DOI: 10.7860/JCDR/2018/32425.11434

Short Communication

Microbiology Section

Exudative Pharyngitis by *Corynebacterium pseudodiphtheriticum*-A Diagnostic Challenge during Diphtheria Epidemic

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ABSTRACT

Corynebacterium pseudodiphtheriticum is a common commensal of the upper respiratory tract which can mimic mild diphtheria clinically and can be a challenge for treating physicians, especially in the setting of a diphtheria epidemic. We report eight cases of pharyngitis caused by macrolide resistant *Corynebacterium pseudodiphtheriticum.*

Keywords: *Corynebacterium diphtheriae*, Macrolide resistance, Throat swabs

INTRODUCTION

Corynebacterium pseudodiphtheriticum, a member of genus *Corynebacterium*, is a common upper respiratory tract commensal. However, this bacterium is increasingly being reported as a cause of various infections ranging from upper and lower respiratory tract infections, endocarditis, cutaneous and ocular infections [1,2]. Most of these patients are immunocompromised or have other comorbidities [1,3]. *C. pseudodiphtheriticum* can cause exudative pharyngitis which sometimes may mimic diphtheria [4,5]. This can pose a challenge for the treating physicians, especially in the setting of a diphtheria epidemic.

MATERIALS AND METHODS

A retrospective descriptive study was carried out at the Department of Microbiology, Government Medical College, Manjeri, Kerala, India from January 2017 and July 2017. A waiver of informed consent was obtained from Institutional Ethics Committee (IEC/GMCM/09/17). Patient anonymity was strictly maintained throughout the study. Patient details were collected from laboratory and medical records.

Throat swabs collected from clinically suspected cases of diphtheria were included in the study. swabs sent for other reasons like follicular tonsillitis were excluded. Cases were determined as 'suspected' according to the interim guidelines released by the Government of Kerala [6].

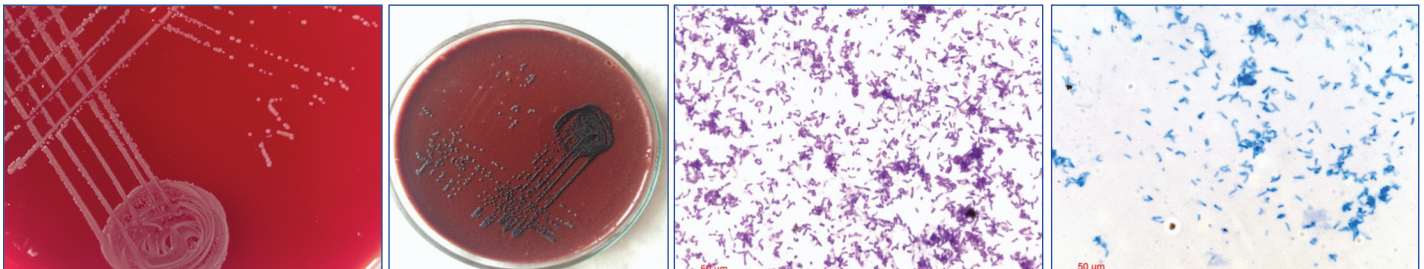
Culture and Identification Procedures

Throat swabs were collected from each patient. Gram's smears were examined for predominant presence of Gram positive bacilli. Swabs were inoculated onto Blood Agar (BA) and Potassium Tellurite Blood Agar (PTBA) and incubated aerobically at 37°C for 48 hours. The BA plates were checked for the presence of white, opaque, non-haemolytic colonies and PTBA plates for black colonies [Table/Fig-1,2]. Only those specimens which yielded pure growth of black colonies on PTBA were included in the study. Gram's and methylene blue smears were prepared from the growth [Table/Fig-3,4]. Colonies of Gram positive bacilli with cuneiform arrangement and metachromatic granules were reported provisionally as 'Organisms morphologically resembling *C. diphtheriae* grown in culture'.

All isolates morphologically resembling *Corynebacterium diphtheriae* were sent to State Public Health Laboratory (SPHL) Thiruvananthapuram for further identification and '*tox*' gene study. At SPHL, identification was done by standard biochemical tests [7]. Polymerase Chain Reaction (PCR) for the presence of '*tox*' gene was done by amplification of specific sequences using primers described previously [8]. Elek's test was performed to confirm the expression of '*tox*' genes using modified Elek's test protocol [9]. We received the final report from SPHL within 7-10 days.

Antibiotic Sensitivity Testing (ABST) was done on Mueller Hinton agar supplemented with 5% blood by Kirby Bauer method. As final identification of the isolates took about 7-10 days, we proceeded with ABST on 'Organisms morphologically resembling *C. diphtheriae'* considering the gravity of the disease. Inoculum was prepared according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. Penicillin (10 U), Ampicillin (10 µg), Erythromycin (15 µg) and Azithromycin (15 µg) discs (Hi Media) were used for testing. CLSI do not provide zone diameter breakpoints for disc diffusion method of antibiotic sensitivity testing for *Corynebacterium spp*. CLSI determined breakpoints for staphylococci were used for interpretation [11,12].

After collecting throat swabs, patients were given Erythromycin (40 mg/Kg/day in four divided doses) for 14 days from the outpatient clinics. Immediately after receiving the presumptive



[Table/Fig-1]: Opaque, grey, smooth, convex non haemolytic colonies on blood agar after 48 hours incubation at 38°C. **[Table/Fig-2]:** Slightly dry, convex, grey/black colonies on Potassium tellurite blood agar after 48 hours incubation at 38°C. **[Table/Fig-3]:** Gram's stain (100X) *C. pseudodophtheriticum*-Gram positive bacilli in cuneiform arrangement. **[Table/Fig-4]:** Methylene blue stain (100X) *C. pseudodophtheriticum*-metachromatic granules and cuneiform arrangement.
(All Images left to right)

report of a morphological diagnosis, the patients were called back by the primary physicians and were referred to Government Medical College, Kozhikode for further management as the hospital did not have the facility to administer Anti-diphtheria antiserum in required cases. Erythromycin was discontinued and patients were treated with Crystalline Penicillin (200000 IU/Kg in four divided doses) for 14 days.

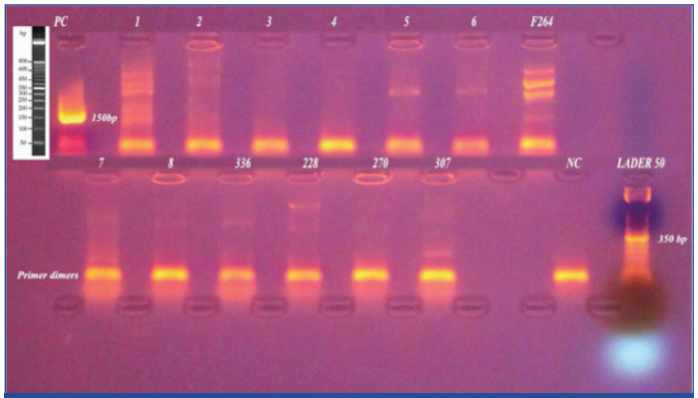
Few isolates resembling *C.diphtheriae* obtained between May-July 2017 were resistant to macrolides but sensitive to Penicillin and Ampicillin. These isolates were sent to Aster MIMS Microbiology laboratory for identification and '*tox*' gene detection by PCR. Species identification was done by VITEC-2 Compact system (bioMérieux) and '*tox*' gene detection by PCR [8]. Antibiotic sensitivity tests were done with E-strips (bioMérieux) for Penicillin (0.016-32 µg/mL), Ampicillin (0.016-32 µg/mL), Erythromycin (0.016-32 µg/mL), Azithromycin (0.016-32 µg/mL) and Clindamycin (0.016-32 µg/mL). The CLSI determined breakpoints were used for interpreting MIC of Penicillin, Erythromycin and Clindamycin [10]. For Ampicillin and Azithromycin, CLSI determined breakpoints for staphylococci have been used [11].

RESULTS

A total of 341 throat swabs were processed between January 2017 and July 2017 of which 152 were from males and 189 from females.

Of the 341 suspected cases, a provisional report of 'Organisms morphologically resembling *C. diphtheriae* have grown in culture' was issued for 33 (9.6%) patients. A total of 20 isolates among these were confirmed as *C. diphtheriae* from SPHL of which 17 were toxigenic strains. Three isolates were identified as *C. minutissimum*, one *C. jeikeium* and one *Arcanobacterium*.

A total of 8 out of 33 isolates were identified as *C. pseudodiphtheriticum*. All tested negative for the presence of '*tox*' gene [Table/Fig-5]. All eight patients were children below 10 years of age and had presented to the outpatient department with features of fever and exudative pharyngitis. Apart from the current illness all eight children were healthy and there was no history suggestive of a compromised immune system.



[Table/Fig-5]: Lanes1-8 test strains *C. pseudodiphtheriticum*. Negative for '*tox*' gene. 150 bp denotes '*tox*' gene.
PC: Positive control, NC: Negative control

All patients recovered from the illness. Details like age, sex, primary immunisation status, presenting complaints and presence or absence of signs of toxicity of eight patients from whom *C. pseudodiphtheriticum* were isolated are given in [Table/Fig-6].

DISCUSSION

The Northern districts of Kerala, especially Malappuram and Kozhikode witnessed a resurgence of diphtheria which started in June 2015. By 2016 the numbers of diphtheria cases rose to epidemic proportions [13]. Government Medical College, Manjeri serves a large number of patients belonging to Malappuram district. The index case during the 2015 outbreak was reported

S. No	Age/ Sex	Immunisation status	Fever	Sore throat	Pseudo membrane	Clinical signs of toxicity	ADS *given
1	10/M	Fully immunised	+	+	+ R tonsil	_	_
2	10/M	Partially immunised (till 1.5 years)	+	+	+	_	+§
3	8/M	No records available	+	+	+ R tonsil	_	+§
4	10/F	Partially immunised (till 1.5 years)	+	+	+	_	_
5	10/F	Completed (till 5 years)	+	+	+	_	_
6	7/F	Fully immunised	+	+	+	_	_
7	9/M	Fully immunised	+	+	+	_	_
8	10/F	Partially immunised (till 1.5 years)	+	+	+ R tonsil	_	_

[Table/Fig-6]: Clinical details of 8 patients including immunisation status.
M: Male; F: Female
*Anti diphtheria antiserum
§Patients who received ADS
Two patients received ADS, but discontinued due to hypersensitivityreaction

from this district. All suspected cases of diphtheria from this area were being referred to Government Medical College, Kozhikode till June 2016 after which laboratory started basic culture of throat swabs for diphtheria.

Diagnosis of diphtheria was made based on clinical features including the presence of a pseudo-membrane [6]. Swabs collected from suspected cases of diphtheria were sent to microbiology laboratory for culture. Although membranous pharyngitis can be caused by several organisms including bacteria, viruses and Candida, diphtheria remains the first differential diagnosis in the setting of an epidemic [4].

During the study period, 33 isolates were reported presumptively as *C. diphtheriae* of which 8 (24%) were identified as *C pseudodiphtheriticum*. All were from children below 10 years of age. Among them four were males and four were females. Pathogenic potential of *C. pseudodiphtheriticum* is well known but is usually associated with an immunocompromised state or other underlying illness [14-16]. All the eight patients included in this study presented with signs and symptoms of severe upper respiratory tract illness. There was no history of previous chronic illnesses, invasive procedures or transplants. Metachromatic granules are absent or are minimally seen in *C. pseudodiphtheriticum* [17]. Among the eight isolates in present series, five had minimal metachromatic granules. With prolonged incubation, granules were more prominent. In the eight cases that we have reported, *C. pseudodiphtheriticum* was grown in pure culture in PTBA and was the predominant growth in BA. This confirms the association of the isolate with exudative pharyngitis. All isolates were negative for *tox* gene; hence, the pathogenicity cannot be attributed to toxins. Though, a common commensal of upper respiratory tract, *C. pseudodiphtheriticum* has the potential to breach epithelial cell barrier, invade deeper tissue and elicit pro-inflammatory responses which could explain the signs and symptoms [4,18].

Although, the beginning of the current outbreak was in 2015, *C. pseudodiphtheriticum* was isolated for the first time in May 2017 with clustering of cases till July. From August 2017-till date we have not isolated *C.pseudodiphtheriticum* even though we continue to isolate *C. diphtheriae. C. pseudodiphtheriticum* shows variable susceptibility to macrolides and lincosamides, whereas susceptibility to beta lactams has been near universal [1,2,19]. In the present study all isolates were sensitive to beta lactams; however resistance to macrolides and clindamycin was 100%. Resistance to macrolides

is reported in other *Corynebacterium* spp. like *C. amycolatum, C. urealyticum*, *C. afermentans and C. auris. C. jeikeium* shows inducible resistance to macrolides [3]. *C. minutissimum* and *C. jeikeium* isolated from the present patients were sensitive to all antibiotics tested. Resistance to beta lactam or macrolides has not been reported in *C. diphtheriae* isolated from North Kerala during the present outbreak since 2015. In this scenario, resistance to macrolides should raise a suspicion that the isolate may not be *C. diphtheriae.*

For the eight patients with a presumptive diagnosis of diphtheria, prompt treatment had to be initiated according to the protocol without waiting for lab confirmation which took about 7-10 days. Patients had to be shifted to a hospital in a different town anticipating treatment with ADS. ADS administered for two out of eight patients had to be discontinued due to hypersensitivity reactions. In addition to the considerable inconvenience incurred by patients and family, administrative machinery had to be mobilised for tracing contacts to institute prophylactic treatment which was not required, had a final confirmation of the isolate been possible without time delay.

Microbiology laboratories in public sector centres catering to this area need to be equipped with facilities for rapid confirmation of identity of isolates so that unnecessary delay in diagnosis, unwanted hospital admissions and treatments can be avoided.

LIMITATION

We were unable to confirm the identity of the probable *Corynebacterium* isolates before proceeding to antibiotic sensitivity tests due to resource constraints. There was clustering of isolates during the months of May, June and July. We did not investigate whether these patients came from the same locality. Epidemiological typing was not done to establish clonal origin of the isolates.

CONCLUSION

C. pseudodiphtheriticum presenting as exudative pharyngitis can mimic mild diphtheria clinically and pose a diagnostic challenge in the setting of a diphtheria epidemic. Macrolide resistance provides a clue that the isolate may not be *C. diphtheriae.*

ACKNOWLEDGEMENTS

Department of Paediatrics, Government Medical College, Kozhikode provided clinical information of the patients.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Sep 17, 2017**
Date of Peer Review: **Nov 23, 2017**
Date of Acceptance: **Feb 13, 2018**
Date of Publishing: **Apr 01, 2018**