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# Non-diphtheriae *Corynebacterium* Species as Emerging Pathogens: Case Series from a Tertiary Care Hospital in Western Nepal

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## ABSTRACT

**Background:** *Corynebacteria* other than *C diphtheriae* are often regarded as commensals of the skin and mucosa. However, these *Corynebacterium* species were recently recognized as emerging pathogens, causing systemic infections. Six cases due to non-diphetherial *Corynebacteria* as primary pathogens are reported here.

**Methods:** This was an observational study. Therapeutic outcome along with demographic and clinical details were obtained from the patients' records. Organisms were identified and antibiotic sensitivity testing was performed by conventional techniques.

**Results:** *C. xerosis* was isolated from three patients: one with non-healing wound over the face following scald injury; second, a post-operative wound infection; and third, a case of neonatal sepsis. Two cases with acute exacerbation of COPD (case 3 and 5), yielded *C bovis* and *Corynebacterium* spp. respectively. The later had a prolonged hospital stay showing concurrent infections with *Acinetobacter* and *Candida spp. C falsenii*, an unusual pathogen was recovered from blood of a 2 year septicemic child . The patients responded to appropriate antibiotic therapy and made substantial recovery.

**Conclusions:** This case series highlights the importance of non *C. diphtheriae Corynebacteria* as primary pathogens. Non-diphtherial *Corynebacteria* should not be discarded as commensals or contaminants. Timely identification, proper clinical correlation and appropriate therapeutic intervention can lead to favorable outcome.

***Keywords: Non-diphtherial Corynebacteria, COPD, Sepsis, Wound infection***

## INTRODUCTION

The genus *Corynebacterium* comprises *C. diphtheriae* and non-diphtherial *Corynebacteria,* known as diphtheroids. Diphtheroids have traditionally been considered part of the normal commensal flora of the skin and mucous membranes, most importantly the mucosa of the urinary tract and conjunctiva. It was estimated that about 12-30% of humans carry diphtheroids as part of the normal skin flora (Funke *et al*., 1997). However, recent data show that diphtheroids, including *C. urealyticum, C. jeikeium* and *C. striatum* can be incriminated as the etiologic agents of diseases in the very young, the elderly, and in individuals who are immunocompromised or have other associated pathological conditions of urinary or respiratory tract (Lee, Ferguson and Sarubbi, 2005; Superti *et al*., 2009).

Being ubiquitous in both nature and animals, it is not surprising that some of the diphtheroids can be isolated as pathogens if they are looked for with high index of suspicion in certain groups of patients or from particular anatomical sites.

Here, we describe series of infections due to different *Corynebacterium* species. These patients had clinical evidences of systemic as well as localized skin and soft tissue infections, and were either admitted to various wards or attended the out patients department of the Manipal Teaching Hospital, a tertiary care hospital in Western Nepal.

## CASE REPORTS

Case 1.

A 14 day old female baby was admitted with neonatal

respiratory depression. Her heart rate was 84/minutes with severe bradycardia. She was resuscitated with ambu bag followed by oxygen supplement through face mask. She developed fever of 103°F on the next day. On suspicion of septicemia, blood for culture was collected onto biphasic medium. After 24 hours, growth was flagged due to turbidity of the liquid medium. Gram staining from the broth revealed Gram positive coccobacilli. Subculture on blood agar exhibited confluent pure growth of cream colored non haemolytic colonies. A gram stain from these colonies showed gram positive club shaped pleomorphic bacilli with palisade arrangement. The colony was inoculated onto Loeffler's serum slope (LSS). Albert's stain from the LSS after 6 hours of incubation showed thin bacilli with metachromatic granules (Fig.1). As depicted in table 1, the organism was identified as *Corynebacterium xerosis* by interpretation of various phenotypic characters (Forbes, Sahm and Weissfeld, 2007). This isolate was sensitive to erythromycin, ampicillin, ciprofloxacin, amikacin, cephazolin, chloramphenicol, cotrimoxazole, gentamicin and vancomycin. The patient was treated with IV ampicillin 50 mg/kg TID and IV gentamicin 1.7mg/kg TID. After 4 days the patient became afebrile, with vital functions, including cry, returning to normal. The patient was kept under follow up and was advised universal immunization schedule.

Case 2

A 2 year young male child was admitted as a case of acute bronchitis and suspected septicemia. The parents had noted that child brought out small quantity of blood stained sputum. Blood culture after 24 hours of incubation showed distinct turbidity. On subculture, tiny and dry nonhaemolytic colonies grew on blood agar after overnight incubation at 370 C. The organisms were subsequently identified as *Corynebacterium falsenii* based upon phenotypic studies (table 1). Antibiotic sensitivity pattern revealed that the organism was sensitive to erythromycin, cefotaxime, cotrimoxazole, vancomycin, while it was resistant to cephazolin, cefoxitin, ofloxacin and gentamicin. The child was treated with cefotaxime 5mg IV tds along with syrup paracetamol 5ml TID. He was symptom free on day 5 and was discharged from the hospital.

Case 3

An 81 year old male patient was hospitalized for shortness of breath and severe discomfort in the chest for the last one day. He was diabetic and had past history of chronic obstructive pulmonary disease (COPD) for the last five years. He also gave history of tobacco exposure in the form of cigarette smoking for more than past twenty years. He had chronic cough which was productive in nature and had frequent episodes of cough in the past few years lasting for 3-4 months in each episode. Besides, he complained of shortness of breath. On examination, the patient was afebrile with respiration rate of 22/minute, pulse rate of 70/minute. His BP was of 160/100 mm Hg. There were mild crepitations and rhonchi on both the lung fields, especially in the infrascapular areas. There were typical features of increased frequency in the shortness of breath and increase in the severity of coughing along with copious amount of expectoration. Thus the case was diagnosed as acute exacerbation of COPD.

Sputum was sent for examination in the laboratory. Before processing, the sample was subjected to gram staining to confirm that it was a proper representative of the lower respiratory tract in accordance with the Murray and Washington criteria (Murray and Washington, 1975). Gram staining of the specimen showed gram positive short bacilli with palisade type of arrangement along with pus cells. The sample was inoculated onto 5% sheep blood agar and chocolate agar. After overnight incubation at 37°C a confluent pure growth of tiny off white opaque non haemolytic colonies developed on the blood agar plate. Gram stain of the colony exhibited microscopic morphology comparable to the one observed under direct microscopy resembling diphtheroids. Albert's staining of the specimen showed short bacilli with metachromatic granules. The organism was catalase positive, oxidase negative, did not reduce nitrate, did not hydrolyse either urea or esculin, and fermented only glucose, and thus was speciated as *Corynebacterium bovis* as per the criteria laid down in table 1 (Forbes, Sahm and Weissfeld, 2007) . Antibiotic sensitivity testing showed that this organism was sensitive to amoxicillin clavulanic acid, erythromycin; but resistant to ciprofloxacin, ceftazidime, ceftriaxone and tetracycline. The patient had already been

administered cefixime IV TID and azithral 500mg IV OD empirically due to the acute exacerbation. In addition, nebulisation was given every 6 hourly basis along with IV hydrocortisone 100mg TID and Pantocil 40mg IV OD. After 9 days staying in the hospital, he was discharged with considerable improvement in his signs and symptoms.

Case 4

A male child aged 5 years was admitted to the Manipal Teaching Hospital with 7% scald over the face. In spite of regular wound dressing and care, as well as topical application of antibiotics, healing was poor that necessitated wound debridement. The debrided material was sent to the microbiology laboratory for culture and sensitivity.

Gram stained smear of the material showed gram positive cocci arranged mostly in clusters, as well as gram positive club shaped bacilli with pleomorphic morphology and palisade arrangement. The sample was inoculated onto blood agar and Mac Conkey's agar medium. After overnight incubation at 37°C blood agar showed confluent growth of non haemolytic cream colored tiny colonies and golden yellow colored opaque round colonies after overnight incubation at 37°C. A gram stained preparation from the same colonies revealed pleomorphic gram positive bacilli (GPB) and gram positive cocci. LSS was inoculated with the GPB like colony from the blood agar plate and incubated at 37°C for 6 hours. Albert's stain made from the growth on the serum slope exhibited thin bacilli with abundant metachromatic granules.

The diphtheria like organism was identified as *C xerosis* on the basis of the characteristics mentioned for Case 1, above (table 1). Following debridement, the patient was treated with injection fluclox 300mg QID, local application of ointment gentamicin, aciloc 300mg IV OD and ointment supar BC (zinc oxide topical) at bed time. Patient recovered well and the wound healed in a 12 days' time. He was then referred to Sushma Memorial Hospital, Kathmandu for cosmetic therapy.

Case 5

A 61 year male patient was admitted to the hospital with the complaints of shortness of breath which was of acute onset and productive cough since past two days. On examination, the patient was afebrile with respiration rate of 20/minute, pulse rate of 78/minute and BP of 148/100 mm Hg. There were mild crepitations and rhonchi on both lung fields, heard over both the infrascapular regions. Besides, he had typical features of severity in the shortness of breath and increase in the severity of cough and in the volume of expectoration. Thus the case was diagnosed as acute exacerbation of COPD. The patient also had pulmonary hypertension. Sputum for culture was sent on the day of admission. The gram stained smear of the sputum samples was analyzed first for its suitability on the basis of standard criteria (Riegel *et al*., 1996). The smear showed gram positive short bacilli with palisade type of arrangement along with plenty of pus cells. The sample was inoculated onto 5% sheep blood agar and chocolate agar. After overnight incubation at 37°C a confluent pure growth of tiny off white opaque nonhaemolytic colonies developed on the blood agar plate. Gram stain of the colony exhibited morphology quite comparable to the one observed under direct microscopy resembling diphtheroids. Albert's stain performed from the growth on Loeffler's serum slope inoculated with a colony from blood agar showed short bacilli with metachromatic granules. The organism was catalase positive, oxidase negative, urease negative, reduced nitrate to nitrite, did not hydrolyse esculin, and fermented glucose and maltose. On the basis of the afore mentioned criteria, it was identified as *Corynebacterium* species (table 1) and antibiotic sensitivity pattern showed this organism to be sensitive to ciprofolxacin, but resistant to erythromycin, azithromycin, cefotaxime, amoxycillin clavulanic acid.

The patient was treated with IV azithral 500mg OD, nebulisation every 6 hourly and suction SOS. However, his condition deteriorated. Four days later, blood and the tip of the suction tube were sent for culture. None of the samples grew any organism. His condition worsened and after a period of another 3 days, repeat samples of sputum, blood and urine were sent for culture. Though blood and urine were sterile, sputum showed mixed growth of *Acinetobacter* species and *Candida* species. *Acinetobacter* was sensitive to ciprofloxacin, imipenem, ceftriaxone, piperacillin, piperacillin tazobactam, but resistant to cefazoline and amikacin. There was improvement in his condition after the antibiotic regime was modified to IV ceftriaxone. He was discharged from the hospital after a stay of 20 days with the advice to come for regular

follow up for COPD.

Case 6

A primigravida aged 25 years underwent left sided caesarian section (LSCS) at 38 weeks of gestation. She had past history of bronchial asthma and allergic rhinitis for which she was regularly being treated at the Manipal Teaching Hospital. There were no obstetrics complications and she delivered a full term baby weighing 2.8 kg .She was discharged from the hospital on the second day following child birth. Ten days later, she presented with serous discharge from the operated wound. On examination, her body temperature was 98.60 F, BP was 110/70 mm Hg. All other vital functions were within normal limits. The discharging wound was indurated, tender, nonfloctuant, and there was obvious gaping of the wound.

A wound swab was collected and sent to the laboratory for culture and sensitivity, and she was started with capsule fluclox 500mg QID, tablet chymoral forte BD and tablet chewvit 1 OD. The swab on gram staining showed gram positive club shaped bacilli with pleomorphic morphology and palisade arrangement. Culture showed confluent growth of nonhaemolytic cream colored tiny colonies on the blood agar after overnight incubation at 37°C.

A gram stained preparation from the same colonies revealed gram positive pleomorphic bacilli resembling diphtheroids. A Loaffler's serum slope was inoculated with the colony from the blood agar plate and incubated at 37°C for 6 hours. Albert's stain made from the serum slope exhibited thin bacilli with abundant metachromatic granules. The diphtheria like organism was identified as *C. xerosis* on the basis of the characteristics mentioned above (table 1). The organism was sensitive to ciprofloxacin, but resistant to erythromycin, ampicillin, azithromycin, and penicillin. The patient improved with the aforementioned treatment and the wound healed.

***Table 1:***
***Cultural characteristics biochemical properties and antibiotic sensitivity pattern of the isolates***

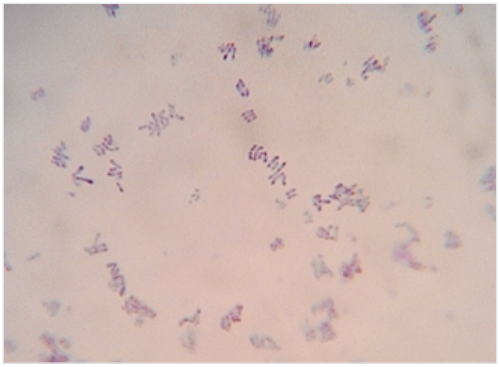
| Case Sl No. | Colonies on SBA and microscopic features of Gram and Albert stained smears | Urea hydrolysis | Nitrate reduction | Esculin hydrolysis | G | M | S | Identification* | Sensitivity pattern | Resistance pattern |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Creamy nonhemolytic, Pleomorphic GPB, with metachromatic granules | -ve | +ve | -ve | F | F | F | *C xerosis* | Erythromycin, ciprofloxacin, amikacin, cephazolin, chloramphenicol, cotrimaxozol, gentamicin, vancomycin | None |
| 2 | Tiny, dry nonhemolytic, GPB, with metachromatic Granules | +ve | +ve | +ve | F | NF | NF | *C falsenii* | erythromycin, cefotaxime, cotrimoxazole, vancomycin | cephazolin, cefoxitin, ofloxacin, gentamicin |
| 3 | Tiny, off white opaque, nonhemolytic, GPB,with Metachromatic granules | -ve | -ve | -ve | F | NF | NF | *C bovis* | amoxicillin-clavulanic acid, erythromycin | ciprofloxacin, ceftazidime, ceftriaxone, and tetracycline |
| 4 | Tiny, creamy, nonhemolytic, GPB, with metachromatic Granules | -ve | +ve | -ve | F | F | F | C xerosis | Erythromycin, ciprofloxacin, amikacin, cephazolin, chloramphenicol, cotrimaxozol, gentamicin, vancomycin | None |
| 5 | Tiny off white, opaque, nonhemolytic, GPB, with Metachromatic granules | -ve | +ve | -ve | F | F | NF | *Coryne-bacterium spp* | ciprofolxacin | erythromycin, azithromycin, cefotaxime, and amoxycillin-clavulanic acid |
| 6 | Tiny, creamy, Nonhemolytic, GPB,with Metachromatic granules | -ve | +ve | -ve | F | F | F | C xerosis | ciprofloxacin | erythromycin, ampicillin, azithromycin, and penicillin |

SBA= sheep blood agar, GPB=Gram positive bacilli, G=glucose, M=maltose, S=sucrose, F=fermented with production of acid only, NF=not fermented
*All isolates were catalase positive and oxidase negative

## DISCUSSION

*C. diphtheriae* is a primary pathogen of the respiratory tract and skin with clinical presentation due to the effects of its toxin on target organs (Fig.1). *Corynebacteria* other than *C diphtheriae* are not paid enough attention as these are often part of normal skin and eye flora. Sometimes growths of *Corynebacteria* other than *C diphtheriae* from specimen of blood, wound swab, sputum are dismissed as contaminants and are not studied further.

***Figure 1: Albert's stain of the growth on Loaffler's serum slope showing pleomorphic short bacillary forms with distinct metachromatic granules***



During the study period from 14.12.2014 to 27.11.2015 six cases (table 2 showing all patient details) were found affected by *Corynebacteria* other than *C. diphtheriae.* In two cases, the organisms were isolated in pure culture from blood, in two cases from sputum and in one case from pus specimen. In one case *C. xerosis* was obtained as co infecting agent with *Staphylococcus aureus* from postoperative wound infection.

The present series of cases demonstrated that non-*C. diphtheriae Corynebacteria* also deserved attention as these could be primary pathogens. All these isolates were obtained from long standing open wound infections, respiratory tract infections and blood stream infections in the elderly, debilitated, and immunocompromised individuals. Multidrug resistance among the isolates also has a remarkable finding as reported previously (Riegel et al., 1996; Brandenburg *et al*., 1996). Thus isolation of non-*C diphtheriae Corynebacteria* from clinical specimens, especially in the above mentioned settings should not be ignored in view of its propensity to establish nosocomial infections (Brandenburg *et al*., 1996). Although, the mortality rates in most infectious episodes were observed to be low, mild skin and soft tissue infections might lead to bacteraemia. Even bacteremia, in immunocompromised patients and in those dealing with medical devices in the ICUs, emphasizes the need for prompt and accurate laboratory diagnosis and timely therapeutic intervention (Soriano *et al*., 1998).

In this study, different non- *C. diphtheriae Corynebacteria* from various clinical specimens have been reported. There were *C. xerosis* in three patients, *C. bovis* in one, *C falsenii* in one and unspeciated diphtheroid in one patient. Though *C. xerosis* has been shown as a normal inhabitant of human skin and eye, there has been an increased number of case reports during the past few years, claiming an association of *C. xerosis* with conditions such as septicemia,

***Table 2. Clinical Details of the Cases***

| Case No.<br>Age<br>gender | Hospital stay | Clinical presentation | Diagnosis | Treatment | Specimen(s) cultured | Organism (s) identified |
|---|---|---|---|---|---|---|
| Case 1<br>14 days<br>female | 4 days | Poor respiratory effort | Neonatal depression, sepsis | Ampicillin, Gentamicin | Blood | *C. xerosis* |
| Case 2<br>2 years<br>male | 5 days | Cough, blood tinged sputum, Fever | Bronchopneumonia , septicemia | Cefotaxime | Blood | *C. falsenil* |
| Case 3<br>81 years<br>Male | 9 days | Shortness of breath | Acute exacerbation of COPD | Cefixime , Azithromycin | sputum | *c. bovis* |
| Case 4<br>4 years<br>M ale | 12 days | Scald over face 7% | Scald over face 7 % | Flucloxacillin | Debrided tissue | *C. xerosis , S aureus* |
| Case 5<br>61 years<br>Male | 20 days | Shortness of breath,<br>Cough with expectoration | Acute exacerbation of COPD | Azithromycin, Ceftriaxone | Sputum<br>Urine<br>Blood | *Corynebacterium spp*<br>*(a). Acinetobacter spp*<br>*(b). Candida spp*<br>No growth<br>No growth |
| Case 6<br>25 years<br>female | Out-patient care | Purulent discharge from surgical wound | Wound infection | Flucloxacillin | Wound swab | *Corynebacterium xerosis* |

endocarditis, pleuropneumonia, peritonitis, osteomyelitis, septic arthritis, mediastinitis, meningitis especially in the immunocompromised patients and in those recovering after measure surgeries (Pessantha *et al*., 2003; Cattani *et al*., 2000).

During the study period, *C. xerosis* was detected in one neonate with bacteremia and having two deep wounds such as scald and LSCS. In two of these cases of wound infections, the samples were obtained from the deeply inflamed site; in one it was the debrided wound specimen and in the other it was the abscess drain. Thus chances of skin flora *C. xerosis* contaminating the specimen were less. One of our patients (case No.1) was a neonate with very poor respiratory effort at birth. During the hospital stay, the baby developed fever and *C. xerosis* was isolated from the blood. *C. xerosis* has been implicated in the past in causing blood stream infections in the neonates (Cattari *et al*., 2000; Poorschen, Goodmen and Rafai, 1977).

The patient in Case 2 was a child aged 2 years presenting with acute respiratory infection and high grade fever. In this child sputum did not grow any pathogen. However blood culture revealed growth of C falsenii. It is a zoonotic pathogen, producing oral lesions in avian species (Fernandez *et al*., 2003). Tam *et al*. (2010) isolated *C. falsenii* from the blood sample of a 13 month old baby who was suffering from unexplained febrile episode where vancomycin was administered through the central vascular line for treating infected skin lesion in the thigh. This case study describes the importance of *C falsenii* blood isolates. Therefore, before discarding these pathogens as mere contaminants, one should know and have proper judgment regarding its clinical significance.

In this series, there were two cases of COPD with acute exacerbations. In both these cases, sputum culture yielded *Corynebacteria*; *C. bovis* in one and *Corynebacterium* sp in the other. *C bovis* as a causative agent of eye and soft facial tissue infection was recently documented (Chow, Bui and Clarridge, 2015). Previously COPD patients were shown to have superadded infection due to *C striatum*, an emerging pathogen having potential to cause outbreaks of various other respiratory as well as skin and soft tissue nosocomial infections (Funke *et al*., 1997; Lee, Ferguson and Sarubbi, 2005). It was, therefore, emphasized that speciation ought to be attempted in all cases where there was heavy and pure growth of *Corynebacteria* on culture (Fernandez et al., 2003; Chow, Bui and Clarridge, 2015).

## CONCLUSION

It is concluded that non-*C diphtheriae Corynebacteria* are emerging potentially pathogenic micro-organisms, some of them showing multidrug resistance. This case series highlights the growing importance of these bacteria as nosocomial pathogens. However, timely diagnosis and prompt therapeutic intervention can lead to favorable outcome.

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