

Corynebacterium amycolatum: An underestimated pathogen presenting as chronic nodules in the concha and external auditory canal– An unique case report from central Kerala

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Abstract

Aim and background: Corynebacterium amycolatum, is an emerging pathogen in various infectious diseases especially in case of ear infections. Here we are reporting a rare case which presented as multiple oozing nodules in the right external auditory canal and surrounding conchal region.

Case description: A 46 year old male, presented with multiple nodular lesions in the right external auditory canal and concha with a 15 year history of itching, oozing and bleeding from the nodules, which temporarily subsides on starting medications only to recur again. He had undergone incision and drainage for the same earlier only for the nodules to reappear.

Surgical excision of the nodules was done and the specimen send for microbiological evaluation. Routine culture grew moderate growth of Corynebacterium spp and scanty growth of Staphylococcus aureus. Corynebacterium spp was identified as C. amycolatum by matrix associated laser desorption ionisation time of flight (MALDI TOF). Treatment was given with regular dressing and oral medication.

Conclusion: Evaluation of chronic ear infections should include uncommon causative agents and the presence of non diphtheria Corynebacterium should be given due diligence before rejecting it as part of normal Microbiota. Correct antibiotic treatment should be initiated based on species susceptibility.

Clinical significance: This case report provides information that *C. amycolatum* could be a potential ear pathogen especially in patients who have had previous surgical interventions. It is likely that many infections are missed as this organism is often mistaken as normal skin flora or a contaminant. Our findings suggest that *C. amycolatum* is a potential pathogen even when it is isolated along with another pathogenic organism (*S. aureus*) *S. aureus* must be contributing to the acute nature while *C. amycolatum* contributes to the chronic presentation.

Keywords: Ear Infections, Mixed Infection, *Corynebacterium Amycolatum*, Antibiotic Resistance, MALDI TOF

Introduction

Non diphtheria *Corynebacterium* species are a group of Gram positive bacilli long regarded as mere commensals of the skin and mucous membranes, and are often interpreted as contaminants in clinical specimens.¹ It is widely distributed in environments such as soil, water, plant and animal material. *C. amycolatum* was first identified by Collins and Burton on human skin in 1988. It is widely recognized as an emerging pathogen in infectious diseases, especially in nosocomial settings and among Immunocompromised patients.²

C. amycolatum derives its name from lack of mycolic acid in its cell wall unlike other *Corynebacteria*. Despite the absence of corynemycolic acid in the cell wall, 16S rRNA sequencing confirmed its inclusion in *Corynebacterium* genus.³ Treating *C. amycolatum* infections is challenging due to frequent multidrug resistance as most isolates are resistant to penicillins, clindamycin, erythromycin, and fluoroquinolones, but may only be susceptible to vancomycin and linezolid.²

Case Report

A 46 year old Immunocompetent man, who is a welder by profession, presented in our Department of Otorhinolaryngology at Caritas Institute, a tertiary care hospital in Kottayam, central Kerala, India, with intense itching, oozing and bleeding from multiple nodules in the conchal region and external auditory canal of right ear. He has no history of diabetes mellitus, hypertension or any other chronic illness other than the presenting condition. He gives a 15 year history of symptoms, which temporarily subsides on starting medications only to recur again. He had undergone incision and drainage for the same earlier only for the nodules to reappear. After acute inflammation had settled, the nodules were surgically excised and a portion of the excised tissue specimen was sent to the Department of Microbiology. On microscopy, moderate polymorphonuclear cells with numerous Gram positive bacilli which were arranged in palisades resembling the typical morphology of the diphtheroids and scanty Gram positive cocci arranged singly and in pairs were seen. No granules were seen in the diphtheroids. Acid fast staining revealed no acid fast organisms. Smears for fungal elements were also negative. Due to the chronic clinical presentation aerobic, anaerobic as well as fungal cultures were mounted. Aerobic culture was done on blood, chocolate and MacConkey agar. All 3 cultures showed moderate growth of fine colonies and scanty larger white colonies after overnight incubation. After 48 hours of incubation, the fine minute colonies were found to become flat, dry, creamy white, non lytic colonies, which showed Gram positive bacilli in palisade arrangement and scanty, larger, beta hemolytic golden yellow colonies which showed Gram positive cocci arranged in clusters on Gram staining. The organisms were identified

presumptively as *Corynebacterium* spp and *Staphylococcus aureus* by biochemical characterisation. *Corynebacterium* species produced catalase and oxidase was negative. It did not reduce nitrate, hydrolyse esculin or utilize urease. *Staphylococcus aureus* produced catalase, was slide and tube coagulase positive and produced yellow colonies on mannitol salt agar. Traditional bacterial identification methods, such as phenotypic and biochemical techniques, often fail to reliably identify *Corynebacterium* spp. at the species level. As MALDI-TOF MS is a reliable method for identifying *Corynebacterium* spp the isolate was sent for MALDI TOF and was identified as *Corynebacterium amycolatum*.

Antibiotic susceptibility testing was done by Kirby Bauer disk diffusion method according to CLSI M 100 36th edition guidelines for *Staphylococcus aureus* and EUCAST version 16 guidelines for *Corynebacterium* species.^{4,5} *S. aureus* showed susceptibility after 24 hours of incubation to penicillin, cefoxitin, oxacillin, cotrimoxazole, clindamycin, doxycycline, ciprofloxacin, levofloxacin, mupirocin, gentamicin, vancomycin and linezolid whereas erythromycin was resistant. *Corynebacterium amycolatum* showed susceptibility after 48 hours of incubation to tetracycline, rifampicin, moxifloxacin, vancomycin and linezolid. It showed resistance to penicillin, clindamycin and ciprofloxacin. Histopathology report shows features consistent with angiolymphoid hyperplasia with eosinophilia.

Treatment and follow up

Patient was treated with surgical excision of the nodules and was discharged the same day following which dressing was done regularly with mupirocin ointment. Initially oral empirical treatment was started with T. Linezolid which was deescalated to T. Doxycycline and

continued for a period of 1 month. Now patient is asymptomatic, there is no itching or new nodular formation and comes for regular follow up.

Figure 1: first one before surgery



Figure 2: second one after surgery



Figure 3: Gram staining from tissue sample showing moderate Gram positive bacilli and scanty Gram positive cocci arranged singly and in pairs.

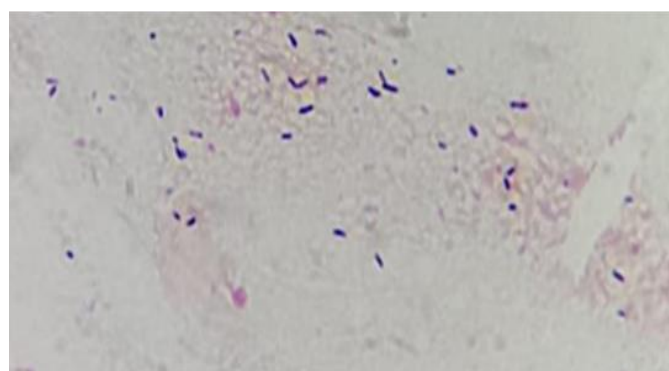


Figure 4: Chocolate agar showing fine, dry, non lytic colonies and larger white opaque, non lytic colonies after 24 hours of incubation.

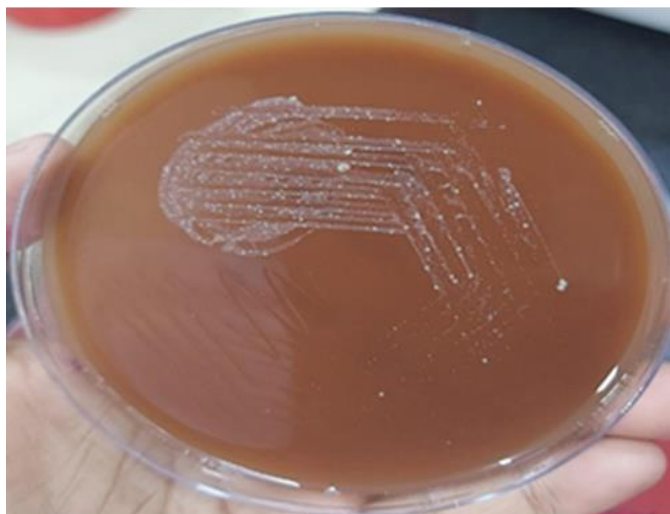


Figure 5: Antibiotic susceptibility testing on blood agar showing inducible clindamycin resistance in *Corynebacterium amycolatum*



Discussion

Corynebacteria are Gram-positive bacilli with a characteristic club-shaped appearance and swollen ends, typically arranged in palisades or in formation resembling “Chinese letters” pattern. They are catalase-positive, non-motile, non-spore forming bacteria with an aerobic or facultatively anaerobic metabolism. The *Corynebacterium* genus comprises two major groups: *Corynebacterium diphtheriae*, the etiologic agent of diphtheria, and the non-diphtherial *corynebacteria* (ND-C), also referred to as “coryne form” bacteria. The latter represent a heterogeneous group of species widely

distributed within the human Microbiota, particularly on the skin and respiratory and genital mucosa. For a long time, these bacteria were regarded as harmless commensals lacking clinical significance, and their isolation from clinical specimens was often interpreted as mere colonization or contamination rather than true infection. However, recent evidence highlights their emerging role as opportunistic and nosocomial pathogens responsible for a wide range of human infections. This pathogenicity is thought to be associated with several virulence factors, including the presence of pili, microcapsules, cell wall components, enzymes and toxins, which enable these microorganisms to adhere to epithelial surfaces, multiply in vivo, and evade host defense mechanisms.¹ It is a biofilm-forming bacterium with strong adherence capacity, which protects it from antibiotics and the immune response. Conversely, immune activation in response to its biofilm can cause collateral tissue damage due to the release of phagocytic enzymes, free radicals, and the formation of immune complexes.⁶

Corynebacterium amycolatum is considered a potential multi drug resistant opportunistic pathogen that can cause various infections, such as bacteremia, peritonitis, endocarditis, and wound infections, especially in nosocomial settings and among immunocompromised patients.² It has been implicated in a wide variety of infections like neonatal sepsis, nosocomial pneumonia, mastitis, nosocomial endocarditis, post endophthalmitis corneal ulcer, subcutaneous abscesses, emphysematous chronic osteomyelitis etc^{1,2,3,7,8,9,10}

According to Mitchell et al, coryneforms encountered in clinical microbiology labs are either contaminants, colonizers, or part of polymicrobial infections where general empirical therapy is most appropriate and the

criteria for identifying these bacteria in cultures should be based on knowledge of which patient populations and clinical situations are at risk for infections from *Corynebacterium* species.¹¹ However in a case series by Sengupta et al conducted in Vellore, they detected 12 cases of *C. amycolatum* in individuals with ear infection.

These patients were either Immuno compromised or have had previous surgical interventions as in our case. It becomes difficult to distinguish between infection, colonization, and contamination when these organisms are isolated from ear specimens. Out of the 12 patients seven had pure growth of *C. amycolatum* while the rest had growth of other organisms like *Pseudomonas aeruginosa* and *Staphylococcus aureus*.¹² In another study by Ibarra Armenta G et al on organisms causing chronic Suppurative Otitis media *Corynebacterium* species were isolated in 18 cultures, representing 19.3% of all cultures, of which *C. amycolatum* accounted for the majority of identified isolates. Among the 12 positive cultures, eight were polymicrobial, with *C. amycolatum* coexisting with other bacteria.⁶ In our case also the presentation was that of a polymicrobial one. According to Funke et al guidelines to be followed are: (a) A positive direct Gram stain and a strong leukocyte reaction and (b) pure cultures. The predominance of coryneform bacteria in material from normally sterile sites should also be considered an indicator for a possible disease association.¹³

The clinical relevance of *Corynebacterium amycolatum* has long been overlooked, mainly due to difficulties in accurate identification and its frequent confusion with *C. xerosis*, *C. minutissimum* and *C. striatum*, which share highly similar biochemical characteristics.^{1,13} *C. amycolatum* strains are predominantly misidentified as

C. xerosis, because both taxa have dry colonies whereas *C. minutissimum* and *C. striatum* strains produce moist colonies.¹³

Matrix-assisted laser Desorption /ionization time-of-flight mass spectrometry (MALDI-TOF MS) allows for the accurate identification of most pathogenic coryneform Gram-positive rods using an FDA-cleared method.¹¹ This is a tool, whereby proteins liberated from bacteria are ionized and detected by a mass spectrometer (MS), the spectrum is analyzed, and its pattern is compared to entries found in a database, giving rise to a degree of match. Protein fingerprints are deemed to provide identification to genus and species with an accuracy which approaches that of genetic methods.¹⁴ Studies have suggested that *Corynebacterium amycolatum* is a zoonotic agent.¹⁴ In a study, by Aalbaek et al., *C. amycolatum* was found to be associated with canine otitis externa. On basis of their study, coryneform bacteria should be regarded as potential secondary pathogens able to proliferate in the environment of an inflamed ear canal.¹⁵ However our patient did not give us any history of contact with dogs or cats.

C. amycolatum demonstrates more antibiotic resistance than other similar organisms.¹¹ So it is important to recognize their pathogenic potential, especially when isolated from sterile sites, in large numbers in pure cultures and when there is an associated direct microscopic evidence in the form of Gram-positive bacilli.⁸ Studies have shown high resistance to β -lactams, macrolides, fluoroquinolones, and clindamycin, while maintaining susceptibility to Glycopeptides and oxazolidinones such as vancomycin and linezolid.⁶ If targeted antimicrobial therapy is indicated for wounds and sterile body tissue/ fluid samples, vancomycin and linezolid are reliable options for empirical coverage of

Corynebacterium species.¹¹Therapeutic approaches for C. amycolatum and other bio film-forming bacteria must be based on understanding the effects of antibiotics on their structure. Inadequate treatment may lead to rapid bacterial growth, detachment, and dissemination into the bloodstream and surrounding tissues.⁶

Conclusion

In recent times, many species once thought to be non-pathogenic, have emerged as opportunistic pathogens, Coryneform bacteria are an excellent example of this.⁸Our findings suggest that C. amycolatum could be a potential ear pathogen even when it is isolated with other pathogens. The burden of proving Corynebacterium isolated in the laboratory as the causative agent lies upon the microbiologist. Treating physician should keep this in mind while interpreting the laboratory report. MALDI-TOF MS has emerged as a robust and reliable tool for species identification among Corynebacteria.³

Clinical significance

C. amycolatum is a relatively rare cause of ear infections. As it demonstrates more antibiotic resistance, careful identification with appropriate antibiotic susceptibility testing and right duration of treatment is required in managing these infections.

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