≥16 | 0 | 11        | 11 (17.5%)   |             |
| Ciprofloxacin       | 0.015–16    | 0.125 | >16   | ≤1        | 2  | ≥4  | 21| 2         | 23 (36.5%)   |             |
| Moxifloxacin        | 0.06–16     | 0.06  | >16   | ≤16       | ≤1| ≥2  | 15| 7         | 22 (34.9%)   |             |
| Penicillin          | 0.06–64     | 1     | 16    | ≤0.125    | 1  | ≥2  | 52| 0         | 52 (82.5%)   |             |
| Cefotaxime          | 0.25–256    | 2     | 16    | ≤1        | 2  | ≥4  | 28| 10        | 38 (60.3%)   |             |
| Rifampicin          | 0.015–64    | 0.015 | 16    | ≤1        | 2  | ≥4  | 14| 2         | 16 (25.4%)   |             |
| Vancomycin          | 0.06–1      | 0.25  | ≤0.25 | ≤2        | 0  | 0   | 0 | 0         | 0             |             |
| Linezolid           | 0.015–1     | 0.25  | 0.5   | ≤4        | ≥8 | 0   | 0 | 0         | 0             |             |
| Daptomycin          | 0.015–1     | 0.125 | 0.25  | ≤1        | 0  | 0   | 0 | 0         | 0             |             |

Table 1. Susceptibility of 63 Corynebacterium striatum clinical isolates to 16 antimicrobial agents. Isolates were classified as resistant, intermediate, or susceptible, according to criteria defined by CLSI38. MIC, minimum inhibitory concentration; MIC50/90, MIC that inhibits 50% and 90% of the isolates, respectively.

The MIC90 values of vancomycin, daptomycin and linezolid were in the range 0.25–0.5 mg/L, and none of the 63 isolates were resistant to them. Considering the MIC90 values, the most active compounds were vancomycin and daptomycin (MIC90 = 0.25 mg/L for both compounds) followed by linezolid (MIC90 = 0.5 mg/L). Among the 5 aminoglycosides tested, amikacin and gentamicin were the most active compounds (MIC90 values of 1 and 2 mg/L, respectively). Only three C. striatum were resistant to both compounds (MICs > 64 mg/L). Tobramycin was also very active (56 isolates were susceptible). Kanamycin and streptomycin showed low activity against our C. striatum (MIC90 > 64 mg/L). The compounds of the MLSB group were poorly active against our C. striatum (MIC90 of 8 and >64 mg/L for erythromycin and clindamycin, respectively). Rifampicin was poorly active too (MIC90 = 16 mg/L). Ciprofloxacin and moxifloxacin also showed low activity against our isolates (MIC90 > 16 mg/L for both compounds). A high number of the isolates were resistant to the β-lactams penicillin and cefotaxime (MIC90 = 16 mg/L).

The 59 C. striatum resistant to at least one of the tested compounds were divided in 29 resistance profiles (Fig. 1). The most frequently encountered resistance phenotype was penicillin (12 strains), followed by penicillin-cefotaxime and erythromycin-clindamycin-penicillin (6 strains each). Multidrug resistance (MDR), defined as non-susceptibility to at least one agent in three or more antimicrobial categories (as defined for other microorganisms39), was observed in 31 (49.2%) strains. Among the 31 MDR isolates, 19 resistance profiles could be distinguished. Eight MDR isolates showed resistance to eight or more compounds, belonging to seven main classes of antibiotics (aminoglycosides, ansamycins, macrolides, lincosamides, fluoroquinolones, penicillins and cephalosporins).

**Molecular detection of resistance genes.** The *aph(3′)-Ic* gene was detected in the 10 strains resistant to kanamycin (MIC > 64 mg/L). However, fourteen strains susceptible to kanamycin were also *aph(3′)-Ic*-positive. The genes *aph(3′)-Ib* and *aph(6)-Id* were detected in 8 isolates, five of them resistant to streptomycin (MICs > 64 mg/L), and the other three showed MICs of streptomycin in the range 1–4 mg/L. The gene *aac(3′)-XI* was found in 7 isolates. Two of them were susceptible to both gentamicin and tobramycin (MICs < 0.06 mg/L) whereas the other five isolates showed MICs of 8 and 16 mg/L for gentamicin and tobramycin, respectively.
Twenty out of the 24 *C. striatum* resistant to erythromycin carried the *erm(X)* gene. Ten out of 20 carried *erm(X)* plus the *erm(B)* gene. From the 24 clindamycin-resistant isolates 22 were positive for the gene *erm(X)* and 10 out of 22 were also *erm(B)*-positive. The *mef(A-E)* gene was not found in any strain tested.

The sequences of the QRDR region of the *gyrA* gene of 21 isolates categorized as resistant or intermediate to fluoroquinolones were compared to that of the quinolone-susceptible *C. striatum* ATCC 6940 (GenBank accession number AY559038). The relationships between the MICs of ciprofloxacin and moxifloxacin and the mutations in the *gyrA* QRDRs of the 21 isolates are summarized in Table 2. Eleven strains showing MICs = 16 mg/L for these two compounds carried a double mutation at resistance hotspots Ser-87 and Asp-91 (*C. striatum* numbering), generating a change from Ser-87 to Phe and another change from Asp-91 to Gly or Ala. In two double

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**Figure 1.** PFGE patterns and antibiotic resistance profiles of the 63 *C. striatum*. An: amikacin; Gm: gentamicin; Km: kanamycin; Sm: streptomycin; Tob: tobramycin; Rif: rifampicin; Eri: erythromycin; Clin: clindamycin; Cip: ciprofloxacin; Mox: moxifloxacin; Pen: penicillin; Ctx: cefotaxime.
In 11 MDR strains, six of which were isolated in the neonatology ward. The remaining 20 MDR clinical isolates coexist simultaneously in the same cell. Enzymatic inactivation of the antibiotic molecule is the most prevalent in its side effects during the long courses of treatment required for hardware or device-associated infections must be considered as an alternative to vancomycin for treatment of high-level daptomycin resistance has been recently reported25, 26. 

were susceptible to vancomycin. More recently, Gomila et al.20 reported that their 52 isolates showing resistance to penicillin were positive for the gene bla, encoding a class A β-lactamase. The C. striatum bla gene encodes a serine hydrolase belonging to the class A β-lactamase family protein. Forty-six out of the 63 C. striatum were positive for the ampC gene, encoding a class C β-lactamase. Forty-two of the 46 ampC-positive isolates were penicillin-resistant whereas 4 isolates were sensitive. The ampC gene was detected in 22 of the 28 cefotaxime-resistant C. striatum. The 52 penicillin-resistant isolates had MICs of cefotaxime in the range 1–256 mg/L.

Discussion

One of the most serious problems related to treatment of the infections caused by C. striatum is the isolation of multidrug-resistant strains from clinical material and the selection of the appropriate antibiotic therapy for a given type of infection. C. striatum infections should be treated according to the results of the susceptibility tests. Multiple drug resistance is caused by the interplay of multiple resistance mechanisms that emerge via the acquisition of extraneous resistance determinants or spontaneous mutations. This work highlights the high prevalence of multi-resistant strains and resistance genes among the C. striatum isolated in a hospital in Tunisia, in particular resistance to aminoglycosides, compounds of the MLSβ group, fluoroquinolones, and β-lactams.

The 63 C. striatum analysed in this study were susceptible to vancomycin and linezolid. In an earlier report using the disk diffusion method, Martinez et al.35 showed that 31 C. striatum isolated from clinical samples were susceptible to vancomycin. More recently, Gomila et al.26 reported that their 52 C. striatum isolated from patients with chronic obstructive respiratory disease were susceptible to vancomycin too. Vancomycin is still active today and therefore it represents an adequate option for treatment of severe infections caused by C. striatum. Linezolid has also shown an excellent activity, with MICs routinely below 0.5 mg/L25. Gómez-Garcés et al.36 showed that vancomycin and linezolid were equally active against 30 clinical C. striatum (MIC₉₀ of both compounds = 0.5 mg/L). Linezolid can be considered as an alternative to vancomycin against C. striatum, although its side effects during the long courses of treatment required for hardware or device-associated infections must be pondered. All our C. striatum were susceptible to daptomycin (MIC₉₀ = 0.25 mg/L). Daptomycin has also been proven to be active against C. striatum, alone23 or combined with rifampicin24. Therefore, daptomycin can also be considered as an alternative to vancomycin for treatment of C. striatum infections, although rapid emergence of high-level daptomycin resistance has been recently reported25, 26.

Aminoglycosides are used as complementary antibiotics to treat serious infections caused by diphteroids. Among aminoglycosides, amikacin and gentamicin showed good activity “in vitro” against our C. striatum (MIC₉₀ = 1 and 2 mg/L, respectively). Aminoglycoside resistance occurs through several mechanisms that can coexist simultaneously in the same cell. Enzymatic inactivation of the antibiotic molecule is the most prevalent in

| Number of strains | Ciprofloxacin | Moxifloxacin |
|-------------------|--------------|--------------|
|                   | MIC (mg/L)   | Phenotype    | MIC (mg/L) | Phenotype |
| 8                 | >16          | R            | >16        | R         |
| 3                 | >16          | R            | >16        | R         |
| 1                 | >16          | R            | 8          | R         |
| 1                 | >16          | R            | 8          | R         |
| 1                 | >16          | R            | 4          | R         |
| 1                 | 8            | R            | 1          | I         |
| 1                 | 4            | R            | 1          | I         |
| 2                 | 2            | I            | 1          | I         |
| 1                 | 8            | R            | 1          | I         |
| 1                 | 4            | R            | 1          | I         |

Table 2. Relationship between mutations in the QRDR regions of the gyrA gene and the MICs for 21 C. striatum classified as resistant or intermediate to fluoroquinolones.
the clinical setting. The aac(3)-XI gene, encoding an aminoglycoside 3-N acetyl transferase conferring resistance to gentamicin and tobramycin in C. striatum, was not found in our 3 gentamicin-resistant isolates, suggesting that resistance is mediated by another mechanism. Kanamycin and streptomycin are not used in clinical practice in Tunisia but were tested in this study because they are good markers for detecting the presence of the aac(6)-Id gene. Eight strains carried the aac(6)-Id gene, encoding an aminoglycoside 6-O-phosphotransferase implicated in resistance to kanamycin, neomycin, paromomycin, ribostamycin and lividomycin, is part of a larger DNA region containing the aph(3′)-Ib-aph(6)-Id tandem pair of resistance genes conferring streptomycin resistance in Corynebacterium spp. Identical aminoglycoside resistance regions were found in the plasmid pTP10 from C. striatum in close vicinity to the erythromycin and chloramphenicol resistance regions. As expected, the 10 C. striatum resistant to kanamycin carried the aph(3′)-Ic gene. However, this gene was also detected in strains susceptible to kanamycin, probably due to mutations affecting its coding sequence or its promoter. These results confirm that the aph(3′)-Ic gene is widespread in Corynebacterium spp. Resistance to streptomycin in Corynebacterium spp. is related to the presence of the tandem of genes aph(3′)-Ib and aph(6)-Id, encoding for aminoglycoside-3′-phosphotransferase [APH (3′)-Ib] and aminoglycoside-6′-phosphotransferase [APH (6)-Id], respectively. Five of the 8 isolates resistant to streptomycin carried the aph(3′)-Ib and aph(6)-Id genes, and the remaining three had MICs for streptomycin in the range of 1 to 4 mg/L, probably as a consequence of mutations in the above mentioned genes or in their promoter. Other mechanisms such as active efflux of the antimicrobial and reduced intake into the bacterial cell can contribute to streptomycin resistance in these isolates. Amikacin is eventually prescribed in combinative therapy against severe infections caused by C. striatum at the FHU hospital. Our results indicate that amikacin is the preferable aminoglycoside for treatment of C. striatum infections whereas gentamicin could be a valid alternative. However, the occurrence of resistant strains requires continual vigilance.

Erythromycin and clindamycin were inactive against the majority of our C. striatum, in particular clindamycin, with a MIC of eight times greater than that of erythromycin. This fact confirms the previously reported high prevalence of resistance to compounds of the MLS2 group among Corynebacterium spp., including C. striatum. MLS resistance in Corynebacterium spp. is most often mediated by two mechanisms: target-site modification mediated by ribosomal RNA methylases codified by the so-called erm genes and active drug-efflux mediated by a membrane efflux pump encoded by the mef(A-E) gene. Our results confirmed those of previous studies which pointed out that erm(X) is the most important gene implicated in MLS resistance in Corynebacterium spp. For the first time we have detected the erm(B) gene encoding the ribosomal RNA methylase Erm(B) in C. striatum. The gene erm(B) confers high-level resistance to macrolides in Campylobacter coli and other relevant pathogens but is exceptional in Corynebacterium spp. Ten of our strains carried the erm(B) and erm(X) genes simultaneously, a characteristic previously reported only in one strain of C. urealyticum.

One third of our C. striatum showed intermediate or high-level resistance to ciprofloxacin and moxifloxacin. Fluoroquinolones have been extensively used at the FHU hospital during the last two decades. Upon antibiotic administration, a selective pressure is created in body organs where fluoroquinolones tend to accumulate. Exposure to fluoroquinolones selects for spontaneous mutants in large bacterial populations, including those that colonize the skin and mucous membranes such as corynebacteria. Thus, fluoroquinolone resistance emerged in clinical isolates of C. striatum and C. amycolatum. Resistance to fluoroquinolones in Corynebacterium spp. is caused by mutations in the QRDR of the gyrA gene. In our strains, single amino-acid substitutions in position 87 of the GyRA protein generated ciprofloxacin resistance but double mutations in the gyrA gene leading to changes in positions 87 and 91 were necessary for high level resistance to ciprofloxacin and moxifloxacin. In four of the 21 fluoroquinolone-resistant C. striatum, increases of MICs of ciprofloxacin and moxifloxacin until 16 mg/L were related to a double non-conservative mutation at positions 87 and 91. Sierra et al. reported double mutations at positions 87 and 91 in the gyrA gene of six of their C. striatum, although the MICs of moxifloxacin for their strains were lower (6–8 mg/L). Five of our strains with single mutations at positions 87 or 91 are still resistant to ciprofloxacin although with lower MICs (in the range 2–8 mg/L) whereas the MICs of moxifloxacin remained at 1 mg/L. Single mutations in the residue Ser-87 or in the residue Asp-91 described by Sierra et al. increased the MICs of ciprofloxacin to 1–6 mg/L, whereas remaining susceptible to moxifloxacin. The higher level of moxifloxacin resistance in our strains suggest the existence of a resistance mechanism additionally to mutations in gyrA. In two strains no changes in their QRDRs were detected, indicating that resistance was mediated by a different mechanism.

β-lactams are the most broadly used class of antimicrobials. Successful treatments of C. striatum infections with penicillin or amoxicillin have been reported. However, low susceptibility to penicillin and cefotaxime among other β-lactams have been communicated, although the genetic mechanism of resistance has not been characterized so far. Considering the MICs of values (16 mg/L), penicillin and cefotaxime showed the same low activity against our C. striatum. The fact that the rate of penicillin-resistant strains is higher than that of cefotaxime-resistant strains is explained because the CLSI susceptibility breakpoint for penicillin has recently been dropped from 1 mg/L to 0.125 mg/L. Hydrolysis of β-lactam antibiotics by β-lactamases is the most common mechanism of resistance for this class of antibacterial agents in clinically important bacteria. The β-lactamases are classified by protein sequence in four molecular classes, A, B, C, and D, based on conserved and distinguishing amino acid motifs. Fifty-two of our strains were resistant to penicillin and this resistance was related to the presence of a bla gene encoding a class A β-lactamase. The chromosones of Corynebacterium jeikeium K411, Corynebacterium urealyticum DSM 7109, and Corynebacterium resistent DSM 45100 encode the corresponding counterparts of the C. striatum bla gene, although it has not been associated with resistance to β-lactams in these species. The ampC gene, encoding a class C β-lactamase, was detected in 42 of the 52 penicillin-resistant C. striatum. The ampC genes, which are widely distributed among the Enterobacteriaceae, encode enzymes active on both penicillins and cephalosporins. We show here that two β-lactamase-encoding genes, bla and ampC, are present in β-lactam-resistant C. striatum. Our data revealed high resistance rates to β-lactams and a high
prevalence of the bla and the ampC genes among the C. striatum isolated in our hospital. These data are of value to practitioners, discouraging the use of β-lactam compounds for the treatment of infections caused by C. striatum.

PFGE is considered the gold standard in epidemiological studies of pathogenic microorganisms, providing important insights into their population structure49. Our results showed 22 distinct PFGE patterns from 63 C. striatum strains. The high diversity of genotypes among the 63 C. striatum revealed that they are mainly not closely related. Therefore, C. striatum at the FHU hospital may originate from different lineages and sources instead of expansion of a single clonal lineage. This corresponds to the pathogenic condition of C. striatum as an opportunistic pathogen that causes occasional disease in predisposed patients. Some PFGE patterns were more frequently isolated, suggesting the existence of a few more prevalent clones. We consider patterns E and A as closely related. Therefore, surveillance of MDR C. striatum should be continued.

In conclusion, this study highlights the relevance of C. striatum as an emerging multidrug-resistant nosocomial pathogen at the FHU hospital. The C. striatum isolates showed 100% susceptibility to vancomycin, linezolid and daptomycin and high rates of resistance to rifampicin, compounds of the MLSB group, fluoroquinolones and β-lactams. Among the several clones of C. striatum circulating at the FHU hospital, the most prevalent were the most resistant. Therefore, surveillance of MDR C. striatum should be continued.

Methods

Bacterial strains and growth conditions. During the period 2011–2014, 90 strains recovered from clinical specimens submitted for routine culture to the microbiology laboratory of the FHU hospital were assigned to the genus Corynebacterium on the basis of colony morphology, Gram staining, and catalase production. They were isolated in pure culture except the 7 specimens from vaginal swabs, where the Corynebacterium were the predominant microorganisms in a poly-microbial culture. In that cases the Corynebacterium were considered of clinical significance since they were associated with a strong leukocyte reaction in Gram staining45. In all cases the Corynebacterium were isolated after two different culture sets. Sixty-three out of the 90 strains were identified as putative C. striatum using API Coryne V2.0 strips (bioMérieux, Marcy l’Etoile, France). C. striatum was differentiated from C. amycolatum by additional phenotypic tests (tyrosine hydrolysis, N-acetylglucosamine assimilation, phenylacetic acid assimilation and susceptibility to the vibriostatic agent O/129). Identification was confirmed by MALDI-TOF using the Vitel MS (bioMérieux) system, in accordance with manufacturer’s instructions. The anatomic sites of specimens from whom the 63 C. striatum were isolated and the clinical diagnosis for the infected patients are shown in Table 3. All strains were grown on blood agar plates at 37 °C and kept frozen at −80 °C in Brain Heart Infusion broth with 20% glycerol until use.

Antimicrobial susceptibility assays. Antimicrobial susceptibilities were determined by micro-dilution in cation adjusted Müller-Hinton broth and interpreted following Clinical and Laboratory Standards Institute (CLSI) guidelines49. Sixteen antimicrobials were tested: amikacin, gentamicin, kanamycin, streptomycin, tobramycin, rifampicin, erythromycin, clindamycin, doxycycline, ciprofloxacin, penicillin, cefotaxime, vancomycin (all of them purchased from Sigma Aldrich, Madrid, Spain), moxifloxacin (Discovery Fine Chemicals, Dorset, United Kingdom), linezolid (Pfizer, Bilbao, Spain) and daptomycin (Cubist, Madrid, Spain). Daptomycin broth was supplemented to 50 mg/L calcium for determinations of susceptibility to that drug. Of note, CLSI susceptible interpretive criteria for penicillin has been recently dropped from 1 mg/l to 0.125 mg/L39. MIC values of the antibiotics not considered in CLSI guidelines for Corynebacterium spp. (amikacin, tobramycin, kanamycin, moxifloxacin and linezolid) were interpreted in accordance to criteria defined by CLSI for Staphylococcus aureus49. Since the CLSI lacks breakpoints of streptomycin for staphylococci we have considered the MIC breakpoints values proposed by the French Society of Microbiology (http://www.sfmmicrobiologie.org/UserFiles/files/casfm/CASFM2013vjuin.pdf) to classify the C. striatum as susceptible, intermediate or resistant to streptomycin.

Table 3. Sources of specimens from whom C. striatum were isolated and clinical diagnosis for the 63 infected patients. CVC: Central Venous Catheter; IU: Intrauterine.
Escherichia coli ATCC 25922 and Streptococcus pneumoniae ATCC49619 were used as control strains for susceptibility testing assays.

**Amplification and sequencing of genes related to resistance.** The presence of aminoglycoside modifying enzyme (AME) genes common in Corynebacterium spp. [aph(3′)-Ic, aac(3)-XI], and the tandem of genes aph(3′)-Ib and aph(6)-Id] was investigated by PCR. Resistance to compounds of the MLS group was investigated by amplification of the erm(X), erm(B) and mef(A-E) genes. Resistance to quinolones in Corynebacterium spp. is related to point mutations in the sequence of the quinolone resistance-determining region (QRDR) of the gyrA gene. Thereby, the QRDR at that gene was amplified and sequenced as previously described. The 63 C. striatum were analysed for the presence of the bla gene, encoding a class A β-lactamase involved in resistance to penicillins and cephalosporins in Corynebacterium spp. Primers used to amplify the above mentioned genes are listed in Supplementary Table S1. PCR reactions were performed as previously described. PCR products were purified using the QiAquick PCR Purification kit (Qiagen, Madrid, Spain). Purified DNA was sequenced by Macrogen (Seoul, Korea) with the primers outlined in Table S1. Mutations in gyrA were identified by aligning sequences of resistant isolates to the sequence of C. striatum ATCC6940 (GenBank accession number A559038) using the Clustal W program.

**Pulsed-field Gel Electrophoresis and dendrogram analysis.** We obtained XbaI macro-restriction patterns of the 63 C. striatum with a published protocol and a CHEF-DR31 variable angle system (Bio-Rad, Hercules, California, USA). The PFGE patterns were analysed with Fingerprinting II v4.5 software (Bio-Rad). Each isolate was compared with all other isolates using the Dice similarity coefficient and the unweighted pair Group method with arithmetic means (UPGMA), with 1% of optimization and tolerance. Isolates were classified as indistinguishable if they showed 100% similarity, as closely related subtypes if they showed 95–99% similarity, and as different strains if they showed <95% similarity.

**Ethics statement.** This study was performed in accordance with the ethical guidelines of the Declaration of Helsinki (1975). Written informed consent was obtained from each patient from whom samples were taken.

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Acknowledgements
This work was supported by Plan Nacional de I + D + i 2013–2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía, Industria y Competitividad, Spanish Network for Research in Infectious Diseases (REIPI RD12/0015) - co-financed by European Development Regional Fund “A way to achieve Europe” Operative program Intelligent Growth 2014–2020, and grants from the Farhat-Hached University Hospital and the University of Carthage (Tunisia).

Author Contributions
S.A., J.B., L.M.M. and J.N. conceived and designed the study, S.A. and A.F. collected and identified the clinical isolates, S.A., M.E.M., M.E.C. and J.N. conducted the experiments, S.A., M.F.M., J.N. and M.E.C. analysed the data, S.A., L.M.M. and J.N. drafted the manuscript. All authors reviewed and approved the manuscript.

Additional Information
Supplementary information accompanies this paper at doi:10.1038/s41598-017-10081-y

Competing Interests: The authors declare that they have no competing interests.

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