Panacea Journal of Medical Sciences 2024;14(2):593–601

Content available at: https://www.ipinnovative.com/open-access-journals

Panacea Journal of Medical Sciences

Journal homepage: http://www.pjms.in/

Case Series

Unusual presentations of chronic osteomyelitis caused by unusual pathogens: A case series from a tertiary care hospital of eastern India

Ananda Sanchayeeta Mandal[1], **Deboprio Das**[2], **Hirak Jyoti Raj**[3]*

[1]*Sarat Chandra Chattopadhyay Government Medical College and Hospita, Howrah, West Bengal, India*
[2]*Nil Ratan Sircar Medical College and Hospital, Kolkata, West Bengal, India*
[3]*Dept. of Microbiology, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India*

ARTICLE INFO

Article history:
Received 11-08-2022
Accepted 17-11-2022
Available online 13-08-2024

Keywords:
chronic osteomyelitis
unusual
pathogen

ABSTRACT

Chronic osteomyelitis is multifactorial in its occurrence, type, severity and prognosis. Presentation of chronic osteomyelitis may be varied even very unlike of its prototype. Cases of osteomyelitis were encountered caused by Nocardia brasiliensis, Actinomyces spp., Aspergillus flavus, Staphylococcus aureus, Morganella morganii with unusual presentations. The organisms except Staphylococcus aureus were among the seldomly reported ones. Two of the cases were found to be biofilm associated as studied by biofilm detection throughatomic force microscopy and methods of Anderl et al and Stepanovic et al. Only one case,c aused by biofilm producer strain of No cardia brasiliensis, required surgical correction with amputation. Rest four cases were recovered with antimicrobial therapy. Strong degree of suspicion of biofilm development and involvement of unusual etiological agents were required to diagnose and treat the cases of chronicity. It could put aside the drastic decisions like amputation which might be associated with immense eco-socio-psychological impact onthe patients and their families.

1. Introduction

Chronic osteomyelitis is an inflammatory event affecting bone causing destruction of osseus tissue and formation of sequestrum. It may present with periods of waxing and waning of variable duration. Its occurrence, type, severity and prognosis depend on several factors. Positive microbiological culture from the relevant specimen remains the gold standard in diagnosis of this entity. The most widely-used classification system of chronic osteomyelitis in adults is the Cierny–Mader classification.[1] The types are medullary osteomyelitis, superficial osteomyelitis, localized osteomyelitis and diffuse osteomyelitis. Systemic factors responsible for osteomyelitis are Malnutrition, Renal or hepatic failure, Diabetes mellitus, Chronic hypoxia, Immune disease, Immunosuppression, Immune deficiency, Alcohol abuse, Malignancy.[2] Some local factors are also responsible for chronic osteomyelitis, e.g. chronic lymphoedema, venous stasis, major vessel compromise, arteritis etc.[2] Infectious agents may find a portal of entry into the bone by following mechanisms: an open injury to the bone, a minor trauma, which can lead to a blood clot around the bone and then a secondary infection from seeding of bacteria, bacteremia deposits bacteria in a focal area of the bone and gives rise to hematogenous osteomyelitis. This predominately occurs in pediatric population.[3,4] In adults, it typically occurs from a distal site of infection. The commonest primary sites of infection are distant areas like the urinary tract, intravenous catheters, heart tissues etc. Osteomyelitis when gets disseminated via bloodstream in adults, mostly affects vertebrae.[3] The causative pathogens vary according patient-related parameters such as age, immune status, history of trauma and geographical

* *Corresponding author*.
E-mail address: dr.Sanchayeeta@gmail.com (H. J. Raj).

https://doi.org/10.18231/j.pjms.2024.106

location.[5] Generally, hematogenous osteomyelitis is caused by one infectious agent only, while contiguous-focus osteomyelitis is frequently polymicrobial.[6] Adult chronic osteomyelitis is most commonly caused by far Staphylococcus aureus.[7] Methicillin-resistant S. aureus (MRSA) has also been increasingly isolated from chronic osteomyelitis lesions.[8] Other causative pathogens include Staphylococcus epidermidis, Pseudomonas aeruginosa, Serratia marcescens and Escherichia coli.[9] Mycobacterial and fungal infections are generally uncommon and are often associated with immunodeficiency. However, the present study group, during the period of January 2021 to December 2021, encountered five cases of chronic osteomyelitis caused by either very uncommon and unusual pathogen in unusual sites or with uncommon presentation like biofilm formation or with all three taken place altogether. The case series also mentions the treatment and fate of the cases as well.

In all five cases in this story, the specimens like bone sequestrum, sinus discharge, scrapping collected from the suspected lesions were sent to the department of Microbiology as part of daily routine works. Cases were traced including reports of other specialties on retrospective basis. The study was conducted with the due approval of institutional ethics committee. Processing of the specimens was done following the methodology of standard text book.[10] Scrapings were rinsed thrice with sterile PBS to remove any non-adherent organisms. This was followed by inoculation of the specimens onto various culture media like 5% Sheep blood agar (SBA) and Lowenstein Jensen media. Any growth in these media was further processed accordingly by conventional and automated identification systems like VITEK-2 compact whenever needed. Relevant history and other clinical findings, clinical parameters like blood profile, radiological findings were collected from the orthopedics department of the institute for the purpose of corroboration. Biopsy samples from the lesions, were grossed according to the protocol; and formalin fixed paraffin embedded sections were prepared. Sections were stained with Hematoxylin and Eosin and special stains for fungus like Gomori's Methenamine Silver (GMS) wherever required. Referral to other specialties for relevant information were suggested to the clinicians.

2. Case 1

A55-year-old gentleman, bus conductor by occupation, attended the orthopedics Out Patient Department with pain and tenderness in the left foot for six months. On examination, there were multiple discharging sinuses on the medial aspect of left foot. Local temperature was not raised. History revealed that he had a history of Type 2 diabetes mellitus for last 15 years. But the blood glucose level had not been monitored for last two years. Roentgenogram revealed sequestrum formation with a thick involucrum surrounding it. Gram's and ZN staining of the discharge material and the tissue biopsy specimen revealed occasional Gram-positive branching filamentous structures and pus cells. (Figure 2 a,b). Microbiological culture of the discharge from the site and the biopsy specimen were put up in SDA, Nutrient agar, 5% SBA and LJ medium. One set of nutrient agar and 5% SBA was also placed for anaerobic incubation in Gas pack system. Dry, wrinkled, colonies grew in Lowenstein Jensen (LJ) media after 10 days of aerobic incubation (Figure 1) with no growth in the anaerobic set. Staining from the colonies showed acid-fast long filamentous bacteria in ZN staining with 1 % sulphuric acid used as decolorizer and Gram-positive filamentous branching bacteria in Gram staining respectively. This was suggestive of Nocardia spp. Species identification was done by conventional biochemical methods including the tests of growth at 42^0C after 3 days (-ve), gelatin hydrolysis (+ve), acid from rhamnose(-ve), hydrolysis of casein(+ve), urea hydrolysis(+ve) and diagnostic AST by Kirby-Bauer disc diffusion method showing susceptibility to gentamicin, tobramycin and amikacin and resistance to erythromycin, respectively. As per the results the species was inferred to be Nocardia brasiliensis.[11]

Histopathological examination of the affected tissue revealed necrotic bone with infiltration of polymorphs and lymphocytes and micro-abscess formation. Fite's stain revealed presence of acid-fast bacilli (Figure 2 a). The chronicity and the history of refractoriness of treatment with co-trimoxazole, amikacin and imipenem raised the suspicion of probable involvement of biofilm formation in this case.

2.1. Study of biofilm

It is done by polycarbonate membrane method as devised by Anderl et al. for Nocardia spp.[12] Ten drops of bacterial suspension of Nocardia spp. obtained from the patient grown in BHI broth adjusted to 10^6CFU /mL was used to seed black polycarbonate membrane filters (25 mm diameter; pore size 0.22 μM, Millipore, Germany) placed on Brain heart Infusion (BHI agar) agar plates. The plates were inverted and incubated at 37°C for 48 hours only. The membrane-supported biofilms were then transferred to fresh culture medium in every 24 hoursly. After a total 14 days' incubation, growths on the membranes got washed with PBS (Phosphate buffer saline) having a pH of 7.2, by agitation at 180 rpm for 1 min to remove the non-adherent cells. Atomic Force Microscopy was then done without further sample treatment in the tapping mode from an outside centre.[13] We used the tapping mode as it was suitable for soft materials and analysis was performed with WSxM software. In result, the growth of Nocardia brasiliensis showed colony biofilm formation on polycarbonate membrane which was demonstrated by atomic force microscopy. (Figure 3). The patient ultimately

had to undergo an amputation of his left foot.

3. Case 2

A 50-year-oldhousewife attended orthopedics OPD with pain and swelling of left leg below knee for last one month. She has undergone open reduction and internal fixation (ORIF) of both bone fracture of left leg three months back (Figure 5). Local examination revealed solitary sinus with purulent discharge containing-yellow-white grains. The grains were collected and crushed in between two slides followed Gram staining. On microscopy the stain revealed central filamentous Gram-positive mycelium encircled by a peripheral zone of swollen radiating club shaped structures mimicking sun-ray appearance (Figure 6). On ZN staining acid fast clubs were detected suggestive of infection by actinomycetes. Microbiological culture from the sequestrum (Figure 4) sent to the Dept. of microbiology was scooped out and crushed grains were put up in both aerobic and anaerobic conditions with 5-10% CO_2 at 37^0C in 5% SBA and Brain Heart Infusion agar (BHI Agar) and in LJ medium. The specimens were also inoculated in thioglycolate broth. Growth appeared in 5% SBA in anaerobic incubation set with small smooth, flat, opaque colonies somehow resembling 'molar tooth' in appearance. In thioglycolate broth the growth appeared as small fluffy balls below the surface of the medium. LJ medium categorically didn't grow anything when observed for first two weeks. It was followed up for eight weeks and found to be sterile. Biopsy from the specimen showed bacterial colonies comprising of basophilic radiating filaments on Hematoxylin-Eosin-stained sections, a finding consistent with actinomyces (Figure 7). Growth identification was done by colony morphology, gram staining, ZN staining, urea hydrolysis test, nitrate reduction test. Gram positive, nonacid-fast, non-sporing, branching, beaded, thin filamentous rods, urea hydrolysis test negative, ability to reduce nitrate to nitrite and fermentation test positive in glucose, maltose, sucrose, xylose, CAMP test negative, led towards the diagnosis of Actinomyces spp.[14] X-ray of left leg was suggestive of sequestrum formation (Figure 5). The patient received the treatment of intravenous PenicillinG6-hoursly for six weeks with curettage and debridement of tissue sloughs from the wound periodically. This was followed by prolonged oral amoxicillin-clavulanic acid therapy for eight weeks. She eventually made a complete recovery.

4. Case 3

A 3-year-old girl was brought to Pediatric Medicine OPD with history of pain, tenderness in right leg and lower thigh and restriction of mobility of right knee joint. Patient was a known case of Acute Lymphoblastic Leukemia (ALL) on remission and continuation phase of chemotherapy. She also had recent history of fever and vomiting. Overlying skin of the affected region appeared red, shiny, edematous with rise in local temperature. On examination there is tumefaction and occasional discharge of purulent materials. There was no history of trauma, instrumentation or previous sore throat to point towards onset of acute rheumatic fever. Blood picture showed raised C-reactive protein and erythrocyte sedimentation rate. The discharge material was collected and looked for any grain in both wet mount and ZN staining; in vain, twice. Drainage of pus was done then and cultured in 5% SBA, Nutrient agar, LJ medium and SDA, both aerobically and anaerobically wherever feasible. Growth of gram-positive cocci was obtained (Figure 8a,b), which was later diagnosed to be Methicillin Resistant Staphylococcus aureus having susceptibility to vancomycin, teicoplanin, levofloxacin, ciprofloxacin, amikacin, gentamicin, tigecycline as detected conventional disk diffusion method and by using E-strip for vancomycin and cefoxitin (Figure 9). X-ray of right knee-joint and thigh revealed deep seated soft tissue swelling and demineralization of bone. However, she was diagnosed to be a case of hematogenous osteomyelitis borne of adjacent soft tissue cellulitis. The Patient was started on intravenous Vancomycin. She eventually made a full recovery from her osteomyelitis.

5. Case 4

A 22-year-old short statured female attended microbiology laboratory with history of non-healing chronic discharging sinus on both upper limbs at elbow region (Figure 10a,b). She was a follow up case of mixed connective tissue disorder for more than four years and received various types of immunosuppressive drugs including corticosteroids. Of importance, at the time of presentation, she was on Category I Anti-Tubercular Drugs (ATD) based on clinical suspicion only without any microbiological evidence. On examination there was pus coming out from the sites of protracted skin, immobilization and loss of function of both the elbow joints. There was no history of previous injury or any surgical intervention in the involved joints. Radiological investigation viz. MRI of left elbow showed erosion and collection suggesting septic arthritis.

Microbiological investigation began with collection of pus from both the elbow sites. The pus was examined by direct Gram staining and ZN staining. It showed presence of Gram-positive branching septate hyphae (Figure 11). The sample pus was cultured on SDA medium, which reveled growth greenish yellow fluffy wooly colonies (Figure 12) which on lactophenol cotton blue (LPCB) teased wet mount showed features suggestive of Aspergillus flavus (Figure 13). Biopsy was done from affected site and H&E and GMS-stained sections showed necrotic bone, hemorrhage and clumps of septate fungal hyphae with branching at acute angle (Figures 14 and 15).

ZN stain didn't show any acid-fast bacilli and CB-NAAT was negative, virtually ruling out the suspicion of tuberculosis for which Category I ATD was started. The ATD was withdrawn as per the suggestions of microbiologists and the patient was admitted and started on intravenous Liposomal Amphotericin B. She eventually made a full recovery.

6. Case 5

A 45-year-old gentleman, traffic police by occupation attended general surgery OPD with discharging sinus associated with pain and tenderness in right foot for last one month. He had a history of bone injury consequent to a penetrating trauma in right foot received in a motorcycle accident around three months back. On examination, a large ulcer with blood mixed purulent discharge was visibly developed exposing part of the underlying bone. The affected area was swollen, erythematous, with raised local temperature. The bony exposure took place around two weeks back. The patient had a history of high spiking fever for last seven days. He was a known case of type II diabetes mellitus with poor glycemic control. Hematological study showed a total leucocyte count of 18,000/ mL with 75% neutrophil count and CRP raised to11.5. Conventional X-ray imaging showed obvious osteolytic lesions, periosteal thickening, blurring of soft tissue images suggestive of osteomyelitis.

Discharge from ulcer cum discharging sinuses was sent for microbiological studies, which on Gram and ZN staining did not reveal any decisive picture per se. Bacteriological culture revealed pure colonies of a non-lactose fermenting organism. On further work up, it was found to be motile, gram negative bacilli which was catalase positive, oxidase negative, positive for phenylalanine deaminase, urea hydrolysis, indole production and citrate non-utilizer. The organism was diagnosed as Morganella morganii whose identity was further confirmed by VITEK-2 compact system. No atypical mycobacteria or fungus was grown. AST revealed susceptibility to imipenem and meropenem besides aminoglycosides. He was put on a combination of imipenem and gentamicin IV for 10 days following admission in hospital. Regular surgical dressing and physiotherapy was done but the discharge remained unresolved and a repeat microbiological culture revealed the same organism. The chronicity and that the organism was quite rare to cause osteomyelitis raised the suspicion of probable involvement of biofilm colonization in this case. The study group performed biofilm study in the isolated organism i.e. Morganella morganii by Stepanovic et. al method as described below.[15]

6.1. Study of biofilm

It was done by 96 well tissue culture plate method of Stepanovic et al.[15] Bacterial growth was sub-cultured into BHI broth and incubated at 37^0C overnight. The density of bacterial suspension was adjusted to give an optical density of 0.5 at 600 nm. It was then diluted in well of the microtiter plate [Figure 16] in 1: 100 ratio, i.e, 2 μL of culture suspension in 198μL of BHI broth to make a total of 200μL liquid. The control well contained uninoculated sterile BHI broth. The microtiter plate was then subjected to incubation at 37^0 C in a shaker incubator at 220 rpm for 4 hours followed by discarding of the broth and rinsing of the plate with phosphate buffer saline (PBS) and fixation of adhered cells with cold methanol fixation for 15 minutes. Again, the plate was washed with PBS and then stained with crystal violet stain at room temperature. The stain was removed by washing with phosphate buffer saline/ normal saline. The plate was then allowed to dry. 220 μL of decolorizing solution 70 % ethanol was added to each well for 15 mins at room temperature to quantify the cells. The absorption of the eluted stain was measured at 500-600nm wavelength filter and the result was as follows:

OD of sample > 4 times OD of control; suggestive of strong biofilm producer as per Stepanovic classification of biofilm producer organism. Hence, the causative agent of chronic agent of the chronic osteomyelitis was found to be a biofilm producer explaining the refractoriness of the lesion per se. The patient underwent debridement of the lesions with radicle excision of diseased section of bone under antibiotic coverage and eventually attained remission from the problem. The patient was in uneventful follow up both clinically and microbiologically till recently.

Figure 1: Lowenstein-Jensen media showing growth ofNocardia spp.

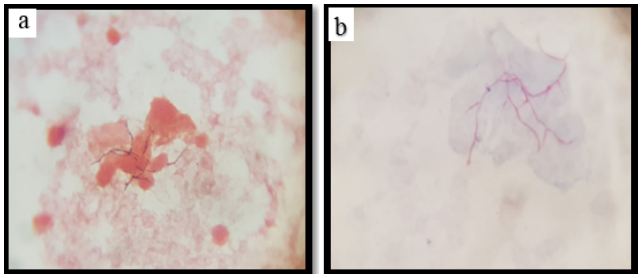

Figure 2: a,b: Shows weakly staining gram positive branching rods on grams stain & partially acid-fast beaded branching filaments on modified ziehl-neelsons stain resembling nocardia spp. respectively.

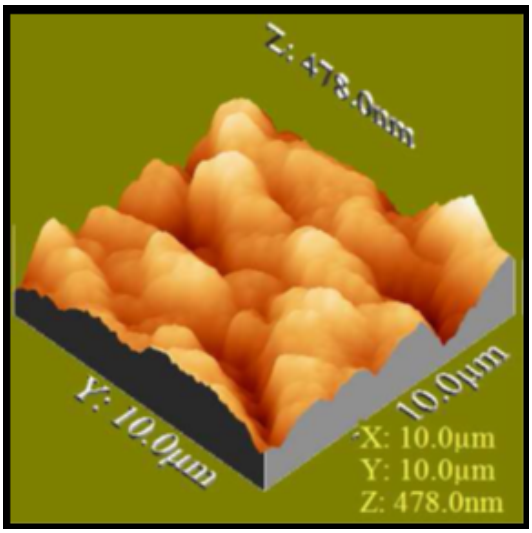

Figure 3: Atomic force image of nocardia spp. biofilm

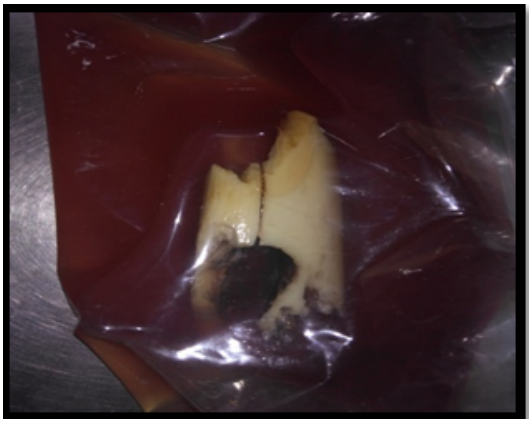

Figure 4: Showing sequestrum sent to our department.

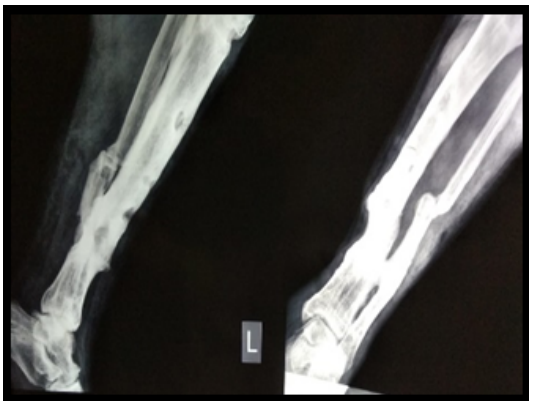

Figure 5: Showing x-ray left lower limb showing sequestration

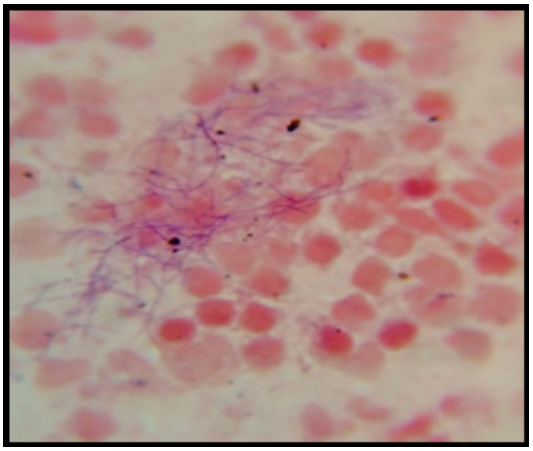

Figure 6: Showing direct gram stain gram positive filamentous bacteria.

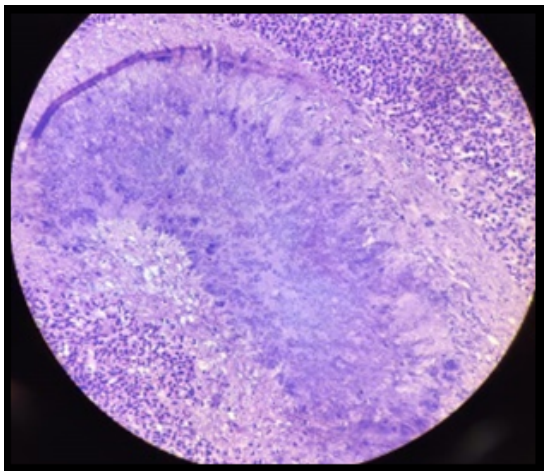

Figure 7: Biopsy shows a sulphur granule composed of basophilic radiating filamentous bacteria. (400x, H&E)

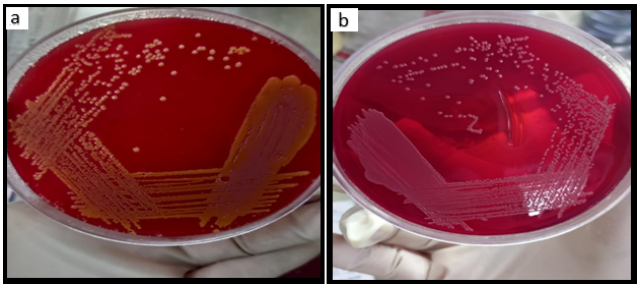

Figure 8: a,b: Showing growth of staphylococcus aureus on blood agar & macconkey agar respectively

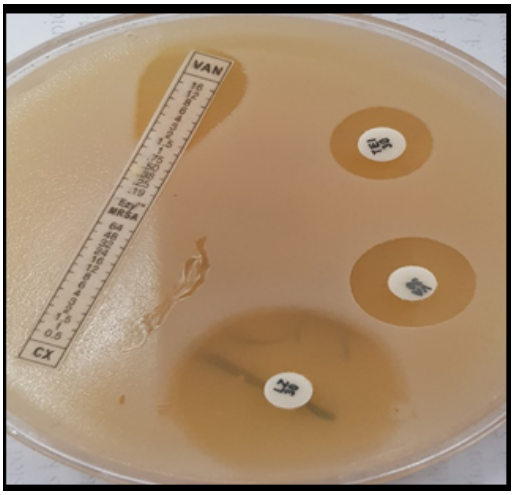

Figure 9: Kirby-bauer disc diffusion with e-strip & discs

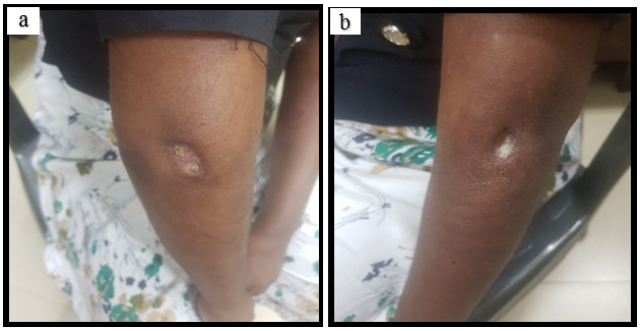

Figure 10: a,b: Showing non-healing chronic discharging sinus of right and left elbow respectively.

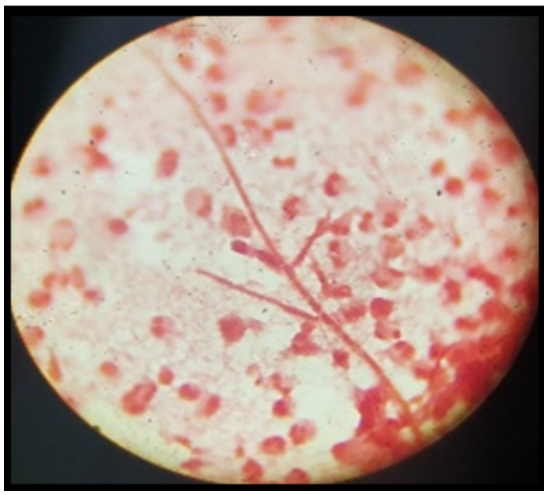

Figure 11: Gram -stained direct smear from the lesion showing hyphae with acute angled branching

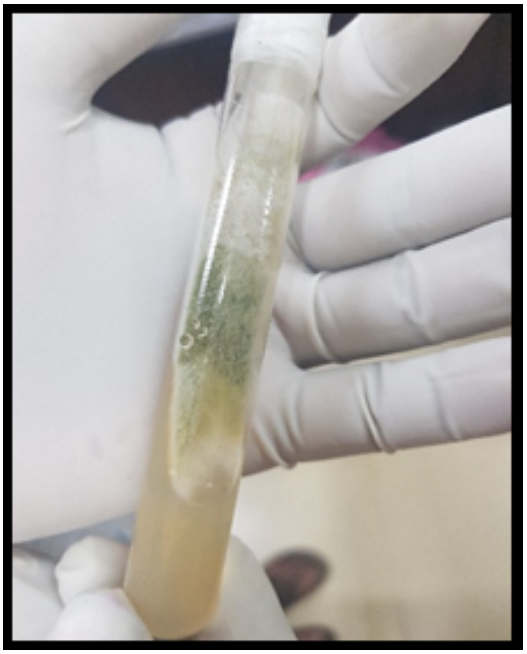

Figure 12: Showing typical yello wish-green powdery growth resembling aspergilus spp. on obverse side.

7. Discussion

Despite advances in surgical and medical management of osteomyelitis, it is still considered one of the most difficult to treat infectious diseases. Progressive destruction of the bone and the formation of sequestrum are characteristics of this disease. The detection of osteomyelitis is first done based on clinical pictures. Confirmation is usually done by radiologic, microbiological and pathological tests analyzed in combination. Complications begin mostly after 10 to 14 days from the onset. Sophisticated investigations include CT scan, MRI scan which are considered as standard of care in diagnosis of osteomyelitis. False positive results have been encountered in degenerative joint disease, bone tumors, and recent surgery. Actinomycetes and Nocardia spp. both are considered as rare etiologic agents of chronic osteomyelitis. De A. et al. reported chronic discharging sinus in a case of mycetoma caused by biofilm producer Nocardia spp.[16] Any osteomyelitis case caused by biofilm producer Nocardia spp. is yet to be reported from this geographical region. Vanegasa S. et al reported a case

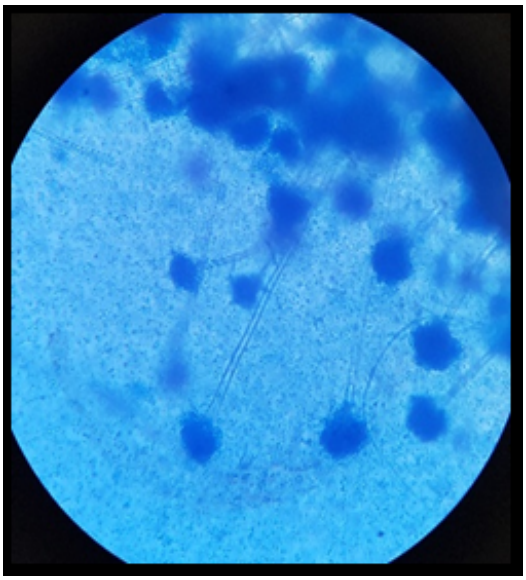

Figure 13: Showing LPCB mount with hyaline septate hyphae, metulae covering three quarters of the surface of the vesicle. [400X]

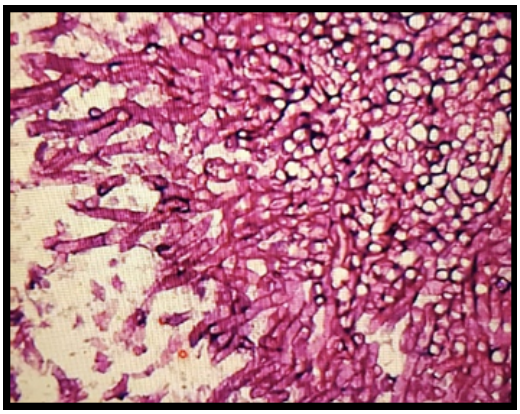

Figure 14: HP section showing broad septate hyphae with acute angle branching resembling aspergillus spp. [400X]

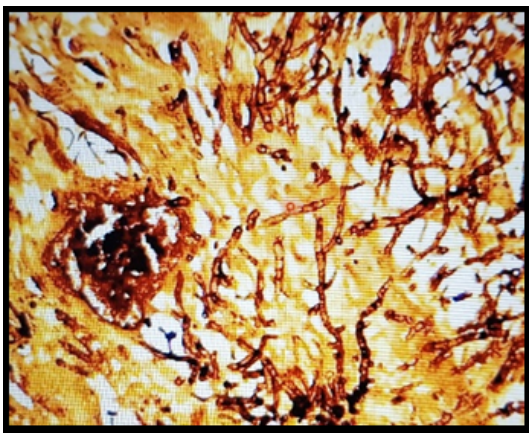

Figure 15: Gomori methanamine silver stain showing aspergillus spp. On HP section [400X]

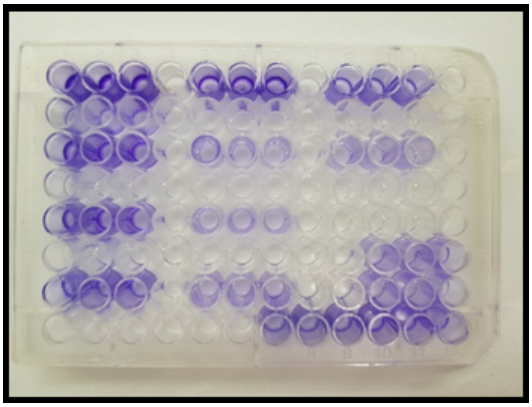

Figure 16: Biofilm demonstration using Microtiter plate method.

of femorotibial osteomyelitis associated with Nocardia brasiliensis, but without any biofilm study.[17] Instead, they planned for long term antimicrobial therapy. We question the rationale of prolonged therapy in osteomyelitis without ascertaining the biofilm status of the causative pathogen. We found another report of spinal form of Nocardia brasiliensis osteomyelitis from India in immunosuppressed hosts[18]. However, there was no association of biofilm in the case. Surgical intervention has to be done in the present case for the factor of biofilm.

Osteomyelitis caused by Actinomyces spp. is quite uncommon, that also in long bones. However, few cases of lower extremity actinomycotic osteomyelitis have been reported. But diagnostic confusion apparently prevailed in all those cases. Ryu DJ et al reported a case of long bone actinomycotic osteomyelitis in an immunocompetent woman with history of trauma.[19] All microbiological diagnostic modalities including polymerase chain reaction for Mycobacterium tuberculosis was negative as there was a diagnostic confusion. Unlike them, our case was quite free of diagnostic dilemma and came out positive in microbiological culture for Actinomyces spp. However, the exact species could not be fixed by biochemical reactions alone. Actinomyces israeli was the most probable bet of ours. As the treatment decision didn't rest upon the species, we suggested intravenous Penicillin G.

Osteomyelitis of a fungal etiology is seldom seen even in an immunocompromised host. Among such cases Candida spp. is the most common causative agent identified. Osteomyelitis caused by Aspergillus spp is even rarer, with an incidence of less than 3%. Spine is the most common site involved, amounting to about half of such cases, with features often mimicking tuberculous osteomyelitis. Hematogenous infection arising from a pulmonary focus occurs mainly in immunosuppressed patients and is caused almost exclusively by Aspergillus fumigatus.[20] Gabrielli E. et al. reviewed 310 individual cases of osteomyelitis associated with Aspergillus spp.They found that the median

age of patients was 43 years (range, 0–86 years), and 59% were males. Predisposing factors associated with were chronic granulomatous disease, hematological malignancy, diabetes mellitus, transplantation, steroid therapy.[21] Site predilection for osteomyelitis were spine followed by base of the skull, jaw, ribs, long bones, sternum. Most commonly effective antifungal drug was liposomal amphotericin B followed by voriconazole and itraconazole. As our patient went well and recovered with monotherapy of liposomal amphotericin B, the literature review found that 62% received combination of antifungal therapy and surgical intervention, 26% were treated with antifungal alone and 9% received only surgery.[22] The duration of antifungal treatment for fungal aspergillosis has not been established or streamlined. The IDSA guidelines recommend a minimum of 6–8 weeks of antifungal therapy in non-immunocompromised patients with Aspergillus osteomyelitis.[23] The recommended length of therapy in the IDSA guidelines for Candida osteomyelitis is 6–12 months.[24] Our patient received antifungal therapy for three months, successfully.

Bone infection by Morganella morganii is seldomly encountered. However, a case of Morganella morganii associated osteomyelitis with biofilm development has been reported around six years back by De A et. al from the same institute the present workers belong to.[16]

There are only handful reported cases in available literature, of which the majority belongs to sporadic septic arthritis without bony involvement in diabetic patients. Immunosuppressed state, autoimmune disorder, long term steroid intake, drug abuse, local trauma and surgical interventions are predisposing factors for Morganella morganii infection.[25–31] Patients with one or more of the risk factors cause high mortality. Predisposing factors in our case was trauma. Biofilm colonization by the bacterial strain was an important pathogenic factor for chronicity. Altered environment, metabolism and suppressed growth rate of bacteria in biofilm Extra-polymeric substances (EPS) render the bacteria less susceptible to antibiotics in biofilm state in vivo in spite of susceptibility in vitro testing; and hence chronicity of the infection.[32] This discrepancy largely owes to the poor antimicrobial penetration through the biofilm matrix to hit the microorganisms. Biofilm is thought to be responsible for many non-responding bacterial infections such as cystic fibrosis, pneumonia, musculo-skeletal infections, necrotizing fasciitis, osteomyelitis, melioidosis, infectious kidney stones, bacterial endocarditis, airway infections, otitis media, chronic rhinosinusitis, biliary tract infections, chronic bacterial prostatitis and infections related to medical devices.[33]

8. Conclusion

The cases are rare and hence worth mentioning with regard to both the causative pathogens, presentations and biofilm production as well. Strong degree of suspicion from clinicians and laboratory physicians as well is key for success. In our present study we could not include molecular studies and sophisticated radiological investigation. Nevertheless, the diagnoses of these rare reports affirm the importance, potency and efficacy of conventional laboratory methods. The cases were eye openers in their own fields emphasizing the need of inquisitiveness and laboratory detailing to solve the unsolved mysteries.

9. Source of Funding

None.

10. Conflict of Interest

None.

References

1. Cierny G, Mader JT, Penninck JJ. Clinical staging system for adult osteomyelitis. *Clin Orthop Relat Res*. 2003;Sep(414):7–24.
2. Momodu II, Savaliya V. Osteomyelitis. 2022 Jan 24. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
3. Hatzenbuehler J, Pulling TJ. Diagnosis and management of osteomyelitis. *Am Fam Physician*. 2011;84(9):1027–33.
4. Dahl LB, Hoyland AL, Dramsdahl H, Kaaresen P. Acute osteomyelitis in children: a population-based retrospective study 1965 to 1994. *Scand J Infect Dis*. 1998;30(6):573–7.
5. Calhoun JH, Manring MM, Shirtliff M. Osteomyelitis of the long bones. *Semin Plast Surg*. 2009;23(2):59–72.
6. Schmitt SK. Osteomyelitis. *Infect Dis Clin North Am*. 2017;31(2):325–38.
7. Lew DP, Waldvogel FA. Osteomyelitis. *N Engl J Med*. 1997;336(14):999–1007.
8. Arnold SR, Elias D, Buckingham SC, Thomas ED, Novais E, Arkader A, et al. Changing patterns of acute hematogenous osteomyelitis and septic arthritis: emergence of community-associated methicillin-resistant Staphylococcus aureus. *J Pediatr Orthop*. 2006;26(6):703–8.
9. Carek PJ, Dickerson LM, Sack JL. Diagnosis and management of osteomyelitis. *Am Fam Physician*. 2001;63(12):2413–21.
10. Tille PM. Specimen management. In: Bailey & Scott's Diagnostic Microbiology. St. Louis, Missouri: Elsevier; 2017. p. 56–71.
11. Tille PM. Nocardia, Streptomyces, Rhodococcus, and Similar Organisms. In: Bailey & Scott's Diagnostic Microbiology. St. Louis, Missouri: Elsevier; 2017. p. 319–28.
12. Anderl JN, Franklin MJ, Stewart PS. Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. *Antimicrob Agents Chemother*. 2000;44(7):1818–24.
13. Chatterjee S, Biswas N, Datta A, Dey R, Maiti P. Atomic force microscopy in biofilm study. *Microscopy (Oxf)*. 2014;63(4):269–78.
14. Tille PM. Overview of Anaerobic Organisms. In: Bailey & Scott's Diagnostic Microbiology. St. Louis,Missouri: Elsevier; 2017. p. 519–20.
15. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter plate test for quantification of staphylococcal biofilm formation. *J Microbiol Method*. 2000;40(2):175–9.
16. De A, Raj HJ, Maiti PK. Biofilm in Osteomyelitis caused by a Rare Pathogen, Morganella morganii : A Case Report. *J Clin Diagn Res*. 2016;10(6):6–8.
17. Vanegasa S, Franco-Cendejas R, Cicero A, López-Jácome E, Colin C, Hernández M. Nocardia brasiliensis-associated femorotibial osteomyelitis. *Int J Infect Dis*. 2014;20:63–5. doi:10.1016/j.ijid.2013.11.002.

18. Lakshmi V, Sundaram C, Meena A, Murthy J. Primary cutaneous nocardiosis with epidural abscess caused by Nocardia brasiliensis: a case report. *Neurol India*. 2002;50(1):90–2.
19. Ryu DJ, Jeon YS, Kwon HY. Actinomycotic osteomyelitis of a long bone in an immunocompetent adult: a case report and literature review. *BMC Musculoskelet Disord*. 2019;20:185. doi:10.1186/s12891-019-2576-2.
20. Vinas FC, King PK, Diaz FG. A Spinal Aspergillus osteomyelitis. *Clin Infect Dis*. 1999;28(6):1223–9.
21. Gabrielli E, Fothergill AW, Brescini L, Sutton DA, Marchionni E, Orsetti E, et al. Osteomyelitis caused by Aspergillus species: a review of 310 reported cases. *Clin Microbiol Infect*. 2014;20(6):559–65.
22. Gamaletsou MN, Rammaert B, Bueno MA, Moriyama B, Sipsas NV, Kontoyiannis DP. Aspergillus osteomyelitis: epidemiology, clinical manifestations, management, and outcome. *J Infect*. 2014;68(5):478–93.
23. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis*. 2008;46(3):327–60.
24. Pappas PG, Kauffman CA, Andes D, Benjamin DK, Calandra TF, Edwards JE, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2009;48(5):503–35.
25. Koyuncu W, Ozan F. Morganella morganii osteomyelitis complicated by secondary septic knee arthritis: a case report. *Acta Orthop Traumatol Turc*. 2012;46(6):464–7.
26. Katz LM, Lewis RJ, Borenstein DG. Successful joint arthroplasty following Proteus morganii (Morganella morganii) septic arthritis: a four-year study. *Arthritis Rheum*. 1987;30(5):583–5.
27. Cetin M, Ocak S, Kuvandik G, Aslan B, Temiz M, Aslan A, et al. Morganella morganii-associated arthritis in a diabetic patient. *Adv Ther*. 2008;25(3):240–44.
28. Gautam V, Gupta V, Joshi RM, Sawhney G, Duhan S. Morganella morganii-associated arthritis in a diabetic patient. *J Clin Microbiol*. 2003;41(7):3451. doi:10.1128/JCM.41.7.3451.2003.
29. Amat AS, Carbonell RP, Rocha LA, Viladomiu AS. Osteomyelitis of the rib due to Morganella morganii. *An Med Interna*. 2004;21(9):464. doi:10.4321/s0212-7199200400090001.
30. Schonweter RS, Orson FM. Chronic Morganella morganii arthritis in an elderly patient. *J Clin Microbiol*. 1988;26(7):1414–5.
31. Deutsch M, Foutris A, Dourakis SP, Mantzoukis D, Alexopoulou A, Archimandritis AJ, et al. Morganella morganii associated acute osteomyelitis in a patient with diabetes mellitus. *Infect Dis Clin Pract*. 2006;14(2):123. doi:10.1097/01.idc.0000198460.70991.57.
32. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G, et al. The application of biofilm science to the study and control of chronic bacterial infections. *J Clin Invest*. 2003;112(10):1466–77.
33. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. *Science*. 1999;284(5418):1318–22.

Author biography

Ananda Sanchayeeta Mandal, Senior Resident https://orcid.org/0000-0002-3643-3188

Deboprio Das, Senior Resident https://orcid.org/0000-0002-3845-6295

Hirak Jyoti Raj, Associate Professor https://orcid.org/0000-0002-8360-2052

Cite this article: Mandal AS, Das D, Raj HJ. Unusual presentations of chronic osteomyelitis caused by unusual pathogens: A case series from a tertiary care hospital of eastern India. *Panacea J Med Sci* 2024;14(2):593-601.