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Identification and antibiogram of clinically relevant non-diphtheriae *Corynebacterium* species

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ABSTRACT

Background: With the increase in immunosuppressed patients and antimicrobial misuse, Non-diphtheriae *Corynebacterium* species (NDC spp.) have risen as opportunistic pathogens in hospitalized patients. The current work aimed at comparing Vitek 2C automated identification cards versus MALDI-TOF (Matrix-associated laser-induced desorption ionization-time of flight) as the gold standard method for the identification of different NDC spp. and determining the antimicrobial susceptibility of NDC spp. by the disc diffusion method for proper management of patients. **Methods:** Thirty NDC spp. isolates were subjected to identification by MALDI-TOF MS and Vitek 2C and antibiotic susceptibility testing by the disc diffusion method. **Results:** Our study showed that *Corynebacterium (C.) striatum* was the most commonly isolated species (83.3%), followed by *C. amycolatum* (10%). 36.7 % of the isolates were recovered from blood culture while 20 % were recovered from deep wounds. As regard isolate identification by Vitek-2C ANC ID CARD compared to MALDI-TOF as the gold standard method, the agreement was 86.7%. Regarding the antimicrobial susceptibility by disc diffusion method, all the isolates showed increased resistance against penicillin (100%), ciprofloxacin (70%), gentamicin (93.3%), tetracycline (76.6%), and clindamycin (70%). On the other hand, vancomycin and linezolid showed promising results where 100 % of the isolates were susceptible. **Conclusion:** We spotlight that NDC spp. is not viewed as a contaminant organism anymore, particularly in immunosuppressed patients. Timely identification, proper clinical correlation, and appropriate therapeutic intervention can lead to favorable outcomes.

Introduction

In the last couple of decenniums, Non-diphtheriae *Corynebacterium* species (NDC spp.) was associated with multiple diseases such as skin and soft tissue infections, prosthetic joint infections, respiratory infections, meningitis, surgical joint infections, urinary tract infections, bacteremia and endocarditis [1].

Still, they are frequently neglected upon recovery from the clinical samples and regarded as skin flora. Many studies reported that these organisms can be of paramount importance, especially when isolated from patients with immunocompromising diseases, using medical devices, and on broad-spectrum antibiotics. Additionally, *Corynebacterium* (C.) species like *C.*

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jeikeium and *C. urealyticum* were found to be the causative agent of some infections among immunocompetent individuals [2].

Identifying the *Coryneform* isolates to the species level is mandatory to offer the ultimate therapeutic option because certain species, like *C. amycolatum*, *C. jeikeium*, and *C. urealyticum*, confer high resistance to antimicrobials. Despite that, some routine laboratories identify most of the isolates to the genus level because of the difficulty of the phenotypic identification of NDC [3].

Laboratory identification of NDC clinical isolates can be executed using diverse techniques such as conventional methods, including their appearance in Gram-stained films, colonial morphology, and biochemical characteristics. Automated identification systems are available as API Coryne strip and the Vitek 2C. Other advanced methods for identifying NDC are molecular assays and matrix-associated laser-induced desorption ionization-time of flight mass spectroscopy (MALDI-TOF MS) [4].

The present study aimed to compare the Vitek 2C automated identification cards with the gold standard MALDI-TOF for identifying different NDC clinical isolates and to determine their antimicrobial susceptibility profile using the disc diffusion method for proper management of cases.

Methods

Study design and study population

This cross-sectional study was conducted at the Central Microbiology Laboratory of Ain Shams University Hospitals during the period between December 2018 and December 2019. This research was approved by the Ethical Research Committee, Faculty of Medicine, Ain Shams University (Ethical approval number: FMASU MD 99/74 2019, FWA 000017585).

This study included thirty non-duplicate NDC spp. isolates recovered from different clinical specimens and submitted to the Central Microbiology Laboratory of Ain Shams University Hospitals for routine culture and antimicrobial susceptibility testing.

Inclusion criteria

We selected isolates suggested to be pathogenic if fulfilling all the following criteria: Pure or predominant growth, *Coryneform* appearance observed in the direct Gram-stained specimen with the presence of polymorphonuclear leukocytes, positive in multiple blood culture sets,

associated with risk factor as an intravenous central line, prosthetic valve, dialysis catheter, and CNS drainage device/shunt (all correlating with high colonization rate with greater risk of developing an infection in immunocompromised patients), accompanied by clinical evidence such as symptoms suggesting infections like bacteremia and deep-seated wound infections, associated with a history of immunosuppression such as the use of immunosuppressive drugs and those with debilitating diseases.

Regarding the blood culture sets, three sets were obtained, where one set was withdrawn from the central line, and the others were simultaneously withdrawn from the peripheral vein. Isolates recovered from positive blood culture from either the peripheral vein or both the peripheral vein and central line were considered pathogenic.

Exclusion criteria

we excluded isolates if patients' data were incomplete, mixed infections with no predominant growth, and positive central line blood culture only.

Isolates were preliminarily identified as *Corynebacterium* spp. by colonial morphological features (i.e. shape, pigmentation, odor, and hemolysis on blood agar), Gram-stained film, and biochemical tests (catalase test and urease test) in accordance with the identification procedures followed at the microbiology laboratory [5]. All the isolates were stored on tryptone soya broth at -70° C. All media were purchased from (Oxoid, UK).

All the 30 isolates of NDC spp. were subjected to the following:

A. Identification by the following:

1. Matrix-associated laser-induced desorption ionization-time of flight mass spectroscopy (MALDI-TOF MS)

All 30 isolates of NDC spp. were identified by MALDI-TOF MS (gold standard) (bioMérieux, France) [6]. Isolates were subcultured on 5% sheep blood agar and incubated for 24 h at 35°C in 10% CO_2. Then, we used the VITEK PICKMETM pen and nibs to pick a thin film of a fresh colony and smear it directly onto a well of the VITEK MS-DSTM48-welled target slides plate. Then we added 1 μL of ready-to-use α-cyano-4-hydroxycinnamic acid (VITEK MS-CHCATM matrix) to the organism on the target slide and allowed it to dry for 1-2 minutes to absorb energy from the VITEK MS laser and transfer it to the microorganisms to enable ionization. As recommended by the manufacturer's

instructions, we used *Escherichia coli* ATCC 8739 strain as a calibrator and internal ID control.

2. Vitek 2C ANC ID card for *Corynebacterium* species (bioMérieux, France)

The bacterial suspensions were prepared from fresh colonies in 5 ml of sterile saline to be adjusted using the VITEK2 DensiChek (BioMérieux, France) to a McFarland standard of 3.0. Then, we inoculated each suspension to an ANC card, and then cards were filled automatically in the VITEK vacuum chamber, sealed, and incubated at 35.5 °C, and read automatically every 15 min for 14 h.

B. Antibiotic susceptibility testing

The antibiotic susceptibility testing was conducted on all isolates by Kirby Bauer disc diffusion method according to EUCAST (2018), using penicillin (1 unit), tetracycline (30 ug), gentamicin (10 ug), ciprofloxacin (5 ug), clindamycin (2 ug), vancomycin (5 ug) and linezolid (10 ug) discs (Oxoid, UK).

Data management and statistical analysis

The collected data were analyzed using IBM SPSS Statistics for Windows, Version 26.0. Data were expressed as both numbers and percentages for categorized data. The following tests were done:

1. Chi-square test to study the association between every 2 variables or comparison between 2 independent groups as regards the categorized data.

2. Cohen's Kappa statistics were used to measure the agreement between every two techniques. The agreement was interpreted as follows: Kappa's 0.80-1.00 is excellent, 0.61 to< 0.80 is good, 0.40-0.60 is moderate, Fair if 0.21-< 0.40, and poor if below 0.20.

3. The probability of error at 0.05 was considered significant, while 0.01 and 0.001 are highly significant.

4. Diagnostic validity test includes agreement and disagreement between 2 methods or techniques (> 85% is excellent).

Results

Regarding the demographic data, the patients in the present study ranged from 55 to 85 years with a mean of 46.7 years. Seventeen out of 30 isolates (17/30, 56.7 %) were collected from female patients and 13 (13/30, 43.3%) from male patients.

As for the hospital departments, 63% (19/30) of isolates were collected from ICU patients, 10% (3/30) from Oncology department patients, 7% (2/30) from Geriatrics department patients, 7% (2/30) from Hematology department patients, 7% (2/30) from Pediatric department patients, 3% (1/30) from Urology department patients, and 3% (1/30) from Neurosurgery department patients.

As regards the types of samples in our study, 36.7 % (11/30) were isolated from blood culture from peripheral vein, 20% (6/30) were isolated from deep wounds, 16.7 % (5/30) were isolated from sputum culture, 16.7 % (5/30) were isolated from midstream urine culture, 6.7 % (2/30) were isolated from central line culture, and 3.3 % (1/30) was isolated from cerebrospinal fluid culture (from shunt, patient show signs and symptoms of meningitis). The distribution of the different NDC species among the various types of clinical specimens is shown in **figure 1.**

The results of identification of the 30 isolates of NDC spp. by Vitek-MS (MALDI-TOF) revealed that 25/30 (83.3%) were *C. striatum*, 3/30 (10%) were *C. amycolatum*, 1/30 (3.3%) was *C. jeikeium*, and 1/30 (3.3%) was *C. afermentans*.

Regarding identification by the Vitek 2C system ANC cards, 73.3 % (22/30) were *C. striatum*, 10% (3/30) were *C. amycolatum*, 3.3% (1/30) was *C. jeikeium*, 6.7% (2/30) were *Turicella otitidis*, and 6.7% (2/30) were unidentified.

As for the agreement in the identification results of the NDC spp. between Vitek MS and Vitek 2C system methods, **table 1** showed a statistically excellent agreement (Kappa = 0.866). It was noted that Vitek 2C failed to identify or misidentified 4 isolates: three isolates (no: 8, 25, 26) were identified by Vitek MS as *C. Striatum* and one (no: 28) was identified as *C. afermentans* (this strain is not present in the database of the Vitek 2C).

As regards the antimicrobial susceptibility by disc diffusion method, the tested isolates exhibited the highest resistance against penicillin (100 %). On the other hand, vancomycin and linezolid showed promising results where 100 % of the isolates were susceptible. **Figure 2** summarizes the results of the disc diffusion method of the thirty studied isolates.

Table 1. Agreement study between Vitek 2C and Vitek MS for the identification of 30 NDC isolates:
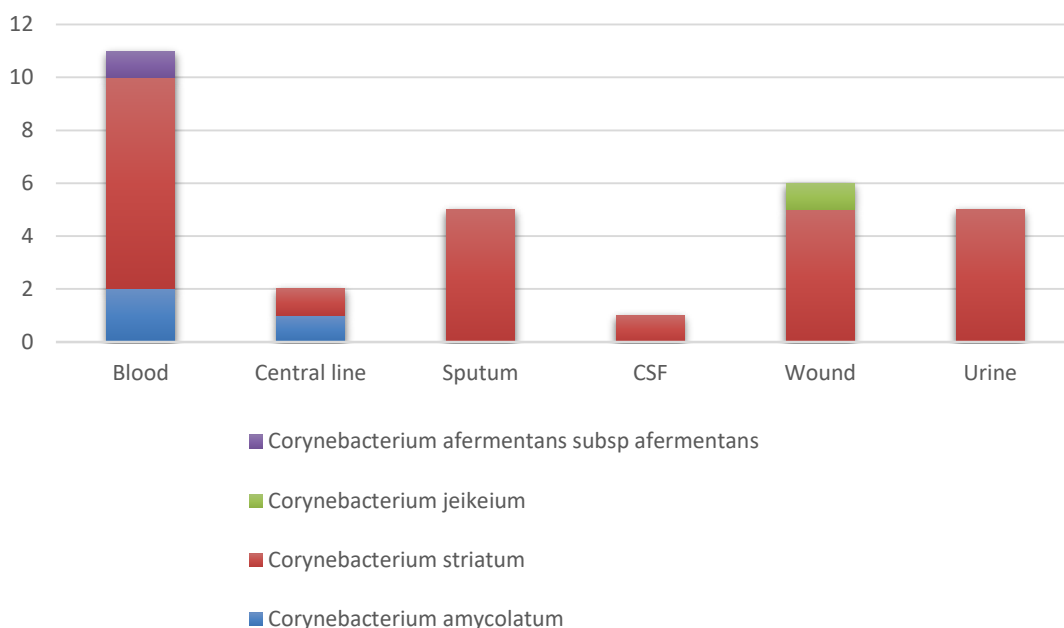
			Vitek MS				Total	Kappa agreement	P value
			C. afermentans	*C. amycolatum*	*C. jeikeium*	*C. striatum*			
Vitek 2C	*C. amycolatum*	Count (%)	0 (0.0%)	3 (100.0%)	0 (0.0%)	0 (0.0%)	3 (10.0%)	0.866	0.000
	C. jeikeium	Count (%)	0 (0.0%)	0 (0.0%)	1 (100%) 100.0%	0 (0.0%)	1 3.3%		
	C. striatum	Count (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	22 (88%)	22 73.3%		
	Turicella otitidis	Count (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (8%)	2 6.7%		
	Unidentified	Count (%)	1 (100%)	0 (0.0%)	0 (0.0%)	1 (4%)	2 6.7%		
Total		Count (%)	1 (100%)	3 (100%)	1 (100%)	25 (100%)	30 (100%)		

Table 2. The results of antimicrobial susceptibility testing by disc diffusion method of the 30 isolates included in the present study.

Sample no./species	Antibiotics	Pencillin	Ciprofloxacin	Gentamycin	Tetracycline	Clindamycin	Vancomycin	Linezolid	Sample type
1. *C. amycolatum*		R	S	S	S	R	S	S	Blood
2. *C. striatum*		R	R	R	R	R	S	S	Blood
3. *C. striatum*		R	R	R	S	R	S	S	Blood
4. *C. striatum*		R	R	R	R	R	S	S	Blood
5. *C. striatum*		R	R	R	S	R	S	S	Blood
6. *C. amycolatum*		R	R	R	R	R	S	S	Central line
7. *C. striatum*		R	R	R	R	R	S	S	Sputum
8. *C. striatum*		R	R	R	R	R	S	S	CSF
9. *C. striatum*		R	R	R	S	S	S	S	Blood
10. *C. striatum*		R	R	R	S	R	S	S	Sputum
11. *C. striatum*		R	R	R	R	R	S	S	Urine
12. *C. striatum*		R	R	R	S	R	S	S	Urine
13. *C. striatum*		R	R	R	R	R	S	S	Wound
14. *C. jeikeium*		R	R	R	R	R	S	S	Wound
15. *C. striatum*		R	R	R	R	R	S	S	Wound
16. *C. striatum*		R	R	R	R	R	S	S	Blood
17. *C. striatum*		R	R	R	R	R	S	S	Wound
18. *C. striatum*		R	R	R	R	R	S	S	Sputum
19. *C. striatum*		R	R	R	R	R	S	S	Wound
20. *C. striatum*		R	R	R	R	R	S	S	Urine
21. *C. striatum*		R	R	R	R	R	S	S	Wound
22. *C. striatum*		R	R	R	R	S	S	S	Urine
23. *C. striatum*		R	R	R	R	R	S	S	Blood
24. *C. amycolatum*		R	R	R	R	S	S	S	Blood
25. *C. striatum*		R	R	R	S	R	S	S	Central line
26. *C. striatum*		R	R	S	R	R	S	S	Sputum
27. *C. striatum*		R	S	R	R	R	S	S	Urine
28. *C. afermentans*		R	S	R	R	R	S	S	Blood
29. *C. striatum*		R	R	R	R	R	S	S	Sputum
30. *C. striatum*		R	R	R	R	R	S	S	Blood

S = Susceptible; R = Resistant.

Figure 1. The distribution of the different NDC species among the various types of clinical specimens in the current study.

■ Corynebacterium afermentans subsp afermentans

■ Corynebacterium jeikeium

■ Corynebacterium striatum

■ Corynebacterium amycolatum

Figure 2. Antibiotic susceptibility by disc diffusion method in different NDC clinical isolates in the current study. (penicillin (P), ciprofloxacin (CIP), gentamicin (CN), tetracycline (TE), clindamycin (DA), vancomycin (VA), and linezolid (LNZ)).
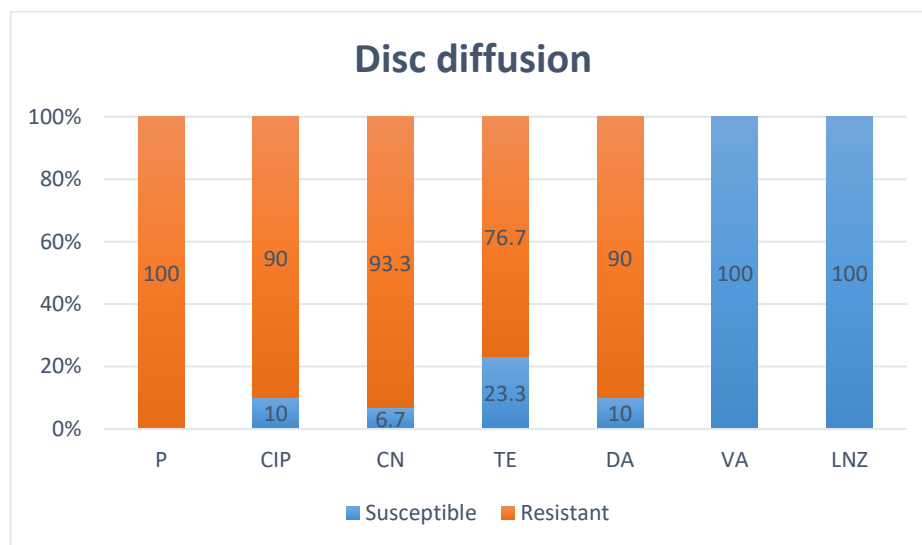
Discussion

For many decades, the pathogenic potential of NDC spp. was ignored, principally due to troublesome identification and differentiating between colonization and infection [3]. However, some studies reported that different NDC spp. have been described as causal agents of infections with high morbidity and mortality rates [7].

The present study aimed to compare the Vitek 2C automated identification cards with the gold standard MALDI-TOF for identifying different NDC clinical isolates and to determine their antimicrobial susceptibility profile using the disc diffusion method for proper management of cases.

The present study was conducted during the period between December 2018 and December 2019. A total of 30 NDC spp. isolates were recovered from different clinical specimens submitted to the Central Microbiology Laboratory of Ain Shams University Hospitals for routine culture and antimicrobial susceptibility testing.

Isolates of NDC spp. were identified phenotypically following the microbiology laboratory procedures (Gram stain, catalase, and urease) and were subjected to further subspecies identification by MALDI-TOF (reference method) and Vitek 2C ID cards. Also, their antimicrobial susceptibility profile to different antimicrobial agents was done by disc diffusion method in accordance to the recommendations of the (EUCAST, 2018) to suggest recommendations for NDC treatment.

In the present study, *C. striatum* was the most commonly identified isolate by MALDI-TOF (83.3 %) and Vitek 2C (73.3 %). 36.7 % of the isolates were recovered from blood culture while 20 % were recovered from deep wounds. The age of the patients included in the current study ranged from 55 to 85 years with a mean of 46.7 years.

Seventeen out of 30 isolates (17/30, 56.7 %) were collected from female patients and 13 (13/30, 43.3%) from male patients.

Our findings were comparable to other researchers. In a study conducted by **McMullen and his team**, they found that out of 256 isolates recovered from a variety of clinical specimens, *C. striatum* isolates were reported most commonly in wound and blood culture specimens from patients ranging from 50 to 69 years of age [8].

Also, in Canada, **Neemuchwala and his colleagues** noted that 47% of their NDC isolates belonged to *C. striatum*. Isolates were predominantly isolated from blood (24.3%), followed by sputum (19.4%). Unlike our results, their isolates were recovered mainly from male patients (51.3%), mostly from patients aged 65 or older [1].

Moreover, in China, in a study conducted by **Sun and his coworkers**, a total of 45 strains of NDC were identified by VITEK 2C and MALDI-TOF MS. Among NDC strains, *C. striatum* was the most prevailing species (15/45, 33.3%) [9].

In another study, in Sweden, **Bläckberg and his team** stated that, using the Microflex MALDI-TOF MS, *C. striatum* was the most common isolated species followed by *C. jeikeium* among patients suffering from infective endocarditis. Unlike our results, the median age of their patients was 71 years (interquartile range, 60–76) and 77% were male [10].

Likewise, **Yanai and his team** reported that C. *striatum* was the species most frequently identified in patients with bacteremia. But, they stated that the overall median age was 73 years and men constituted 74.6% of cases [11].

The difference noticed from our findings, can be attributed to many reasons such as a high number of isolates, different geographical distribution, and different clinical conditions of patients.

In the current study, the comparison of the Vitek 2C and the Vitek MS (reference method) identification results, showed that there was 86.7% agreement (26/30 isolates) for all isolates tested. It was noticed that Vitek 2C failed to identify four isolates, three isolates (no. 8, 25, 26) were identified by Vitek MS as *C. striatum,* and one (no. 28) was identified as *C. afermentans*, this strain is not available in the database of Vitek 2C. To our knowledge, no studies have made such a comparison between the two instruments regarding NDC identification, so it is difficult to compare the results presented in this study.

The different techniques show increasing performance from VITEK 2C to the most recent diagnostic system VITEK MS. This could be explained by the quality of databases, the number of species within the databases (impacting misidentification and no identification), and the individual resolution of the methods (number of substrates in the phenotypic system reducing this species discrimination) [12].

In the current study, regarding the antimicrobial susceptibility by disc diffusion method, all the isolates showed increased resistance against penicillin (100%), ciprofloxacin (70%), gentamicin (93.3%), tetracycline (76.6%) and clindamycin (70%). On the other hand, vancomycin and linezolid showed promising results where 100 % of the isolates were susceptible.

In a similar fashion to our results, in China, **Sun and his coworkers** determined the minimum inhibitory concentration (MIC) of gentamicin, rifampicin, erythromycin, clindamycin, ciprofloxacin, penicillin, cefotaxime, tetracycline, vancomycin and linezolid using broth microdilution method as per CLSI M45. All their 45 isolates were susceptible to vancomycin and linezolid. However, the majority of isolates were resistant to erythromycin (93.3%), ciprofloxacin (93.3%), clindamycin (91.1%), and penicillin (66.7%), respectively [9].

In another study in Sweden, **Bläckberg and his team** determined the MIC of penicillin G, gentamicin, rifampicin, and vancomycin, according to EUCAST, using E-test strips. Resistance to penicillin G was present in most isolates (70%), and resistance to rifampicin was detected in 50% of the isolates but all isolates were susceptible to vancomycin [10].

Moreover, in Japan, **Yanai and his team** performed antimicrobial susceptibility tests by broth microdilution using the CLSI breakpoints. All isolates were susceptible to minocycline, vancomycin, and teicoplanin. Most isolates were resistant to penicillin, imipenem/cilastatin, erythromycin, clindamycin, and levofloxacin [11].

Similarly, **McMullen and his coworkers** in their study in the USA, found that all NDC spp. were susceptible to Vancomycin and linezolid using gradient diffusion E-test [8].

Also, **Neemuchwala and his colleagues**, stated that in their study, according to the CLSI guidelines, all NDC isolates were susceptible to vancomycin and linezolid using commercial broth microdilution Sensititre GPALL1F plates [1].

The high level of resistance to penicillin (100%) in our study, in comparison to a much lower resistance observed in the above studies, may be due to differences in the geographical distribution of NDC species, uncontrolled usage of antibiotics, and varying methods used in its determination as MIC. Additionally, the interpretation may differ between different guidelines.

Conclusion

We must appreciate the importance of NDC spp. as primary pathogens. It should not be discarded as commensals or contaminants.

Regarding isolate identification by Vitek-2C ANC ID CARD compared to VITEK MS (MALDI-TOF-MS) as the gold standard method, the agreement was 86.7%. The VITEK MS is a faster diagnostic alternative and has demonstrated accurate analysis for difficult or new species.

Corynebacterium striatum was the most common and resistant among the NDC spp. In the present work, different NDC spp. displayed a high resistance pattern towards most of the tested antibiotics. However, fortunately 100% of the isolates were susceptible to vancomycin and linezolid. So, these antibiotics could be recommended as empirical treatment in bacteremia and severely ill patients.

Due to the increasing multidrug resistance of NDC spp., identification to species level is mandatory for antimicrobial susceptibility testing. Additionally, we should consider different prevention and control measures to decrease nosocomial *Corynebacterium*-related infections, which can be contracted through contact with hospital personnel colonized with multidrug-resistant NDC.

Further studies remain necessary to investigate clinical, epidemiological, and microbiological features concerning NDC infections to prevent future problems and guarantee continued vigilance by laboratories and the medical community.

Disclosure of potential conflicts of interest

The authors declare that they do not have any conflict of interest.

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Authors' contributions

All authors read and approved the final manuscript.

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