Chandra et al | Article 8812
J Pure Appl Microbiol. 2024;18(2):980-986. doi: 10.22207/JPAM.18.2.12
Received: 28 June 2023 | Accepted: 22 March 2024
Published Online: 27 April 2024

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Identification and Speciation of Non-diphtheritic Corynebacteria (NDC) and their Antimicrobial Susceptibility Pattern by Broth Microdilution

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Abstract

Non-diphtheritic Corynebacteria (NDC), originally considered contaminants in clinical samples, have now emerged as nosocomial pathogens, emphasising the importance of their identification and prompt reporting. 120 non-diphtheritic corynebacteria isolated from pus were chosen for examination. A battery of tests identified isolates and minimum inhibitory concentration (MIC) was detected by broth micro-dilution and interpreted as per Clinical & Laboratory Standards Institute (CLSI) and British Society for Antimicrobial Chemotherapy (BSAC) guidelines. *C. amycolatum* 28 (31%), followed by *C. striatum* 18 (20.5%) was the predominant isolate. Cephalosporins were least effective followed by Gentamycin. However, all isolates were sensitive to Vancomycin and Linezolid. Our research highlights the necessity of implementing clinical antimicrobial therapy protocols for *Corynebacterium* spp. Empirical treatment with vancomycin or linezolid is recommended until *in vitro* susceptibility results become accessible.

Keywords: Non-diphtheritic Corynebacteria, NDC Identification, Speciation of NDC, Corynebacterium Species, *Corynebacterium* spp.

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Citation: Chandra P, Ravi GS, Pasha SAR, Kumar R. Identification and Speciation of Non-diphtheritic Corynebacteria (NDC) and their Antimicrobial Susceptibility Pattern by Broth Microdilution. *J Pure Appl Microbiol.* 2024;18(2):980-986. doi: 10.22207/JPAM.18.2.12

INTRODUCTION

Skin and mucous membranes typically contain non-diphtheritic *Corynebacterium* (NDC), a commensal bacterium. They have often been considered contaminants when isolated from clinical specimens because the discrepancy between colonisation and pathogen has yet to be thru in each case.[1]

Recent data indicate these are new hospital-acquired infections, and most show antibiotic resistance.[1-5] Lipsky *et al.* evaluated the literature on non-diphtheritic *Corynebacterium* (NDC) species infections in 1982, focusing on the clinical and microbiological features. These coryneforms are now being recognised as opportunistic agents in specific situations, such as in individuals who are immunocompromised, with prosthetic devices, or who have spent much time in the hospital.[6] These microorganisms have been connected with a range of infections, including catheter-related bloodstream infections, infections affecting prosthetic valves, bone-related conditions like osteomyelitis and septic arthritis, neurological complications such as meningitis, neurosurgical shunt infections, and brain abscesses, lung involvement resulting in pneumonia and empyema, as well as urinary tract infections and peritonitis.[7] New *Corynebacterium* species have been reported more frequently, as well as their prevalence in countless human infections.[1] When coryneform bacteria are isolated in pure culture from clinical samples or serve as the dominant organisms in generally sterile materials, species-level identification should be obtained. According to von Graevenitz *et al.*, it is best to categorise coryneforms to species level to spot unknown species and show the possible pathogenicity (adherence and possible invasiveness) of previously believed to be non-pathogenic species and describe the unrecognised species.[8]

Clinical microbiologists need to understand the NDC's potential role in human infections more than ever because these pathogens can be major issues in the clinical management of infections. In particular, antibiotic-resistant species can be overlooked because reports have shown an increasing trend in antibiotic resistance to common antibiotics like penicillin, macrolides, and fluoroquinolones.[7,9] The isolation of NDC from clinical samples and their susceptibility patterns to antibiotics have recently been studied globally.[9] Our study aims to identify and speciate non-diphtheritic corynebacteria isolated from wound tissues, evaluate their clinical significance, and determine their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Study design

An unblinded prospective study commenced after obtaining approval from the institutional ethics committee (IEC No -532/L/11/12/Ethics/ESICMC&PGIMSR/Estt.Vol.III) during the period from January 2018 to June 2019 at the Department of Microbiology, ESIC Medical College and Research Centre, Bangalore.

Source of data

All pus, swabs, secretions, and biopsy specimens from the skin and soft tissue infections collected in sterile containers aseptically and submitted to the diagnostic Microbiology laboratory were included.

Study subjects

Inclusion criteria

1. Samples collected from skin and soft tissue infections in sterile containers and sterile swabs.
2. Diphtheroids isolated from skin and soft tissue either in pure culture or with other organisms.

Exclusion criteria

1. Samples collected in unsterile containers and swabs.
2. Diphtheroids isolated from clinical specimens other than skin and soft tissue specimens.

Laboratory methodology

Gram stain was performed on direct smears to evaluate the quality of specimens and occurrence of microorganisms. Samples were inoculated onto blood agar and McConkey agar. When isolated in pure growth or when detected in conjunction with other bacteria, the diphtheroids were deemed clinically important and subjected to additional processing.[10] Isolate identification relied on various criteria, including colony

Table 1. MIC Breakpoints[13,14]

Antibiotic (µg/ml)	CLSI Breakpoints		BSAC Breakpoints	
	Susceptible	Resistant	Susceptible	Resistant
Vancomycin	≤2 µ/ml	-	≤2 µ/ml	≥2 µ/ml
Linezolid	≤2 µ/ml	-	≤2 µ/ml	≥2 µ/ml
Meropenem	≤0.25 µ/ml	≥0.5 µ/ml		
Gentamicin	≤4 µ/ml	≥8 µ/ml	≤1 µ/ml	≥1 µ/ml
Cefotaxime	≤1 µ/ml	≥2 µ/ml		
Ceftriaxone	≤1 µ/ml	≥2 µ/ml		

The resistance break point to Imipenem diphtheroids is not defined, so the cut-off value of Meropenem is considered.

morphology, pigmentation, hemolysis, presence of metachromatic granules in Albert's stain, motility, and biochemical tests such as catalase, Hugh-Leifson's oxidative-fermentative test, Voges-Proskauer (VP) test, arginine hydrolysis, nitrate reduction, urease production, aesculin hydrolysis, CAMP test, and fermentation of glucose, maltose, and sucrose.[11-13]

Antimicrobial susceptibility testing

Antimicrobial Susceptibility testing was done by Broth Microdilution method as per CLSI M45-A.[14] Vancomycin, Linezolid, Imipenem, Gentamicin, Ceftriaxone, and Cefotaxime were tested using microdilution techniques utilising "Mueller Hinton broth enhanced with 5% lysed horse blood in microtiter plates". The breakpoints were adopted from "CLSI M45-A, and antibiotics for which CLSI has not defined any susceptibility criteria were followed as per BSAC guidelines (Table 1).

RESULTS

A total of 7261 pus/swab samples were processed, 120 (1.6%) of these samples yielded diphtheroids in either pure or mixed form. Of these, 88 (73.33%) were pure growth, and 32 (26.67%) were mixed growth. Male patients constituted 53.3% (n=64), and 46.7 % (n=56) were female. Among the male patients, the majority were in the age group 41-50 (n=16, 13.3%) years followed by 51 -60 years (n=16. 13.3%) and among females 21-30 years (n=19, 15.8%) years followed by 31-40 years (n=13, 10.83%). It was statistically not significant with a p value of > 0.05 using Pearson chi-square test. (Table 2).

Table 2. Sex-wise and Age-wise Distribution of samples

Age Group	Female	Male
0-10 years (n=7)	3	4
11-20 years (n=5)	2	3
21-30 years (n=22)	19	3
31-40 years (n=20)	13	7
41-50 years (n=23)	7	16
51-60 years (n=25)	9	16
61-70 years (n=13)	1	12
71 years and above (n=5)	2	3
TOTAL (n=120)	56 (46.7%)	64 (53.3%)

The majority of samples 68 (56.7%) were from the Surgery ward, among these the highest number 21 (30.9%,) were related to wound discharges followed by Diabetic foot ulcers, 16 (23.5%), Postoperative wound infections, 10 (14.7%) and 7 (10.3%) were from cellulitis leading to a prolonged hospital stay (Table 3).

Overall antibiotic resistance pattern of the isolates showed a high frequency of resistance to Cephalosporins and Aminoglycosides. Excellent activities were shown by vancomycin and linezolid. An average of 60.2% of diphtheroids were sensitive to gentamicin. However, the susceptibility pattern varied from 25% (1) (*C. minutissimum*) to 100% (2) (*C. afermentans).*

Susceptibility to Cefotaxime was 53.4% in the present study. Sensitivity to ceftriaxone is alarming, as >50% of the isolates were resistant, and only 40.9% were sensitive. Gentamicin, ceftriaxone, and cefotaxime were ineffective against the less frequent isolates, such as *C. xerosis* (1) and *C. renale* (1). However, they were sensitive to vancomycin, linezolid and imipenem. (Table 4)

Table 3. Distribution of cases infected with Diphtheroids with a clinical diagnosis

Ward	Clinical Diagnosis	Frequency (Percentage)
General Surgery (n=68)	Wound Discharge	21 (30.9%)
	Diabetic foot	16 (23.5%)
	Post-operative wound infection	10 (14.7%)
	Cellulitis	7 (10.3%)
	Abscess	5 (7.4%)
	Discharging sinuses	4 (5.9%)
	Post laparotomy	2 (2.9%)
	Dry gangrene	2 (2.9%)
	Carbuncle	1 (1.5%)
Obstetrics & Gynaecology (n=31)	Post-LSCS wound infection	23 (74.2%)
	Post-abdominal hysterectomy wound	6 (19.4%)
	Post Episiotomy wound	1 (3.2%)
	Umbilical Discharge	1 (3.2%)
Orthopaedics (n=6)	PRTA	4 (66.7%)
	Open reduction & internal fixation wound	2 (33.3%)
Plastic Surgery (n=3)	Fasciotomy wound	1 (33.3%)
	Soft tissue Infection	2 (66.7%)
Intensive care unit (n=7)	Diabetic ketoacidosis with abscess	3 (42.9%)
	Lower limb cellulitis	2 (28.5%)
	Blunt trauma abdomen	1 (14.3%)
	Fournier's gangrene	1 (14.3%)
NICU (n=5)	Umbilical Discharge	5 (100%)

DISCUSSION

Since Corynebacteria are commensals of skin flora, it is challenging to distinguish between infection, colonisation, and contamination when these bacteria are isolated from purulent specimens. Its role in disease is supported when neutrophils/polymorphonuclear leukocytes are noticed in the sample and gram-positive bacilli, especially without any other pathogen.[13] Both acute and chronic wound infections can be caused by *Corynebacterium* spp and NDC if isolated from a clinical sample; additional microbiological testing should be done before reporting it as a contaminant.[13,15]

In the present study, majority of samples were contributed by male patients constituting 53.3% (64/120) and 46.7% (56/120) by female patients. This gender and age group predominance could be due to a higher occurrence of skin and soft tissue infections in this demographic seeking medical care. In the present study, among the 120 culture-positive pus samples, 88 (73.3%) yielded pure bacterial (mono-microbial) isolates, and 32 (26.7%) yielded mixed infection (two or more organisms- polymicrobial). However, in contrast, Mathavi *et al*. and Shravani V *et al.* have reported only 4.9% (42 out of 857) and 36.8% (445 out of 1206) of pure growth, respectively.[7,16] A study by Reddy *et al*. reported isolating 32.4% of diphtheroids from pus samples.[16] This variation in rates of isolation of NDC across different studies is attributed to various factors such as sampling method, geographical variation, study population and diagnostic awareness.

The majority of clinical samples yielding NDC were from surgical wards constituting (56.7% n=68), followed by Obstetrics and Gynecology (25.8% n=31), ICU (5.8% n=7), Orthopedics (5% n=6), NICU (4.2% n=5) and Plastic Surgery (2.5% n=3). In a related study, Rudresh *et al*. observed that the majority of the cases came from surgical wards (36%, n = 9), post-operative gynecology wards (24%, n = 6), reconstructive surgery wards (24%, n = 6), ICUs (8%, n = 2), and general medicine wards (8%, n = 2).[13]

The most common isolate found in this study was *C. amycolatum* 28 (31%), followed by *C. striatum* 18 (20.5%), together constituting 52.3% (46 out of 88). However, *C. jeikeium* and

Table 4. Antibiotic sensitivity pattern of Diphtheroids [n = 88]

Organism	VA	LZ	IPM	GEN	CTR	CTX
C. amycolatum (28)	28(100)	28(100)	19(67.9)	17(60.7)	13(46.4)	16(57.1)
C. striatum (18)	18(100)	18(100)	12(66.7)	13(72.2)	9(50)	10(55.5)
C. jeikeium (13)	13(100)	13(100)	6(46.1)	8(61.5)	5(38.5)	5(38.5)
C. ulcerans (9)	9(100)	9(100)	6(66.7)	5(55.6)	3(33.3)	5(55.6)
C. glucuronolyticum (5)	5(100)	5(100)	5(100)	3(60)	1(20)	0(0)
C. minutissimum (4)	4(100)	4(100)	4(100)	1(25)	0(0)	1(25)
C. pseudodiphtheriticum (3)	3(100)	3(100)	2(66.7)	2(66.7)	2(66.7)	2(66.7)
C. urealyticum (3)	3(100)	3(100)	2(66.7)	1(33.3)	1(33.3)	1(33.3)
C. afermentans (2)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)
C. accolens (1)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)
C. xerosis (1)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)
C. renale (1)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)
Total (88)	88(100)	88(100)	61(69.3)	53(60.2)	36(40.9)	47(53.4)

[VA-Vancomycin, LZ-Linezolid, IPM-Imipenem, GEN-Gentamycin, CTR-Ceftriaxone, CTX-Cefotaxime.]

C. ulcerans constituted 15 (14.9%) and 9 (10.3%), respectively. The most frequently isolated species in a related study by Reddy *et al*. and Shravani V *et al.* was *C. amycolatum*, which accounted for 35.9% of all isolates. CDC group G came in second with 15.7% of all isolates.[16,17] Even in a comparable study by Rudresh *et al*., *C. striatum* (16%) and *C. amycolatum* (20%) were the two most common species.[13] Reshmi Chandran *et al*., on the other hand, identified 5 of the 19 samples (26.31%) as *C. pseudotuberculosis*, while *C. renale* was second with 21.05%, followed by *C. bovis* at 15.78%, *C. ulcerans*, *C. minutissimum*, and *C. pseudodiphtheriticum* at 10.52%. *C. ulcerans* was isolated in 14 of the 43 wound infection isolates (32.5%), along with *C. pseudotuberculosis*, *C. renale*, *C. striatum*, *C. minutissimum*, *C. haemolyticum*, *C. pseudodiphtheriticum* (both 23.25%).[12] This contrasts with the characteristics of the isolates found in the current investigation. Mathavi *et al*., in a similar survey, reported *C. jeikeium* (45.24%) as the predominant species, followed by *C. ulcerans* (30.95%).[7] However, Leal *et al*. found aerobic wound cultures yielding 184 diphtheroid isolates; the most common isolate was *C. striatum* (28%).[18] A review of different studies revealed varying epidemiological prevalence of the most common prevailing isolates among other studies in different geographical areas. This could be because of the higher prevalence of these causative organisms in the restricted location and the change in local epidemiological factors.

Table 5. Organisms isolated along with diphtheroids (n=120)

Type of growth	Organism grown	No.	%
Pure growth		88	73.33
Diphtheroids along with other bacteria	*Escherichia coli*	8	25
	Staphylococcus aureus	5	15.6
	Klebsiella spp.	5	15.6
	Pseudomonas aeruginosa	4	12.5
	Citrobacter spp.	4	12.5
	Enterobacter spp.	2	6.25
	Enterococcus spp.	2	6.25
	2 (6.25%)		
	Providencia spp.	1	3.1
	Morganella spp.	1	3.1

In the current investigation, *Escherichia coli* 8 (25%), *Klebsiella* spp. 5 (15.6%), and *Staphylococcus aureus* 5 (15.6%) were the most prevalent pathogens associated with diphtheroids (Table 5). *Escherichia coli* (24%) has been identified by Rudresh *et al*. as the most prevalent bacterium linked to diphtheroids, next to *Klebsiella* spp. (12%) with *Pseudomonas aeruginosa* (12%).[13]

CLSI recommends MIC as the primary method for assessing antibiotic sensitivity, while BSAC suggests combining MIC determination with disc diffusion testing for select antibiotics. Imipenem's and vancomycin resistance break point

for diphtheroids is yet to be established, rendering its prudent use in clinical practice imperative.[14,19] The drugs with the most excellent efficacy against diphtheroids in the current trial were vancomycin (100%) and linezolid (100%), next to imipenem (69.3%) and gentamicin (60.2%). Similar findings were noted by Reddy *et al*., and Rudresh *et al*. study on Nondiphtherial Corynebacteria where the majority of the isolates were susceptible to vancomycin, linezolid, and imipenem.[13,17] A similar study by Mathavi *et al*. on the Characterization of Nondiphtherial Corynebacteria isolated from clinical samples and their Antimicrobial Susceptibility Pattern found similar findings however, he noted resistance to beta-lactam antibiotics like penicillin, ampicillin and ceftriaxone which is similar to our study.[7]

Chronic non-healing ulcers, advanced age, diabetes, and prolonged antibiotic therapy (Table 3) were some of the associated factors found with diphtheroid's isolation in our study which is in concordant with Rudresh *et al*. study where Chronic non-healing ulcers, prolonged antibiotic therapy, diabetes, more extended hospital stays, and advanced age were some of the associated factors suggestive of nosocomial acquired infection.[13] Coyle et al., in their study on Coryneform Bacteria in Infectious Diseases, showed diphtheroids spread in hospitals from person to person and airborne modes using Plasmid profiling as an epidemiological tool.[6] The Hospital environment contamination could serve as a frequent origin of infections. Implementing appropriate infection control measures and surveillance protocols is essential to manage and prevent such occurrences.

CONCLUSION

We conclude that non-diphtheritic corynebacteria, especially when isolated from a sterile site, provide a concern as nosocomial pathogens in acute or chronic skin and soft tissue infections demanding thorough management and potentially expensive treatments. Due to the considerable resistance of NDCs, it's crucial to conduct identification and antibiotic susceptibility testing on typical antibacterial agents. We recommend Vancomycin and Linezolid to be used as empirical antibiotics in treating wound infections caused by NDCs; however, a definitive antibiotic is to be chosen after performing in vitro antibiotic susceptibility testing.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, ESIC-Medical College & Post Graduate Institute of Medical Sciences & Research, Bengaluru, India, with IEC NO – 532/L/11/12/ETHICS/ESICMC&PGIMSR/ESTT.VOL..III.

REFERENCES

1. Ramana KV, Vikram G, Padma Wali P, et al. Non-Diphtheritic Corynebacteria (NDC) and Their Clinical Significance: Clinical Microbiologist's Perspective. *Am J Epidemiol Infect Dis.* 2014;2(3):83-87. doi: 10.12691/ajeid-2-3-3
2. Funke G, von Graevenitz A, Clarridge JE 3rd, Bernard KA. Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev.* 1997;10(1):125-59. doi: 10.1128/CMR.10.1.125
3. Funke G. Corynebacteria and rare coryneforms. In: Borriello SP, Murray PR, Kunke G, Eds. Topley and Wilson's Microbiology and Microbial Infections, Bacteriology, Tenth Edition ASM Press; 2010;2:977-97. doi: 10.1002/9780470688618.taw0039
4. Bernard K. The genus *Corynebacterium* and other medically relevant coryneform-like bacteria. *J Clin Microbiol.* 2012;50(10):3152-3158. doi: 10.1128/JCM.00796-12
5. Purbasha G, Mangal KK, Sharma YK, Misra RN, Dash KN. Co-infection of Herpes genitalis with *Corynebacterium*

amycolatum: A rare case report from the district of Western Maharashtra, India. *JCDR*. 2012;6(7):1298-1300.
6. Coyle MB, Lipsky BA. Coryneform Bacteria in Infectious Diseases: Clinical and Laboratory Aspects. *Clin Microbiol Rev.* 1990;3(3):227-246. doi: 10.1128/CMR.3.3.227
7. Kalt F, Schulthess B, Sidler F, et al. Corynebacterium Species Rarely Cause Orthopedic Infections. *J Clin Microbiol*. 2018; 27;56(12):e01200-18. doi: 10.1128/JCM.01200-18
8. Funke G, Graevenitz AV, Clarridge III JE, Bernard KA. Clinical Microbiology of Coryneform Bacteria. *Clin Microbiol Rev.* 1997;10(1):126-159. doi: 10.1128/CMR.10.1.125
9. Asgin N, Otlu B. Antimicrobial Resistance and Molecular Epidemiology of *Corynebacterium striatum* Isolated in a Tertiary Hospital in Turkey. *Pathogens.* 2020;9(2):136. doi: 10.3390/pathogens9020136
10. Lagrou K, Verhaegen J, Janssens M, Wauters G, Verbist L. Prospective Study of Catalase-positive Coryneform Organisms in Clinical Specimens: Identification, Clinical Relevance, and Antibiotic Susceptibility. *Diagn Microbiol Infect Dis.* 1998;30:7-15. doi: 10.1016/S0732-8893(97)00193-4
11. Olmos CMF, Sancho JJA, Cortes JI, Ivorra JAR. Septic arthritis of the shoulder due to *Corynebacterium striatum*. *Rheumatol Clin*. 2013;9(6):383. doi: 10.1016/j.reumae.2013.02.006
12. Chandran R, Puthukkichal DR, Suman E, Mangalore SK. Diphtheroids Important Nosocomial Pathogens. *J Clin Diag Res.* 2016;10(12):DC28-DC31. doi: 10.7860/JCDR/2016/19098.9043
13. Rudresh SM, Ravi GS, Alex AM, Mamatha KR, Sunitha L, Ramya KT. Non-Diphtheritic Corynebacteria: An Emerging Nosocomial Pathogen in Skin and Soft Tissue Infection. *J Clin Diag Res.* 2015;9(12):19-21. doi: 10.7860/JCDR/2015/15580.6977
14. Clinical and Laboratory Standards Institute (CLSI). M45-A. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. Wayne, PA: CLSI; 2006.
15. Hollis DG, Weaver RE. Gram-positive organisms: A guide to identification. Special Bacteriology Section. Atlanta, Georgia: Centers for Disease Control and Prevention. 1981.
16. Shravani V, Rahman SS, Bindu H, Kapur I. Isolation of Non-Diphtherial *Corynebacterium* (Diphteroids) and its Antibiogram from Various Clinical Samples. *Ann Int Med Den Res.* 2016;2(1):151-156.
17. Reddy BS, Chaudhury A, Kalawat U, Jayaprada R, Reddy GSK, Ramana BV. Isolation, speciation, and antibiogram of clinically relevant non-diphtherial Corynebacteria (Diphtheroids). *Indian J Med Microbiol.* 2012;30(1):52-57. doi: 10.4103/0255-0857.93033
18. Leal SM, Jr, Jones M, Gilligan PH. Clinical significance of commensal Gram-positive rods routinely isolated from patient samples. *J Clin Microbiol.* 2016;54(12):2928-2936. doi: 10.1128/JCM.01393-16
19. British Standards for antimicrobial Chemotherapy. BSAC Methods for Antimicrobial Susceptibility Testing. Version 13. Approved Standard. Version 13 BSAC Document; 2014. http://bsac.org.uk/wp-content/uploads/2014/06/BSAC-disc-susceptibility-testing-method-June-2014.pdf