

Original Article

Rapid transmission of multidrug-resistant *Corynebacterium striatum* among susceptible patients in a tertiary hospital in China

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Abstract

Introduction: With increasing reports about *Corynebacterium striatum*, its potential pathogenicity and clinical significance are drawing more attention in recent years.

Methodology: During a 14-month period, *Corynebacterium striatum* strains were routinely isolated in lower respiratory tract samples of the inpatients in a tertiary hospital in China, and the suspected isolates were identified with VITEK-2 ANC card and 16S rRNA sequencing technique, respectively. Pulsed-field gel electrophoresis (PFGE) was employed to discriminate different clones, and biofilm-producing abilities of different strains were compared.

Results: A total of 82 strains of *Corynebacterium striatum* were mainly isolated from neurosurgery patients (45.1%, 37/82). Three epidemic clones (type D, F, and I) were identified, accounting for 82.9% (68/82) of strains. All 82 *C. striatum* strains were all sensitive to vancomycin, and resistant to ceftriaxone, imipenem, and ciprofloxacin. One week before *C. striatum* isolation, 89.0% (73/82) patients showed lower levels of hemoglobin, 93.9% (77/82) of patients received treatment of several kinds of antibiotics, and 41.5% (34/82) patients with glucocorticoid, and 46.3% (38/82) of patients showed disturbed consciousness at different levels.

Conclusions: *Corynebacterium striatum* strains isolated during this study were mainly multidrug resistant, and the predominant clones could rapidly transmit among susceptible inpatients. *Corynebacterium striatum* seems to be an important pathogen for patients with specific risk factors, especially for the ones with lower hemoglobin levels who are being treated with broad-spectrum antibiotics.

Key words: *Corynebacterium striatum*; nosocomial transmission; multidrug resistance; risk factors.

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Introduction

Corynebacterium striatum (*C. striatum*) is a kind of Gram-positive bacteria, deemed to be a normal microbiota and widely distributed on skin and mucous surfaces [1]. In recent years, *C. striatum* has been found to be a potentially important bacterial pathogen for humans, since it has been isolated from several kinds of sterile samples, including cerebral fluid, arthroedema, and others [2-6]. However, most of the *C. striatum* strains reported were isolated from the lower respiratory tracts of patients with chronic obstructive pulmonary diseases as well as other specific risk factors [7-9]. The other well-known risk factors include a longer hospitalization period and previous use of beta-lactam antibiotics and invasive devices for diagnosis and treatment [7-9]. Furthermore, most of the *C. striatum* strains reported show multidrug-resistant phenotypes [6-9]. Moreover, multidrug-resistant *C.*

striatum can lead to nosocomial transmission and outbreaks among inpatients with specific risk factors and has been confirmed to be positively correlated with increasing mortality among these patients [8]. All the studies referenced above demonstrated that *C. striatum* deserves more attention for specific groups of patients. In the current study, we aimed to further investigate molecular and epidemiological features and clinical significance of multidrug-resistant *C. striatum*, and to further provide more data for better understanding its potential pathogenicity.

Methodology

Patient enrollment

The patients enrolled in this study were mainly hospitalized in the neurosurgery department, intensive care unit (ICU), and respiratory medicine department. The infection states of these patients were evaluated

based on the Centers for Disease Control and Prevention (CDC)'s criteria for lower respiratory tract infection [10]. To accurately verify *C. striatum* acquisition or infection, microscopy examination was employed to evaluate the quality of the lower respiratory tract samples from these patients and to examine the existence of intracellular phagocytosis of Gram-positive bacilli. Only the patients whose lower respiratory tract samples showed the following features were included in this study: < 10 epithelial cells per low power field (LPF), more than 25 white blood cells (WBC)/LPF, and intracellular Gram-positive bacilli. The medical records of the enrolled patients were reviewed to explore the putative risk factors for *C. striatum* acquisition or infection. This study was conducted at the Affiliated Hospital of Inner Mongolia Medical University, a 1,800-bed general tertiary care hospital in Inner Mongolia, China.

Collection of lower respiratory tract samples

The lower respiratory tract samples were collected by a sterile mucus extractor or under a fiber bronchoscope. The fiber bronchoscope technique was used on the patients who showed serious clinical and imaging manifestations of chest infection. For throat and tracheal surface samples, local anesthesia was administered using 2% lidocaine with an ultrasonic nebulizer. Next, the fiber bronchoscope was inserted into the trachea, bronchus, or deeper through the nasopharynx, to collect suspected samples. For the other patients, sterile normal saline was used to clean their mouths for about three minutes before sample collection. Next, a sterile mucus extractor was inserted to suction sputum through the nasopharynx or oropharynx. For the patients who produced small volumes of sputum, the induced sputum collection technique was employed. Generally, 5 mL of 3% to 5% hypertonic saline solution was administered by ultrasonic nebulizer for up to 10 minutes, and then a sterile mucus extractor was inserted to suction sputum through the nasopharynx or oropharynx.

C. striatum isolation and identification

From November 2013 to December 2014, all the expectorated sputum samples were analysed by microscopy and microbiology techniques in order to ascertain that they were effectively defined as sputum samples and therefore providing true picture of lower respiratory tract [11]. Furthermore, all the cultures suspected to be *C. striatum* were routinely identified with a VITEK-2 ANC card (BioMerieux, Lyon, France) and a 16S-rRNA sequencing technique. Only one *C.*

striatum strain from the same patient was analyzed in this study; repeated isolates from the same patient were excluded.

In vitro drug susceptibility assay

For all the *C. striatum* strains, in vitro susceptibility to the following antibiotics was tested using the broth microdilution method: ceftriaxone, imipenem, erythromycin, clindamycin, gentamycin, tetracycline, vancomycin, ciprofloxacin, sulfamethoxazole, and rifampin (Meilune, Dalian, China). The results were assessed according to the Clinical and Laboratory Standards Institute guidelines [12].

16S-rRNA sequencing

The *C. striatum* strains were routinely cultured on blood agar plates (Tianjin Jinzhang Science and Technology Development Co., Ltd., Tianjin, China) for 48 hours at 37°C. The cultures were collected, *C. striatum* DNA was extracted using a bacterial DNA extraction kit (Qiagen, Hilden, Germany), and amplification of 16S-rRNA gene was performed as previously described [13]. The amplification products were sequenced and the obtained sequences were compared with that of *C. striatum* ATCC6940.

Pulsed-field gel electrophoresis (PFGE)

The same *C. striatum* DNA was extracted as that for 16S-rRNA sequencing.

Macrorestriction (XbaI) patterns were analyzed using Dice coefficient with

Bionumerics software, version 5.0 (Applied Maths, Kortrijk, Belgium).

The classification criteria for PFGE analysis were subjectively designated, and the clones with similarity indexes $\geq 95\%$ were classified as a single type and named with a capital letter.

Biofilm production assay

Fibronectin were pre-coated in sterile 96-well sterile plates as described previously [14]. Briefly, 100 μ L fibronectin (Sigma-Aldrich, St. Louis, USA) at 0.1 mg/mL concentration was added to each well overnight, while 20 mg/mL bovine serum albumin (BSA) was added to three wells as blank control wells. The wells were washed three times with 125 μ L BSA in phosphate-buffered saline (PBS), and blocked for one hour at room temperature.

Before adding bacteria solution, each well was washed three times with PBS. Aliquots of 100 μ L of bacterial suspensions (2 McFarland) were added to each

well. The plates were then incubated at 37°C for 24 hours, and the contents of each well were aspirated slowly and washed three times with 150 µL of PBS. Next, 150 µL of methanol (99%) was added to each well to fix the attached bacteria, and subsequently the bacteria were stained with 1% crystal violet for 1 minute, and then the unbound crystal violet was washed with PBS. Next, 150 µL of glacial acetic acid (33%) was added to each well to dissolve the bound crystal violet, and the optical densities (ODs) of the solution were measured at λ = 570 nm using an enzyme immunosorbent assay reader (Thermo Fisher Scientific, Cleveland, USA). Only trypticase soy broth (TSB) was added to the blank control wells.

Results

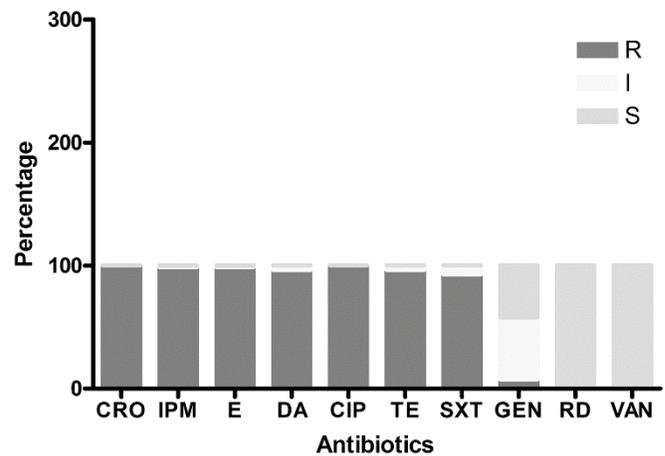
Basic information about the enrolled patients

The average hospitalization period and average age of the 82 patients were 20 days and 61 years, respectively, and the male and female patients accounted for 78.0% (64/82) and 22.0 (18/82), respectively. One week before *C. striatum* isolation, 93.9% (77/82) of patients received anti-infection treatment with different kinds of antibiotics; the most frequently prescribed antibiotics were cephalosporins, carbapenems, and quinolones. Hypohemoglobinemia was observed in 89.0% (73/82) of patients during this study, 17.8% (13/73) of whom died during their hospitalization. Furthermore, 46.3% (38/82) of patients had disturbed consciousness one week before *C. striatum* isolation, 52.6% (20/38) of whom were in a deep coma. Moreover, 41.5% (34/82) of patients were prescribed glucocorticoid one week before *C. striatum* isolation. For invasive medical devices, 36.6% (30/82) patients received endotracheal intubation or tracheotomy before *C. striatum* isolation. The other potential risk factors observed among these 82 cases are shown in Table 1.

Isolates identification and antibiotic susceptibility testing

All the strains were identified to be *C. striatum* by VITEK-2 ANC card, with a confidence level between 93% and 97%. Compared with *C. striatum* ATCC6940, the similarities of the 82 *C. striatum* strains were all ≥ 99.5%. *In vitro* antibiotic susceptibility testing showed that all the strains were sensitive to vancomycin but resistant to ceftriaxone, imipenem, and ciprofloxacin. The resistance of the 82 *C. striatum* strains to the other antibiotics showed a diverse feature (Figure 1).

Figure 1. Resistance rates of the 82 *C.striatum* strains to 10 antibiotics.



S: Sensitive; I: Intermediate; R: Resistant; CRO: ceftriaxone; IMP: imipenem; E: erythromycin; DA: clindamycin; CIP: ciprofloxacin; TE: tetracycline; SXT: sulfamethoxazole; GEN: gentamycin; RD: rifampin; VAN: vancomycin.

Table 1. Resistance pattern of 82 *C. striatum* strains to 10 antibiotics

| Resistance pattern (n) | Antibiotics | | | | | | | | | |
|------------------------|-------------|-----|---|----|-----|----|-----|-----|----|-----|
| | CRO | IPM | E | DA | CIP | TE | SXT | GEN | RD | VAN |
| I (41) | R | R | R | R | R | R | R | R/I | S | S |
| II (32) | R | R | R | R | R | R | R | S | S | S |
| III (1) | R | R | R | R | R | R | R | R | R | S |
| IV (1) | R | R | R | R | R | R | R | S | R | S |
| V (2) | R | R | R | R | R | R | S | I | S | S |
| VI (1) | R | R | R | R | R | S | R | R | S | S |
| VII (1) | R | R | R | R | R | S | S | I | S | S |
| VIII (1) | R | R | R | S | R | R | S | I | S | S |
| IX (1) | R | R | R | S | R | R | S | S | S | S |
| X (1) | R | I | S | S | R | S | S | S | S | S |

S: sensitive; I: intermediate; R: resistant; CRO: ceftriaxone; IMP: imipenem; E: erythromycin; DA: clindamycin; CIP: ciprofloxacin; TE: tetracycline; SXT: sulfamethoxazole; GEN: gentamycin; RD: rifampin; VAN: vancomycin.

Based on the resistance features, 82 *C. striatum* strains can be classified into 10 patterns, designated as I, II, III, IV, V, VI, VII, VIII, IX, and X. Among these patterns, pattern I and II were the predominant ones and showed multidrug resistance features. Only 1 strain (pattern X) was resistant to ceftriaxone and ciprofloxacin and had intermediate resistance to imipenem; this strain belonged to PFGE type M. The resistance features of the other patterns varied significantly, as shown in Table 2.

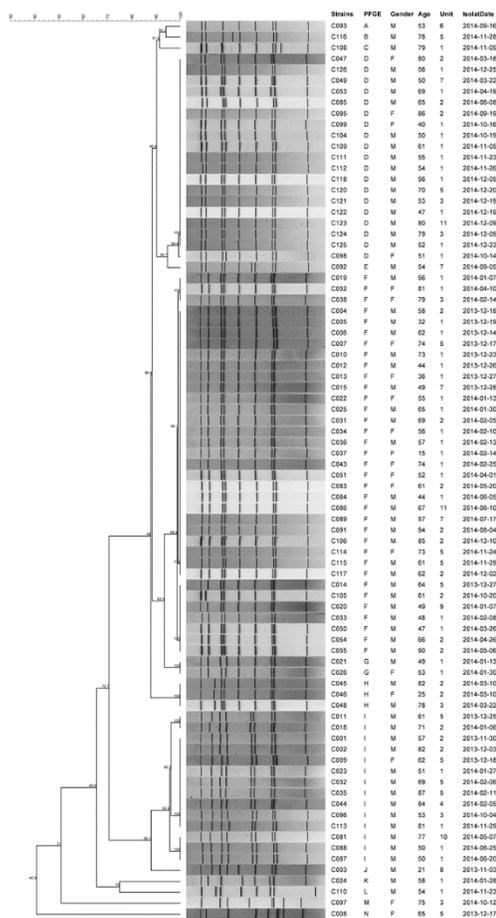
Microbial culture results

Of 82 samples from which *C. striatum* were cultured and isolated, 32.9% (27/82) were pure culture for *C. striatum*. The other samples showed multiple microbes plus *C. striatum*. Among these samples, 45.1% (37/82) of the samples were positive for *C. striatum* plus Gram-negative bacteria, and 19.5% (16/82) of the samples were positive for *C. striatum* plus methicillin-resistant *Staphylococcus aureus*, and 2.5% (2/82) of the samples were positive for *C. striatum* plus *Candida albicans*.

PFGE results

A total of 14 PFGE types were identified, among which types D, F, and I were the predominant clones, accounting for 23.2% (19/82), 42.7% (35/82), and 17.1% (14/82) of samples, respectively. As shown in Figure 2, these three clones were mainly distributed in the neurosurgery unit and ICU. Type F persisted since December 2013 and was distributed among seven departments; 97.1% (34/35) of the strains belonged to resistance patterns I and II, while one strain (C084) was

Figure 2. PFGE results of 82 *C. striatum* strains.



1-Neurosurgery department; 2-Intensive Care Unit; 3-Department of neurology; 4-CCU; 5-Department of Respiratory medicine; 6-Department of orthopaedics; 7- Department of Emergency; 8-General surgery; 9- Department of stomatology; 10- Department of cardiothoracic surgery; 11- Department of rehabilitation.

Table 2. Analysis of potential risk factors for *C. striatum* colonization/infection.

| Potential risk factors | | Hemoglobin | |
|--|---------------------|-------------|---------------------|
| | | Normal (9) | < Normal level (73) |
| Average hospitalization period (days) | | 20.6 | 27.3 |
| Antibiotics usage | Cephalosporins | 77.8% (7/9) | 69.9% (50/73) |
| | Carbapenem | 33.3% (3/9) | 27.4% (20/73) |
| | Quinolones | 33.3% (3/9) | 31.5% (23/73) |
| | Aminoglycosides | 0.0% (0/9) | 5.5% (4/73) |
| | Glycopeptides | 22.2% (2/9) | 12.3% (9/73) |
| | Sulfonamides | 0.0% (0/9) | 1.4% (1/73) |
| Disturbance of consciousness | | 33.3% (3/9) | 47.9% (35/73) |
| Death | | 0.0% (0/9) | 17.8% (13/73) |
| Endotracheal intubation/tracheotomy | | 2.2% (2/9) | 38.4% (28/73) |
| Glucocorticoid usage | | 11.1% (1/9) | 45.2% (33/73) |
| Underlying conditions | Diabetes | 0.0% (0/9) | 4.1% (3/73) |
| | Cerebral infarction | 22.2% (2/9) | 4.1% (3/73) |
| | Cerebral hemorrhage | 33.3% (3/9) | 41.1% (30/73) |
| | COPD | 11.1% (1/9) | 8.2% (6/73) |
| | Malignant tumor | 11.1% (1/9) | 12.3% (9/73) |

COPD: chronic obstructive pulmonary disease.

classified into resistance pattern IX. For types D and I, 87.9% (29/33) of the strains belonged to resistance patterns I and II, while two strains (C085 and C095) belonged to pattern V, and C081 and C018 belonged to patterns IV and VI, respectively.

In vitro biofilm production assay

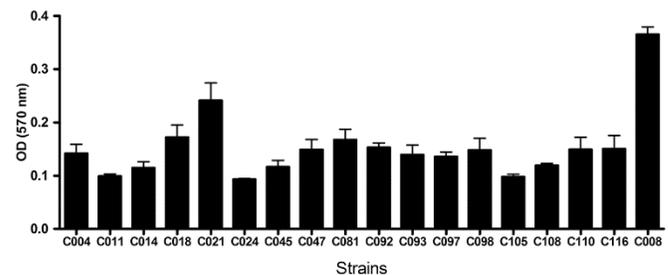
In the present study, 18 *C. striatum* strains with different PFGE types were selected to compare their biofilm production abilities. After 24 hours of incubation, 18 strains showed diverse biofilm production abilities, among which two non-predominant strain (types N and K) showed the highest and lowest biofilm production abilities, respectively. For three predominant strains (C004, C014, and C105) in PFGE type F, the biofilm production abilities were moderate to weak, as shown in Figure 3.

Discussion

In recent years, *C. striatum* has emerged as an opportunistic pathogen leading to various types of healthcare-associated infections, especially lower respiratory tract infection [7-9]. In the current study, we found that some specialties are worthy of attention.

A higher percentage of patients with disturbed consciousness was observed, which might be a key risk factor for *C. striatum* acquisition; this has not been reported in other studies. One week before *C. striatum* isolation, 27 patients in the neurosurgery unit and 10 cases in the ICU were in a deep coma. Moreover, lower hemoglobin was another common feature for these patients, especially the patients with pure *C. striatum* culture. For the 13 deceased patients, the average level of hemoglobin was significantly lower than that of the others. The results suggested that iron acquisition may also be important for *C. striatum* survival, proliferation, or pathogenicity, which was observed in some other common bacterial pathogens, including *Staphylococcus aureus* [15] and *Listeria monocytogenes* [16]. Furthermore, other possible risk factors identified in our study include receiving broad-spectrum antibiotic treatment and diverse invasive medical devices, findings that are consistent with previous reports [8,9]. However, only four patients were diagnosed with chronic obstructive pulmonary diseases (COPD) in this study, which is inconsistent with previous investigations [8,9]. In the present study, previous use of broad-spectrum antibiotics was again proven to be closely correlated with *C. striatum* acquisition or infection, while 69.5% patients were prescribed β -lactam antibiotics and 31.7% patients were prescribed quinolones one week before *C. striatum* isolation.

Figure 3. Absorbance of 18 *C. striatum* strains with different PFGE types on the surface of human fibronectin.



Given the limited number of the patients, the possible differences in the risk factors for *C. striatum* acquisition or infection among patients in different regions require further investigation.

During the period of this study, several instances of *C. striatum* outbreak were observed, and the dates all aggregated during the months with lower temperatures, especially winter and spring, consistent with a previous report [8]. The predominant F clones persisted over 13 months and were isolated in different units, even units apart from each other. Furthermore, the predominant clones D, F, and I also showed higher antibiotic-resistant features. On the contrary, the other non-predominant clones had fewer resistance features. More importantly, the predominant clones accounted for 82.9% (68/82) of the strains tested, the resistance features of which were comparable to that of methicillin-resistant *Staphylococcus aureus* (MRSA) [17-19]. However, the minimum inhibitory concentration values of only 2 *C. striatum* strains were 2 $\mu\text{g/mL}$ to vancomycin, while the other strains were all lower than 0.5 $\mu\text{g/mL}$. Besides, the total resistance rates of the 82 strains seemed to be even more severe, while some reports showed that non-predominant strains were less resistant – even sensitive – to penicillin [8].

To examine the potential pathogenicity and environmental adaptation abilities of different *C. striatum* clones, biofilm production abilities of different *C. striatum* strains were compared. The results showed that the biofilm production abilities among different *C. striatum* strains varied significantly, but the predominant clones did not show the strongest biofilm-producing abilities, which is inconsistent with the findings of a recent report [20]. In contrast, the predominant strains showed moderate to weak biofilm production abilities. Also, the strains with the highest and lowest biofilm production abilities were all non-predominant clones. These findings suggest that the environmental adaptation ability or pathogenicity of *C.*

striatum may be mainly due to the changes of some specific factors of the hosts. The true mechanism of nosocomial transmission or pathogenicity of the predominant *C. striatum* strains requires further investigation.

Conclusions

C. striatum strains analyzed in the present study showed significant multidrug-resistant features and could persist in healthcare-associated environments for a long period, especially the predominant clones. Lower levels of hemoglobin and disturbed consciousness were found to be potential risk factors for *C. striatum* acquisition or infection. Therefore, more attention should be paid to *C. striatum* and the patients with these potential risk factors, and its potential pathogenicity and clinical significance should be further explored.

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