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Clinical characteristics of *Corynebacterium simulans*

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ABSTRACT

Corynebacterium simulans was first reported in 2000. Its characteristics such as isolation frequency, specimen types, and antimicrobial susceptibilities are poorly understood, because identification is difficult using conventional methods. We performed a retrospective observational study of 13 and 317 strains of *C. simulans* and *C. striatum*, respectively, isolated from consecutive patients at Nagoya University Hospital from January 2017 to December 2018. We analyzed patients' backgrounds, types of specimens, and antimicrobial susceptibilities. Antimicrobial susceptibilities were compared with those of *C. striatum*. The frequencies of isolation of *C. simulans* and *C. striatum* were 3.9% and 96%, respectively. *C. simulans* was not detected in specimens associated with mucous membranes, such as sputum and secretions from the craniocervical region, which were frequent for *C. striatum*. *C. simulans* was mainly detected in the skin (61.5%). All *C. simulans* isolates were susceptible to anti-MRSA drugs, as well as to numerous other antibiotics, including those that are orally administered. For example, *C. simulans* was significantly more susceptible to penicillin G, ceftriaxone, and ciprofloxacin than *C. striatum* (respective susceptibilities: 66.7% vs 5.4%, 50.0% vs 4.0%, 66.7% vs 5.9%). There was no significant difference between meropenem and erythromycin, although susceptibility to each was relatively high (100.0% vs 31.7%, 50.0% vs 11.9%). *C. simulans* was susceptible to numerous orally administered antibiotics and more susceptible to antimicrobial drugs than *C. striatum*. *C. simulans* was detected less frequently than *C. striatum* and was infrequently detected in specimens associated with mucous membranes. These characteristics will aid the selection of optimal antimicrobial therapies.

Keywords: *Corynebacterium simulans*, *Corynebacterium striatum*, antibiotic susceptibility, MALDI-TOF MS

Abbreviations:
MALDI-TOF MS: matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry
CLSI: Clinical and Laboratory Standard Institute
MRSA: methicillin- resistant *Staphylococcus aureus*
PCG: penicillin G
CTRX: ceftriaxone
MEPM: meropenem
EM: erythromycin
TC: teicoplanin
DOXY: doxycycline
CPFX: ciprofloxacin
ST: sulfamethoxazole trimethoprim

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VCM: vancomycin
LZD: linezolid
RFP: rifampicin
ADL: activities of daily living

INTRODUCTION

Corynebacterium simulans, which was isolated and identified in 2000 by Wattiau et al,[1] resides in the skin and may cause infections.[2-4] The nucleotide sequence of the genome of *C. simulans*[1] is 98.0% and 96,9% identical to those of *C. striatum* and *C. minutissimum*, respectively, which are highly resistant to numerous antibiotics such as β-lactams, cephems, carbapenems, and quinolones.[5-9] Anti-MRSA drugs are recommended to treat infections caused by *C. striatum*.[6] Such treatment typically requires long-term intravenous infusion. Linezolid serves as an alternative that is internally administered, although it causes many adverse effects and is expensive.

Tests to identify *C. simulans* are not easily conducted in routine clinical practice.[10] For example, *C. simulans* is not included in available databases of conventional tests such as the VITEK 2, API Coryne,[11] and RapID CB Plus, which misclassify *C. simulans* as *C. striatum*.[6] Therefore, methods such as matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS), 16S-rRNA sequencing, or both are required.[11-13] For these reasons, there are few published reports of infectious diseases caused by *C. simulans*.[2-4] Moreover, the relevant clinical characteristics of *C. simulans*, such as frequency of isolation and sites of infection are unknown. Furthermore, there are few reports of the antimicrobial susceptibilities of *C. simulans*.[6,14]

The number of opportunistic infections of older people and those with immunodeficiencies caused by *Corynebacterium* spp. is increasing,[5,6] indicating that the availability of effective treatment may become more important. Therefore, we conducted a study of patients' backgrounds, specimen types, and antimicrobial susceptibilities of *C. simulans* compared with those of its closest relative, *C. striatum*.

PATIENTS AND METHODS

We conducted a retrospective observational study from January 2017 to December 2018 at Nagoya University Hospital, Nagoya, Japan. There were 12,833 new hospitalizations and 550,379 outpatients in 2018. The target samples were 14 strains of *C. simulans* and 605 strains of *C. striatum* isolated from all cultured samples collected from outpatients and inpatients. When the same strain was isolated multiple times from the same patient, only the first isolate was used in this analysis. Medical records, which were collected when the target strain was first detected, included age, sex, inpatient or outpatient, specimen type, *Corynebacterium* species, and antimicrobial susceptibilities.

Isolates were analyzed using the VITEK MS MALDI-TOF MS system (bioMérieux's, Lyon, France). We confirmed that the probabilities of identification of all samples ranged from 60% to 99.9%. When identification was not possible using the VITEK MS, or probability of detection was low, tests were performed using the VITEK 2 (ANC ID card) (bioMérieux). If identification was still not possible, the API Coryne (bioMérieux's) was used. The VITEK 2 or API Coryne systems are unable to identify *C. simulans*, which was therefore detected using the VITEK MS.

When an isolate was suspected as a *Corynebacterium* sp. in normally sterile body fluids,

all samples from the patient were tested. When bacteria were detected in specimens in which *Corynebacterium* spp. were considered to be resident, identification tests were sometimes not performed according to the judgment of the supervising microbiologist, the patient's medical history, and the amount of bacteria detected.

Antimicrobial susceptibility tests were performed using a broth microdilution method according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI). We used a dry plate (NG0M/NG1M, custom-panel; Eiken Chemical Co., Ltd., Tokyo, Japan). The breakpoint of the minimum inhibitory concentration (MIC) was based on M45-Ed3.[15] The test was omitted when the bacterial species were considered to be resident bacteria according to the patient's medical history and the amount of detected bacteria.

Statistical analysis

Fisher's exact test was performed to evaluate susceptibilities to each antibiotic. Tables (2 × 2) of *C. simulans* and *C. striatum* were used, and scores were defined as good (Susceptible) or other (Intermediate, Resistant, Nonsusceptible). We conducted a one-sided *t* test according to the assumption that *C. simulans* was more susceptible than *C. striatum*. The significance level was adjusted using the Bonferroni method, which was used for multiple comparisons. All analyses were performed using used IBM SPSS ver. 26.0 (IBM Corp., Armonk, USA).

RESULTS

Patients' backgrounds and bacterial isolates

C. simulans and *C. striatum* were identified in 14 and 605 samples, respectively. Excluding samples from which the same strain was isolated from the same patient, 13 (3.9%) and 317 (96%) samples were positive for *C. simulans* or *C. striatum*, respectively (Table 1). The median

Table 1 Comparison of patients' background and sources of isolates

	C. simulans (n=13) n, %	*C. striatum* (n=317) n, %
Age (Median years, range)	58 (45–78)	70 (0–97)
Male	9 (69.2)	199 (62.8)
Female	4 (30.8)	118 (37.2)
Inpatient	4 (30.8)	231 (72.9)
Outpatient	9 (69.2)	86 (27.1)
Sources of specimens		
Blood	1 (7.7)	14 (4.5)
IV catheter	0 (0.0)	1 (0.3)
Skin	8 (61.5)	71 (22.4)
Urine	2 (15.4)	79 (24.9)
Vagina and vulva	0 (0.0)	5 (1.6)
Septum	0 (0.0)	63 (19.9)
Digestive system (stool, bile, other)	1 (7.7)	11 (3.5)
Head and neck (eye, ear, pharynx)	0 (0.0)	16 (5.0)
Drainage (thoracic/abdominal cavity, postoperative wound)	0 (0.0)	41 (12.9)
Others	1 (7.7)	16 (5.0)
Frequency of isolation (%)	3.9	96.1

ages of patients with samples positive for *C. striatum* (62.8% male) or *C. simulans* (69.2% male) were 58 and 70 years, respectively. *C. simulans* was more frequently isolated from outpatients (69.2%). *C. simulans* was most frequently isolated from the skin (61.5%), followed by urine (15.4%), although it was not isolated from specimens associated with mucous membranes, such as sputum and secretions from the craniocervical region. In contrast, *C. striatum* was most frequently isolated from urine (24.9%), followed by skin (22.4%) and sputum (19.9%). Patients isolated *C. simulans* from skin had cellulitis with abscesses, including foot necrosis and pressure ulcers, or peritoneal dialysis catheter insertion site infection. Patients isolated *C. simulans* from urine had cystitis, and from blood had bacteremia and pyogenic spondylitis. Otherwise, patient isolated *C. simulans* from digestive system (postoperative bile drainage) were asymptomatic. All but one of the patients with bacteremia had a mixed infection.

Antimicrobial susceptibilities of C. simulans

All *C. simulans* isolates were susceptible to anti-methicillin-resistant *Staphylococcus aureus* (MRSA) drugs (vancomycin (VCM), linezolid (LZD), rifampicin (RFP), and teicoplanin (TC)) (Figure 1); 4/6 (67%) and 3/6 (50%) were susceptible to penicillin G (PCG) and ceftriaxone (CTRX), respectively, and more than 50% of strains were susceptible to β-lactam and cephem antibiotics. Susceptibilities to oral antibiotics were as follows: doxycycline (DOXY) 5/6 (83%), ciprofloxacin (CPFX) 4/6 (67%), and sulfamethoxazole trimethoprim (ST) 5/6 (83%).

	PCG	CTRX	MEPM	GM	EM	CLDM	TC	DOXY	CPFX	ST	VCM	LZD	RFP
MIC 0.06													
0.12	4												
0.25	1		3		3								
0.5													3
1		3					2	5	4	1	6		
2		2				3		1		4		6	
4				6		1	1		2	1			
8	1					2							
16					2								
32					1								
64		1											
S	4	3	3	6	3	0	3	5	4	5	6	6	3
(%)	**66.7**	**50.0**	**100.0**	**100.0**	**50.0**	**0.0**	**100.0**	**83.3**	**66.7**	**83.3**	**100.0**	**100.0**	**100.0**
I	1	2	0	0	0	3			0				
(%)	**16.7**	**33.3**	**0.0**	**0.0**	**0.0**	**50.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
R	1	1	0	0	3	3	0		2	1	0	0	0
(%)	**16.7**	**16.7**	**0.0**	**0.0**	**50.0**	**50.0**	**0.0**	**0.0**	**33.3**	**16.7**	**0.0**	**0.0**	**0.0**
NS								1					
(%)								**16.7**					
total	6	6	3	6	6	6	3	6	6	6	6	6	3

Fig. 1 Antibiotic susceptibilities of *C. simulans*

Minimum inhibitory concentrations (MICs): white, susceptible; light-gray, intermediate; dark-gray, resistant and non-susceptible.

PCG: penicillin G
CTRX: ceftriaxone
MEPM: meropenem
GM: gentamicin
EM: erythromycin
CLDM: clindamycin
TC: teicoplanin
DOXY: doxycycline
CPFX: ciprofloxacin
ST: sulfamethoxazole trimethoprim
VCM: vancomycin
LZD: linezolid
RFP: rifampicin
S: susceptible
I: intermediate
R: resistant
NS: nonsusceptible
MIC: Minimum inhibitory concentration.

[a] MIC: white, susceptible; light-gray, intermediate; dark-gray, resistant and nonsusceptible.

Comparison of antimicrobial susceptibilities of C. simulans and C. striatum

Table 2 shows the drug susceptibilities of *C. simulans* and *C. striatum*. *C. simulans* was significantly (Fisher's exact test) more susceptible to PCG, CTRX, and CPFX than *C. striatum*. There was no significant difference between susceptibilities to meropenem (MEPM) and erythromycin (EM), although they were generally good. There were no significant differences between the susceptibilities of the two species to the other antibiotics.

Table 2 Comparison of the antibiotic-susceptibility rates between *C. simulans* and *C. striatum*

	C. simulans	(%)	*C. striatum*	(%)	P[a]
PCG	4/6	66.7	11/202	5.4	0.000[b]
CTRX	3/6	50	8/202	4	0.002[b]
MEPM	3/3	100	64/202	31.7	0.034
GM	6/6	100	176/202	87.1	0.444
EM	3/6	50	24/202	11.9	0.03
CLDM	0/6	0	8/202	4	0.887
TC	3/3	100	141/202	69.8	0.344
DOXY	5/6	83.3	135/202	66.8	0.361
CPFX	4/6	66.7	12/202	5.9	0.000[b]
ST	5/6	83.3	131/202	64.9	0.289
VCM	6/6	100	201/202	99.5	0.971
LZD	6/6	100	201/202	99.5	0.971
RFP	3/3	100	191/202	94.6	0.847

PCG: penicillin G
CTRX: ceftriaxone
MEPM: meropenem
GM: gentamicin
EM: erythromycin
CLDM: clindamycin
TC: teicoplanin
DOXY: doxycycline
CPFX: ciprofloxacin
ST: sulfamethoxazole trimethoprim
VCM: vancomycin
LZD: linezolid
RFP: rifampicin

[a] P calculated using a one-sided Fisher's exact test and corrected ($P < 0.00385$ [$= 0.05/13$]) using Bonferroni's method.

[b] Significant difference.

DISCUSSION

Here we report the clinical characteristics of *C. simulans* with focus on a comparison with *C. striatum*. A major finding is that *C. simulans* isolates, compared with those of *C. striatum*, were generally more susceptible to antibiotics (e.g. anti-MRSAs, β-lactams, and cephems), particularly

those that can be orally administered. Further, *C. simulans* was isolated much less frequently (25-fold) from our cohort of 330 consecutive patients than *C. striatum* and was not isolated from samples associated with mucous membranes, such as sputum and head and neck secretions, in striking contrast to *C. striatum* (Table 1). Although the genomes of *C. simulans* and *C. striatum* are highly related, *C. simulans*, in contrast to *C. striatum*, was significantly more susceptible to PCG, CTRX, and CPFX as well as to MEPM and EM (Table 2).

These findings have important implications for treating infections caused by *C. simulans*. For example, *C. simulans* is likely closely linked to infections that require long-term treatment, such as pyogenic spondylitis, infections of prosthetic joints, and infectious endocarditis.[2-4] Similarly, *C. striatum* causes infectious endocarditis[6] and infectious orthopedic diseases.[16] *C. striatum* is generally less susceptible than *C. simulans* to antimicrobials and is likely resistant to β-lactams, cephems, carbapenems, and quinolones.[5-8] Therefore, many patients with infections caused by *C. striatum* must undergo long-term intravenous antibiotic treatment because of resistance to oral antibiotics.

Here we identified numerous antibiotics, including highly bioavailable oral antibiotics, for effectively treating infections caused by *C. simulans*. Therefore, when long-term treatment of an *C. simulans* infection is required, switching from an intravenous to an oral antibiotic may improve patients' activities of daily living (ADL) and shorten hospitalization.

C. simulans may be more susceptible to antibiotics than *C. minutissimum*, which is genetically closely related to *C. striatum*. We did not study *C. minutissimum*, because it was isolated from one sample. *C. minutissimum* represents approximately 50% of strains susceptible to quinolones, but with low susceptibility to β-lactams, cephems, and macrolides as well as to other antibiotics.[5,9] The differences in susceptibilities to antimicrobials among *C. simulans*, *C. striatum*, and *C. minutissimum* may be caused by a few genetic differences[1] through an unknown mechanism that regulates susceptibility to antimicrobials.

C. simulans was detected less frequently than *C. striatum*, mainly in the skin, and less frequently in specimens associated with mucous membranes. Specifically, the frequency of detection of *C. simulans* vs *C. striatum* in the present study was 3.9%. Other studies found that the frequency of detection of *C. simulans* alone vs that of the number of *C. simulan*s isolates divided by the total number of *C. simulans* plus *C. striatum* isolates, range from 1.9% to 27.8%.[11,12,14,16] It is important to note that in each of the studies cited, only 1 to 5 patients were infected with *C. simulans*, consistent with our present findings.

In the present study, *C. simulans* was isolated from 13 samples. Compared with *C. striatum*, *C. simulans* was mainly detected in the skin, and as stated above, none of the isolates was acquired from sources associated with mucous membranes, such as sputum and secretions from the head and neck, which are relatively common sources of *C. striatum*.[17,18] Consistent with our present findings, among *Corynebacterium* spp., *C. striatum* is often reported in the lungs and bronchi, although *C. simulans* is not isolated from respiratory organs.[19] These findings suggest differences in host-tissue specificity between *C. simulans* and *C. striatum*.

There are three limitations to this study. First, a small number of patients in a single hospital underwent antimicrobial susceptibility testing for *C. simulans*. Therefore, patient variability and regional bias must be considered and future studies must be conducted for longer times and at multiple sites. However, we believe our study contributes clinically significant information regarding the susceptibility of *C. simulans* to numerous antibiotics. Second, clinical microbiology laboratories decide whether to identify *Corynebacterium* spp. Judgment is made according to clinical information and the amount of bacteria in the absence of a uniform standard. Therefore, the actual ratios between sites of isolation may not be reflected by the detection frequencies of the isolates described here. However, our comparison between *C. simulans* and *C. striatum* does

not detract from the argument that the characteristics of the sites of isolation differ, because the conditions between the two groups were uniform. Third, we did not determine the pathogenicities of the *C. simulans* isolates. In the present study, *C. simulans* was the only detectable bacterial species in 1 of 13 individuals, and the patient was therefore treated with the appropriate antibiotics. This is consistent with the findings of our search of PubMed that found only three reports of infectious diseases caused by *C. simulans*. Nevertheless, we believe that our present findings should persuade clinicians to test for *C. simulans* when patients present with drug-resistant infections at the sites identified here.

Although infrequent, the identification of *C. simulans* may allow the use of more antibiotics, including those that can be orally administered, which will likely improve the ADL of patients and shorten hospitalization. The widespread use MALDI-TOF-MS to rapidly and economically identify bacteria in clinical specimens will likely increase the number of infections caused by *C. simulans*. We believe therefore that the present study will facilitate the selection of the optimal treatment of infections caused by *C. simulans*.

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CONFLICT OF INTERESTS

None

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