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Brief Original Article

Corynebacterium striatum: an emerging respiratory pathogen

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Abstract

Introduction: *Corynebacterium* spp. are primarily considered normal flora and dismissed when isolated from clinical specimens. In recent years, *Corynebacterium striatum* has emerged as a multi-drug resistant human pathogen which can cause nosocomial outbreaks. The organism has infrequently been noted to cause respiratory infections. A retrospective study was conducted to identify the clinical and microbiological features of respiratory infection by *Corynebacterium striatum*.

Methodology: *C. striatum* isolates from clinical and surveillance samples were tested for susceptibility to antimicrobials and typed by Random Amplification of Polymorphic DNA (RAPD). Clinical data was obtained through a retrospective review of records.

Results: 15 isolates from clinical and surveillance samples of 11 hospitalised patients were included. The patients suffered from either an exacerbation of COPD (n = 9) or pneumonia (n = 2). The isolates were all multi-drug resistant. RAPD typing found no evidence of an outbreak/ transmission between patients.

Conclusions: *Corynebacterium* spp. must be considered potential pathogens. Suspicious isolates should be identified to the species level since *Corynebacterium striatum* is often multi-drug resistant.

Key words: *Corynebacterium*; diphtheroid; respiratory infection; MDR; RAPD.

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Introduction

Corynebacterium spp. are non-sporing gram-positive bacilli that are found in the environment and as part of normal skin flora. They are largely considered insignificant contaminants when isolated from clinical specimens. However, some of these organisms may unusually be a cause of human disease. *Corynebacterium striatum* is one such species. It was first described as a pathogen in 1980 [1] and has since been implicated as a cause of meningitis, endocarditis and bacteremia. It has all been infrequently described as a cause of respiratory infections [2-4]. Due to relatively few studies reporting *C. striatum* respiratory infection, the syndromes caused, risk factors and other demographic and clinical features remain undefined. It is an important emerging pathogen which is often multi-drug resistant and may cause nosocomial outbreaks [2]. We identified *C. striatum* as a cause of respiratory infection/ colonization in several patients and conducted a literature review to describe respiratory infections caused by this organism.

Methodology

The study was conducted at Vallabhbhai Patel Chest Institute, a respiratory diseases hospital. It was approved by the Institute Ethics Committee. All significant isolates of *C. striatum* recovered from routine diagnostic cultures of clinical samples between Jan 2016 and June 2017 were included. Significant isolates were defined as sputum and tracheal aspirate samples showing > 25 pus cells and < 10 epithelial cells/ low power field, plenty of gram-positive bacilli under the oil immersion lens on microscopic examination and > 10^5 CFU/mL on culture [3]. The sputum samples were processed by a semi-quantitative method using a calibrated loop (10 µL) after treatment with equal volume of N-acetyl cysteine. Tracheal aspirates were processed using a calibrated loop without any pre-treatment. They were cultured on sheep blood agar and MacConkey agar plates (Hi Media, Mumbai, India). After overnight incubation at 37° C in 5% CO_2 atmosphere, > 10^5cfu/ mL non-hemolytic 2 mm in diameter, greyish white, colonies were grown which were identified as *C. striatum* by Vitek 2 compact system (Biomerieux, Marcy-l'E´toile, France). Some of these were further confirmed by MALDI TOF MS (Bruker Corporation, Billerica, Massachusetts, USA). Surveillance samples - throat swabs and nasal swabs- were collected from two of these patients. These were considered as colonizing isolates. Hospital

environmental samples - air, water, surface swabs and swabs from the hands of healthcare workers were also collected as part of routine surveillance measures. Clinical and demographic data of these patients were obtained through a retrospective review of records.

Antimicrobial susceptibility to penicillin, gentamicin, ciprofloxacin, clindamycin, vancomycin, tetracycline, linezolid was tested using Kirby Bauer's disk diffusion method and the results were interpreted as per the EUCAST guidelines [4]. Multi-drug resistance was defined as resistance to 3 or more classes of antimicrobials.

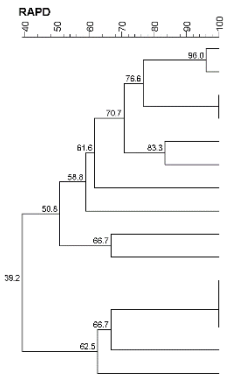
Genomic DNA was isolated using HiPurATM Bacterial Genomic DNA purification Kit (Hi Media, Mumbai, India, cat no. MB505-50PR) following the manufacturer's instructions. Random Amplification of Polymorphic DNA (RAPD) was performed using the ERIC2 primer [5] The banding pattern obtained on electrophoresis of PCR products was analyzed using Gelcompar II (Applied-Maths, Kortrijk, Belgium) and UPGMA generated dice coefficients were used to generate dendrograms.

Results

All coryneform bacteria identified were *C. striatum*. In total, 15 isolates of *C. striatum* from clinical and surveillance cultures were included. They were isolates from 10 sputum samples, 2 tracheal aspirates, 2 nasal swabs and 1 throat swab. No *C. straitum* isolates were recovered from environmental samples. Two of the clinical isolates were recovered from the sputum of a single patient 4 months apart. All patients had fever, raised leucocyte counts and > 25 pus cells/ hpf observed on microscopy of sputum samples.

Patient age ranged between 55 to 70 years. All of them were male. All patients were admitted for treatment, with the length of hospital stay ranging from 4 to 27 days. Three of the patients were admitted to the Intensive Care Unit for at least part of this duration. Two patients had an endotracheal tube in situ. All but one patient became culture positive more than 48 hrs. after admission, with the mean time to culture positivity being 5.9 days (range 1 - 21 days). No other potential pathogens were isolated along with *C. striatum*. All patients made a complete recovery after antibiotic treatment. 9 patients suffered from an exacerbation of Chronic Obstructive Pulmonary Disease (COPD), while the remaining 2 were diagnosed with pneumonia. 4 patients had a history of pulmonary tuberculosis, which may be a marker for impaired immunity. Their demographic and clinical details are shown in Table 1.

Figure 1. UPGMA clustering dendrogram indicating percentage similarities between RAPD pattern of *Corynebacterium straitum* isolates.



Key	Lab No	sample	Patient	Type
1	225/17	sputum	11	Clinical
2	3388/16	sputum	11	Clinical
3	2367/16	sputum	4	Clinical
4	E89/16	Nasal swab	4	Surveillance
5	2065/16	sputum	3	Clinical
6	1544/16	sputum	2	Clinical
7	1289/16	sputum	6	Clinical
8	3516/16	sputum	7	Clinical
9	4156/16	ET Aspirate	8	Clinical
10	2553/16	sputum	6	Clinical
11	2496/16	sputum	5	Clinical
12	E90/16	Nasal swab	5	Surveillance
13	E91/16	Throat swab	5	Surveillance
14	657/17	ET Aspirate	9	Clinical
15	785/17	sputum	9	Clinical

The isolates were all Multi-drug resistant. All isolates were susceptible only to vancomycin and linezolid, except the two isolates recovered from the same patient which were susceptible to tetracycline as well. All 15 isolates were typeable by RAPD (Figure 1). Two clusters of 100% similarity were found. These were clinical and surveillance isolates from the same patients. The two isolates recovered from the sputum of the same patient four months apart showed 96% similarity indicating that it was the same strain.

Discussion

Corynebacterium striatum, along with other coryneform bacteria, is part of the normal flora of skin and mucous membranes. It has traditionally been considered a part of the normal flora of the respiratory tract. *C. striatum* was first recognized as a potential pathogen in 1980 [1] when it was described as the cause of a pleuro-pulmonary infection in a leukemic patient. This was followed by only a few sporadic case reports of respiratory infections till a cluster of cases from an Intensive Care Unit was reported in 1996 [6]. Since then, a number of studies have described infections, transmission and risk factors associated with infection caused by this organism.

The identification of *C. striatum* from clinical specimens requires a high index of suspicion since colonies resemble coagulase-negative staphylococci and may be discarded as commensals. Further, species identification of diphtheroids is important since some of these are now recognized as potential pathogens. Identification of *C. striatum* has presented a problem, particularly for resource-poor settings because conventional phenotypic tests are time-consuming and insufficiently discriminative. In the past, the API Coryne and RapID CB Plus systems were widely used

[6-9] due to more reliable identification. More recently, automated systems such as the Vitek 2 have become more commonly used. *C. striatum* was not included in the Vitek 2 database, leading to inaccurate identification as *Kocuria kristinae* with 99.9% probability [10]. The MicroScan and Pasco systems may misidentify *C. striatum* as *Staphylococcus* spp. or *Micrococcus* spp. with low probability scores [7]. Therefore, reliable identification was only possible through molecular techniques such as 16srRNA and *rpoB* gene sequencing. The current Vitek 2 Compact database includes the organism, though no large studies on identification of *Corynebacterium* spp. have been published. With MALDI-TOF mass spectrometry now widely available, identification of *Corynebacterium* species has become rapid and reliable. We found both Vitek 2 Compact and MALDI-TOF MS identified all our isolates with good probability scores. MALDI-TOF mass spectrometry with both the Bruker and Vitek MS databases show excellent identification of *C. straitum* [11,12]. Drug susceptibility of *C. striatum* has been tested against a variety of antimicrobial classes in most reports. However, due to the absence of internationally agreed upon breakpoints, often breakpoints for various gram-positive organisms have been used such as viridans streptococci [13], *listeria* and organisms except *Neisseria* and *Haemophilus* defined under NCCLS guidelines [7]. At present, breakpoints defined by both the Clinical and Laboratory Standards Institute (M45-A2) and the European Committee on Antimicrobial Susceptibility Testing [4] are available. However, relatively few studies use these criteria making it difficult to identify a clear resistance profile. Many studies find multi-drug resistant strains, though

Table 1. Clinical features of patients included in the study.

Patient	Age , (years)	Diagnosis	ICU admission	Clinical sample	Time to culture positivity (days)	Antibiotic treatment before infection	Smoking*	Co-morbidities	Past tuberculosis*
P1	59	Exacerbation of COPD, Cor-pulmonale	No	Sputum	21	Linezolid	Yes	Diabetes mellitus, Hypertension	Yes
P2	66	Exacerbation of COPD, OSA, type 2 respiratory failure	No	Sputum	6	Piperacillin-Tazobactam, Levofloxacin	No	Diabetes mellitus, Hypertension	Yes
P3	73	Exacerbation of COPD, Allergic rhinitis	No	Sputum	3	Amoxicillin-Clavulanic Acid, Azithromycin	No	-	No
P4	67	Exacerbation of COPD	No	-Sputum -Nasal swab	12	Piperacillin-Tazobactam, Azithromycin	No	-	Yes
P5	55	Exacerbation of COPD, Cor-pulmonale, Type 2 respiratory failure	No	-Sputum -Nasal swab -Throat swab	6	Piperacillin-Tazobactam, Levofloxacin	No	-	No
P6	74	Exacerbation of COPD, Type 2 respiratory failure	No	Sputum	3	Piperacillin-Tazobactam, Azithromycin	Yes	Diabetes mellitus	No
P7	68	Exacerbation of COPD, Emphysema	No	Sputum	9	Piperacillin-Tazobactam, Azithromycin	Yes	-	Yes
P8	71	Exacerbation of COPD, Cor-pulmonale, Type 2 respiratory failure	Yes	Tracheal aspirate	1	-	Yes	-	No
P9	55	Right sided pneumonia, COPD	Yes	Tracheal aspirate	4	Piperacillin-Tazobactam, Azithromycin, Clindamycin	Yes	-	No
P10	72	Bilateral pneumonia, COPD, Type 2 respiratory failure	No	Sputum	7	Piperacillin-Tazobactam, Levofloxacin	Yes	-	No
P11	70	Exacerbation of COPD, Benign Prostatic hypertrophy	No	Sputum	1	-	No	Hypertension	No
			Yes	Sputum	4	Piperacillin-Tazobactam, Azithromycin			

COPD- Chronic Obstructive Pulmonary Disease, OSA- Obstructive Sleep Apnea,

methods of testing vary widely. Varying levels of resistance to most classes of antibiotics has been reported, though isolates remain uniformly susceptible to vancomycin, teicoplanin, and linezolid. A case of bacteremia where the development of daptomycin resistance during therapy has been reported [14]. A recent study which included a large number of isolates (n = 179) found 72% isolates to be resistant to all oral antimicrobials [15]. All our isolates were multidrug resistant sensitive to only Linezolid and vancomycin. We found no significant difference in the resistance profile of colonizing V/s infection causing isolates or between in-patients and out-patients. All our isolates, pathogens and colonizers, were multi-drug resistant, with most isolates showing susceptibility only to Linezolid and Vancomycin.

Several studies investigating nosocomial outbreaks of *C. striatum* infection have been published. The first such outbreak was reported in 1993, where a brown pigmented strain was found to infect 9 patients, 7 of whom were intubated, and 6 had been hospitalized for at least 2 weeks previously. These strains were found to be identical by RFLP. In 1996, the investigators of a nosocomial outbreak in the Netherlands were able to isolate *C. striatum* from the hospital environment, including air and surfaces in the vicinity of the patient as well as from the hands of healthcare workers [6]. Outbreaks of MDR *C. striatum* infections have been described more recently [10,13,16]. Outbreaks of respiratory infection by *C. striatum* have also been reported. In Brazil, a nosocomial outbreak was reported where one MDR PFGE type was responsible for 7 respiratory infections [17], including a patient from whom the same strain was isolated from tracheal aspirate as well as CSF. An outbreak among patients of COPD was reported which infected 21 patients over 18 months, at one point affecting a third of admitted patients [18]. Three deaths were attributed to *C. striatum* infection. Unfortunately, no source/ chain of transmission could be identified and genotyping was not done. The isolates were found to be multi-drug resistant, though due to the absence of breakpoints for Corynebacteria, breakpoints for *Staphylococcus* and *Listeria* were used. Though small sample sizes make statistical analyses difficult, risk factors such as prolonged hospitalization, foreign medical devices, co-morbidities and antibiotic therapy have been identified in a number of studies[6,8,9,13,19–21]. All patients in this study were hospitalized patients and all but one acquired the infection in the hospital. Four of the patients had co-morbidities like diabetes and/or hypertension which may have made them susceptible to the infection.

Typing methods such as PFGE, RAPD, ribotyping, repetitive sequence-based PCR and MALDI-TOF MS-based typing have been used [6, 13, 16]. In our study, we did not find any outbreak or evidence of transmission between patients though most acquired the strain during hospital stay. One patient (P11) was found to be persistently colonized by the same strain which was implicated as the cause of an exacerbation of COPD. Both patients who harbored the organism on their nasal/ pharyngeal mucosa had the same strain isolated from sputum, which may indicate progression from colonization to infection.

Though it is increasingly being identified as a pathogen, the virulence properties of *C. striatum* are poorly understood. Recently, *C. striatum* was shown to produce mature biofilms in vitro similar to other pathogenic organisms [22]. These isolates also showed enhanced biofilm formation in the presence of human fibrinogen. Multi-drug resistant strains were stronger biofilm producers. Interestingly, isolates from intubated patients showed the strongest biofilm production. Biofilm production has also been demonstrated by other studies on clinical *C. striatum* isolates [21,23]. Microbe-microbe interactions between *Staphylococcus aureus*, a common commensal and potential pathogen of the respiratory tract, and *C. striatum* were described in a recent study. *S. aureus* was found to exhibit a decrease in virulence and an increase in adhesion on in vitro co-culture with *C. striatum*. In vivo co-infection with these organisms in a murine model showed a 6-fold decrease in *S. aureus* CFUs and a 20-fold increase in *C. striatum* CFUs. This may facilitate a shift towards chronic *S. aureus* infection and provide an advantage to *C. striatum* over mono-infection [24].

Our study is one of the few describing respiratory infections caused by *C. striatum*. It is the first such study from India. The strengths of our study are the inclusion of detailed clinical data, robust identification of isolates and RAPD typing to rule out an outbreak. However, our study also has some limitations. Clinical data was collected retrospectively and patients were not followed after discharge. Most patients were not screened for colonization. No source of infection could be identified, though endogenous source cannot be ruled out. MICs of the tested antimicrobials were not determined.

Conclusion

In conclusion, *Corynebacterium* spp. must be considered opportunistic pathogens. In case of an

infection, they must be identified to the species level since *C. striatum* is often multi-drug resistant. This will lead to the rapid institution of appropriate chemotherapy and may prevent potential outbreaks.

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