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Original Article

Microbiology Section

# Non Diphtheritic *Corynebacteria*: An Emerging Nosocomial Pathogen in Skin and Soft Tissue Infection

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## ABSTRACT

**Introduction:** Non-diphtheritic *corynebacteria* are normal inhabitants of skin and mucous membrane. When isolated from clinical specimens they are often considered as contaminants. Recent reports suggest their role as emerging nosocomial pathogens.

**Aim:** To speciate non-diphtheritic *corynebacteria* isolated from wound specimens, to correlate their clinical significance and to determine their invitro antimicrobial susceptibilities to 9 antimicrobial agents.

**Materials and Methods:** Twenty five non-diphtheritic *corynebacteria* from skin and soft tissue infections were selected for study. Isolates were identified by battery of tests and minimum inhibitory concentration (MIC) was detected by Clinical & Laboratory Standards Institute (CLSI) described broth microdilution method. MIC was interpreted according CLSI and British Society for Antimicrobial Chemotherapy (BSAC) guidelines.

**Results:** *C. amycolatum* was the predominant species (20%) followed by *C. striatum* (16%). Penicillin was least effective invitro followed by clindamycin and ciprofloxacin. Excellent activities were shown by vancomycin, linezolid and imipenem. Multidrug resistance was found in all the species.

**Conclusion:** Non-diphtheritic *corynebacteria* are potential nosocomial pathogens among acute/chronic complicated skin and soft tissue infection. Vancomycin or linezolid can be used empirically to treat such infections until the invitro susceptibility results are available.

**Keywords:** *C. amycolatum*, Diphtheroids, Multidrug resistance

## INTRODUCTION

Non-diphtheritic *corynebacteria* are aerobic and anaerobic, non-acid fast, pleomorphic, nonbranching, gram-positive rods that do not form spores. They are also called diphtheroids because of their morphologic resemblance with *Corynebacterium diphtheriae*. Human skin flora is very rich in coryneform bacteria [1]. Hence when isolated from clinical specimens they are often neglected as skin contaminants. But recent reports of increased rate of isolation evidenced their potential as emerging nosocomial pathogens among immunocompromized patients (malignancy), patients on medical device, patients receiving broad spectrum antimicrobial therapy and after invasive procedures [2-4]. Some of the species like *C. jeikeium* and *C. urealyticum* can cause infections among immune-competent persons and are true pathogens.

Emergence of antimicrobial resistance among various species of diphtheroids demands their species level identification [5]. But identification of diphtheroids to species level by routine as well as reference laboratories is confounded even after consulting all the available identification schemes [2]. Most of the research works concentrate on case reports and on particular species of diphtheroids [5].

## AIM

The aim of the present study was to speciate non-diphtheritic *corynebacteria* isolated from wound specimens, to correlate their clinical significance and to determine their invitro antimicrobial susceptibilities to 9 antimicrobial agents.

## MATERIALS AND METHODS

A prospective study on clinical samples from skin and soft tissue infections (pus, wound swab and tissue bits) submitted to the Department of Microbiology of a tertiary care medical college in south India between August 2014 and January 2015 were studied. Detailed clinical history with emphasis on prior antibiotic treatment, comorbid conditions, duration of hospital stay and previous hospitalizations was collected.

Samples were streaked on to blood agar and MacConkey's agar. Grams stain was performed on direct smears to assess the quality of specimens and presence of microorganisms. The diphtheroids were considered as clinically significant and further processed, when they were isolated in pure growth or their predominance when they are found in association with other microorganisms [6]. The identification of isolates was done based on colony morphology, pigmentation, hemolysis, presence of metachromatic granules in Albert's stain, motility and biochemical tests like catalase test, Hugh-Leifsons oxidative fermentative test, VP test, arginine hydrolysis, nitrate reduction, urease production, aesculin hydrolysis, CAMP test, and fermentation of glucose, maltose, sucrose [7-10].

**Determination of MIC:** MIC was detected by CLSI recommended Micro dilution method using Mueller Hinton broth enriched with 5% lysed horse blood in microtiter plates against vancomycin, linezolid, imipenem, gentamycin, ceftriaxone, cefotaxime, ciprofloxacin, clindamycin and penicillin. The break points [Table/Fig-1] were adopted from CLSI M45-A [11] and antibiotics for which CLSI has not defined any susceptibility criteria were adopted from BSAC guidelines [12]. Break points for susceptibility and resistance are represented in [Table/Fig-1]. Quality control was achieved by *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

Beta-lactamase detection was done using nitrocefin discs (Fluka analytical, Sigma-Aldrich, Switzerland) according to manufacturer's instructions. Briefly, nitrocefin discs were moistened with one drop

of deionized water and using a sterile applicator stick several well isolated and similar colonies were smeared onto the surface. Development of red color within 5 minutes is considered positive for Beta-lactamase production.

## STATISTICAL ANALYSIS

Statistical analysis was done using Epi Info™ 7.1.4 software program developed by Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (USA). Simple frequencies were tabulated. Chi square test was done to determine the statistical significance. A p-value of $< 0.05$ was considered as statistically significant.

## RESULTS

A total of 634 clinical samples from skin and soft tissue infections were studied. Twenty five single-patient isolates of genus *Corynebacterium* were included in the study (15 males and 10 females; mean age 47 years with range 22 to 76 years). Organisms were isolated from chronic non-healing ulcers, post slit skin graft raw areas, diabetic foot and post LSCS wound infection [Table/Fig-2]. All the patients were on broad spectrum antibiotics for prolonged period prior to sampling. The mean duration of hospital stay of patients was 14.5 days (range 7 to 30 days). Diabetes was found in 8 out of 25 cases.

Among the 25 isolates, 6 were obtained as pure growth and remaining were obtained as mixed growth, with diphtheroids as the predominant isolate [Table/Fig-3]. Direct smears stained with Gram's stain showed inflammatory cells with gram positive bacilli in 28% of the samples. Twelve different species of *corynebacterium* were isolated [Table/Fig-4]. *C. amycolatum* was the predominant species (20%) followed by *C. striatum* (16%).

Overall antibiotic resistance pattern of the isolates showed high frequency of resistance to penicillin followed by clindamycin and ciprofloxacin. Excellent activities were shown by vancomycin, linezolid and imipenem [Table/Fig-4]. Beta-lactamase production was detected in 40% of isolates.

| Antibiotic | Sensitive | Resistant |
|---|---|---|
| Penicillin | ≤1 µ/ml | ≥4 µ/ml |
| Ciprofloxacin | ≤1 µ/ml | ≥4 µ/ml |
| Cefotaxime | ≤1 µ/ml | ≥4 µ/ml |
| Ceftriaxone | ≤1 µ/ml | ≥4 µ/ml |
| Imipenem | ≤4 µ/ml | ≥16 µ/ml |
| Gentamycin | ≤4 µ/ml | ≥16 µ/ml |
| Vancomycin | ≤4 µ/ml | - |
| Linezolid | 0.06 µ/ml | - |
| Clindamycin | ≤0.5 µ/ml | ≥4 µ/ml |

**[Table/Fig-1]:** Breakpoints for *Corynebacterium* spp.

| Antibiotic | Sensitive | Resistant |
|---|---|---|
| Surgery male ward | 8 | Cellulitis of limbs with non-healing ulcers, diabetic foot, road traffic accidents with crush injuries, post-operative wound infections |
| Plastic surgery | 6 | Post slit skin graft wound infection |
| Gynecology ward | 6 | Post LSCS wound infection |
| Medical ward | 2 | Cellulitis of limb with wound |
| ICU | 2 | Post-operative wound discharge, diabetic keto-acidosis with carbuncle |
| Surgery female ward | 1 | Cellulitis |

**[Table/Fig-2]:** Distribution of cases infected with diphtheroids in different wards and their clinical conditions.

| Type of growth | Organism grown | Number | % |
|---|---|---|---|
| Pure growth of diphtheroids | | 6 | 24 |
| Diphtheroids along with other bacteria | *E coli* | 6 | 24 |
| | *Klebsiella* | 3 | 12 |
| | *Pseudomonas aeruginosa* | 3 | 12 |
| | *Staph aureus* | 3 | 12 |
| | *Moraxella* spp | 2 | 8 |
| | *Enterococcus* | 1 | 4 |
| | Group C *Streptococcus* | 1 | 4 |
| | Total | 25 | 100 |

**[Table/Fig-3]:** Organisms isolated along with diphtheroids.

## DISCUSSION

*Corynebacterium* spp can cause both acute and chronic wound infection [4,13]. When a non-diphtheritic *corynebacteria* is isolated from clinical specimen, detailed patient profile and repeat microbiological analysis should be done before reporting it as contaminant [2,10]. Antimicrobial susceptibility patterns of *corynebacteria* are not predictable and hence detection of antimicrobial susceptibility may be necessary in order to obtain the best therapeutic results.

In the present study all the diphtheroids were isolated from inpatients. Twenty four percent of samples grew diphtheroids in pure growth and 76% of isolates were obtained along with other bacteria. *E coli* (24%) was the commonest bacteria associated with diphtheroids followed by *Klebsiella* spp (12%), *Pseudomonas aeruginosa* (12%), *Staphylococcus aureus* (12%), *Moraxella* spp (8%), *Enterococcus* spp (4%) and Group C *Streptococcus* spp (4%). Initially cases were treated according to the susceptibility pattern of associated bacteria considering diphtheroids as possible skin commensals. After complete course of antibiotics, wound did not showed any signs of healing and repeat culture obtained same diphtheroids. Customized therapeutic combinations were designed according to the sensitivity pattern of diphtheroids and associated microorganisms for each patient. Treatment with such regimen showed very good response. The isolation of *Corynebacterium* species from clinically apparent skin and soft tissue infection and healing of lesions after appropriate antibiotic therapy suggest the pathogenic role of these organisms in our patients.

Current CLSI guidelines recommend detection of MIC as standard method of determining antibiotic sensitivity. Though BSAC recommend MIC as the standard method, it also recommends use of disc diffusion testing for few antibiotics [12]. CLSI recommends detection of MIC using Mueller Hinton broth enriched with 5% lysed horse blood in microtiter plates as standard method [11]. Many laboratories fail to determine MIC because of its complex procedure and lack of technical expertise. Automated systems like Vitek 2/ Phonix/API can determine sensitivity of diphtheroids [14]. But very few laboratories have this facility.

In the present study vancomycin (100%) was the most active drug against diphtheroids invitro followed by linezolid (96%) and imipenem (92%). Penicillin (0%) was least active drug followed by clindamycin (28%) and ciprofloxacin (32%). Similar findings were made by Soriano et al., and Camello et al., [5,15]. Though vancomycin is the most active drug, resistance to the drug has been reported in *C. aquaticum* and CDC group B1 [15]. An isolate of *C. riegelii* showed an MIC of 16 µg/ml against linezolid. Resistance break point to linezolid for diphtheroids is not defined and hence judicious use of this drug is essential in clinical practice. Imipenem resistance was noted in one isolate of CDC group G from a case of post slit skin graft wound infection and one isolate of *C. urealyticum* from diabetic ketoacidosis with carbuncle. Both the patients were previously treated with carbapenems for non-healing ulcers leading to selection of imipenem resistant corynebacteria. Multidrug resistance was found in all *Corynebacterium* spp. Nosocomial outbreak of clonal multidrug resistant *Corynebacterium* spp has been recently reported [16].

| Organism (n) | Percentage susceptible {n (%)} | | | | | | | | | Beta-lactamase positive |
|---|---|---|---|---|---|---|---|---|---|---|
| | Va | LZ | IPM | Gen | CTX | CTR | CIP | CD | P | |
| *C. amycolatum* (5) | 5 (100) | 5 (100) | 5 (100) | 4 (80) | 4 (80) | 3 (60) | 3 (60) | 3 (60) | 0 (0) | 2 (40) |
| *C. striatum* (4) | 4 (100) | 4 (100) | 4 (100) | 3 (75) | 3 (75) | 3 (75) | 3 (75) | 3 (75) | 0 (0) | 1 (25) |
| *C. simulans* (3) | 3 (100) | 3 (100) | 3 (100) | 2 (67) | 2 (67) | 2 (67) | 0 (0) | 0 (0) | 0 (0) | 2 (67) |
| CDC group G (3) | 3 (100) | 3 (100) | 2 (67) | 1 (33) | 1 (33) | 1 (33) | 1 (33) | 1 (33) | 0 (0) | 1 (33) |
| *C. confusum* (2) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 2 (100) |
| *C. glucuronolyticum* (2) | 2 (100) | 2 (100) | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (50) |
| C. argentoratense (1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 1 (100) |
| *C. riegelii* (1) | 1 (100) | 0 (0) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 0 (0) |
| C. *sanguinis* (1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| C. *accolens* (1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| *C. urealyticum* (1) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| C. *aurinucosum* (1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Total (25) | 25 (100) | 24 (96) | 23 (92) | 17 (68) | 16 (64) | 15 (60) | 8 (32) | 7 (28) | 0 (0) | 10 (40) |

**[Table/Fig-4]:** Antibiotic susceptibility of pattern of isolates.
Va=vancomycin, LZ=linezolid, IPM=imipenem, GEN=gentamycin, CTR=ceftriaxone, CTX=cefotaxime, CIP=ciprofloxacin, CD=clindamycin and P=penicillin

In this study, chronic non-healing ulcers, advanced age, diabetes, longer duration of hospital stay and prolonged antibiotic therapy were the risk factors for diphtheroids infection. Most of the cases were from male and female surgical wards (n=9), plastic surgery ward (n=6) gynecology post-operative ward (n=6), intensive care unit (ICU) (n=2) and medical wards (n=2). Higher occurrence of cases in particular wards and multi-drug resistance among the strains, suggest the probable nosocomial origin of these bacteria. Coyle et al., using plasmid profiling as an epidemiological tool showed diphtheroids spread in hospitals from person to person and airborne modes [2]. Environmental contamination of wards may be the common source for infection. Proper infection control and surveillance activities are needed to control such infections.

## LIMITATION

Limitation of the present study was smaller sample size. Further study has to be done on isolates obtained from larger geographical area to know the exact prevalence of skin and soft tissue infections due to non-diphtheritic corynebacteria.

## CONCLUSION

We conclude that non-diphtheritic *corynebacteria* are potential nosocomial pathogens among acute/chronic complicated skin and soft tissue infection. Wound infection with such organism causes delayed healing, raised treatment costs, and resource demanding wound management practices. We recommend vancomycin or linezolid be used empirically to treat complicated skin and soft tissue infections with non-diphtheritic *corynebacteria* and the ultimate therapeutic regimen against these organisms should be chosen according to the invitro susceptibility results, the site of the infection and associated microorganisms.

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