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CASE REPORT

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Isolation of *Corynebacterium freneyi* from a case of exudative pharyngitis, a close mimicker of *Corynebacterium diphtheriae*

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Abstract

Corynebacterium freneyi is a recently described coryneform bacteria. It is only rarely identified from clinical specimens and its pathogenic significance has not been well studied. Here we report the isolation of the species from the throat swab of a patient with suspected diphtheria. The morphology on direct microscopy and culture also closely resembled *Corynebacterium diphtheriae*, which almost led to misidentification. The prompt clinical and microbiological response suggests a probable pathogenic role. This is the first report of the isolation of this species from an oropharyngeal sample.

INTRODUCTION

The genus *Corynebacterium* consists of diverse species, among which *Corynebacterium diphtheriae* is considered to be the most pathogenic. Other species have been isolated from various environmental as well as human sources [1]. *Corynebacterium freneyi* is a recently described species and is closely related to *Corynebacterium xerosis* [2]. It was first described by Renaud *et al.* following the isolation of three strains that closely resembled *C. xerosis* and *C. amycolatum* phenotypically. However, on further genotypic and phylogenetic analysis, they were found to be different and hence given the status of new species. All three were isolated from wound exudate or pus samples, but their clinical relevance was not described [3]. Reports of the isolation of this species from clinical samples are rare. Here we report an interesting case, where *C. freneyi* was isolated from the throat swab of a patient with a clinical picture resembling diphtheria.

CASE REPORT

A 55-year-old lady presented to the medicine outpatient department with a history of sore throat over the past 10 days. There was no associated fever, dysphagia, hoarseness of voice or neck swelling. There were no similar complaints in any family members. On examination, the patient was afebrile, mild pallor was present and blood pressure was 150/90 mm Hg. Examination of the oral cavity and pharynx revealed bilateral tonsillar enlargement (grade 2 on left side, grade 1 on right side) and a whitish patch over the left tonsil. Pharyngeal walls appeared normal. Other general and systemic examination findings were within normal limits.

Investigations

A throat swab was sent to the microbiology laboratory for Albert stain and culture. Albert stain revealed plenty of green coloured bacilli with metachromatic granules studded along the bacilli arranged in cuneiform pattern (Fig. 1). This was reported immediately to the clinician with suspicion of diphtheria. The patient was admitted as a suspected case of diphtheria and was started on erythromycin and diphtheria antitoxin administration was planned. Simultaneously throat swab culture was performed on 5% sheep blood agar, potassium tellurite agar and Loeffler's serum slope, which was further subcultured onto potassium tellurite agar after 6 h. Following overnight incubation of the culture plates, dry, rough, white, nonhaemolytic colonies with irregular margins were observed on the blood agar (Fig. 2). Greyish black, dry, rough, colonies with irregular margins were seen on the potassium tellurite agar, which on further incubation of up to 48 h turned to black coloured colonies (Fig. 3). A catalase test was performed from both the plates and it was positive. Gram stain of the colonies revealed club-shaped Gram-positive bacilli that were not uniformly stained and arranged in cuneiform pattern. Albert stain revealed green coloured bacilli with metachromatic granules and arranged in cuneiform pattern. Colonies were identified to be

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Keywords: *Corynebacterium freneyi*; pharyngitis; diphtheria.

Abbreviations: *C. amycolatum*, *Corynebacterium amycolatum*; *C. diphtheria*, *Corynebacterium diphtheria*; *C. freneyi*, *Corynebacterium freneyi*; *C. xerosis*, *Corynebacterium xerosis*; MALDI TOF MS, matrix assisted laser desorption ionisation time of flight mass spectrometry.

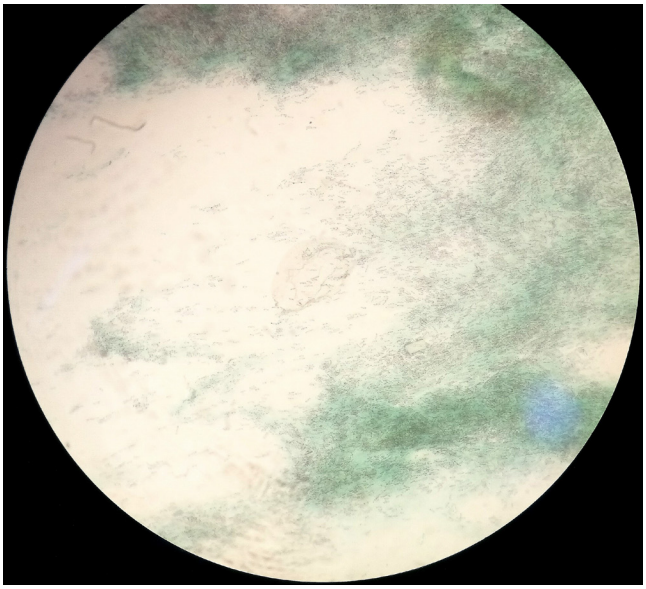

Fig. 1. Direct Albert stain from the throat swab showing abundant green coloured bacilli with metachromatic granules arranged in cuneiform pattern.

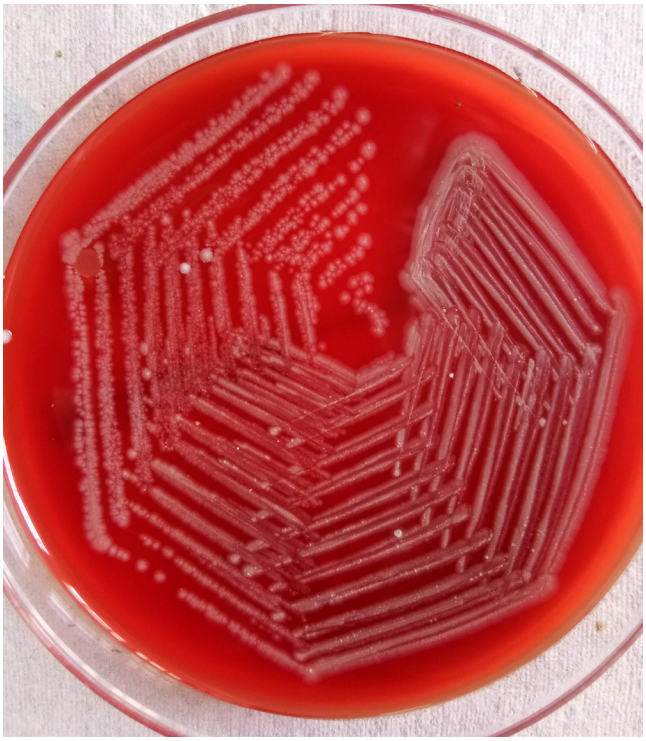

Fig. 2. Dry, rough, white, nonhaemolytic colonies with irregular margins observed on the blood agar plates.

C. freneyi using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI TOF MS) with a 99.9% confidence level. Conventional biochemical tests were performed to identify its differences from other *Corynebacterium* spp., especially *C. diphtheriae*. Urea was not hydrolyzed, and glucose, maltose and fructose were fermented. Fermentation of fructose is not a feature of *C. diphtheriae*.

Treatment

As *C. diphtheriae* was ruled out by culture and the patient did not have any toxic features, antitoxin administration was suspended. The patient was continued on the antibiotic erythromycin (tablets, 500 mg, four times a day).

Outcome and follow-up

The patient improved symptomatically within 2 days of antibiotic administration. A repeat throat swab was sent after 3 days of antibiotics, which again showed green coloured bacilli with metachromatic granules in the Albert stain, but no organisms were grown in the culture. The patient was discharged on the third day and was advised to continue oral antibiotics.

DISCUSSION

Coryneform bacteria are part of normal flora, particularly in the skin. Therefore, they are generally considered to be contaminants in clinical samples, except under certain conditions. Their presence should not be dismissed in immunocompromised patients, as many of the species are emerging as opportunistic pathogens, notably *Corynebacterium striatum, Corynebacterium jeikeium* and *C. xerosis* [1].

Coryneform bacteria are also a common component of oral flora. Their presence in the oral cavity should only be a cause of concern in the presence of clinical manifestations resembling diphtheria, which is mainly caused by toxigenic C. *diphtheriae* followed by *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*. Other coryneform species have been reported to be capable of mimicking the clinical manifestations and morphological characteristics of *C. diphtheriae*, for example *Corynebacterium pseudodiphtheriticum* [4]. In our case, the patient had sore throat and a whitish patch over the left tonsil, requiring diphtheria to be ruled out. The morphology on direct Albert stain and the characteristic black colonies that grew on the potassium tellurite agar almost confirmed a diagnosis of diphtheria. Surprisingly, it turned out to be *C. freneyi* following identification by MALDI TOF MS. This emphasizes the fact that there are many more coryneform bacteria that can mimic *C. diphtheriae*. Misidentification could have led to unnecessary antitoxin administration to the patient, which is expensive and potentially dangerous. It also would have led to inappropriate public health measures, such as contact tracing and prophylaxis.

C. freneyi is a rarely reported species from clinical samples and has never been reported from oral or pharyngeal samples. A previous study described a case of bacteraemia due to *C. freneyi* in an adult man following vascular surgery, which has been claimed to be the first report of this species causing human clinical infection [5]. Funke *et al.* conducted

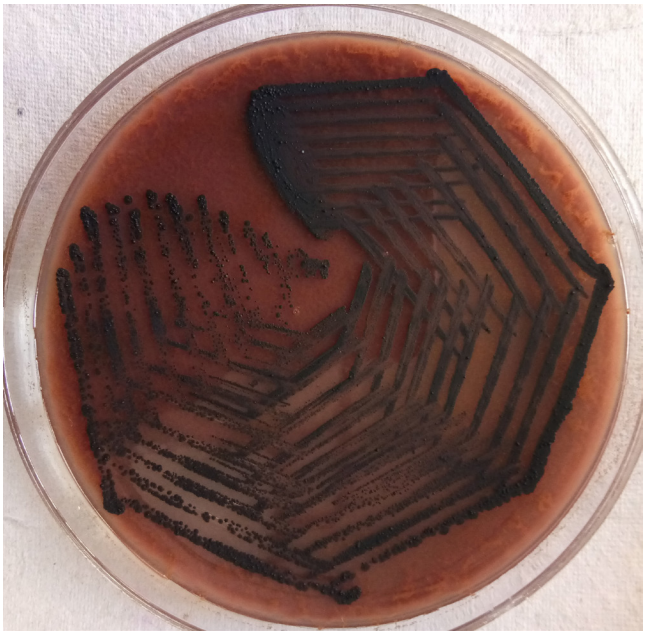

Fig. 3. Black, dry, rough, colonies with irregular margins observed on the potassium tellurite agar.

an extensive study on this species, in which they isolated *C. freneyi* from 18 clinical samples over a period of 3 years, with the majority (13/18) being from urogenital tract, but the clinical significance of these isolations was not well studied [2]. None of the isolates in the above studies were from the oral cavity or throat. In our study, the patient had clinical symptoms and the organism was isolated as a single pathogen in significant quantity. The symptoms were promptly relieved with oral erythromycin and the culture performed after 3 days of therapy was negative. However, the direct Albert stain from the repeat sample still showed organisms, which may have been dead bacilli, as they failed to grow in culture. The prompt clinical and microbiological response suggests that the organism may be the causative pathogen and not just part of the normal flora. A previous study showed that ~62% of isolates of *C. freneyi* were sensitive to erythromycin [2]. We could not perform an antimicrobial susceptibility test for our isolate, which is a limitation of our case report.

Coryneform bacteria are considered to be contaminants most of the time when they are isolated from clinical samples. However, several species of coryneform bacteria have recently been identified as probable pathogens. There is probably significant underreporting, as species-level identification is not possible using conventional methods. Routinely dismissing coryneform bacteria as contaminants may not be appropriate and some species may be pathogenic in specific clinical conditions.

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Author contributions
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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient.

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