Original Article

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Non-diphtheriae Corynebacteria Causing Breast Abscess in Nonlactating Females-Seeking to Avoid Pitfalls in Diagnosis of Chronic Breast Infections Through Diagnostic Stewardship

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ABSTRACT

Background: Consideration of non-diphtheriae Corynebacteria as an infective organism in mastitis, is usually neglected. So, infections due to Corynebacterium species may remain undiagnosed or misdiagnosed. The aim of our study was to focus on non-diphtheriae Corynebacterium as causative organisms in breast infections using scientific logic and technology of Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDITOF MS) to categorize the isolates upto species level. The clinical correlation and response to management especially targeted antimicrobials was also recorded.

Methods: All the consecutive pus/tissue samples from Breast abscess received during study period were processed as per the standard guidelines. The identification of the isolate was done by automated methods. The cytopathological/histopathological and clinical details of the patients with infection due to Corynebacterium sp. were recorded and analyzed.

Results: Out of 52 non-duplicate samples, five showed growth of non-diphtheriae Corynebacteria. These were identified as C. kroppenstedtii and C. amycolatum. The antimicrobial susceptibility testing showed 100% susceptibility to Amoxicillin-clavulanate, Tetracycline, Vancomycin and Linezolid for all the five isolates. Histopathological examination was suggestive of chronic inflammatory mastitis/Granulomatous mastitis.

Conclusion: Non diphtheriae Corynebacteria particularly lipophilic Corynebacteria have a predilection to cause infections of breast tissue and Breast abscess which may mimic as tubercular abscess or chronic non-specific mastitis leading to unnecessary usage of antimicrobials. So, these isolates from breast tissue/pus should not be ignored rather definitely identified This would also ensure diagnostic stewardship.

Keywords: *Breast Abscess, Corynebacteria, MALDITOF MS, Diphtheroids, Lipophilic Corynebacteria*

Introduction

Breast infections may be caused by a variety of microorganisms. The usual mode of entry of the microorganism is through a break or crack in the skin on the nipple. So, these type of infections are more common in lactating women. The infection usually involves the fatty tissue of the breast which leads to swelling which pushes on the milk ducts. The result is pain and lumps in the infected breast. In non-lactating females, Breast infections are supposed to be uncommon but are on the rise and need special attention. The etiological agents of breast abscess mainly include *Staphylococcus aureus*, others being Streptococci, *Enterobacteriaceae*, Anaerobes like *Peptostreptococcus*, *Bacteroides* etc. Abdelhadi MS et al found most frequent isolation of *Staphylococcus aureus* in cases of non-lactational breast abscess. Other organisms isolated were Streptococci, *Mycobacterium tuberculosis*, *Enterobacteriaceae*.[1]

Consideration of *Corynebacterium* sp. as an infective organism in mastitis, is usually neglected in a clinical Microbiology laboratory, because of three important reasons *viz* existence of these microorganisms as skin colonizers/ contaminants, longer incubation period for growth of few lipophilic Corynebacteria which usually exceeds the normal incubation period for routine aerobic bacteria culture and difficul identification up to species level in routine Microbiology lab. So, infections due to *Corynebacterium* species may remain undiagnosed or misdiagnosed. A focus on these non-diphtheriae *Corynebacterium* as causative organisms in breast infections in non-lactational females led us to use scientific logic and technology of Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDITOF MS) to categorize the isolates upto species level, clinical correlation and response to management especially targeted antimicrobials in order to ascertain significan e and properly position their role.

Material and Methods

This was an observational prospective study between August 2018 to June 2019. All the consecutive pus/tissue samples from Breast abscess received for Aerobic Bacterial culture were processed as per the standard guidelines. Cytological/ Histopathological examination of pus/tissue specimen was recorded. Histopathological examination findings were correlated. Gram stain examination from the sample was done and culture was done on Columbia Sheep Blood agar and MacConkey agar. Culture plates were incubated at 37^0C but incubation time was increased from usual two days to seven days. Pure growth of the isolated bacteria was identified by MALDITOF-MS (Biomerieux, France) and/or Vitek-2 (Biomerieux, France) using ANC card and antimicrobial susceptibility testing was performed by Disc diffusion and/or E-test method (Figure-1). CLSI (Clinical and Laboratory Standards Institute) guidelines for *Staphylococcus aureus* was used for interpretation of the antimicrobial susceptibility. Other investigations like Real Time PCR for Tuberculosis and/or GeneXpert assay for Tuberculosis, Real Time PCR for Brucella, Fungal smear and/or Fungal culture, AFB stain and/or AFB culture were also performed. The clinical details of the patients with infection due to *Corynebacterium* sp. were recorded and analyzed. Institutional permission was taken for collection of data of the patients and Informed verbal consent was taken from the patients with infection.

Results

A total of non-duplicate 52 samples of patients either admitted or attending outpatient clinics at our tertiary health care set-up were studied between August 2018 to June 2019. These were pus/tissue samples from patients of suspected breast infections. Corynebacterium species was isolated as pure growth, in samples from five female patients presenting with clinical features of mastitis. The median age of the patient was 35 years. In the present study, out of five patients, two were diabetic and one was being treated for malignancy.

Gram stain examination of Pus/tissue sample showed many pus cells and few Gram positive bacilli (Figure-2). Growth on Sheep blood agar was noticed after 48-72 hours of incubation under ambient aerobic atmosphere. The colonies were small, grayish white, smooth, dry and non-haemolytic (Figure-3). Gram stain examination of the colonies showed Gram positive bacilli arranged in parallel and v-shaped suggesting Diphtheroids like morphology. The organisms were identified both by MALDITOF MS and Vitek-2. The Histopathological examination (HE) of breast tissue was suggestive of chronic inflammatory mastitis/ Granulomatous mastitis (Figure-4). The clinical details of the patients along with species of *Corynebacterium* are listed in Table-1.

The antimicrobial susceptibility testing showed 100% susceptibility to Amoxicillin-clavulanate, Tetracycline, Vancomycin and Linezolid for all the five isolates

Discussion

Non-lactational breast abscesses are often sub-areolar and are associated with chronically draining sinuses and

Table 1: Clinical, Diagnostic and Management details of patients with non diphtheriae Corynebacteria isolated from Breast tissue/pus samples.

Case no.	Age/ Gender	Clinical details/ Risk factor	HPE	Gram stain	Culture	Treatment	Follow-up
1	35/F	Swelling Right breast, Discharge present from periareolar area present for 8-10 months, Non diabetic	Chronic active Inflammation-Infiltration by inflammatory infiltrate in background of fibrosis, no granulomas, no evidence of malignancy	Many pus cells with Gram positive bacilli	*C. kroppenstedtii*	Drainage and debridement of right breast abscess and dead necrosed tissue with biopsy of underlying tissues Amikacin 1 gm I/V OD and Amoxycillin+ clavulanic acid 1.2 gm I/V BD for 5 days followed by oral Amoxycillin+Clavulanic acid 625 mg BD for 5 days followed by Doxycycline 100 mg BD for 7 days	Right breast lump does not show recurrence. Patient presented with left breast lump after 1 year which was suggestive of inflammatory lesion and possibility of idiopathic granulomatous mastitis was suggested.

2.	55/F	Left breast abscess since last 12 days. History of Right breast abscess 1 year back for which Incision and drainage was done, Diabetic, Hypertension, Depression	Acute on chronic inflammatory pathology with abscess formation. No granulomas seen.	Many pus cells with few Gram positive bacilli	*C.amycolatum*	Incision and drainage of breast abscess followed by Clarithromycin 500 mg BD and Levofloxacin 750 mg OD for six days	No history of recurrence till one year
3.	49Y/F	Heaviness in left breast Retroareolar left breast lump for 3 weeks, Diabetic	Extensive acute on chronic inflammatory cell infiltration, with surrounding areas of fibrosis. Focal areas of histiocytic giant cell reaction.	Many pus cells with Gram positive bacilli	*C.amycolatum*	Incision and drainage and debridement of breast lump followed by administration of Linezolid 600 mg BD for six days	Patient developed non-healing wound for which debridement of necrotic tissue was done followed by administration of Ofloxacin 200 mg BD and Cefuroxime 500 mg BD for 5 days Patient did not develop recurrence till one year
4.	38/F	History of recurrent breast abscess present, Known case of carcinoma breast, Multiple Incision and drainage , On antitubercular therapy (ATT) for more than one year without any clinical response	Dense sheets of mixed inflammatory cells in a dense necrotic background consistent with suppurative inflammation, no malignant cells seen.	Many pus cells and Gram positive bacilli	*C. kroppenstedtii*	Incision and drainage of breast abscess, Trimrthoprim/ Sulfamethoxazole 160mg/800 mg BD and Clarithromycin 500 mg BD for 7 days followed by Doxycycline 100 mg BD for 10 days	No history of recurrence till one yea
5.	26/F	Lump and pain in breast for last 15 days	NA	Many pus cells and Gram positive bacilli	*C. kroppenstedtii*	Incision and drainage of breast abscess, Amoxycillin+clavulanate 625 mg BD followed by Doxycycline 100 mg BD	No history of recurrence till one year

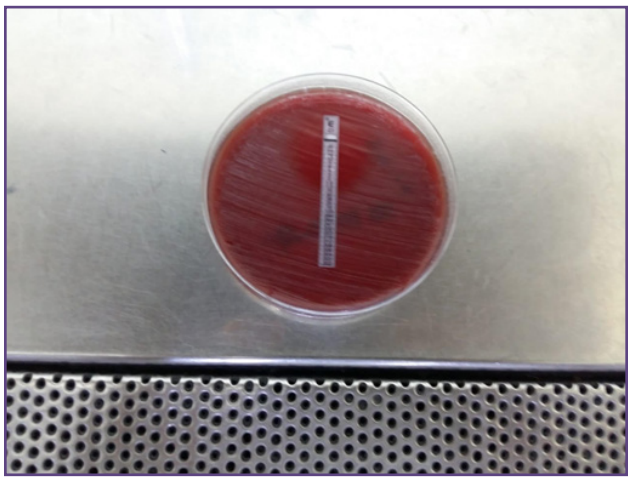

Fig. 1: Antimicrobial susceptibility by E-test method.

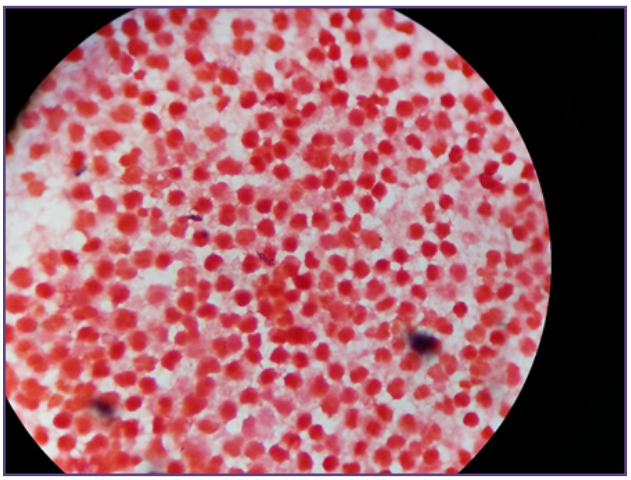

Fig. 2: Gram stain examination (1000X)- Plenty of pus cells with Gram positive bacilli.

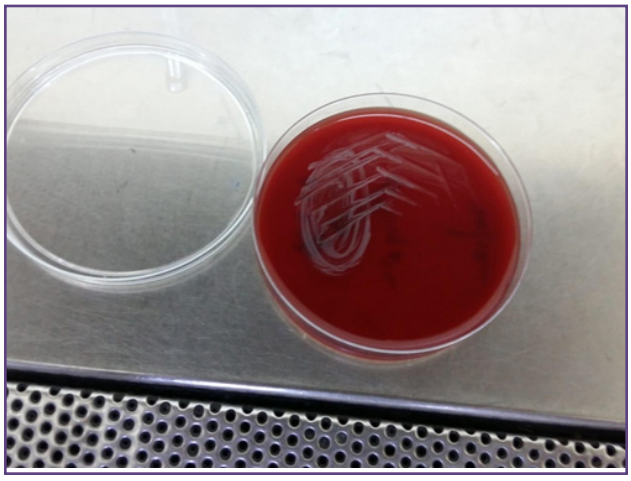

Fig. 3: Columbia Sheep blood agar showing growth of Corynebacteria.

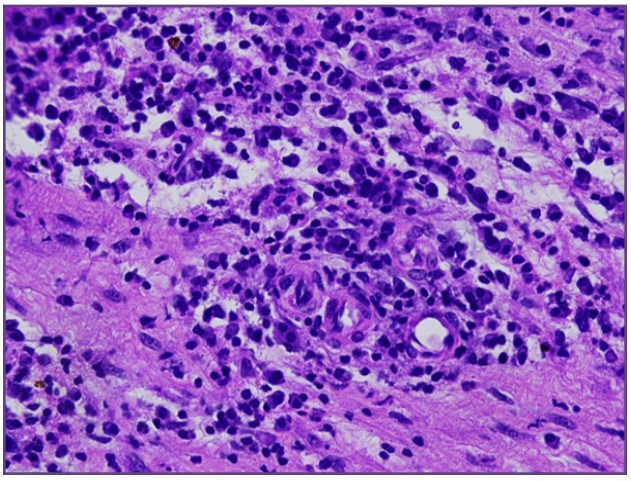

Fig. 4: 400X HE showing lymphocytes, plasma cells, fibroblastic and vascular proliferation.

abscess formation. These abscesses have a chronic course, and have a tendency to form extensive fistulas. Smoking and Diabetes mellitus are significant risk factors for non-lactational abscesses.[2] In our case series, all the five patients were non-lactating females.

The isolation of *Corynebacterium* species from the clinical specimens is usually considered as contamination as it is the normal skin flora. In case of breast abscess, the isolation of *Corynebacterium* species should not be ignored because mammary area is the favourable site for the proliferation of lipophilic *Corynebacteria*.[3] In our case series of breast abscess, *C. kroppenstedtii* was the most common isolate accounting for 60% cases. *C. kroppenstedtii* does not contain mycolic acids and needs lipids for its growth. This is why, the mammary areas, rich in lipids, are favourable to its development and its proliferation.[3] Similar to ours, Kutsuna et al also reported cases of mastitis in females caused mainly by *C. kroppenstedtii*.[4] In the study by Poojary et al, out of the 10 isolates four isolates were identified as *C. kroppenstedtii*; one isolate as C. striatum and five isolates were identified as *C. amycolatum*/*C. xerosis*.[5] Ang LMN et al reported isolation of *C.accolens* from a case of breast abscess with granulomatous mastitis.[6]

In our case series, the histopathological examination of breast abscess correlated very well with findings suggestive of chronic inflammatory mastitis/Granulomatous mastitis. Abdelhadi MS et al also found association of Granulomatous mastitis in 60% cases of non-lactational breast abscess.[1] Chronic inflammatory mastitis/ Granulomatous mastitis have been found to be associated with autoimmunity, trauma, hypersensitivity, breast feeding and microbial

infections.[7] Hyperprolactinemia has been found to be a modifiable risk factor for the development of Breast abscess.[8]

In view of recurrent breast abscess and diagnostic dilemma, most of our patients were investigated for Tuberculosis, fungal and *Nocardia* infections also. Three of our patients were already on antitubercular therapy.

Breast infections due to *Corynebacterium* species may occur in both immunocompetent and immunocompromised patients. In the present study, out of five patients, two were diabetic, one was being treated for malignancy and two did not furnish any significant history of comorbidities.

Idiopathic granulomatous mastitis is a known but usually forgotten clinical entity and may mimic as breast cancer and patient may present as recurrent breast abscess.[9] Amongst microbial infections, infections due to *Corynebacterium* sp. especially *C. kroppenstedtii* has been found to be closely related to pathogenesis of granulomatous mastitis in a metagenomic analysis.[10] Hence, the possibility of Corynebacteria in these cases should always be kept in mind and microbiological studies should always be pursued. Also, *Corynebacterium* species should not be ignored in breast abscess and the definitive identification of *Corynebacterium* species should be done for the proper management of the cases. Rapid and reliable identification techniques like MALDITOF MS thus help in the antimicrobial stewardship by curtailing the use of anti-tubercular and other empirical non-targeted therapy. At the same time, meticulous direct smear examination of Gram stain is very important for further processing of samples. In our case series the isolation of different Corynebacteria from the breast abscess was possible only because of suspicion on Gram stain examination which has resulted in the use of appropriate culture media and incubation conditions. We have earlier reported a case of breast abscess with fistula formation due to *C.amycolatum* at our institute.[11] We believe that in the earlier years we would have isolated Corynebacteria from breast specimens but it would have been considered as of doubtful significance because of non-definitive identification of the isolate. MALDITOF MS has thus played a vital role in understanding the significance of these species from breast pus/tissue specimens.

Corynebacteria survive in lipid filled vacuoles surrounded by a reactive neutrophilic granulomatous infiltrate. Lipophilic agents which have a high volume of distribution, like rifampin, clarithromycin, help to achieve adequate tissue concentrations for bactericidal activity, which are also active in other granulomatous infections, such as mycobacteria.[12] In our study, isolates were sensitive to Tetracyclines, Vancomycin and Linezolid. We suggest use of antimicrobials other than beta lactams like doxycycline/tetracycline, co-trimoxazole or Vancomycin for empirical treatment of atypical breast infections with sincere attempts to seek out lipophilic Corynebacterium as causative organism especially *C. kroppenstedtii*, including prolonged incubation and usage of MALDITOF or other technologies and ruling out other organisms of chronic mastitis, as far as possible by molecular techniques.

We were able to stop ATT in such patients, thus moving towards helping stewardship program

Conclusion

Non-diphtheriae Corynebacteria may be an important cause of non-lactational breast abscess. Non diphtheriae Corynebacteria particularly lipophilic Corynebacteria have a predilection to cause infections of breast tissue and Breast abscess which may mimic as tubercular abscess or chronic non-specific mastitis leading to unnecessary usage of antimicrobials. So, Non diphtheriae Corynebacteria isolated from breast tissue/pus specimens should not be ignored rather definitely identified. Clinical microbiology laboratories can identify organisms to genus and species level accurately, with advanced technologies like MALDI-TOF. This would also ensure diagnostic stewardship .

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Competing interest

None

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